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A quantitative evaluation of health care system in US, China and Sweden

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Abstract

This study is mainly aimed at evaluating the effectiveness of current health care systems of several representative countries and improving that of the US. To achieve these goals, a people-oriented non-linear evaluation model is designed. It comprises one major evaluation metric and four minor metrics. The major metric is constituted by combining possible factors that most significantly determine or affect the life expectancy of people in this country. The four minor metrics evaluate less important aspects of health care systems and are subordinate to the major one. The authors rank some of the health care systems in the world according to the major metric and detect problems in them with the help of minor ones. It is concluded that the health care system of Sweden scores higher than the US and China's system scores lower than that of the US. Especially, the health care system of US lags behind a little bit compared with its economic power. At last, it is reasonable for the American government to optimize the arrangement of funding base on the result of goal programming model.

Key words: Health care system, evaluation.

Introduction

A satisfactory health care system of one country is supposed to provide its residents with effective health care, so that a majority of citizens can enjoy a security and high-quality life, with maximized social equality and minimized total medical expenditure. The complexity of the health care systems makes it difficult to evaluating the health care system by taking into account only a few factors.

The considerations of previous research tend to emphasize the financial efficiency of health care systems. Controlling of the cost has been reported as the key factor of this system [1,2]. However, health care system is slightly different from financial system [3,4]. Medical care is a necessity rather than a commodity for citizens of a country. The quality of medical care of patients is much more important than controlling of cost in health care system [5]. In health care system, health insurance covers most large medical expenses, but there is no institution in a financial system would insurance the high consumption. Thus, we developed a people-oriented comprehensive evaluation system and pay more attention to make sure quality of health care of people. In this evaluation system, the quality of health care to the patient has a higher priority rather than the financial efficiency.

In addition, previous researchers fail to consider the relationship between health care system and medical research institution [1-3]. The health care system is heavily influenced by the development of biomedical research. The investment to the medical research institution can improve the operational efficiency of the health care system, discover new drugs, revise the therapies, and improve the life quality of patients. It is no doubt that increase of investments on medical research has positive impact on health care system in developed country. However, for a developing country, it seems reasonable to pay more attention on other part of health care system such as development medical insurance system, building new hospitals and medical education [6].

Several studies described the qualitative analysis of patient satisfactory of health care and

government investment to medical system [7, 8]. However, since there is a complex non-linear relationship between increase of government investment and improvement patient satisfactory of health care system[9,10,11], it is hard to optimize the amount of investment to current health care system; a quantitative analysis model is highly desired. In this study, we are going to conduct a quantitative model to evaluate the health care system of the United States, Sweden, and China. We will also develop a goal programming model to evaluate the best government funding allocation to health care system.

Definitions and Key Terms

A *health care system* is the organization and the method by which health care is provided.

The *potential of health care* (P_{hc}) of a country shows the power of medical researches and development supported by the government at the present time. It is positively correlated with the size of medical research staff and the quantity of funding from the government.

The health of a citizen is *perfectly ensured*, if his/her income plus aid from the government (plus the financial compensation from medical insurance system if he/she is covered by it) is large enough to prevent and/or cure diseases, ignoring the irreversible damage to health that is beyond the ability of current medical technology.

The *quality index of life* represents the relief from possible diseases or accidentally physical injury based on economic aid offered by medical insurance, and a certain quantity of medical resources provided by current health care system, as well as scientific potential of medicine realized by government-funding researches. This index can approximately imply the quality of life.

The *life expectancy* represents the average life span of a newborn and is an indicator of the overall health of the population of a country.

Practical effect of medical resources is the quantity of all categories (medical doctors, nurses, beds) of medical aid that is practically distributed to each citizen in a country on average.

The medical care resources are divided into *essential health care* and *complementary health care resources*. *Complementary care* is the kind of services that offer holistic benefits that complement or enhance the health care received from the physicians or hospital, and essential health care embraces all the other kinds.

The *matching degree* of a health care system in a country measures whether the system is massive enough to keep up with the development of the country. The health of a citizen is *perfectly ensured*, if his/her income plus aid from the government (plus the financial compensation from medical insurance system if he/she is covered by it) is large enough to prevent and/or cure diseases, ignoring the irreversible damage to health that is beyond the ability of current medical technology.

The *fairness index* represents how well a health care system distributes its resources to everyone who needs it, both rich and poor, urban and rural residents.

The *life index* of a nation is a general and comprehensive figure that describes how much life of high quality is enjoyed by all the citizens in one country. It is positively correlated with *quality index of life* and average *life expectancy.*

Universal health care refers to delivery by a combination of public and private systems. In most cases, the law says that everyone must have access to health care. Germany and Sweden, for example, has universal coverage, and social insurance plans cover the majority of people. Symbols are listed in Table 1.

Assumptions

People around the world have the same susceptibility to diseases, whichever country they are in.

- Medical personnel and scientific researchers are all competent for their job.
- The per capita GDP of one country can denote how rich and developed the country is.
- Every health care system possesses approximately equal ability of emergency management.
- Every type of disease occurs to people in all countries with the same possibility.
- If a resident is covered by the health care insurance, he/she will be able to afford his/her medical expenditure.
- The investment into scientific medical researches is always effectively used.
- The investment into science researches will pay off (be transformed into applied technology) 25 years later averagely.

Table 1. Symbols of evaluation model

Symbols	Definitions & Descriptions
L_{index}	Life index
E_{life}	Life expectancy
Q_{life}	Life quality index
P_{mr}	Practical effect of medical resources
P_{ei}	Perfect ensurence index
P_{tech}	The current power of medical technology
P_{hc}	Potential of health care
R_e	Essential health care resources
R_c	Complementary health care resources
D_{un}	Unnecessary degree
D_{ne}	Necessity degree
N_{in}	Number of residents who are covered by medical insurance
k_{gov}	The proportion of government reimbursement in medical expense
$X_{med,i}$	One's medical expenditure which is submitted to Poisson distribution
$X_{inc,i}$	one's net income which is submitted to normal probability distribution
E_e	Average essential expenditure to maintain everyday life
$X_{inc,i}$	one's income which is submitted to normal probability attribution
$N_s(t)$	Number of medical researchers
$M_s(t)$	Quantity of funding going to medical research
τ	Time delay

Model Design

The Major Evaluation Metric
General Analysis

Since the service object of the health care system is people, we perceive that the evaluation metric should reflect how well the length and quality of people's life are guaranteed by the system through providing health care, which is represented by a general concept called *life index*. Then we decompose *life index* into two parts that are mutually independent: *quality index of life* and *life expectancy*, which measure the quality and quantity of residents' life, respectively. We whereafter keep breaking down *quality index of life* into concrete concepts and simple factors. In so doing, the evaluation system model is concretized and operationalized, because: 1) *life index* is quantified and hence computable; 2) it is easier to search and identify associated sources of data.

As we attach great emphasis on the practical effectiveness of health care systems, the *life index* (L_{index}) is the final metric that decides whether a health care system is good or not. According to our definition, we have:

$$L_{index} = Q_{life} \times E_{life}\ , \quad \text{..... (1)}$$

where

Q_{life} is standardized life quality index, and E_{life} is the average life expectancy of the population in one country.

E_{life} of countries in the world, as a basic and useful kind of data, can be easily found from more than one reliable sources of information, but Q_{life} is comparatively abstract and complicated to measure.

Since it is unreasonable to limit E_{life} to a fixed range, L_{index} is not standardized here.

Obviously, a health care system can help promote Q_{life} in many different ways, but we notice

that almost every way is realized through one of the following three channels:

I every country organizes and provides health care resources to its citizens;
II medical insurance and government offer economic aid so that the patients have access to medical care service; and
III the government invests money into medical researches so that we will have more advanced medical technology that can cure the currently incurable diseases and prevent unpredictable diseases in the future.

Using three corresponding variables P_{mr}, P_{ei} and P_{tech} to measure the effectiveness of the above three channels, we find that Q_{life} is positively correlated with each one of them. Therefore, it is reasonable to define:

$$Q_{life}=(P_{mr}+P_{ei}+P_{tech})/k_q, \quad \text{..... (2)}$$

where

P_{mr} is the *practical effect of medical resources*,

P_{ei} is *perfect ensurence index*,

P_{tech} is the current power of medical technology (all of them are standardized indexes), and k_q is a coefficient to standardize Q_{life}.

To get Q_{life}, we have to obtain the value of P_{mr}, P_{ei} and P_{tech} one by one.

Quantify and Calculate P_{mr}

Since the medical care resources are divided into essential health care and complementary health care resources, P_{mr} should be broken down into two corresponding parts: the practical effect of essential health care and that of complementary health care. Thus, we get

$$P_{mr}=\prod_{i=1}^{3}\frac{R_{e,i}}{R_{e,i}+k_{e,i}}+\prod_{i=1}^{3}\frac{R_{c,i}}{R_{c,i}+k_{c,i}}, \quad \text{..... (3)}$$

Where R_1, R_2 and R_3 respectively refer to the number of medical doctors, nurses and the beds in hospitals,

R_e is a standardized index that denote the essential health care resources,

R_c is a standardized index that denote the complementary health care resources,

$k_{e,i}$ and $k_{c,i}$ are empirical coefficients, and the relationship among them is expressed by

$$R_{e,i}\times k_{e,i}=R_{c,i}\times k_{c,i}$$

Note that:

when $R_{e,i}\to 0$, we have

$$\frac{R_{e,i}}{R_{e,i}+k_{e,i}}\to\frac{1}{k_{e,i}}R_{e,i},$$

which means the practical effect of medical resources is decided by the quantity of medical resources completely (directly proportional to it);

when $R_{e,i}\to\infty$, we get $\frac{R_{e,i}}{R_{e,i}+k_{e,i}}\to 1$,

which means excessive medical resources contributes little and will cause a great waste;

we multiply the monomial $\frac{R_i}{R_i+k_i}$ (i=1, 2, 3)

because the lack of any one of R_i will bring serious difficulty to any health care system.

Calculate P_{ei}

The population of one country can be divided into two categories: those who are covered by medical care insurance (N_{in}) and those who are not (N_{un}). So the proportion of insured people (P_{insure}) is given by

$$P_{insure}=\frac{N_{in}}{population}=\frac{N_{in}}{N_{in}+N_{un}}, \quad \text{..... (4)}$$

The perfect ensurence index (P_{ei}) actually measure how many people have their health well ensured through either joining health care insurance or paying by their sufficient income. Thus, it is reasonable to finally define P_{ei} as

$$P_{ei}=1-P_{uninsure}\times\frac{total\ medical\ expenditure\ shortage}{total\ medical\ expenditure}, \quad \text{..... (5)}$$

which in fact is

$$P_{ei}=1-(1-P_{insure})\times\frac{\sum_{i=1}^{N_{un}}\left[X_{med,i}\times(1-k_{gov})-\left(X_{inc,i}-E_e\right)\right]\times I_A}{\sum_{i=1}^{n_{Au}}X_{med,i}\times(1-k_{gov})},$$

$$I_A=\begin{cases}1 & ,i\in A\\ 0 & ,i\notin A\end{cases},$$

$$A=\left\{X_{M,i},X_{inc,i}\,\middle|\,X_{M,i}\times(1-k_{gov})>\left(X_{inc,i}-E_e\right)\right\},$$

..... (6)

where

k_{gov} is the proportion of government reimbursement in medical expense,

E_e is the average essential expenditure to maintain everyday life,

$X_{med,i}$ is the medical expenditure of someone in the country, which is submitted to Poisson distribution, and

$X_{inc,i}$ is the net income of someone in the country which is submitted to normal distribution.

Calculate P_{tech}

The current power of medical technology (P_{tech}) can be well deduced by the potential of health care (P_{hc}) years ago, which means P_{tech} can be estimated as P_{hc} with a time delay (τ), because it takes a period of time to transfer scientific investment into scientific products. Some scholars believe that τ=20–30 years and we make it 25 [6] years here.

Firstly, we calculate P_{hc} based on its definition:

$$P_{hc}=\frac{N_s(t)}{k_N+N_s(t)}\times\frac{M_s(t)}{k_M+M_s(t)} \quad \text{..... (7)}$$

where

$N_s(t)$ is the number of medical researchers, and

$M_s(t)$ is the quantity of funding going to medical research.

Note that both of the two factors, medical researchers and money, can enhance P_{hc}, but excessive investment (medical researchers and money) gives only limited effect to P_{hc}. This truth supports our idea to define P_{hc} this way.

Secondly, we incorporate τ into P_{hc} to get P_{tech}

$$P_{tech}=\frac{N_s(t-\tau)}{k_N+N_s(t-\tau)}\times\frac{M_s(t-\tau)}{k_M+M_s(t-\tau)} \quad \text{..... (8)}$$

Medical research plays an important role to improve the math expectation of residents' life in future.

$$E_{life}=E_0+k_{lt}\times P_{hc} \quad \text{..... (9)}$$

where E_0, k_{lt} are coefficients.

With Eq (1), (2), (3), (4), (6), and (8), L_{index} can be expressed by a complicated equation which involves a series of variables.

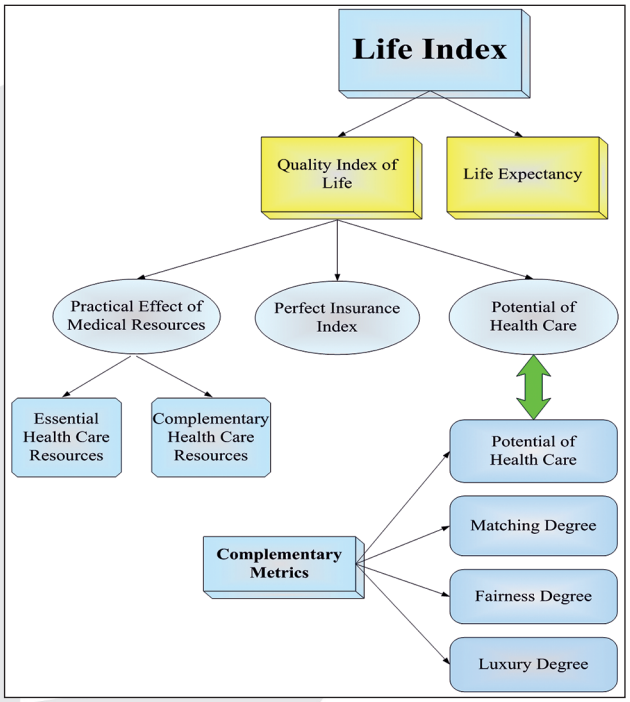

Figure 1. The diagram showing the organization of evaluation of health care system

Subordinate Metrics

Potential of Health Care (P_{hc})

Eq(7) gives the expression of P_{hc} which is actually a standardized index that predicts the power of medical technology (P_{tech}) in the future.

Matching Degree

To get *matching degree* of each health care system, two factors need to be taken into account: the how well residents' health is ensured and how wealthy the country is. A rich country has the ability to maintain a large scale of health care system that provide abandon health care resources, while a developing country can only afford a smaller and cheaper one. This fact implies that it is harder for a developed country to maintain a matching health care system, because the country has to invest more (money, etc) into its health care system. Therefore, *matching degree* is given by

$$Matching\ Degree=\frac{10}{\ln(per\ capita\ GDP)-\ln(L_{index})} \quad (10)$$

where

R_e is the essential health care resources,

R_c is the complementary health care resources, and

Per capita GDP is per capital Gross Domestic Product.

Fairness Degree

The attribution of medical resources cannot possibly be absolutely fair. We tend to believe that wealthy people have more chance to accept medical aid then the poor. Here we compare urban people with those living in rural areas by measuring the quantities of health care resources attributed to them respectively.

$$Fairness\ Degree = \frac{rural\ medical\ resouces}{urban\ medical\ resouces} \quad ..(11)$$

Luxury Degree

Considering some parts of a health care system may not play the most essential role or cannot bring immediate benefits to the residents, we define Complementary health care resources and potential of health care to be "unnecessary", while essential health care resources and perfect ensurence index to be "necessary". Thus, if we continue to *define unnecessity degree* (D_{un}) and *necessary degree* (D_{ne}) as:

$$D_{un} = R_c + P_{hc} \quad ..... (12)$$

$$D_{ne} = R_e + P_{ei} \quad ..... (13)$$

we will arrive at:

$$Luxury\ Index = \frac{D_{un}}{D_{ne} + D_{un}} \quad ..... (14)$$

Revise the spending plan for US

Although the health care system of US ranked considerably high in the world, is still far from ideal. In this part, we try to revise the previous model to optimize the health care system of US to give more detailed suggestions, as we realize that improving such a complicated system requires further investigation.

Suppose the government has already given it the funding shortage (the health care system of U.S. needs extra 300 billion dollars to push its *matching degree* to as high as Sweden's) and therefore the total budget expands, how shall the health care system spend the extra 300 billion dollars wisely? We argue that a wise spending plan should maximize the *life index*. As we have stated above, *life index* is positively correlated with *quality index of life* and average *life expectancy*. However, *life expectancy* varies very slightly as time elapses, hence we prescribe that an ideal spending plan is the one that maximizes *quality index of life*. Now our aim is to revise the previous model to solve this non-linear programming problem.

We identify nine symbols representing nine major expenditures in Table 2:

Table 2. Symbols representing major expenditures

Symbols	Definition
$F_{gov,\ 1}$	Economic aid to patients
$F_{gov,\ 2}$	Salary of research staff
$F_{gov,\ 3}$	Funding to support medical researches
$F_{gov,4}$	Salary of medical doctors in essential medical source
$F_{gov,5}$	Salary of medical nurses in essential medical source
$F_{gov,6}$	Equipment in essential medical source
$F_{gov,7}$	Salary of medical doctors in complementary medical source
$F_{gov,8}$	Salary of medical nurses in complementary medical source
$F_{gov,9}$	Equipment in complementary medical source

The Objective function is

$$Max\ L_{index} = (P_{ei} + P_{mr} + P_{hc}) \times (E_0 + k_{lt} \times P_{hc})/k_q \quad ..... (15)$$

$$subject\ to \sum_{j=1}^{9} F_{gov,j} = F_{total}$$

where

$$P_{ei} = 1 + \left(\frac{F_{income}}{F_{med} - F_{gov,1}} - 1 \right) \times P_{uninsure} EI_A$$

$$P_{hc} = \frac{F_{gov,2}}{k_N S_{salary} + F_{gov,2}} \times \frac{F_{gov,3}}{k_M + F_{gov,3}}$$

$$P_{mr} = \prod_{i=1}^{3} \frac{F_{gov,i+3}}{F_{gov,i+3} + n_{e,1} k_{e,1}} + \prod_{i=1}^{3} \frac{F_{gov,i+6}}{F_{gov,i+6} + n_{c,1} k_{c,1}}$$

The process of deduction shown as following:

$$P_{ei} = 1 - P_{uninsure} \times \frac{\sum_{i=1}^{n_{Au}} (X_{M,i} \times (1 - k_{gov}) - X_{F,i}) \times I_A}{\sum_{i=1}^{n_{Au}} X_{M,i} \times (1 - k_{gov})}$$

$$\Rightarrow P_{ei} = 1 - P_{uninsure} \times \frac{EI_A \times \left[n_{Au} \times EX_{M,i} \times (1 - k_{gov}) - n_{Au} \times EX_{F,i} \right]}{n_{Au} \times EX_{M,i} \times (1 - k_{gov})}$$

$$\Rightarrow P_{ei} = 1 - P_{uninsure} \times \frac{EI_A \times \left[EX_{M,i} \times (1 - k_{gov}) - EX_{F,i} \right]}{EX_{M,i} \times (1 - k_{gov})}$$

$$\Rightarrow P_{ei} = 1 - P_{uninsure} \times \frac{EI_A \times \left[EX_{M,i} \times (1 - k_{gov}) - EX_{F,i} \right]}{EX_{M,i} \times (1 - k_{gov})}$$

$$\Rightarrow P_{ei} = 1 - P_{uninsure} \times EI_A \left[1 - \frac{EX_{F,i}}{EX_{M,i} \times (1 - k_{gov})} \right]$$

$$\Rightarrow P_{ei} = 1 - P_{uninsure} EI_A \left[1 - \frac{}{(1 \quad \frac{gov,1}{med})} \right]$$

$$\Rightarrow P_{ei} = \quad - \left[P_{uninsure} EI_A - \frac{u P_{uninsure} EI_A}{\times (1 - \frac{gov,1}{med})} \right]$$

$$\Rightarrow P_{ei} = \quad - \left[P_{uninsure} EI_A - \frac{n_{Au} \times u P_{uninsure} EI_A F_{med}}{n_{Au} \times \quad \times (F_{med} - F_{gov,1})} \right]$$

$$\Rightarrow P_{ei} = \quad - P_{uninsure} EI_A + \frac{{}_u u P_{uninsure} EI_A F_{med}}{n_{Au} \quad (F_{med} \quad F_{gov,1})}$$

$$\Rightarrow P_{ei} = \quad - P_{uninsure} EI_A + \frac{F_{income} P_{uninsure} EI_A F_{med}}{n_{Au} \quad (F_{med} \quad F_{gov,1})}$$

$F_{med} \quad n_{Au}$

$$\therefore P_{ei} = 1 - P_{uninsure} EI_A + \frac{F_{income} P_{uninsure} EI_A}{F_{med} - F_{gov,1}}$$

$$P_{mr} = \prod_{i=1}^{3} \frac{R_{e,i}}{R_{e,i} + k_{e,i}} + \prod_{i=1}^{3} \frac{R_{c,i}}{R_{c,i} + k_{c,i}}$$

$$\Rightarrow P_{mr} = \prod_{i=1}^{3} \frac{\frac{F_{gov,i+3}}{n_{e,1}}}{\frac{F_{gov,i+3}}{n_{e,1}} + k_{e,1}} + \prod_{i=1}^{3} \frac{\frac{F_{gov,i+6}}{n_{c,1}}}{\frac{F_{gov,i+6}}{n_{c,1}} + k_{c,1}}$$

$$\Rightarrow P_{mr} = \prod_{i=1}^{3} \frac{F_{gov,i+3}}{F_{gov,i+3} + n_{e,1} k_{e,1}} + \prod_{i=1}^{3} \frac{F_{gov,i+6}}{F_{gov,i+6} + n_{c,1} k_{c,1}}$$

$$P_{hc} = \frac{N_s}{k_N + N_s} \times \frac{M_s}{k_M + M_s}$$

$$\Rightarrow P_{hc} = \frac{\frac{F_{gov,2}}{S_{salary}}}{k_N + \frac{F_{gov,2}}{S_{salary}}} \times \frac{F_{gov,3}}{k_M + F_{gov,3}}$$

$$\Rightarrow P_{hc} = \frac{F_{gov,2}}{k_N \times S_{salary} + F_{gov,2}} \times \frac{F_{gov,3}}{k_M + F_{gov,3}}$$

$\Rightarrow$

$$\max \quad L_{index} = \left(P_{ei} + P_{mr} + P_{hc} \right) \times (E_0 + k_{lt} \times P_{hc}) \Big/ k_q$$

$$\begin{cases} P_{ei} = 1 + \left(\dfrac{F_{income}}{F_{med} - F_{gov,1}} - 1 \right) \times P_{uninsure} EI_A \\ P_{hc} = \dfrac{F_{gov,2}}{k_N S_{salary} + F_{gov,2}} \times \dfrac{F_{gov,3}}{k_M + F_{gov,3}} \\ P_{mr} = \displaystyle\prod_{i=1}^{3} \dfrac{F_{gov,i+3}}{F_{gov,i+3} + n_{e,1} k_{e,1}} + \prod_{i=1}^{3} \dfrac{F_{gov,i+6}}{F_{gov,i+6} + n_{c,1} k_{c,1}} \end{cases}$$

$$subject \ to \quad \sum_{j=1}^{9} F_{gov,j} = F_{total}$$

Results and Discussion

Compare the Effectiveness of Health Care Systems

We had the ability to express Q_{life} with variables that are supported by sufficient data. Figure 2 shows the scores of America, China and Sweden in P_{mr}, P_{ei} and P_{tech}. Note that the perfect ensurence index of Sweden is 1 (the largest possible value), because, the Sweden has a universal health care system that

impose medical insurance to every citizen. Moreover, the power of medical science exceeds that of Sweden, which, we perceive, is an inevitable result of large investment into it in the past several decades.

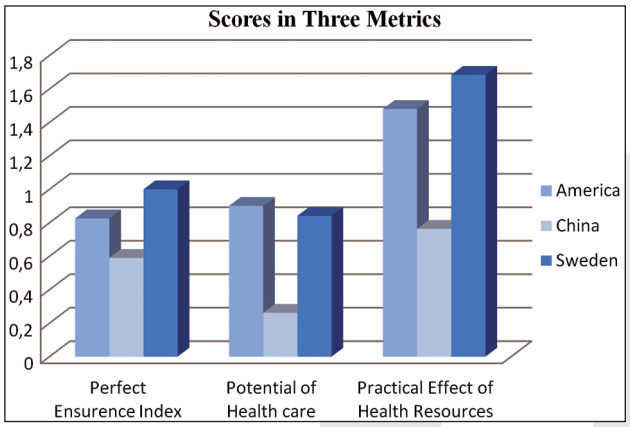

Figure 2. Three countries' scores of P_{mr}, P_{ei} and P_{tech}

Figure 3 provides the comparison of life indexes of US, China and Sweden. Very clearly, the health care system of Sweden is the best among them and the system of China lags behind extremely.

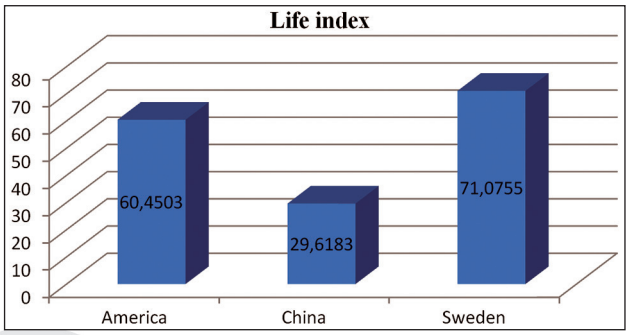

Figure 3. The current life index of U.S., China and Sweden

The analysis of life index of US

Figure 4 shows the life index of US and the change of P_{mr}, P_{ei} and P_{tech} from 1990 to 2008; all of the four variables were roughly increasing except for a bit of ramp-down at the end of the 20th century.

The perfect insurance index fluctuates with time, but the general trend is rising, presumably pushed by the development of domestic economy. Since the year 1992, the index rose but dropped

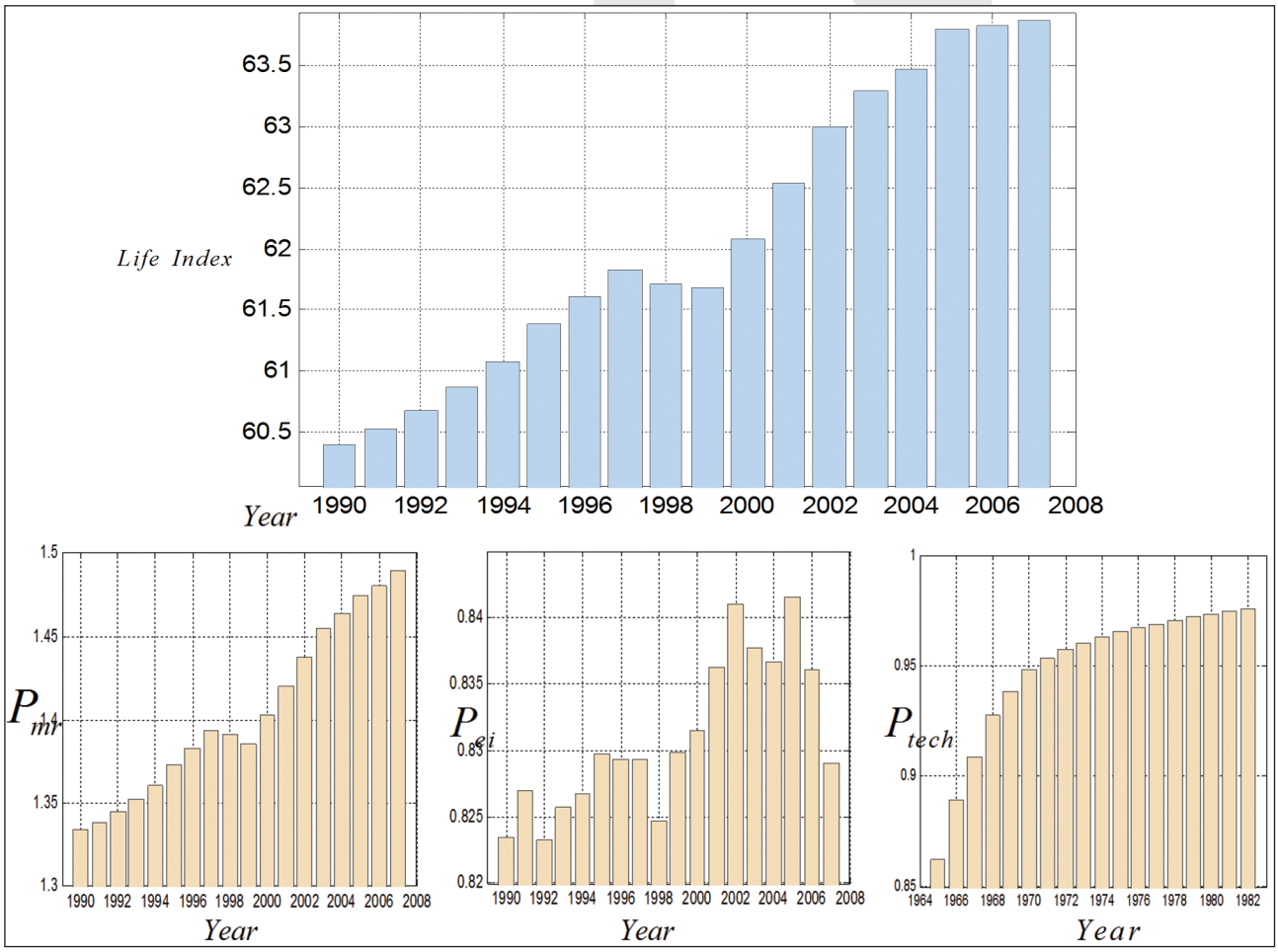

Figure 4. The history trend of life index, P_{mr}, P_{ei} and P_{tech} of US

drastically in 1998, [14] which is a rather puzzling phenomenon. If we take a look back on history of America, we will find that in the 1993, President Clinton issued a new policy about medical insurance aiming at popularizing medical insurance so that every citizen is covered[16]; in 1998, [15] he declared that this policy was suddenly ceased because of some reason. The data coincide with historic changes amazingly.

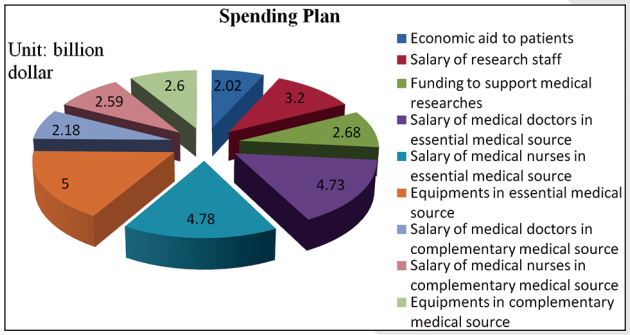

Figure 5. Revised spending plan of US by greedy algorithm

Analyses of Subordinate Metrics

With the most general metric *life index,* we are able to evaluate and rank the health care systems in the world (e.g. the health care system of US is better than that of China but not as good as that of Sweden), but a considerable amount of information is lost or neglected at the same time, which will bring much difficulty in identifying exiting limitations and problems with these health care systems. In order to crack this, we pick out and rearrange some factors to constitute new metrics as complementary metrics (potential of health care, matching degree, fairness degree, & luxury degree). With the help of these complementary metrics, different aspects of one health care system can be evaluated and its limitations become detectable and predictable.

A low *matching degree* may suggest the necessity of investing more money into the health care system so that its scale can be enlarged, while a high one implies that the current health care system is massive enough considering the limited economy scale. Figure 7 implies that the health care system of U.S. should be stronger to match the massive scale of its economy [9]. If we want the matching degree of U.S. to be promoted to 1.66, the government must invest more money into health care system [10]. The *matching degree* implies a slight lack of government investment into health care system [11].

Since it is difficult to obtain all the data to decide their precise quantities, we consider it to be feasible to substitute them with numbers of beds in hospitals in urban and rural areas [12, 13]. The information delivered by Figure 8 is clear: China did a very poor job in health care fairness while that of US could be better.

We consider *luxury index* to be tolerant of subtle conceptual ambiguity, because the slight lack of preciseness in defining concepts may weaken its competence in give an absolute evaluation, but still allows it to serve as a metric to compare different health care systems. (Note that the word 'unnecessary' doesn't mean 'redundant'.)

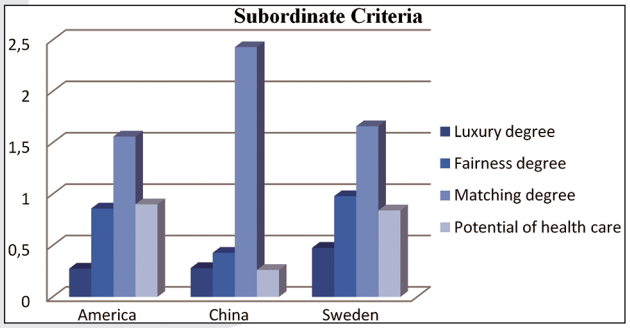

Figure 6. All the Subordinate metrics

It is never easy to give a complicated system properly and a precise evaluation [17], as the result is connected with multiple factors that are interwoven with each other [18]. However, if we establish a model that based on reasonable assumptions and tolerate a certain degree of ambiguity, satisfactory result could be achieved [19]. On the other hand, limitations of our model also mainly originate from the assumptions and ambiguity [20-23].

It is well admitted that few things are perfect in the world, whereas we never stop pursuing ideal health care systems, even though it takes a lot of money, manpower, time and energy to improve, because they are our safe guard that relieve our fear of diseases [24-31].

References

1. *Perleth M, Jakubowski E, Busse R, What is 'best practice' in health care? State of the art and perspectives in improving the effectiveness and efficiency of the European health care systems. Health Policy, 2001(56), 235-250*

2. *Ros CC, Groenewegen PP, Delnoij DM, All rights reserved, or can we just copy? Cost sharing arrangements and characteristics of health care systems, Health Policy, 2000(52), 1-13*

3. *Abelson J, Miller FA, Giacomini M, What does it mean to trust a health system?: A qualitative study of Canadian health care values, Health Policy 2009(91), 63-70*

4. *Calnan M, Towards a conceptual framework of lay evaluation of health care, Social Science & Medicine, 1988(27), 927-933*

5. *Petersen I, Swartz L, Primary health care in the era of HIV/AIDS. Some implications for health systems reform, Social Science & Medicine, 2002(55), 1005-1013*

6. *Chang C, Zhang Y, Deng D, Xiao Y, A comprehensive evaluation model of health care system, Networking and Information Technology (ICNIT), 2010 International Conference, 535 -539*

7. *K. Claxton The irrelevance of inference: a decision-making approach to the stochastic evaluation of health care technologies, J Health Econ. 1999(18), PP. 341-64.*

8. *Yang T, Matthews SA, Understanding the non-stationary associations between distrust of the health care system, health conditions, and self-rated health in the elderly: A geographically weighted regression approach, Health & Place, 2012(18), 576-585*

9. *Smith PC, Stepan A, Valdmanis V, Verheyen P, Principal-agent problems in health care systems: an international perspective, Health Policy. 1997(41), 37-60*

10. *Pons-Vigués M, Puigpinós-Riera R, Rodríguez D, Sanmamed M J., Pasarín MI, Pérez G, Borrell C, Casamitjana M, Benet J, Country of origin and prevention of breast cancer: Beliefs, knowledge and barriers, Health & Place, 2012(18), 1270-1281*

11. *Hollander MJ, Miller JA, Kadlec H, Evaluation of Healthcare Services: Asking the Right Questions to Develop New Policy and Program-Relevant Knowledge for Decision-Making, Healthcare Quarterly, 2010(4), 40-47*

12. *Oliveira MD et al, Modeling hospital costs to produce evidence for policies that promote equity and efficiency, European Journal of Operational Research, 2008(16), 933-947*

13. *Rijsbergen MV et al. Managing the overflow of intensive care patients. European Journal of Operational Research, 2008 (16), 988-1010*

14. *B.X. Qin, Bill Clinton's health care reform. American Research 1994, 7-8*

15. *Congressional Quarterly, Health Care's Hour, 1993, 19-20*

16. *Congressional Quarterly, 1993, 2458-2459*

17. *Wang Q, Liu Y, Mo L, The evaluation and prediction of the effect of AIDS therapy, Proceeding of IEEE/ ICME International Conference, 2007, 1591- 1596*

18. *Wang Q, Liu Y, Pan X, Atmosphere pollutants and mortality rate of respiratory diseases in Beijing, Science of the Total Environment, 2008(391), 143-148*

19. *Wang Q, Liu Y, Zhang B, Economic strategies in the issue of controlling AIDS, Proceeding of IEEE/ ICME International Conference, 2007, 1601- 1608*

20. *Shmueli A, Israelis evaluate their health care system before and after the introduction of the national health insurance law, Health Policy, 2003(63), 279-287*

21. *Kiil A, What characterises the privately insured in universal health care systems? A review of the empirical evidence, Health Policy, 2012(106), 60-75*

22. *Wensing M, Baker R, Szecsenyi J, Grol R, On behalf of the EUROPEP Group, Impact of national health care systems on patient evaluations of general practice in Europe, Health Policy, 2004(68), 353-357*

23. *Gu Xing-Yuan, Tang Sheng-Lan, Reform of the Chinese health care financing system, Health Policy, 1995(32), 181-191*

24. *Yaesoubi R, Roberts SD, Payment contracts in a preventive health care system: A perspective from Operations Management, Journal of Health Economics, 2011(30), 1188-1196*

25. *Avgerinos ED, Koupidis SA, Filippou DK, Impact of the European Union enlargement on health professionals and health care systems, Health Policy, 2004(69), 403-408*

26. *Zu H, Wang Q, Dong M, Ma L, Yin L, Yang Y, Compressed Sensing Based Fixed-Point DCT Image Encoding, Advances in Computational Mathematics and its Applications, 2012(2), 237-240*

27. *Wang Q, Li M, Xia LC, Wen G, Zu H, Gao M(2013), Genetic Analysis about Differentiation of Helper T Lymphocytes, Genetics and Molecular Research, in press*

28. *Xia L, Zhou C, Phase transition in sequence unique reconstruction, Journal of Systems Science and Complexity 2007(20), 18-29*

29. *Zhang SW, Li YJ, Xia L, Pan Q, PPLook: an automated data mining tool for protein-protein interaction, BMC bioinformatics 2011(11), 326*

30. *He PA, Xia L, Oligonucleotide profiling for discriminating bacteria in bacterial communities, Combinatorial Chemistry & High Throughput Screening 2007(10), 247-255*

31. *Steele JA, Countway PD, Xia L, et al., Marine bacterial, archaeal and protistan association networks reveal ecological linkages, The ISME Journal 2011(5), 1414-1425.*

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An analysis of 263 female patients exposed to physical violence that admitted to a hospital emergency department in Turkey

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Abstract

Violence against women is a continuing social issue and there is a need to implement ways of reducing this problem. In our study, the demographic characteristics and trauma zones of women who attended the emergency department of a hospital in Turkey were investigated together with whether the women had received any psychiatric support. 263 of 646 women who were the victims of physical violence attended the Emergency Department of Dişkapi Yildirim Beyazit Training and Research Hospital from 2009 to 2010. The women were contacted by telephone invited to participate in the research. Violence against women is seen most frequently in the 20-40 age group. It is seen from this study that the most common physical violence against women mostly occurs among those who have low levels of education and income, are married and have children. It was also found that 7.6 % of patients had received psychiatric support after being subjected to violence. When the educational level increases, the rate of receiving psychiatric support increases. In terms of violence against women, emergency physicians and also wider society should be informed about the extent of the problem, second there is a need for more training in the recognition of violence to women. In addition the reasons for this violence and ways of resolving the problem should be further investigated.

Key words: Violence against women, emergency department, domestic violence, women, physical violence

Introduction

In 1993, the defines violence against women as "Taking a sexist action that gives or may give physical, sexual or psychological pain or suffering or threatening or enforcing with these kind of actions or withholding from freedom arbitrarily whether publicly or privately" [1]. In Turkey, it is known that 35% of married women and 40% of women living in the Eastern Anatolian Region are exposed to physical violence by their male partners at least once in their lifetime [2].

Although interestingly research in foreign countries, intimate partner violence ratios between men and women seems to be closer, although it appears that psychologically and physically women suffer more from violence [3]. Domestic violence is one of main causes of women suffering injury and can result in their death [4]. The resulting absence from work and increase in medical care constitutes an economic loss. Thus, violence against women is accepted as a public health issue [4].

When women who are abused present to health care providers, the women often will not admit to being subject to physical violence and will have symptoms of somatization such as fatigue, headache, chest pain, gastrointestinal complaints, shortness of breath or pelvic pain. Frequently, medical practitioners will not associate these complaints with the fact that the women have been subjected violence this results in this violence to women being misdiagnosed unregistered and consequently it is hidden [5].

The 24 hour accessibility of an Emergency Department (ED) means that they are the primary point of initial contact for women who have been subjected to physical violence will attend [6]. In this study, demographic characteristics, trauma zones and psychiatric aid status of women who had been subjected to physical violence and attended ED are analyzed and discussed.

Material and method

This is a retrospective, cross sectional descriptive study concerned with the records of 263 from

a total of 646 women who were the victims of physical violence and attended the ED of Dişkapi Yildirim Beyazit Training and Research Hospital from 2009 to 2010. After an attempt to contact all 646 women by telephone 263 women over the age of 18 volunteered to participate in this research. Approval from the ethics committee was obtained.

The following characteristics were investigated. The trauma regions and whether the women had received psychiatric help. The income levels of women were also analyzed by used the classification of income levels distribution in Turkey. According to this classification, women were divided into the following 5 income groups; very low – low –moderate – good and very good [7].

The Statistical Package for Social Science (SPSS) 17.0 for Windows package program was used for data analysis. The continuous variables were; mean, median and standard deviation, the median and mode ordinal variables and the number and nominal variables were expressed as percentages. The continuous variables were evaluated using a histogram and a One-Sample Kolmogorov-Smirnov Test. $P > 0.05$ was considered as a normal distribution. The relationship between nominal variables was evaluated using a Pearson Chi–Square Test and Fisher's Exact Test and $p > 0.05$ was considered as meaningful.

Results

Patients were grouped according to their ages; 79 patients (30.1%) were under the age of 30, 85 (32.3%) were between the ages of 30-40 and 43 (16.3%) between the ages of 40-50 , and 39 (14.8%) 50 -60 years of age , and 17 (6.5%) were over the age of 60. The age distribution of the patients is shown in Figure 1.

The mean age of the patients was 35 ± 13.3 and the median age 35 years (age range: 18-84). Of these patients, 148 (56.30%) were married, 63 (24.00%) unmarried, 52 (19.80%) were divorced.

Of the women subjected to violence 79 (30.00%) did not have children, 184 (70%) patients had one child or more. 89 (33.80%) women worked. 174 (66.20%) patients did not work. 79 (30.00%) patients had no social security. The distribution ratios of the women who had social security was: 115 (43.70%) patients were in the Social Insurance Institution scheme (SSK), 11 (4.20%) were part of the Bag-Kur scheme, 25 (9:50%) had a green card (A type of social security for lower income levels of population in Turkey), 33 (12.50%) had standard state health care.

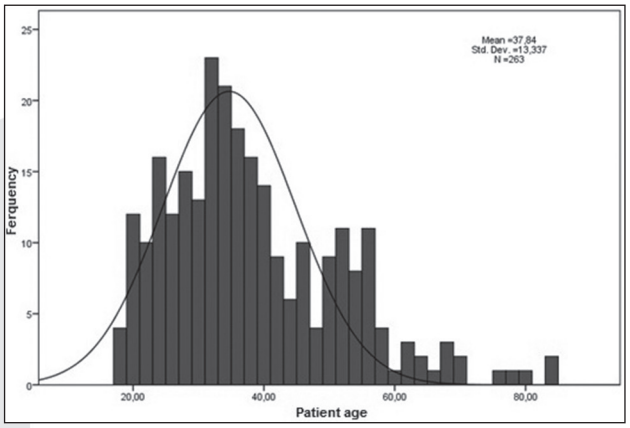

Figure 1. The age distribution of the women exposed to physical violence

91 (34.60%) of the women had a very low income, 108 (41.10%) were in the low income group, 48 (18:30%) had a moderate income, 16 (6.00%) patients had a good income. None of the families there was not in the group of very good income level. In terms of education levels, 21 (8.00%) women were illiterate, 52 (19.80%) women had completed primary schools, 86 (32.70%) had completed secondary education, 82 (31.20%) had completed high school, 22 (8.40%) were university graduates. 217 of the women (82.50%) had been subjected to violence once, 46 (17:50%) had been exposed to violence more than once.

The perpetrator of the violence was as follows: 96 (36.50%) women had been attacked by the wife, 66 (25.10%) by their relatives, 60 (22.80%) by colleagues and 41 (15.60%) women had been subject to violence by an unknown person. According to the perpetrator of the violence, the distribution of educational status of patients shown in Figure 2.

The distribution of parts of the body of the women received a physical assault most commonly isolated head and neck trauma (99 patients, 37.60 %) were present (Figure 3).

Only medical treatment was given the women for physical trauma, although 20 (7.6%) women had received psychiatric help after the assault. The educational background of these women was as follows; 5 (25%) had completed primary scho-

ol, 15 (75%) graduated from high school or university. The relationship between the educational status of the patients not wanting psychiatric assistance is shown in Figure 4.

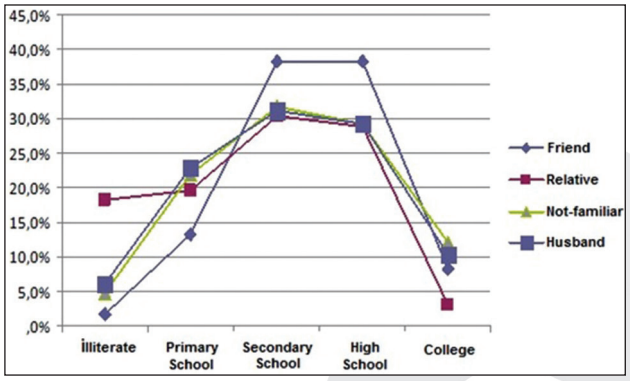

Figure 2. Education levels of the women exposed to physical violence

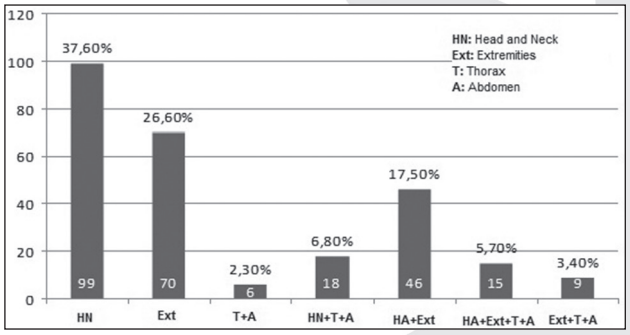

Figure 3. The effected body region of the women exposed to physical violence

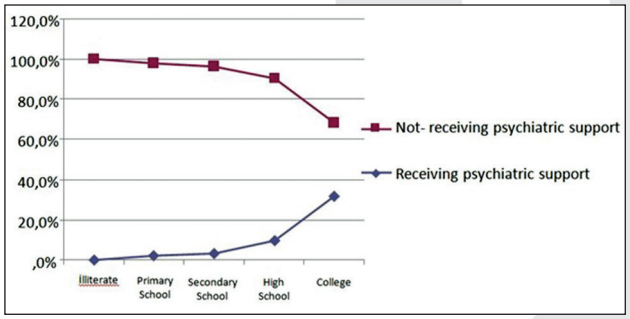

Figure 4. Psychiatric support condition according to education level

Discussion

Approximately one fifth of women attend EDs because of domestic violence. According to studies in Turkey , one in three women experience physical violence at the hands of their husband at least once in their lives [2]. The cause of the trauma to women presenting to the ED are often not attributed to domestic violence by the medical staff. To achieve the correct diagnosis and offer appropriate treatment to women victims of physical violence, it is necessary to take a holistic approach considering not only the signs of physical trauma but also use the details from the forensic report and elicit information from the patient [8]. In addition to the physical damage of physical violence, it can increase the risk of other diseases in the long term. Even if women who have experienced physical violence often do not want to or cannot talk about it, every woman that presents with an injury to a medical institution should be considered as a victim of domestic violence until proven otherwise [6].

According to the results of 48 studies across the world; 10-69 % of women experienced violence by their spouses or partners [2]. In our study, 646 of 2459 patients were women who attended the ED because of physical violence . 56.3% of patients who were subjected to violence were married and 64.9% of the women who were married had been beaten by her husband (Figure 2).

In studies shows that women's educational level is not a factor that determines whether women are the victims of violence, but shows that the educated women are more successful for the break away from violence. In Turkey and almost all countries, women who have lower levels of education are exposed to more domestic violence [9]. 61.4% of the women in the current study only have primary or lower education levels while 39.6% of the women had graduated from high school or university (Figure 2).

Women who are subject to violence can develop many mental disorders such as; post-traumatic stress, depression, suicide attempts, depression, anxiety, mood and eating disorders, alcohol and drug dependence, antisocial personality disorders, psychosis and aggressive behavior towards the children [3, 10]. 50% of female psychiatric patients have a history of violence and 25% of suicide attempts in women there is a history of assault [11]. The fetus of pregnant women who victims of violence are also likely to be injured [3]. This damage may be happen if the woman has received a blow directly to the abdominal region, also may be happen indirectly with other body regions trauma [3]. In a meta-analysis study, women who are exposed to violence during pregnancy have a significantly higher probability of giving birth to low birth weight infants [12]. In

our study, 7.6% of the patients received psychiatric support. Those women with a higher level of education tend to receive psychiatric support more than the women with a lower level of education (Figure 4). In the literature, women exposed to violence had a low level of education and were in a low socioeconomic group and it is interesting to note that they did have not any income and didn't work [13-14]. In our study 199 of the women (75.70%) had a low and very low income, 64 (24.30%) had moderate or high income levels. Our findings were compatible with the literature.

The approach of health workers in relation to violence against women, the diagnosis of violence, treatment and after care are very important. Ramsey et al stated that there was not enough evidence regarding the efficacy of medical interventions in women victims of violence [15]. In our study, after the first examination none of the doctors referred patients for psychiatric assessment. This negligence can cause that the recurrence of violence and the same woman can attend the ED many times and each event can be worse than the previous one and a possibly reaching such an extreme situation that the woman may attempt suicide. In this context, in medical training programs, diagnosis, treatment and psychological support should not be neglected. Also the patient data must be collected in more detail. Furthermore, there is a need for an increased number of studies on this topic.

Conclusion

The staff of an ED are not only charged with provide medical intervention but they should also ensure that the women victims of physical violence are offered the appropriate psychiatric support. Reporting of cases of violence against women, as well as judicial directions in this regard together with the creation of a database, women victims, institutions that provide support for women and that the women can be directed to receive psychological support, are as important as emergency medical assistance.

References

1. *United Nations Declaration on the Elimination of Violence Against Women. Available from: www.un.org/documents/ga/res/48/a48r104.htm.*
2. *Altinay, A.G. and Y. Arat, Türkiye'de kadina yönelik şiddet. 2007, İstanbul: Punto.*
3. *Wathen, C.N. and H.L. MacMillan, Interventions for violence against women: scientific review. JAMA, 2003. 289(5): p. 589-600.*
4. *Edwards, T.A., et al., Stages of change as a correlate of mental health symptoms in abused, low-income African American women. J Clin Psychol, 2006. 62(12): p. 1531-43.*
5. *Fincanci, Ş.K., Kadina Yönelik Şiddete Adli Tip Açisindan Yaklaşim, in Kadina Yönelik Şiddet ve Hekim Sempozyumu. 2003.*
6. *Akin, A., Kadina Yönelik Aile İçi Şiddetin Kadin Sağliğina Etkileri:Kadina Yönelik Aile İçi Şiddetle Mücadelede Sağlik Hizmetleri. 2008, TC Başbakanlik Kadinin Statüsü Genel Müdürlüğü Yayini: Ankara.*
7. *Aydin, K., Türkiye'de Kişisel Gelir Dağiliminin Sosyoekonomik ve Demografik Belirleyicileri. Çalişma ve Toplum, 2012. 1.*
8. *Acil Serviste Kadina Yönelik Şiddetin Tani-Tedavi ve Yönlendirilmesi. 2th ed. 2009, Ankara: Türk Tabipleri Birliği Merkez Konseyi Kadin Hekim ve Kadin Sağliği Kolu.*
9. *Balci, Y.G. and U. Ayranci, Physical violence against women: evaluation of women assaulted by spouses. J Clin Forensic Med, 2005. 12(5): p. 258-63.*
10. *Kaya, M. and B. Kaya, Kadina Yönelik Şiddet; Pandoranin Kirik Kutusu. Sağlik Toplum Siyaset, 2000. 3: p. 50-53.*
11. *Noel, N.L. and M. Yam, Domestic violence. The pregnant battered women. Nurs Clin North Am, 1992. 27(4): p. 871-84.*
12. *Murphy, C.C., et al., Abuse: a risk factor for low birth weight? A systematic review and meta-analysis. CMAJ, 2001. 164(11): p. 1567-72.*
13. *Günay, T., et al., İzmir'de bir gecekondu bölgesinde kadina yönelik aile içi şiddet. Sağlik ve Toplum, 2006: p. 31-37.*
14. *Turhan, E., A. Güraksin, and T. İnandi, Erzurum'da kadina yönelik aile içi şiddet. Sağlik ve Toplum 2006. 16: p. 24-30.*
15. *Ramsay, J., et al., Should health professionals screen women for domestic violence? Systematic review. BMJ, 2002. 325(7359): p. 314.*

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Anesthetic management of Eclampsia/hellp

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Abstract

Objective: Patient anesthesia management remains a matter of debate. Therefore, we aim to present the anesthesia practice on patients in our clinic.

Method: The medical records of 99 patients with the diagnosis of eclampsia that were administered anesthesia due to caesarean section between the dates of September 2005 and September 2010 in Dicle University Hospital were retrospectively analyzed. The patients were classified into two groups: patients that were administered spinal anesthesia (group S), and patients that were administered general anesthesia (group G).

Results: The study included 38 patients that were administered spinal anesthesia and 61 patients that were administered general anesthesia. HELLP syndrome coincided in 12 patients that were given spinal anesthesia and in 33 patients that were given general anesthesia. Spinal anesthesia was performed on patients that had low Hb, Htc, platelet counts and ALT levels preoperatively. The data showed that the general anesthesia group had more blood transfusions than the spinal anesthesia group (p=0.01). In group S, only one patient experienced a complication, whereas in group G, 15 patients experienced complications (p=0.004). In group S nine patients had transfusions, while in group G, 30 patients had transfusions (p=0.019). One patient in group S as well as four patients in group G died postoperatively.

Conclusion: Anesthesia management should be tailored to the patients' condition and laboratory results. Additionally, a regional anesthetic approach should be preferred unless there is a contraindication.

Key words: Eclampsia, spinal anesthesia, general anesthesia.

Introduction

Hypertensive disorders are seen in 3-8% of pregnancies and are one of the major causes of maternal mortality and morbidity (1,2). Eclampsia is among the hypertensive disorders, and it is a life threatening condition, with an incidence that varies within a range of 1/3448 and 1/100. It typically occurs during the third trimester, especially before 32nd gestational week (3). Major maternal complications including hemolysis-elevated liver enzymes and lower platelet count (HELLP) syndrome, placental abruption, disseminated intravascular coagulation, pulmonary aspiration, pulmonary edema, acute renal failure and cardiopulmonary arrest may occur in these patients as well (4, 5). Anesthesia management is a challenge for these patients; hemodynamic instability, mental status and coagulation dysfunction make an anesthetic decision controversial. Ideal anesthetic methods for these patients will not deteriorate organ function and will have a minimal effect on hemodynamic parameters (6). This study aims to compare general anesthesia (GA) and spinal anesthesia in patients diagnosed with eclampsia.

Method

After the consent of the Ethics Committee, the records of 99 patients' that were diagnosed at Dicle University between September-2005 and September-2010 were gathered. The exclusion criteria included hypertension and proteinuria diagnosed before the 20th gestational week; renal, hematologic and cardiac diseases causing proteinuria, hypertension, multiparous women, and patients with hematologic or other diseases with increased hepatic enzyme levels were also excluded. Patients were classified into two groups: patients that were administered general anesthesia (group G), and patients that were administered spinal anesthesia (group S).

The ages of the patients, gravid, parity, gestational week at admission, and length of stay were recorded. Preoperative laboratory results, and postoperative features and complications were also recorded. Additional disorders (HELLP) and any drugs administered preoperatively were also recorded. The

postoperative lab values, vital signs, postoperative 1st and 5th minute APGAR scores of the fetus, and all blood and blood products that were given during the operation were noted as well. A 6 gram loading dose of magnesium sulfate was administered to all patients, followed by a 2 gram maintenance dose 20 minutes later. Postoperative complications and mortality rates were also recorded. Postoperative complications are listed in Table 4.

Statistical analysis

Statistical analysis was performed by SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean values ± standard deviation for continuous variables. P values of less than 0.05 were considered significant. Means and standard deviations (SD) were calculated for continuous variables and subject characteristics and demographics were analyzed descriptively. The normal distribution of the variables was analyzed by the Kolmogorov–Smirnov test. The Chi-square test and the Student's t test were used to evaluate associations between the categorical and continuous variables. All variables were included in the backward stepwise procedure.

Results

Group S contained 38 patients while Group G contained 61 patients. Groups were comparable in age, gravid, parity and gestational age at the time of admission. HELLP syndrome was demonstrated in 12 patients in Group S and 33 patients in Group G (Table 1).

Group S had higher levels of Hb, Hct and ALT levels compared to Group G (p>0.01). Group S had a higher platelet count than Group G but it was not statistically significant. Other preoperative parameters were similar (Table 2).

APGAR scores of neonates were similar in the two groups at the 1st and 5th minutes. Arterial blood pressures (both systolic and diastolic) were also similar. Group G had more transfusions compared to Group S (p=0.01). In Group S, nine patients had transfusions of blood products, while 30 patients in Group G had transfusions (p=0.019). Moreover, total blood transfusion was higher in Group G (Table 3). Postoperatively, only one patient in Group S experienced a complication, whereas 15 patients in Group G experienced complications (p=0.004). During the postoperative period, one patient died from group S; in group G, 4 patients died.

Discussion

Eclampsia is one of the leading causes of perinatal mortality and morbidity in developing countries. Eclampsia causes significant physiologic changes during pregnancy. Caesarean section is required in 11-57% of these cases (7), however the patients are hemodynamically unstable and a possibility of multiple organ failure and insufficient intravascular volume is present; accordingly, the correct type of anesthesia to be utilized is still a matter of debate (6). Additionally, if HELLP syndrome is seen in patients with eclampsia, the decision on which type of anesthesia to use becomes even more challenging (6,8). General anesthesia (GA) seems preferable among patients suffering from coagulation disorders and fetal distress. However, several studies have observed that GA may have several disadvantages. Laryngoscopy while under GA may trigger a hemodynamic response which may provoke intracerebral bleeding and pulmonary complications, in addition to possibly increasing the risk of prolonged intubation and aspiration of gastric contents. General anesthesia may also delay recovery after anesthesia (6, 9, 10, 11).

The use of spinal anesthesia (SA) during coagulation disorders and thrombocytopenia is controversial. Severe hypotension may also result from this technique due to high motor block. Sensitivity against vasopressors used for hypotension can also increase with spinal anesthesia (12). Nevertheless, if no complication exists, these patients will benefit from regional anesthesia. Greater control on hemodynamic stability may be provided by spinal anesthesia (13, 14, 15). Several studies have shown the safety of single dose spinal anesthesia (16, 17, 18, 19). In our study, patients without absolute contraindications for regional anesthesia benefited from SA, while patients with severe bleeding disorders benefited most from GA.

A review of the literature shows that APGAR scores were better in spinal anesthesia groups when compared to patients with general anesthesia (10, 20, 21, 22, 23). In our study, APGAR scores in both groups were comparable (Table 3).

Table 1. Demographic Data

	Group S (n=38)	Group G (n=61)	*p*
Age	31.63±6.81	31.82±7.46	0.900
Gravida	3.84±3.15	3.59±3.08	0.696
Parite	2.42±2.78	2.57±2.88	0.796
Gestational Week of Hospitalization	32.84±3.68	33.39±2.82	0.404
Total length of stay	6.63±2.99	6.64±3.79	0.991
Eclampsia + HELLP n (%)	12(31.6%)	33(54.10%)	0.038

Values are given as mean ± standard deviation. n: number

Table 2. Preoperative Laboratory Values

	Group S (n=38)	Group G (n=61)	*p*
Hct	35.37±7.79	32.44±6.30	0.043*
Hb	12.16±2.60	10.85±2.30	0.011*
Plt	166.87±93.32	131.31±115.18	0.112
ALT	67.68±84.547	157.54±247.470	0.036*
AST	132.27±180.920	204.80±256.241	0.135
LDH	918.43±1074.639	1077.17±819.467	0.420
Bilirubin	3.98±16.047	6.99±40.588	0.677
Blood sugar	120.46±54.100	116.70±36.675	0.684
Urea	27.41±14.532	36.68±35.003	0.130
Creatinine	0.82±0.493	1.12±1.205	0.147
Proteinuria	416.43±157.057	396.43±174.540	0.582
Number of convulsions	1.61±0.887	1.85±0.980	0.209
Mean systolic AT	163.16±9.893	164.59±12.052	0.540
Mean diastolic AT	100.53±8.366	101.97±9.259	0.454

Values are given as mean ±standard deviation. n: number

Table 3. Postoperative Laboratory Values

	Group S (n=38)	Group G (n=61)	*p*
Fetus 1. min APGAR	4.34±2.197	3.73±1.745	0.132
Fetus 5. min APGAR	6.13±2.451	5.48±2.103	0.167
Mean systolic AT	129.74±9.722	131.80±10.410	0.327
Mean diastolic AT	81.32±6.646	82.62±7.938	0.399
RBC Transfuse	1.71±1.113	2.78±2.225	0.233
TDP Transfusion	2.00±0.00	3.24±2.625	0.431
Platelet Suspension	2.00±0.00	2.57±1.718	0.766
Platelet Apheresis	Absent	1.62±1.193	absent
Total amount of Transfusion (U)	0.53±1.41	2.97±5.05	0.01*

Values are given as mean ±standard deviation. AT: Arterial tension
U: Unit min: minute

Table 4. Complications

	Group S (n)	Group G (n)
AKF	1	4
Shortness of breath	-	2
Sepsis	-	1
AKF+ICB	-	2
Shortness of breath + ABY	-	4
Intra abdominal bleeding	-	2

AKF: acute kidney failure ICB; intra cranial bleeding. n: number

General anesthesia may influence uterine vascular resistance or perfusion pressures during caesarean sections thereby altering blood flow and indirectly altering uterine contractions; ultimately, this may lead to an increase in blood loss (24). Afolabi et al compared spinal and general anesthesia techniques in patients that underwent caesarean section and found that blood loss was lower in those that were given spinal anesthesia (25). Another study shows that general anesthesia is correlated with more postoperative blood loss and decreased Htc levels when compared to regional anesthesia in caesarean sections (26). Similarly in our study, Group G had a greater need for blood transfusions during the postoperative period (Table 3).

Patients with eclampsia may be faced with a number of complications ranging from intracranial bleeding, renal and pulmonary impairment to cardiopulmonary arrest (4, 27). In a study conducted by Singh et al, they found that pregnant women with stable eclampsia and without major complications could use spinal anesthesia to avoid possible complications of general anesthesia (6). In our study, there were no significant intraoperative complications in either group, but Group G had more postoperative complications.

Several studies have shown that 7-36% percent of eclampsia cases that occur in conjunction with HELLP syndrome may also have acute renal failure (28, 29, 30). In our study, 11.1% of patient had acute renal failure. One patient (2.6%) and 10 patients (16.4%) had acute renal failure in Groups S and G, respectively. We think that this may be because there are more patients with HELLP in the GA group (n=33; 54.10%) when compared to the SA group (n=12, 31.6%) (p=0.038).

Vigil De Garcia et al found that in developing countries the maternal mortality rate due to eclampsia is 9.4%. In our study, the maternal mortality rate was 5.05% (31).

Several limitations of the study include not randomizing patient selection, as well as not optimizing the anesthesia method individually.

Conclusion

GA should be avoided in eclamptic pregnant women according to their condition and lab results. Spinal anesthesia may be a safer method in the absence of absolute contraindications to regional anesthesia.

References

1. *Geographic variation in the incidence of hypertension in pregnancy. World Health Organization International Collaborative Study of Hypertensive Disorders in Pregnancy. Am J Obstet Gynecol 1988; 158: 80-83.*
2. *Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet 2001; 357: 53-56.*
3. *Gambling D. Principles and Practice: Hypertensive disorders: obstetric anesthesia. Philadelphia: Elsevier; 2004.*
4. *Barton JR, Sibai BM. Cerebral pathology in eclampsia. Clin Perinatol 1991; 18: 891-910.*
5. *Kaplan PW, Repke JT. Eclampsia. Neurol Clin 1994; 12: 565-582.*
6. *Singh R, Kumar N, Jain A, Chakraborty M. Spinal anesthesia for lower segment Cesarean section in patients with stable eclampsia. Journal of Clinical Anesthesia 2011; 23: 202–206.*
7. *Didley GA, Cotton DB, Phelan JP. Critical Care Obstetrics: Complications of PIH. 2nd ed. Oxford: Blackwell, 1991.*
8. *Graciaa PV, Silvab S, Montufara C, Carrolb I, Riosb SL. Anesthesia in pregnant women with HELLP syndrome. International Journal of Gynecology & Obstetrics 2001; 74: 23-27.*
9. *Yuen TS, Kua JS, Tan IK. Spinal haematoma following epidural anaesthesia in a patient with eclampsia. Anaesthesia 1999; 54: 350-354.*
10. *Ramanathan J, Coleman P, Sibai B. Anesthetic modification for hemodynamic and neuroendocrine response to cesarean section delivery in severe preeclampsia. Anesth Analg 1991; 73: 772-779.*
11. *Lavies NG, Meiklejohn BH, May AE, Achola KJ, Fell D. Hypertensive and catecholamine response to tracheal intubation in patients with pregnancy-induced hypertension. Br J Anaesth 1989; 63: 429-434.*
12. *Talledo OE, Chesley LC, Zuspan FP. Renin-angiotensin system in normal and toxemic pregnancies.*

III. Differential sensitivity to angiotensin II and norepinephrine in toxemia of pregnancy. Am J Obstet Gynecol 1968; 100: 218-221.

13. *Gogarten W. Preeclampsia and anaesthesia. Curr Opin Anaesthesiol 2009; 22: 347-351.*

14. *Öz H, Akkor A, Aykaç B, Sun S. Preeclampsia-eclampsia, anesthesia and intensive care.. Perinatal Journal 1993; 1: 50-54.*

15. *Mandal NG, Surapaneni S. Regional anaesthesia in pre-eclampsia: advantages and disadvantages. Drugs 2004; 64: 223-236.*

16. *Botfield C, Howell P. The use of spinal anaesthesia in severe preeclampsia. Fetal Maternal Med Rev 2001; 12: 67-79.*

17. *Visalyaputra S, Rodanant O, Somboonviboon W, Tantivitayatan K, Thienthong S, Saengchote W. Spinal versus epidural anesthesia for Cesarean delivery in severe preeclampsia: a prospective randomized, multicenter study. Anesth Analg 2005; 101: 862-868.*

18. *Dyer RA, Piercy JL, Reed AR. The role of the anaesthetist in the management of the pre-eclamptic patient. Curr Opin Anaesthesiol 2007; 20: 168-174.*

19. *Santos AC. Spinal anesthesia in severely preeclamptic women: when is it safe? Anesthesiology 1999; 90: 1252-1254.*

20. *O'Brien JM, Shumate SA, Satchwell SL. Maternal benefit of corticosteroid therapy in patients with HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome: Impact on the rate of regional anesthesia. Am J Obstet Gynecol 2002; 186: 507-522.*

21. *Moodley J, Jjuuko G, Rout C. Epidural compared with general anaesthesia for caesarean delivery in conscious women with eclampsia. BJOG 2001; 10: 378-382.*

22. *Okafor UV, Efetie ER, Igwe W, Okezie O. Anaesthetic management of patients with pre-eclampsia/eclampsia and perinatal outcome. J Matern Fetal Neonatal Med 2009; 22: 688-692.*

23. *Krishnan L, Gunasekaran N, Bhaskaranand N. Anesthesia for cesarean section and immediate neonatal outcome. Indian J Pediatr 1995; 62: 219-223.*

24. *Yalınkaya A, Güzel Aİ, Kangal K, Uysal E, Erdem S. Comparing the blood values of the patients operated by cesarean under spinal and general anesthesia. Perinatal Journal 2009; 17(2): 70-73.*

25. *Afolabi BB, Lesi FE, Merah NA.Regional versus general anaesthesia for caesarean section.Cochrane Database Syst Rev 2006;4: CD004350.*

26. *Lertakyamanee J, Chinachoti T, Tritrakarn T, Muangkasem J, Somboonnanonda A, Kolatat T.Comparison of general and regional anesthesia for cesarean section: success rate, blood loss and satisfaction from a randomized trial.J Med Assoc Thai 1999; 82: 672-680.*

27. *Kaplan PW, Repke JT. Eclampsia. Neurol Clin 1994; 12: 565-582.*

28. *Celik C, Gezginc K, Altintepe L, Tonbul HZ, Yaman ST, Akyurek C, Turk S: Results of the pregnancies with HELLP syndrome. Ren Fail 2003, 25: 613-618.*

29. *Cavkaytar S, Ugurlu EN, Karaer A, Tapisiz OL, Danisman N: Are clinical symptoms more predictive than laboratory parameters for adverse maternal outcome in HELLP syndrome? Acta Obstet Gynecol Scand 2007, 86: 648-651.*

30. *Gul A, Aslan H, Cebeci A, Polat I, Ulusoy S, Ceylan Y: Maternal and fetal outcomes in HELLP syndrome complicated with acute renal failure. Ren Fail 2004, 26: 557-562.*

31. *Gracia PV. Maternal deaths due to eclampsia and Hellp syndrome. Int J Gynaecol Obstet 2009; 104: 90–94.*

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The influence of -330 IL-2 gene polymorphism and HLA-DRB1*1501 allele on age at onset in Iranian multiple sclerosis patients

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune, multifactorial and complex genetic disease due to demyelination of the central nervous system. It is believed that HLA and cytokines genes are involved in the pathogenesis of MS. Previously, we showed that HLA-DRB1*1501 allele and −330 T/T IL-2 genotype had most susceptibility effect on Iranian MS patients. In this study, we evaluated the influence of susceptible DRB1*1501 allele and -330 IL-2 polymorphism on age at onset of MS. Our results indicated that individuals with T/T genotype (23.8±5 vs. 34.4±10 years, $p<0.0001$) and HLA-DRB1*1501 allele (21.3±3 vs. 26.6±3 years, $p<0.0001$) had significantly lower age at onset of disease than controls. The -330 T/T IL-2- HLA DRB1*1501 haplotype showed the highest significant association to young age at onset. The 330 T/T^- IL-2- HLA DRB1*1501 haplotype had older age at onset of disease compare to 330 T/T IL-2- HLA $DRB1*1501^-$ haplotype, but it was not significant. However, we have provided evidence of an interaction between HLA-DRB1*1501 allele and the -330 T/T IL-2 genotype in age at onset of MS. But additional Studies on large sample size maybe discover the fact of these gene interactions in age of disease onset.

Key words: IL-2, polymorphism, HLA-DRB, age at onset, Multiple sclerosis.

Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory and autoimmune disease due to demyelination of the central nervous system. It is believed to be initiated and mediated by autoreactive T cells directed against myelin antigens. Both genetic and environmental factors are contributed in disease risk (1). For a long time, the only HLA-DRB1 region was known as susceptible genetic factor to MS, and allelic interactions of the HLA-DRB1 locus had been believed (2).

Interleukin 2 was identified as pro and anti inflammatory factor .Interleukin 2 was initially found as an autocrine secretory product from activated T cells with growth factor properties. Later, it was found that IL-2 elicited T-cell proliferation, survival and differentiation of effectors. The interleukin-2 gene was strong functional candidate which involved in immune regulation and function. The main nonredundant role of IL-2 was the maintenance of peripheral T-cell tolerance; it had an important role in regulatory T-cell (Treg) homeostasis. The impairment of Treg cells was believed to be the cause of autoimmunity in the absence of IL-2 (3, 4).

Interleukin-2 (IL-2) is a cytokine involve in the function and regulation of immune system. IL-2 gene was located on chromosome 4q21. Many studies implicated that interleukin-2 (IL-2) played an effective role in the pathology of MS (5-11). Levels of IL-2 increased in the cerebrospinal fluid (CSF) and sera of MS patients (12).

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John and colleagues identified -330 Interleukin-2 promoter single nucleotide polymorphism (13). Matesanz et al. showed that both of -330 G/T and T/T genotypes of IL-2 gene have an association with susceptibility to secondary progressive (SP) course of MS (10). First study on Iranian population identified that −330 G/G IL-2 genotype was significantly more frequent in MS patient group than controls (14). On the contrary, next study was found that there is not significantly difference between MS individuals and healthy controls (15). Our previous study indicated that −330 T/T genotype and HLA-DRB1*1501 allele were significantly more frequent in MS patients than control group (under publish).

Matesanz et al found that -330 IL-2 gene polymorphism did not effect on age at onset of MS disease (10). Several studies up to now showed that HLA-DRB1*15 was correlated with younger age at onset of disease and carriers of DR15 are susceptible to MS development at an earlier age than non carriers (16-20). In contrast, others did not confirm that HLADRB1*15 decreased age at onset of MS disease (21, 22).

In the present study, we assess effect of -330 IL-2 polymorphism genotype and HLA-DRB1*1501 allele on age at onset of MS disease.

Materials and Methods

Subject and control groups

One hundred unrelated patients with relapsing remitting MS were included in this study. They had mean standard deviation age of 27.09±5.917 years and age range of 20-42 years. Patients were selected from medical genetics department of Sarem women hospital and diagnosed by neurologist according to the McDonald criteria (23). One hundred ethnically, age and sex matched healthy controls with no personal or family history of autoimmune disorders was selected. Control group had mean age of 29.8.2±7.801 years and age range of 20-52 years. All individuals were informed of the study and gave their informed written consent.

Genotyping and data collection

DNA was extracted from peripheral blood samples by using salting out method (24). HLA-DRB genotyping was performed by HISTO TYPE SSP Low Resolution Kits (BAG Health care, Germany) in accordance with the manufacturer's recommendations. The sequencing of IL-2 gene containing -330 polymorphism was detected by performing RLFP-PCR according to Matesanz and colleagues (10). According to the result of HLA-DRB typing and -330 IL-2 genotyping, we could determine susceptible alleles and genotypes. Base on age at onset of each individual, the influence of these positions on age of disease onset was analyzed.

Statistical Methods

To evaluate the influence of susceptible DRB1*1501 allele and -330 IL-2 polymorphism on age of disease onset, t-test and Mann-Whitney U test were performed. P value <0.05 was considered as significant. SPSS 18v for windows software was used.

Result

Age at onset of MS in relation to susceptible HLA-DRB1*1501 allele and -330 IL-2 polymorphism genotypes was calculated and their results were shown in Table 1 and 2. As it was demonstrated in table 1, patients with T/T genotype had significantly ($p<0.0001$) lower age at onset of disease than controls (23.8±5 vs. 34.3±10 years). Carriers of HLA-DR1*1501 allele was significantly ($p<0.0001$) associated with lower age at onset in MS individuals than control group (21.3±3 vs. 26.6±3 years) (Table 2). Age at onset of MS in relation to haplotype of HLA-DRB1*1501 and -330 IL-2 gene was shown in Tables 3 and 4. The carriers of -330T/T- DRB1*1501 had the youngest age at onset of disease significantly. Patients with -330T/T - DRB1*1501 haplotype had younger age at onset of disease than carriers of -330T/T$^-$ - DRB1*1501 (20.4±0.5 vs. 34.5±0.7 years, $p<0.002$) (Table 3). Also, patients who carried -330T/T- DRB1*1501 were significantly younger in age at onset of disease than carriers of -330T/T- DRB1*1501$^-$ (20.4±0.5 years vs. 32.4±9 years, $p<0.0001$) (Table 4).

Table 1. Age at onset of MS in relation to -330 IL-2 genotypes

Genotype	Patient N=100	Control N=100	P
GG	27.1±3.38	27.27±4.13	0.875
GT	30.68±5.07	28.82±6.89	0.192
TT	23.83±5.7	34.35±10.43	0.000

Independent two sample t test was used.

Table 2. Age at onset of MS in relation to HLA-DRB1

HLA-DRB1	Patient N=100	Control N=100	P
1501^{+}	21.36±3.6	26.6±3.83	0.000
1501^{-}	29.54±4.9	30.15±8.05	0.55

Independent two sample t test was use.

Discussion

Multiple sclerosis is a chronic inflammatory and autoimmune disease. Most of genes which caused susceptibility to MS are involve in immune response. In autoimmune disease such as MS, one of the most important genes is cytokine genes as IL-2. Many studies have shown that HLA genes, especially HLA II, are the most important susceptible genes to MS. Previously, we studied association of HLA-DRB1 alleles and -330 IL-2 gene polymorphism to MS disease. We found that -330 T/T IL-2 genotype was the most frequent genotype among other genotypes and had significantly association to MS. Also it was shown that the HLA-DRB1*1501 allele had the most susceptibility effect on disease (under published). In this study, we analyzed the effect of HLA-DRB1*1501 allele and -330 IL-2 genotypes on age at onset of MS disease.

Matesanz et al found that -330 IL-2 gene polymorphism did not effect on age at onset of MS disease (10). One study on Norwegian and Swedish MS patients was shown HLA-DRB1*15 was correlated with younger age at onset of disease (P: 0.009) (25). A correlation between HLA-DRB1*15 and younger age at onset of MS has been reported by several studies (16-20). In contrast, other studies did not confirm that HLA DRB1*15 decreased age at onset disease (21, 22).

Our results showed that HLA-DRB1*1501 allele and -330 T/T IL-2 genotype had significantly effect on decrease of age at onset of MS. Individuals who carried HLA-DRB1*1501 allele or -330 T/T IL-2 genotype was significantly younger than non-carriers.

The -330 T/T IL-2- HLA DRB1*1501 haplotype showed the highest significant association to young age at onset of MS. Carriers of -330 T/T IL-2- HLA DRB1*1501 haplotype had 20.4±0.5 years mean age at onset of disease. When in this haplotype, susceptible -330 T/T IL-2 genotype was replaced by -330 T/T^{-} genotype while carried susceptible HLA DRB1*1501 allele, age at onset of MS significantly increased to 34.5±0.7 years (p: 0.002). This demonstrated the major effect of -330 T/T genotype on decreasing age at onset of MS. Also, if susceptible HLA DRB1*1501 allele of -330 T/T IL-2- HLA DRB1*1501 haplotype was replaced with HLA $DRB1*1501^{-}$ allele while carried susceptible -330 T/T genotype, mean age at onset of disease increased to 32.45±9.75 years (p: 0.0001). It showed main effect of HLA DRB1*1501 allele on decreasing age at onset of disease. This comparison

*Table 3. Age at onset of MS in relation to -330 IL-2 genotype and HLA-DRB*1501 allele*

	-330 IL-2 genotype		p
	TT-	TT+	
HLA-DRB1 allele 1501^{+}	34.5±0.7 (2)	20.42±0.57 (28)	0.002[a]
1501^{-}	29.92±4.66 (56)	28.64±6.28 (14)	0.394[b]

[a] *Mann-Whitney U test and Randomization was used;*
[b] *independent two sample t test was used.*

Table 4. Age at onset of MS in relation to -330 IL-2 and HLA-DRB1 haplotype

	HLA-DRB1 allele		p
	1501^{+}	1501^{-}	
-330 IL-2 genotypes			
GG	ND[a]	27.1±3.38 (20)	-
GT	34.5±0.7 (2)	31.5±4.57 (36)	0.41[b]
TT	20.42±0.57 (28)	32.45±9.57(42)	<0.0001[c]

[a] *ND: it was not detected;*
[b] *Mann-Whitney U test and Randomization was used;*
[c] *independent two sample t test was used.*

indicated important effect of both susceptible HLA DRB1*1501 allele and -330 T/T genotype on decreasing age at onset of MS. Since the 330 T/T$^-$ IL-2- HLA DRB1*1501 haplotype had older age at onset of disease (not significantly) in comparing -330 T/T IL-2- HLA DRB1*1501$^-$ haplotype (34.5±0.7 vs. 32.45±9.57 years), -330 T/T genotype maybe have more important effect on decreasing age at onset of MS. As this difference was not significant, studies on large sample size maybe discover the fact.

In conclusion, our result reveals that the susceptible HLA-DRB1*1501 allele and -330 T/T IL-2 genotype had the major effect on age at onset of disease and result in younger age of MS onset . We have provided evidence of an interaction between HLA-DRB1*1501 allele and the -330 T/T IL-2 genotype in age at onset of MS. However, additional studies on large patient collections are necessary to find out these gene interactions in age of disease onset.

References

1. *Oksenberg JR, Barcellos LF. Multiple sclerosis genetics: leaving no stone unturned. Genes Immun. 2005, 5: 375–387.*

2. *DeLuca GC, Ramagopalan SV, Herrera BM, Dyment DA, Lincoln MR, Montpetit A,. An extremes of outcome strategy provides evidence that multiple sclerosis severity is determined by alleles at the HLA-DRB1 locus. Proc Natl Acad Sci U S A. 2007, 52: 20896–20901.*

3. *Malek TR, Bayer AL: Tolerance, not immunity, crucially depends on IL-2. Nat Rev mmunol. 2004, 4: 665–674.*

4. *Malek TR. The biology of interleukin-2. Annu Rev Immunol. 2008, 26: 453–479.*

5. *Lu CZ, Fredrikson S, Xiao BG, Link H. Interleukin-2 secreting cells in multiple sclerosis and controls. J Neurol Sci. 1993, 120: 99–106.*

6. *Gallo P, Piccinno M, Pagni S, Tavolato B. Interleukin-2 levels in serum and cerebrospinal fluid of multiple sclerosis patients. Ann Neurol. 1998, 24: 795–797.*

7. *Gallo P, Piccinno MG, Pagni S, Argentiero V, Giometto B. Immune activation in multiple sclerosis: study of IL-2, sIL-2R, and gamma-IFN levels in serum and cerebrospinal fluid. J Neurol Sci. 1989, 92: 9–15.*

8. *Trotter JL, Clifford DB, McInnis JE, Griffeth RC, Bruns KA. Correlation of immunological studies and disease progression in chronic progressive multiple sclerosis. Ann Neurol. 1989, 25: 172–178.*

9. *Reboul J, Mertens C, Levillayer F, Eichenbaum-Voline S, Vilkoren T, Cournu I, et al. Cytokines in genetic susceptibility to multiple sclerosis: a candidate gene approach. French Multiple Sclerosis Genetics Group. Journal of Neuroimmunology. 2000, 102-107.*

10. *Matesanz F, Fedetz M, Collado-Romero M, Fernandez O, Guerrero M, Delgado MC, et al. Allelic expression and interleukin-2 polymorphisms in multiple sclerosis. Journal of Neuroimmunology. 2001, 119- 101.*

11. *Matesanz F, Fedetz L, Leyva C, Delgado O, Fernández A. Alcina, Effects of the multiple sclerosis associated -330 promoter polymorphism in IL2 allelic expression, J. Neuroimmunol. 2004, 148; 212–217.*

12. *Petitto JM, Streit WJ, Huang Z, Butfiloski E, Schiffenbauer J. Interleukin- gene deletion produces a robust reduction in susceptibility to experimental autoimmune encephalomyelitis in C57BL/6 mice. Neurosci Letter. 2000, 285; 66–70.*

13. *John S, Turner D, Donn R, Sinnott P, Worthington J, Ollier W.E.R., et al. Two novel biallelic polymorphisms in the IL-2 gene, Eur. J. Immunogenet. 1998, 25; 419–420.*

14. *Amirzargar A, Khosravi F, Dianat S, Hushmand F, Maryousef P, Foroushani AR, et al. Profile of cytokine gene polymorphisms in Iranian multiple sclerosis patients. Mult. Scler. 2007, 13; 253–255.*

15. *Shokrgozar MA, Sarial S, Amirzargar A, Shokri F, Rezaei N, Arjang Z, et al. IL-2, IFN-gamma, and IL-12 gene polymorphisms and susceptibility to multiple sclerosis. J. Clin. Immunol. 2009, 29; 747–751.*

16. *Masterman T, Ligers A, Olsson T, Andersson M, Olerup O, Hillert J. HLA-DR15 is associated with lower age at onset in multiple sclerosis. Annals of Neurology. 2000, 48; 211–219.*

17. *Celius EG, Harbo HF, Egeland T, Vartdal F, Vandvik B, Spurkiand A. Sex and age at diagnosis are correlated with the HLA-DR2, DQ6 haplotype in multiple sclerosis. Journal of the Neurological Sciences. 2000, 178; 132–135.*

18. *Hensiek AE, Sawcer SJ, Feakes R. HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis. Journal of Neurology, Neurosurgery and Psychiatry. 2002; 72; 184–187.*

19. *Weatherby SJ, Thomson W, Pepper L. HLA-DRB1 and disease outcome in multiple sclerosis. Journal of Neurology. 2001, 248; 304–310.*

20. *Barcellos LF, Oksenberg JR, Begovich AB, HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. American Journal of Human Genetics. 2003, 72; 710–716.*

21. *Barcellos LF, Sawcer S, Ramsay PP. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. Human Molecular Genetics. 2006, 15; 2813–2824.*

22. *Ballerini C, Guerini FR, Rombola G. HLA-multiple sclerosis association in continental Italy and correlation with disease prevalence in Europe. Journal of Neuroimmunology. 2004, 150; 178–185.*

23. *McDonald WI, Compston A, Edan G. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001, 50; 121– 7.*

24. *Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1998, 16(3); 1215.*

25. *Smestada C, Brynedalb B, Jonasdottirc G, Lorentzena AR, Mastermanb T, Akessonb A, et al. The impact of HLA-A and -DRB1 on age at onset, disease course and severity in Scandinavian multiple sclerosis patients. European Journal of Neurology. 2007, 14; 835–840.*

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Effectiveness of a structered commercial dieting program on weight control

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Abstract

Background and Aim: Overweight and obesity are seriously growing health problems. The goals of a successful weight loss and management program should be to reduce body weight, and to maintain a lower body weight over the long period of time. The commercial dieting program is a structured healthy eating program, which consists of a 4 phase structured individual nutrition plan. Aim of this study was to assess effectiveness of the program on the weight control, and maintenance of weight loss achieved during dieting.

Methods: This is a retrospective cohort study, conducted on the participants of the program, followed between years of 2009-2011 in Turkey. The total number of the participants was 768. A phone call was made to all the participants. Totally 520 participants answered the call and 270 accepted to participate in the study. Ten questions were asked to all participants about their current weight, how many kilograms they lost during dieting, duration and level of adherence to the program, and their opinions about the program.

Results: Mean age of the participants was 43 ± 12.2 years. Baseline body mass index was 35.5 ± 6.8 kg/ m^2; there was no difference between males and females (p=0.528; t=-0.632). In contrast to males who lost an average of 14.2 ± 9.1 kg, females lost 10.8 ± 6.4 kg (t= 2.9, p = 0.04). After the program, both males and females reduced their body mass index significantly ($p < 0.05$). Two-third of the participants reported that the benefits of the program were worth the cost.

Conclusions: The commercial nutrition program is effective for weight reduction and for maintaining weight, easy to comply, and worth the cost. It is successful in achieving lifestyle changes.

Key words: Obesity, reduced daily caloric intake, commercial nutrition program, weight loss.

Introduction

Overweight and obesity are seriously growing health problems, increasing the risk of death and a variety of chronic diseases. However, they are not receiving the attention they deserve from primary care physicians and health workers. Among the reasons cited for not treating obesity is the lack of competent knowledge for counseling (1).

The goals of a successful weight loss and management program should be to reduce body weight and to maintain a lower body weight over a long period of time. A successful weight - loss program should have an initial goal of a 5 to 10% reduction in weight and maintain it at least for one year. A reasonable duration to achieve a 10% reduction in body weight is more than 6 months (1, 2).

Due to high public interest in weight loss programs, different commercial nutrition programs have been developed. Although commercial weight loss programs provide treatment to millions of participants, accurate information about the effectiveness of these programs is rarely available (3). Numerous commercial weight loss programs demonstrate short-term success (4). Although many studies have been done related to weight loss programs, still there are controversies about which form of diet would be the best for treatment of overweight and obesity (5-8). Commercial weight loss programs have been recommended as the second step in a graded, 4-steep model for weight management (9). Some studies showed that in management of obesity, low carbohydrate and high protein diets would be more effective than low-calorie, low-fat diets (10, 11). On the contrary, some other studies did not support this view (12, 13). Some studies retrieve that effectiveness of weight loss programs may depend on the adherence and regular contact with the therapist not only the content and form of the diet (14, 15).

Aim of this study was to assess the effectiveness of the commercial nutrition program (CNP), a

structured commercial healthy eating program on weight control, and maintenance of weight. The main focus of the study is to assess the effectiveness of the CNP program, which relates to how well a treatment works in practice, with BMI as the main outcome variable.

Material and Methods

Study Design and Sample Size

This is a retrospective cohort study. This study was conducted on the participants of CNP, followed between 2009 and 2011 in Turkey. Participants were recruited from existing records of one physician applying the program to his patients. Total number of the participants was 768. Data was subtracted from patient records. A phone call was made to participants to inquire about variables such as patient satisfaction; meanwhile informed consent was also taken. Totally data for 270 participants was available.

Study variables were amount of BMI change during dieting, duration of dieting, level of adherence to the program, and participants' opinions about the program. We also asked the reasons of giving up the program to the participants who dropped out of the program and their weight at the time of leaving. Out of the 270 participants, 215 replied the phone interview. Participants who were younger than 18 years old at the beginning of the program were excluded and in total of 195 participants (78 male, 40%; 117 female, 60%) were included in the analysis. BMI and weight change were the primary outcome measures.

BMI was recorded at three time points: Baseline BMI (BMI-1), at the time of joining the program; Discontinuation BMI (BMI-2), at the time when participants left the program; Current BMI (BMI-3) at the time of phone interview.

Basic principles of the CNP

The CNP consists of a 4-phase structured individual nutrition plan. Trained counselor, who is a medical doctor, compiles medical history and collects blood samples. All results and data are gathered and analyzed, and individual nutrition plan is created. The CNP counselor guides and supports the participants throughout all stages of the program.

Phase 1 is a 2 day preparation and detoxification process. Phase 2 is a minimum of 14 day strict adjustment phase. All the participants will be required to eat 3 meals daily following 8 simple rules. Participants will have an option to remain on this phase until they have reached their desired desired body weight. Phase 3 is a more relaxed adjustment phase where participants begin to integrate additional foods into their daily meal plan.

Phase 4 is the maintenance phase where participants continue to follow basic rules.

The Eight Basic Rules of CNP:

1. Eat 3 meals per day
2. A minimum of 5 hours between meals
3. Meals must not exceed 60 minutes
4. Begin each meal with protein
5. Eat only 1 type of protein with each meal
6. Finish dinner by 9 p.m.
7. Drink at least 2 liters of water per day
8. Eat one apple per day

Statistical Analyses

Data was reported as numbers, percent, mean and standard deviation. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequencies and percentage. All analyses were carried out with the Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Normality of variables was evaluated by the Kolmogorov-Smirnov's test. Student's t test was used to compare BMI and weight differences; paired t test was used to compare the changes in BMI and weight. In order to compare BMI at the three time points, we performed repeated measures ANOVA with "duration of dieting" as co-factor. Pearson correlation analyze was used to determine the relation between lost weight and duration of dieting. Statistical significance level p was taken as less than 0.05.

Results

Mean age (± SD) of the participants was 43 ± 12.2 years. The oldest participant was 74 years old. BMI-1 was 35.5 ± 6.8 kg/m^2; there was no difference between males and females ($p=0.528$; $t=-0.632$). Also there was no difference between the BMI of the male and female subjects at BMI-2 and BMI-3 measurements ($p>0.05$).

Table 1. Descriptive features of the participants

	Minimum	Maximum	Mean	SD
Age (year)	18	74	43	12.2
BMI-1 (kg/m²)	24.4	58.3	35.4	6.7
BMI-2(kg/m²)	20.6	52.6	31.2	5.9
BMI-3 (kg/m²)	20.6	153.8	32.5	10.7
Duration of dieting (week)	1	24	5.9	5.8
Weight loss (kg)	0	50	12.1	7.7

In contrast to males who lost an average of 14.2 ± 9.1 kg, females lost 10.8 ± 6.4 kg (t= 2.9, p = 0.04). After the program, both males and females reduced their initial body weight; their BMI reduced significantly (t and p values 12.1; < 0.001 and 17.6; <0.001, respectively, Table 1).

The number of the participants who fully implemented the program was 142 (72%). Out of 195 participants, 75 (38.4%) were still continuing to the program, 120 discontinued the program due to different reasons. The reasons of quitting the program were: difficulty in adherence (16.4%), disregard the program (13.8%) personal workload (10.3%), get bored with the program (8.7%), having some illnesses unrelated to the program (5.1%), reasons related to the CNP advisor (0.5%) and some other reasons (6.7%).

Only 15 (7.7%) participants tried another weight loss program after quitting CNP. Number of the participants who reported the compliance with the diet as "very easy" was 47 (24.1%), as "easy" was 84 (43.1%), as "difficult" was 45 (23.1%), and as "very difficult" was 19 (9.7%).

Out of the participants, 157 (80.5%) reported that it was worth the money they paid for the CNP and 166 of them (85.1%) would recommend the program to others.

There was no significant difference between male and female subjects in view of adherence to the program (χ^2=0.295; p=0.587). We found a strong correlation between the duration of dieting and amount of weight loss (Pearson r=0.350; p<0.001, Figure 1).

In order to compare BMI between male and female subjects at the 3 measurement times, we performed repeated measures ANOVA by assuming duration of dieting as co-factor. We found that the BMI of both males and females at first measurement decreased significantly in the second measurements (F=124.6; p<0.001). However the difference was not statistically significant (F=2.067; p=0.130), in the subjects who discontinued dieting; while females kept their body weight; males began to gain some weight (Figure 2).

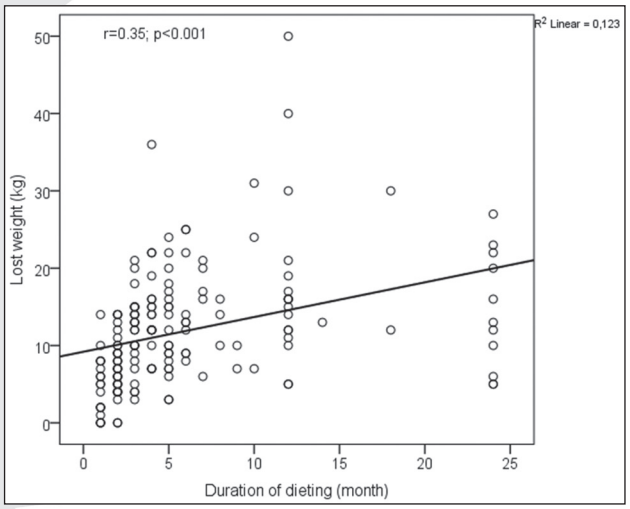

Figure 1. Correlation between weight reduction and duration of dieting

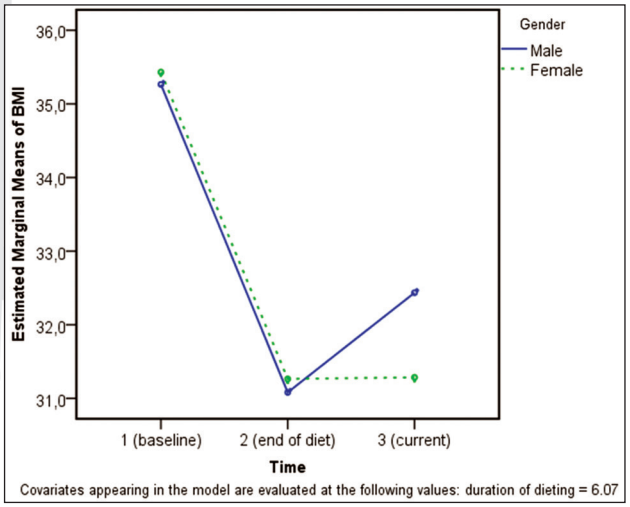

Figure 2. Change of BMI of the participants according to gender and time

Discussion

Results of this study have shown that the CNP is effective in weight loss and preserving the lost weight. At the beginning of the program, there was no difference between BMI of male and female participants.

It was an interesting finding that male participants lost more weight than females. Male participants lost more weight when compared with females, which was statistically significant. It can be attributed to males having more daily activity and work in jobs requiring more physical strength. This finding supports the literature suggesting that dieting combined with increased daily physical activity is more effective in weight lost programs (16, 17).

Nearly 2/3rd of the participants reported that it was easy to implement the program. Generally, implementing dieting programs were reported as difficult by the participants (18). Reasons for adherence and effectiveness of the program may be flexibility of the CNP in choosing the diet content, and frequent supervision/consultation by the counselor, which is consistent with the literature (18).

Vast majority of the participants reported that the cost of the program was worth the money paid and they would recommend it to others. This is not the expected result from commercial dieting programs (19). It is also noteworthy that the cost - effectiveness of the CNP has a very important place in terms of providing patient satisfaction.

There was no difference between male and females in view of compliance to the program. It is well known that expensive programs have high adherence rate. High rate of adherence may be attributed to high cost.

There was a positive correlation between implementation of the program and lost body weight. This result is expected from all dieting programs. Weight control programs should be implemented life-long rather than certain period of time and permanent life style changes that will last a lifetime should be recommended (1).

Although female participants maintained their weight after quitting implementation of the program, male participants tended to get some weight which was not statistically significant. This finding shows that female participants won permanent lifestyle changes and were able to maintain and implement the changes in their daily life. Having no information about the occupation of participants may be a limitation of this study. However, in the Turkish culture, we expect majority of the women to be housewives. Maintaining their weight may be more difficult for men because they have a more outdoor lifestyle.

Conclusion

Weight gain was positively correlated with different unhealthy eating styles and behaviors such as food craving, binge eating disorder and night eating syndrome (20). CNP is effective for weight reduction and for maintaining lost weight. It is easy to implement and worth the cost. This nutritional program is successful in acquiring lifestyle changes. In order to be successful in weight control, reducing daily consumption of calories and increasing physical activity are indispensable basic elements.

Given the apparent substantial, long-term success of CNP in weight reduction, perhaps greater emphasis should be placed on prevention of obesity rather than its treatment.

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References

1. *National Institutes of Health National Heart, Lung, and Blood Institute. North American Association for the Study of Obesity, The Practical Guide Identification, Evaluation, and Treatment of Overweight and Obesity in Adults NIH Publication Number 00-4084, (2000).*

2. *Andeerson GJ, Hensrud DD. Textbook of Family Practice. Philadelphia, PA Elsevier, 2011: 1169.*

3. *Furlow EA, Anderson JW. A systematic review of targeted outcomes associated with a medically supervised commercial weight-loss program. Journal of the American Dietetic Association. 2009; 109(8): 1417-21. Epub 2009/07/28*

4. *Grodstein F, Levine R, Troy L, Spencer T, Colditz GA, Stampfer MJ. Three-year follow-up of participants in a commercial weight loss program. Can you keep it off? Arch Intern Med 1996; 156: 1302-1306.*

5. *Aston LM, Stokes CS, Jebb SA. No effect of a diet with a reduced glycaemic index on satiety, energy intake and body weight in overweight and obese women. Int J Obes (Lond) 2008; 32: 160-165.*

6. *Maki KC, Rains TM, Kaden VN, Raneri KR, Davidson MH. Effects of a reduced-glycemic-load diet on body weight, body composition, and cardiovascular disease risk markers in overweight and obese adults. Am J Clin Nutr 2007; 85: 724-734.*

7. *Brunner EJ, Wunsch H, Marmot MG. What is an optimal diet? Relationship of macronutrient intake to obesity, glucose tolerance, lipoprotein cholesterol levels and the metabolic syndrome in the Whitehall II study. Int J Obes Relat Metab Disord 2001; 25: 45-53.*

8. *Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. Ann Intern Med 2004; 140: 778-785.*

9. *Khaodhiar L BG. Health benefits and risks of weight loss2001. In: Bjorntorp P, ed. International Textbook of Obesity. Chichester, England: John Wiley & Sons; 2001: 413-439.*

10. *Gardner CD, Kiazand A, Alhassan S, Kim S, Stafford RS, Balise RR, Kraemer HC, et al. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. JAMA 2007; 297: 969-977.*

11. *Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, et al. A low-carbohydrate as compared with a low-fat diet in severe obesity. N Engl J Med 2003; 348: 2074-2081.*

12. *Das SK, Gilhooly CH, Golden JK, Pittas AG, Fuss PJ, Cheatham RA, Tyler S, et al. Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y randomized controlled trial. Am J Clin Nutr 2007; 85: 1023-1030.*

13. *Lecheminant JD, Gibson CA, Sullivan DK, Hall S, Washburn R, Vernon MC, Curry C, et al. Comparison of a low carbohydrate and low fat diet for weight maintenance in overweight or obese adults enrolled in a clinical weight management program. Nutr J 2007; 6: 36.*

14. *Meffert C, Gerdes N. Program adherence and effectiveness of a commercial nutrition program: the metabolic balance study. J Nutr Metab 2010; 197-656.*

15. *Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, McManus K, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med 2009; 360: 859-873.*

16. *Akturk Z, Dagdeviren N, Enec FC, Ayemir İ, Tastan K. An Exercise Facility Connected to Family Practice Offices as a Solution for Female Obesity. Turkiye Klinikleri J Cardiovasc Sci 2010; 22.*

17. *Vranesic Bender D, Krznaric Z. Nutritional and behavioral modification therapies of obesity: facts and fiction. Dig Dis 2012; 30: 163-167.*

18. *Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, et al. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003; 348: 2082-2090.*

19. *Spielman AB, Kanders B, Kienholz M, Blackburn GL. The cost of losing: an analysis of commercial weight-loss programs in a metropolitan area. J Am Coll Nutr 1992; 11: 36-41.*

20. *Saka M, Türker PF, Bas M, Metin S, Yılmaz B, Köseler E. An Examination of Food Craving and Eating Behaviour with regard to Eating Disorders Among Adolescent. HealthMED 2012; 6(4): 1331-1340.*

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Adolescence and sexuality

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Abstract

Background: Adolescence is a period of transition between puberty and adulthood. It is a situation in which the subject remakes his concepts about himself and that leads to abandon his infantile image and to project it in the future of his adult life.

Purpose: The purpose of this study was to explore the relationships among individual factors, parental factors and adolescent attitudes about sex.

Methods: MEDLINE, SCIELO and LILLACS were searched through July 2012 to identify relevant articles. Were considered eligible studies on teen and sexuality. **Results:** The complete heterosexual genital relationship occurs in adolescence and the central sexual fantasy that is formulated during childhood takes its final form. At the sam time , define the role of procreative, searching for the love object in the external world. Masturbation is a normal expression of infantile sexuality In search of genital definition, adolescents often go through periods of homosexuality.

Conclusion: In modern society, what have happened is the franchise of sexual practices and perversions in general. The adult created a climate for their children rather promiscuous than permissive and that rather than allow them to serving their demands, have acted as an inhibiting factor , confusion and misuse of the original goals of the sexual instinct .

Key words: Adolescents, sexuality, masturbation, homossexuality.

Introduction

Adolescence is a crucial moment in the life of man and is the decisive step of a process of detachment. When the genital maturity stimulates to relate with the other sex, it is possible the consummation of incest. At the same time, you define your role of procreative, escaping to the incest, the adolescent begins the search for the love object in the external world, which will be realized with the discovery of your pair, if managed the internal detachment of the parents. It is a period of contradictions, ambivalent, painful, characterized by fights with the family and its enviroment.[1]

The sexual characters primary and secondary are in men and women in different ages. In girls, which are more early, the development of the breasts is the first sign of the initiation of sexual maturation. Soon after, they appear the pubic hair, axillary, and among these, the menarche. In the boy, on the contrary, the first sexual character secondary is the pubic hair, since it has already began to increase the size of genital organ. Only after they appear the axillary and finally the facial.

Freud, for tests on the sexual theory, says that "with the arrival of puberty, operating changes intended to give the sexual child life its final normal form. The sexual instinct was until then predominantly self-erotic, now is a sexual object". [2]Adolescence is the time in which under the gaze of the other, the subject will have to take ownership of an image of the body transformed.

Methods

We performed a literature review, based on a manual lifting of books and indexed publications and access to databases: MEDLINE, SCIELO AND LILLACS, provided by EBSCO Publishing Green Initiative.

The descriptors used were: "adolescence, sexuality". Most references date from the period 1987-2012.

The including criteria were: means of publication (books, journals); publication language (english, french, portuguese); articles type (clinical trial, meta-analysis, randomized controlled-trial, review, systematic review); age (13-18 years); references that focus on the approach of adolescence and its development of normal sexuality. Studies addressing homossexuality, masturbation during adolescence and the dilemma of teens today. Were

excluded studies that did not date from the mentioned period.

A general reading was performed in order to obtain an overview of scientific issues involving bioethics and mental health.

Results

The process of sexual adolescent, whether or not there is sexual intercourse between them, you must combine in itself three qualities: it must be a similar, yet different sex; to be desired, it should attract by his body, regarded as an object; it can only be loved if you take the time of the parents as for recent of the words, that is, if it can sustain an ideal figure of the other sex, as the parents have incarnated, for a time, this other absolute.[3]

The adolescent identifies, in his own body, the partial objects attributes of the parent of the same sex, whether they are the breasts or the menstrual blood for the girl, the voice or facial hair for the boy.[4]

The desire of the adolescent is hesitant: directed toward the other sex, he finds the forbidden from Edipo complex; addressed to the similar, he is oriented to a bond more fraternal than sexual.[5] Love the similar is in the foreground. The friendship in relation with the other, the fact of being or not of the same sex, become secondary.[6,7]

For the girl, the puberty marks what can be viewed by others.[8] The image of the body is committed to seeking a conformity to a model socially defined, found in magazines and in the figures of highlights female, as also with the demand of a confirmation by the other that the status of his body has changed. On the other hand, the menarche is, for her and the other, the first sign of their access to genital life.[9]

For the boy, that the animate in relation with the other sex will be the voice and its placing the evidence: the act of counting advantages.[10]

The change of tone causes a voice more serious, similar to the father in reality. Adolescence is certainly the time of a comparison and a confrontation with the image of the parent of the same sex. There is for the adolescent a ownership of the gaze and the voice supposed of another, this look and this voice which comes from the mother in the experience of the stage of the mirror.[11]

The adolescent through his experiences like dating versus first relationships and love versus fraternal tie, tests his sexual identity.[12] Flirting is an exercise that will demonstrate the value of look and voice. Dating is where the sexual games like the kiss on the mouth and touch, make him realize the quality of objects and the body of the other and the own body for the other. The object of love for the adolescent is different, what counts is the loving state.[13]

Adolescent and Masturbation

Masturbation is a normal expression of infantile sexuality, although it can also occur in adults.[14,15] The masturbatory act carries , inside an erotic game, a ratio of two, in which the hand performs the actual level of the role of sex of the other. However, the ghost can repress the Other, may be confined to persons of the same sex, different organs and orifices of the genital organs, the partial objects, such as droppings of the body, or may extend to animals or a world of inanimate and mysterious objects.

Masturbation helps the teen to accept her sex and fight the tendency to consummate incest. This assumes a new meaning to defend the young of the incest, since incestuous ghosts can materialize because there was already maturing of the genital organ. During adolescence the central masturbation fantasy that is formulated during childhood takes its final form and paradoxically must now be directed outward for appropriate object finding and pair matching in the service of procreative aims. This is a step in adaptation that requires a further developmental landmark. The path toward airing these private fantasies is facilitated by chum ship relationships as a step toward further exposure to the social surround.

In normal adolescence, masturbation fulfills the function of the ego to help organize itself around the genital supremacy. If this materializes, there will be an elaboration of the conflict by the loss of the body and identity of children. Masturbation is a testament to the adolescent genital operation and a recognition of the instrument which will enable him to face the genital relationship.[16] The appearance of semen in the boy and menstruation in girls is the starting point of a new stage in the possession of the body. In the psychic plane determines

conflicts and strains that can lead to unconscious rejection of procreation, sterility or serious difficulties to assume a paternal or maternal role.

The onset of puberty intense masturbatory activity means a repeat of maniac denial of the first year of life. The teenager has fantasies about beloved and fulfill these fantasies the same way that the game met during childhood: the need to prepare to have a sexual partner who is denied, what gets through masturbation.[16]

Teen and Perversion

As Freud said: "the sexual instincts gratify themselves through the conservation of the ego instincts".[2] The called polymorphous-perverse phase would begin with the birth of a subject and ended with his early adolescence, the production of final genitalization of their sexuality, which came to be proven and understood by young since puberty. There is a combination of sexual abstinence and losses of object, while appear frequent attempts to reestablish the polymorphous - perverse behavior, conduct that is rejected by adults.[17]\

The complete heterosexual genital relationship occurs in adolescence and is a much more common phenomenon than is considered usual in the world of adults of different social classes who try to deny the genitality of the teenager.[18,19,20]

With the end of the oral bond with the mother, occurs the discovery and manipulation of the genitals and the fantasies of establishing a bond genital. These bond genital fantasies happen with the characteristics of penetrating to male and penetrated to female. Then are the fantasies of penetrating or being penetrated the model of bond that will remain throughout the life of the subject later , as an expression of masculine and feminine.[21,22] For this, the figures of the mother and father are fundamental and essential. The absence or shortage of father figure will be what determines the fixation on the mother and, consequently, will also be the origin of homosexuality, both man and woman.

In search of genital definition, adolescents often go through periods of homosexuality, which may be the expression of a projection of bisexuality lost and desired, in another individual of the same sex.[23,24] Should not scare anyone from homosexuality fleeting situations that adolescents present.[25]

The lack of a father figure makes both the boy and the girl set to become mother.[26] The boy by not having a male figure with which to identify, for a deficit or absence of a father figure, try to look for that figure all his life (looking penis that gives power and masculinity). The girl set the relationship with the mother oral and skin contact repressing and denying the possibility of a relationship with a penis, even in the absence of their early object relations. The root of homosexuality is the fact that the father did not assume their roles or he is absent. Both the boy and the girl are going to homosexuality, because both are thus obliged to keep bisexuality as a defense against incest.[27] Recent research has suggested that the sexual identity development of lesbian, gay, and bisexual (LGB) youths may not follow a single pattern, but may follow a variety of pathways.[28] Although some research documenting variability identity development exists, unclear are the potential individual and social contexts that predict these different patterns, as well as the contexts that predict changes in identity integration over time.[29,30]

Conclusion

There is much talk in the fall of sexual taboos in modern society, when in fact what seems to have happened is the franchise of sexual practices and perversions in general, than the overcoming of prejudices. The adult contemporary, in its desire to free themselves from the yoke of repression of sexual instincts enslaved to them all the time, created a climate for their children rather promiscuous than permissive and that rather than allow them to serving their demands, have paradoxically instinctive acted as an inhibiting factor, confusion and misuse of the original goals of the sexual instinct.

The release of sexual mores in our time and the degree of safety provided by the improvement of contraceptive methods allow the possibility of a contemporary teenager significantly change their sexual behavior in relation to past generations. The media have arguably become the leading sex educator for children and teenagers.[31] Considerable research now exists that attests to the ability of the media to influence adolescents' attitudes and beliefs about sex and sexuality. In addition, new research has found a significant link between exposure to sexual content in the media and earlier onset of sexual intercourse.[32,33]

The sexual dilemma of the young today is undoubtedly based on the values of family crisis and bankruptcy of the contemporary institution of marriage as an instrument to ensure the stability of affective relationships and procreative function of the species.[34]

References

1. *Aberastury A et. al – Adolescência. Porto Alegre, Artes Médicas, 1990.*
2. *Freud S. (1905) – Três ensaios sobre a teoria sexual. Obras completas (vol. VII) . Rio de Janeiro, Imago, 1987.*
3. *Aberastury A; Knobel M – Adolescência normal. Porto Alegre, Artes Médicas, 1989.*
4. *Dolto F. – Psicanálise e pediatria. Rio de Janeiro, Guanabara, 1988.*
5. *Fenichel O. – Teoria psicanalítica das neuroses. São Paulo, Atheneu, 1997.*
6. *Allensworth-Davies D, Welles SL, Hellerstedt WL, Ross MW. - Body image, body satisfaction, and unsafe anal intercourse among men who have sex with men. J Sex Res. 2008 Jan-Mar; 45(1): 49-56.*
7. *Aylwin AS, Reddon JR, Burke AR. Sexual fantasies of adolescent male sex offenders in residential treatment: a descriptive study. Arch Sex Behav. 2005 Apr; 34(2): 231-9.*
8. *Kulik-Rechberger B. - Individual and environmental conditions influencing puberty in girls. Ginekol Pol. 2008 Oct; 79(10): 697-701.*
9. *Agampodi SB, Agampodi TC, Ukd P. - Adolescents perception of reproductive health care services in Sri Lanka. BMC Health Serv Res. 2008 May 3; 8: 98.*
10. *Rassial JJ. – O adolescente e o psicanalista. Rio de janeiro, Companhia de Freud, 1999.*
11. *Rassial JJ. – A passagem adolescente. Porto Alegre, Artes e Oficios, 1999.*
12. *Thomas JJ. Adolescents' conceptions of the influence of romantic relationships on friendships. J Genet Psychol. 2012 Apr-Jun; 173(2): 198-207.*
13. *Ballester Arnal R, Gil Llario MD. - Sexuality in children 9-14 years old. Psicothema. 2006 Feb; 18(1): 25-30.*
14. *Shapiro T. - Masturbation, sexuality, and adaptation: normalization in adolescence. J Am Psychoanal Assoc. 2008 Mar; 56(1): 123-46.*
15. *Strachan E, Staples B. Masturbation. Pediatr Rev. 2012 Apr; 33(4): 190-1.*
16. *Gerressu M, Mercer CH, Graham CA, Wellings K, Johnson AM. - Prevalence of masturbation and associated factors in a British national probability survey. Arch Sex Behav. 2008 Apr; 37(2): 266-78.*
17. *Katz G, Lazcano-Ponce E. - Sexuality in subjects with intellectual disability: an educational intervention proposal for parents and counselors in developing countries. Salud Publica Mex. 2008; 50 Suppl 2: s239-54.*
18. *Haydon AA, Herring AH, Prinstein MJ, Halpern CT. Beyond age at first sex: patterns of emerging sexual behavior in adolescence and young adulthood.J Adolesc Health. 2012 May; 50(5): 456-63. Epub 2011 Nov 17.*
19. *Heilborn ML, Cabral CS. - Sexual practices in youth: analysis of lifetime sexual trajectory and last sexual intercourse. Cad Saude Publica. 2006 Jul; 22(7): 1471-81.*
20. *Mungrue K. - Has early initiation of sexual activity in adolescence had an impact on teenage childbearing in Trinidad and Tobago? Int J Adolesc Med Health. 2008 Jul-Sep; 20(3): 255-60.*
21. *Bos HM, Sandfort TG, de Bruyn EH, Hakvoort EM. - Same-sex attraction, social relationships, psychosocial functioning, and school performance in early adolescence. Dev Psychol. 2008 Jan; 44(1): 59-68.*
22. *De Bruyn EH, Cillessen AH, Weisfeld GE. Dominance-popularity status, behavior, and the emergence of sexual activity in young adolescents.Evol Psychol. 2012 Jun 21; 10(2): 296-319.*
23. *Macdougall J. – Em defesa de uma certa anormalidade, teoria e clínica psicanalítica. Porto Alegre, Artes Médicas, 1991.*
24. *Osório LC – Adolescente hoje. Porto Alegre, Artes Médicas, 1992.*
25. *Arreola S, Neilands T, Pollack L, Paul J, Catania J. - Childhood sexual experiences and adult health sequelae among gay and bisexual men: defining childhood sexual abuse. J Sex Res. 2008 Jul-Sep; 45(3): 246-52.*
26. *Fairtlough A. - Growing up with a lesbian or gay parent: young people's perspectives. Health Soc Care Community. 2008 Sep; 16(5): 521-8.*
27. *Rule NO, Ambady N, Adams RB, Macrae CN. - Accuracy and awareness in the perception and categorization of male sexual orientation. J Pers Soc Psychol. 2008 Nov; 95(5): 1019-28.*

28. *Worthington RL, Navarro RL, Savoy HB, Hampton D. - Development, reliability, and validity of the Measure of Sexual Identity Exploration and Commitment (MOSIEC). Dev Psychol. 2008 Jan; 44(1): 22-33.*

29. *Coyne CA, D'Onofrio BM. Some (but not much) progress toward understanding teenage childbearing: a review of research from the past decade. Adv Child Dev Behav. 2012; 42: 113-52.*

30. *Rosario M, Schrimshaw EW, Hunter J. - Predicting different patterns of sexual identity development over time among lesbian, gay, and bisexual youths: a cluster analytic approach. Am J Community Psychol. 2008 Dec; 42(3-4): 266-82.*

31. *O'Hara RE, Gibbons FX, Gerrard M, Li Z, Sargent JD. Greater Exposure to Sexual Content in Popular Movies Predicts Earlier Sexual Debut and Increased Sexual Risk Taking.Psychol Sci. 2012 Jul 18.*

32. *Chapman EN, Werner-Wilson RJ. - Does positive youth development predict adolescent attitudes about sexuality? Adolescence. 2008 Fall; 43(171): 505-23.*

33. *Strasburger VC. Adolescents, sex, and the media. Adolesc Med State Art Rev. 2012 Apr; 23(1): 15-33.*

34. *Savin-Williams RC, Ream GL. - Prevalence and stability of sexual orientation components during adolescence and young adulthood. Arch Sex Behav. 2007 Jun; 36(3): 385-94.*

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An evaluation on vital organ donation, culture, altruism and informed consent

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Abstract

Altruism has a biological basis of concept, besides of that altruistic behaviors are shaped up by cultural components classifications of the communities and empowers the relationship among the members of the community. Cultural components, individualism and collectivism definitely have a relationship with informed consent and autonomy which take part in medical ethics principles and this relationship must be evaluated in frame of organ donation. That evaluation informs us about what the community actually think about their culture. Because culture includes abstract and concrete values depend on the society, person and the geographical location possibilities. Societies have their own cultural links and their own perception about life in terms of altruistic behaviors. Science and technology help these communities to keep in touch to each other by also affecting their ethics value. Along with the ethics principles that are constructed in an universal frame, cultural components of the societies are applied in different ways.

Key words: Altruism, culture, organ donation, autonomy, informed consent.

Introduction

Communities of Asia, especially Middle East and Europe-Western which can explicate east and west culture are different in terms of abstract and concrete cultures that are called as encounter cultures which mean that culture is a distinctive feature between communities. The cultural structure has different components, their differences especially begin with language structure and symbiotic as their structures and signs effect some phenomenon such as the way of thinking, creating new approach to events, defining the problem, critical thinking, superstitions, religion, interpretation of life and requesting long-term healthy life.[1,2] Knowing that, not only cultural approaches but also health policy and health issues play an important role on medicine. This enables us to observe some of the altruistic behaviors better. In this context, it is said that attitude and realization of the organ donation are invariably depend on cultural component. Cultural life has an impact on health and also it determines attitudes about health applications. Both of collectivism and individualism approaches are determined by their own comprehension of perception toward health and these approaches are steered by some of health applications include vital donor or cadaver[4].

The first point to be indicated is the affect of the ecological conditions on culture. Researches show that a significant effect on communities is geography-ecological conditions-. Ecological conditions have an affect on abstract and concrete complements of culture. The most significant difference between the continents is climate as it determines the cultural components. It know that priority of the community developed as communal life in Asia and Asian communities usually have the idea of collectivism, but in Europe, priority of the individual development-individualism- was accepted more important as a feature.[3,4]

Another point to be discussed is the language differences that form the altruistic applications. However communities have their own tradition, custom, symbolic thoughts, community dynamics and beliefs, it is the language and culture of language which actually create a pathway to the cultures such as English culture, Arabic culture, Turkish culture. Studies on relationship between individualism and collectivism with languages show that language is decision-maker factor[4]. In the world, languages are separated in two major ways: Agglutivenative and non agglutivenative. Both of them are content of languages' grammatical structure which depends on morphology and these forms are of deeply importance on human development for the expression of oneself. These terms were introduced by Wilhem Humbolt in 1836.[1] While the language of Asia or non-Western languages are generally agglutivenative, language of Western is nonagglutivenative.[4]

What is the difference between agglutivenative and nonagglutivenative languages in terms of component of culture? Firstly, agglutinative languages are generally spoken in Asia, and among non-Western communities which give more importance on collectivist life than individual life and it has been used since ancient time (Sumerian, Hurrian). Secondly, non-agglutinative languages are generally spoken by Western communities which have American or Anglo-Saxon culture that evoke individualism.[3]

Both of these language types include signs, visual fields, patterns of symbolism, visual semiotics, and perception of life. However, without a single doubt, communities which use non-agglutivenative language are also very important as it is the core discipline of individualism.[3,4] It is important that opposite views and ideas enable a progression for human development since this progress provide different point of views to people. It can be said that essence of development is based on this dilemma. This fundamental dilemma can be seen as not only language structures like agglutinative and non-agglutinative but also social development theories like individualism and collectivism terms which are used to describe cultural differences in social behavior.

Individualism and collectivism are two components which complete each other like a DNA helix in a symbiotic relationship. They cannot be regarded as separate and independent components as they contribute to the development of the progress of each other. So that it can be regarded as this symbiotic relationship belongs to human contributing to the self-expression in two contradictory ways. So, people find an opportunity for development because comparing and contrast enable creative and critical thinking. In this way, people can think of different possibilities and criticism, they have thought of new ways, experience in various ways, they make synthesis more easily for different usage of symbiotics and language.

This difference is of not only structure of verbal language but also nonverbal language which is actually body language that every community has its own style on verbal and nonverbal language.

Briefly, encounter culture can be defined as west and east culture which has various features, but there is same diversity in custom, tradition, community dynamics and beliefs. Along with that language's grammatical structure plays an important role to shape up human thoughts, attitudes, behaviors, applications, approach to events and so forth. The same features are also the crucial points of organ donation as organ donation includes behavior, attitude, thought, community dynamics, relationship, custom and tradition. This issue has different ways of solving problems as legal and altruistic approach.[6] In the light of the statements above, the frame of organ donation changes depending on communitarian comprehension and individual comprehension.

Altruism

Altruism concept has been the subject of the study of moral philosophers, sociologists and evolutionary biologists. Altruism is described in the free dictionary as; **"**Instinctive cooperative behavior that is detrimental or without reproductive benefit to the individual but that contributes to the survival of the group to which the individual belongs". Altruism concept was clearly introduced as ethical doctrine by Auguste Comte (1798-1857). Comte defined altruism "a tendency or a desire to live for others. Comte described altruism as living for the sake of the others".[8] John Stuart Mill (1806-1873) described altruism as an impetus for action and difference between gradations of pleasure.[9] On the other hand, famous sociologist Emile Durkheim (1858-1917) said that moral collectivism and altruism are perceived as organic nature of society.[10] Pitirim A. Sorokin (1889-1968) developed creative altruism and his foresight is that people who internalize high altruistic values strengthen adaptation and interaction of the societies.[11] Altruism is also defined in evolutionary biology, an organism behave altruistically to benefit from the other organisms, this benefit explains that altruistic behaviors depend on natural selection acts. This biological process probably leads people to altruistic behavioral development for the behalf of other organisms.[12]

Altruistic approaches create human behaviors such as family relationship and family's or society's expectations that are more important than person's own goals or desires. In another words, altruism is highly accepted as an element of prosocial behavior. However it has been known that altruistic behaviors depend on person, group, and, community; causes and conclusions of culture which include various parameters emerge from the applications of these behaviors. These parameters are emerged by basic

components that include geography and human biology and their interaction to each other. We have the knowledge of organism processes which includes biological, physiologic and chemical, but it isn't enough for understanding every mechanism about living organism including human. However, we can understand altruistic behaviors of human with observing and evaluating some situations like process of organ transplantation procedure from vital donor to patient. In this context, in the case of organ donation, donor is usually chosen among kin members in Turkey. There are two basic reasons for this donation: the first one is biological coherence in terms of tissue and blood crossmatching. The second reason is cultural basis. This cultural behavior is shaped up by Turkish family members and leading authorities of society via custom, religion, rule of elders, traditions that presented as his/her own culture. Especially, societies of Asia-Middle East- implement their own life as the most extreme style which they easily sacrifice themselves for an holly aim or they are considered as nationality, ethnicity, religion, and land.

Both of these approaches are sensible but problem is about donor who gives its own organ to kin member without first questioning if the donor is truly enthusiastic or not. Why organ donation emerges as a problem for donor in Turkey? Because, people in Turkey constitute a pride community which appears as a perception of being accepted and approved by the other members of the society.[12] The outlook of the "others" is always more important than their own aspects- especially the thoughts of the family members. If a vital donor commits organ donation, the reason of this commitment will probably be the desire to be accepted by vital donor's own family members and to be applauded and/or honored in the community. But then the vital donor can regret for his own behavior. Therefore, the explanation of the process of organ donation is of crucial importance significant in terms of understanding the fundamental dilemma of conscientious acceptance or the objection of the vital donor.

This contradiction arises because of two reasons. The first reason is collectivism. The acceptance of being a vital donor with the intention of being accepted by the social community is actually a requirement of the collectivist societies. In this respect, as Islam religion includes collectivist approaches, this reason plays a crucial role on this choice. According to collectivist cultural approach, sense of responsibility, shared values, social well-being, accomplishment of community, values of community are more significant than person or vice versa. Individual approaches include more liberal feelings of individual freedom, individual independence, and individual development. Individuality takes place in center as a core on the contrary of community.

The second reason is being a pride community. Pride has two meanings as positive and negative. As for the first meaning, pride describes a feeling of happiness that comes from achieving something. For the other meaning, pride refers to feeling of exceedingly high self-regard which makes people dependent and people can be slave of pride merely. Pride is an important emotion that plays an important role on life of human and it begin from childhood and ends with the death. Pride reinforces prosaically behaviors such as altruism, adaptive behavior and achievement.[13] Pride has positive and negative value, high and low activation and its family, state or /and a group forms. Loss of the pride causes people to be aggressive and enact antisocial behaviors since pride community members suffer from pride.[14] Because worth concept neither belongs to person nor belongs to a community which has like fairly a symbiotic life. The behaviors of the person should be in harmony with the common acceptance of the society. Otherwise, person is isolated from the rest of the society and the family members as the common expectation is to develop an altruistic behavior in accordance with the necessity of the community as it will be stated below.

Altruistic Behaviors in Turkey

Turkish people have altruistic behaviors which have different behavioral styles and they can be evaluated in terms of different characteristic approaches. This situation occurred because of different cultural components which were emerged by geographical positions. These cultural components determine two dimensions: individualism and collectivism.[15] Both of them have abstract and concrete cultural components that include differences in attitudes, values, cognition, communication, behaviors, intention, prediction, approaches, attribution, socialization, self concepts, and practices.[16] In this context, these behaviors are also of equal importance in history. As

its known, life began in Anatolia 600.000 years ago and what we know about Anatolia's written history is that this history had a number of communities.[17] Anatolia is bridge and a settlement and its communities are both individualist and collectivist. For this reason, Turkish people have some values from previous communities that are seen idioms, superstitions, music, fortunetelling, eating etc. In addition to this, Turkish people have culture of Seljuk and Ottoman communities and its own neighbors. This data means that Turkish people are exposed to both west and east culture. This interaction has existed for thousands of years. In this period, Turkey has not only effected its neighbor countries with its own culture but also it has been effected by them.

Turkish people have collectivism and altruism, this situation is handled as a big problem by some of artist or writers like Sinan Çetin. Sinan Çetin who is a cinema master, said that "collectivist and altruistic culture are my pain" and he thinks that collectivism and altruism are big problems for Turkey and the world.[18] Altruistic expectation of collectivism is forced by people who really perform unwilling acts. Besides of altruism, unwilling acts are supported by pride perception. Turkish people have honor culture which is described in two components as inner and outer honor. Turkey perceives honor as it is in Mediterranean and South America. They give more importance outer pride than inner pride. Honor has different kinds: collective honor, feminine and masculine.[13,14] But in Turkey honor has also another characteristic; honor culture is explained that the pride is perceived and objectified as a possession. Therefore, people don't want to lose their pride, so they frequently try to do everything for outer honor. People who give more significance to outer honor than inner honor and it is a problem for individual and individual happiness, peaceful, development for oneself. Outer pride and altruism help behaviors to increase since people fell themselves to be forced for good behavior to the others like organ donation. However they also do misbehaviors such as chastity killings, misconduct and bribe and these misbehaviors are seen more frequently in outer pride community.[19] Because people think that the bad behaviors are not considered as a problem such as bribe and misconduct unless society is aware of one's behaviors. Inner pride is as important as outer pride for building a healthy community.[13] Individualism is a basic element of the society, condition of the individual equally determines the condition of the society. These interactions caused a cultural contradiction that while one side of Turkey is highly tend to the west, the other one is highly tend to the Middle- Eastern so there is a smooth difference between the parts of Turkey. Due to this difference, the societies living in Anatolia have come across with some of cultural dilemmas. Some of people has an '*asiret'* concept that population changes between 300-10000 and they are all relatives. These *asiret's* life mainly base on their *asiret*'s culture, people mostly consider views of their '*asiret'* rather than their own, that is, individuals must fit into *asiret* rules. In this situation, their *asiret*'s views, approaches or practices are more important than individual thought. However, west, south-north west cities highly have western culture and Turkish language is dominant here. Contrary to '*asiret*' culture, family concept and individualism are accepted. Naturally, this situation indicates the reason of the difference in the altruistic behaviors among people in Turkey. On one hand, some people actualize the altruistic behaviors consciously but some people actualize altruistic behavior so as not to be condemned, criticized. On the other hand, they actualize altruistic behaviors, especially organ donation, to prevent the complaints about them and to be appreciated and to be honored by their relatives besides of their close and distant social environment. If a member of the *'asiret'* or community doesn't have precious pride - deny of organ donation like kidney or tissue etc.- the family or members of *asiret's* pride also hurt and they are condemned by social environment.

Informed Consent and Organ Donation

Culture causes comprehension and perception of concepts like autonomy and informed consents. Informed consent is interested in autonomy of concept; the more individual autonomy actualizes, the more informed consent develops and takes place in the life of the individual. At this point, 'decision-making' comes out as an important concept as it also recalls the concept of 'destiny'. So that avoiding of the self responsibilities makes individual to transfer the responsibilities to the "others".

In Turkey, the informed consent can be handled in that way: However patient rights has been accepted since 1998 in Turkey, a contradiction is said to be

existed about autonomy and informed consent based on the cultural components of the society. Medical doctors take the informed consent from the patient in the frame of autonomy but the decision is actually belong to the family or *asiret* members rather than the patient's own decision. Health professionals take lesson about medical ethics concept and applications, besides legal regulation on patient rights call as Patient Rights Legislations". Approaches that take into consideration of the patient autonomy and informed consent are explained in 'Patient Rights Legislations'.[22] Doctors consider the determined rules in the frame of ethic codes, principles and patient rights legislations, as well. Because most of the patients are not equipped with enough psychological, social, and cultural knowledge as they are lack of the sense of individual independence, they are not able to have the ability of making a judgment about autonomy, decision making, and informed consent; that's why this issue emerges as a problem. In this reason, instead of making their own decision, patients or vital donors accept decision of their family members or *asiret*. Patients and vital donor actually don't convey their own decision to the medical doctor. As a conclusion these people who have altruistic behavior are accepted, honored, and respected by social environment or family member; but as a conclusion of this attitude, their behavior turn into an obligation rather than their own intention. The doctors are aware of this situation, but unfortunately they have no other option but to accept their decision because of cultural approaches. The mentioned problem increases especially in the east parts of Turkey. This problem not only emerges in Turkey.

Middle-East communities give more importance on justice than autonomy. Therefore, people consider firstly their rights- patient rights but the important point is that they do not perceive autonomy as individual rights - but in Turkey a minority of groups take into consideration autonomy -informed consent- consciously.

Comprehension of the informed consent which takes place in the frame of patient rights changes depending on people of their connection with west or east culture. People connected with west culture have core family concept and individualism. These people easily understand autonomy and informed consent, so they apply these disciplines in their life and in hospitals.

However, people connected with the east culture don't comprehend individualism perfectly and don't understand autonomy and informed consent. They often accept the idea and decisions of their family or *asiret* as their own. That's why in the period of informed consent, *asiret* or family's idea is accepted as the patient's individual idea. In this context, application of this legal regulation is difficult in real terms because culture of people don't include enough autonomy concept and practices like informed consent.

Briefly, as the members of these *asirets* and large families- are not conscious about the concept of autonomy, they do not give importance about a group of people's justice or right but they don't give importance about individual right, autonomy. It is a big problem since it is not possible for a person to decide on one's own thoughts in terms of autonomy. However doctors are aware of this realism, they are helpless about that dependent and collectivist attitudes which do not include altruism concept.

Conclusion

Turkish people have more collectivist interdependence than individualist self-reliance. Both individualism and collectivism have a complex structure which includes a lot of factors and it is not possible to separate them. Altruism concept and altruistic behaviors, in the frame of organ donation and vital donor in Turkey, explain us individual and collectivist approaches and their applications. Altruism is not important only in communication or in relationship but also important for the development in health fields and technology that presents new hopes to people. Human factor is the most important element of these developments. Vital donor and organ donation have a very close relationship in terms of altruistic comprehension and perception which are, at same time, connected to the component of culture and biology in the way of organ donation. In the context of collectivist communities such as Turkey, vital donor ,who has not enough idea and behavior of autonomy and informed consent, accept ideas or views of their social environment.

In this manner, organ donation and vital donor gain more importance in terms of autonomy and informed consent but components of culture affect the application of these concepts. The behavior of the vital donor, who commits donation with the purpose

of sacrificing his family members, is actually not an altruistic behavior on contrary to the popular belief of the community. In Turkey, organ donation progress includes complex ethical issues such as autonomy, decision making, donors self assessment and donors expectation. In order to create a balance between individual and community, new regulations and education can be put into practice. Authorities take into consideration these problems and doctors have to pay attention on ethical concern and evaluate donors' thoughts since they can actually be vulnerable. In the long run, the most beneficial solution is explaining the importance of being an individual rather than a collectivist.

References

1. *Humboldt W. On Language: On the Diversity of Human Language Construction and its Influence on the Mental Development of the Human Species. Ed. Michael Losonsky.Trans. Peter Heath. Intro. Hans Aarsleff. Cambridge: CUP, 1988. Rpt. 1999. access date: 4 March 2012.*
2. *Moriarty SE. The Symbiotics of Semiotics and Visual Communication Journal of Visual Literacy. Spring 2002; 22: 19-28. http://www.ivla.org. access date: 5 March 2012.*
3. *Oyserman D, Lee WS S, Does Culture Influence What and How We Think? Effects of Priming Individualism and Collectivism Psychological Bulletin American Psychological Association 2008; 134: 311–342. DOI: 10.1037/0033-2909.134.2.311 access date: 7 March 2012.*
4. *Haspelmath M. An Empirical Test of The Agglutination Hypothesis Max-Planck-Institut für evolutionäre Antropologie. Leipzig. http://www.eva.mpg.de/lingua/staff/haspelmath/ access date: 4 March 2012.*
5. *Toth A., "Are all Agglutinative Languages related to one Another, International Mikes. http://www.federatio.org/mikes_bibl.html access date: 5 March 2012.*
6. *Badhwar N. K. Altruism versus self-interest: Sometimes False Dichotomy, In E. F. Paul, F. D. Miller, & J. Paul (Eds.), Altruism Cambridge UK: Cambridge University Press 1993; 90-117.*
7. *Mitteldorf, J.J. and Wilson, D.S. 2000. Population Viscosity and the Evolution of Altruism. J. Theor. Biol. 2004: 481-496. access date: 4 March 2012.*
8. *Comte A. System of Positive Polity, 1-4 London: Longmans, Green and Co. 1875-1877.*
9. *Mill S J, Utilitarianism, 1863. http://www.utilitarianism.com/mill1.htm. access date: 2 March 2012.*
10. *Dubeski N. "Durkheim's Altruism as the Source of His Social Holism: A Discussion of the Vialibility of a Social Basis for Moral Principles" Electronic Journal of Sociology, 2001. http://www.sociology.org/archive.html. web. access date: 4 March 2012.*
11. *Sorokin PA. Studies of the Harvard Research Center in Creative Altruism. B.V. Johnston. Ed. Pitirim A. Sorokin: On the Practice of Sociology Chicago: University of Chicago Press. 1948; pp. 305-316.*
12. *Wilson S.D. and Wilson O.E. Rethinking the Theoretical Foundation of Sociobiology, The Quarterly Review of Biology, 2007; 82; No. 4. access date: 5 March 2012.*
13. *Tracy JL, Robins RW.(2004). Show Your Pride: Evidence for A Discrete Emotion Expression. PsycholSci, 2004; 15: 194–197. access date: 4 March 2012.*
14. *Öner-Özkan B., Gençöz T. (2001) The Importance of Investigation of Turkish Culture from the Point of View of Cultural Pride. Kriz Journal. 2001; 14-3: 19-25. access date: 3 March 2012.*
15. *Fiske A P. (2002). "Using Individualism and Collectivism to Compare Cultures—A Critique of the Validity and Measurement of the Constructs: Comment on Oyserman et al. University of California, Los Angeles, Psychological Bulletin Copyright 2002 by the American Psychological Association, Inc. 128: 78–88. access date: 11 March 2012.*
16. *Páez D. Variation of Individualism and Collectivism within and between 20 Countries A Typological Analysis Jean-Claude Deschamps Journal Of Cross-Cultural Psychology, 2005; 36(3): 321-339.*
17. *Akurgal E. Ancient Civilizations and Ruins of Turkey, Net Publication, Turkey. 1969.*
18. *Soydan M. Political Identity and Cinema Language Relationship: Example of Sinan Cetin (Siyasal Kimlik ve Sinema Dili İliskisi: Sinan Çetin Örneği). Küresel İletisim Dergisi, Bahar 1. 2006, access date: 6 March 2012.*
19. *Coymak A. Isik R. Other East as a Social Representation: Honor and Chastity Killing in Social Psychological Paradigm. 2011.*
20. *http://qub.academia.edu/AhmetCoymak/Papers/1001068/ Other East as a Social Representation Honor and Chasity Killing in Social Psychological Paradigm (CRcsPP Working Paper.1001068) Retrieved (01.04.2012) access date: 9 March 2012.*
21. *Ministry of Health of Patients' Rights Legislations. Turkey (1998) http://sbu.saglik.gov.tr/hastahaklari. access date: 4 March 2012.*

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Cases with critical illness polineuromyopathy

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Abstract

Background: One of the significant causes of difficult weaning of Intensive Care Unit patients from mechanical ventilation is critical illness neuropathy and critical illness myopathy. 50-70% of intensive care unit patients has critical illness neuropathy, 42% of them has critical illness myopathy. It is diagnosed by EMG, there is no specific method for treatment, it is more symptomatic. However, some medicines are reported to be beneficial such as Intra Venous Immune Globulin.

Methods: Some cases were observed retrospectively in terms of mortality that patients diagnosed with critical illness neuropathy and critical illness myopathy who were administered Intra Venous Immune Globulin and not administered Intra Venous Immune Globulin.

Results: In our study 19 patients were included. Critical illness neuropathy was present in 18 out of 19 patients and one patient was critical illness myopathy. Cases were separated in two groups that 6 of the critical illness neuropathy patients were administered Intra Venous Immune Globulin and 12 of the patients were not administered. The age range was 10-84. All patients receiving Intra Venous Immune Globulin were successfully weaned from the mechanical ventilator while 5 of the patients not receiving Intra Venous Immune Globulin could be weaned off, 7 patients were required ventilation and died.

Conclusions: It is surmised that critical illness neuropathy patients receiving IVIG are easier to be weaned from the mechanical ventilator as well as their mortality rate can be decreased. In addition, an efficient rehabilitation programmer will have a positive effect upon the treatment procedure of the critical illness neuropathy/critical illness myopathy patients.

Key words: Critical illness polyneuropathy, critical illness myopathy, electromyography.

Introduction

"Critical illness" frequently develops in patients hospitalized in intensive care units as a complication of an illness, a trauma or a surgery. Muscle weakness might occur in those patients as a result of that critical illness affection. Some of the underlying reasons of muscle weakness acquired in Intensive Care Unit (ICU) patients might be Guillain-Barre Syndrome, Myasthenia Gravis, polyneuropathy.[1] Polyneuropathy develops in 70-80% of the patients with sepsis and multiple organ dysfunction. In 50-70% of those polyneuropathy patients, critical illness neuropathy (CIP) occurs and 42% of them exhibits critical illness myopathy (CIM). Pati et al.[2] observed loss of muscle strength in 25-85% of ICU hospitalized patients who developed polyneuropathy. Hyperglycemia, corticosteroid and neuromuscular blocking agents are the major risk factors while multiple organ dysfunction and systemic inflammatory response syndrome (SIRS) and immobilization are the other various risk factors for intensive care unit acquired weakness.[1]

In our study we will examine the results of intravenous immunoglobulin treatment administered in CIP/CIM patients who are diagnosed through electromyography (EMG) and having difficulty in weaning from the mechanical ventilator.

Material and Methods

After receiving the research ethics committee approval from Yuzuncu Yil University Faculty of Medicine; between January 2005 and December 2010, from 19 patients hospitalized in anesthesia ICU of our hospital were included in our study who were mechanical ventilation dependent and diagnosed with CIP/CIM through EMG. ICU patients were examined in terms of their age, gender, CIP/CIM diagnosis, major risk factors, mortality and treatment alternatives.

Statistical analysis

Descriptive statistics for continuous variables of the overemphasized features were expressed as average, standard deviation, minimum and maximum values while for categorical variables they were expressed as number and percentage. For comparison of groups in terms of continuous variables, Mann–Whitney U test was used. In determining the relationship between categorical variables khi-kare and likelihood ratio test were used. Statistical significance level was 5% in the calculations.

Results

Demographical data and duration of Intensive Care Unit hospitalization of patients were shown in Table 1. Routine intensive care monitorization, blood gas analysis of the patients and trail of weaning from mechanic ventilator were performed. Blood glucose levels of the patients were checked in accordance with the intensive insulin treatment. After EMG 18 patients were diagnosed with CIP and 1 patient was CIM. 11 of the patients (57.9%) used corticosteroid due to major risk factors and 8 of the patients (42.1%) did not. IVIG treatment was administered to 6 of the patients (31.6%) while therapy was not given to 13 of the patients (65.4%). All of the patients given therapy achieved complete response on the treatment. During the IVIG treatment, 0.4gr/kg IVIG (Pentaglobulin 50 mL, Biotest Pharma, Dreieich/ Germany) was administered for 3 days. 7 of the patients with CIP were died, 11 of the patients were survived that 6 of 11 were taken therapy as 5 of 11 were not. Patient with CIM diagnosis was discharged from the hospital. No statistical significance was determined between EMG result and gender (p=0.33), risk factor and gender (p=0.463), mortality and treatment (p=0.127) and risk factor and mortality.

Discussion

Progress in the treatment of critical patients especially who required mechanical ventilator in ICU, lengthened the duration of staying in the intensive care unit. It is reported that muscle weakness and paralysis are very common in ICU and cause CIP/CIM, consequently those two diseases are common determined.[3] CIP/CIM complicates to wean the patients from mechanical ventilator in ICU as well as lengthens the duration of ICU and increases the mortality.

As it was the most remarkable finding identified by Bolton and his colleagues in 1984 that CIP patients have difficulty in weaning from mechanical ventilator, flask and areflexia extremity are observed in the neurological examination.[4] CIP is a distal axonopathy containing both sensation and motor nerves. Pati et al.[1] classified polyneuropathy in 3 groups such as CIP, CIM and CIP/CIM (developing together). In the pathological physiology, complications of sepsis and SIRS triggered by severe trauma and burns might cause impaired perfusion of peripheral nervous system. Also any increase in capillary permeability might cause the transmission of toxicants into peripheral nerve system. In addition, it is indicated that hyperglycemia which occurs as a result of increased insulin resistance cause ischemia in nerve by raising the endoneural vascular resistance and reduce the blood flow.[5-6]

Endoneural hyperkalaemia and/or membrane depolarization associated with hypoxia in motor axones might support the development of CIP.

CIM is a diffuse neuropathy effecting proximal as well as distal muscle groups. Muscle weakness is effective on leg proximal. It may proceed to facial muscles and rarely seen involvement of extra ocular muscles. Deep tendon reflex is tending to be decreased. The most significant complication of CIM is the difficulty in weaning from the mechanical ventilation based upon diaphragmatic weak-

Table 1. Demographical data and duration of intensive care unit hospitalization of patients

	Average	Minimum	Maximum
Age (n:19) (years)	43.37	10	84
Female (n:10) (years)	47.6	24	84
Male (n: 9) (years)	38.67	10	63
Number of days in intensive care	44.89	9	120
Number of days on EMG	21.82	2	52

ness. Hypercatabolism due to sepsis and muscle protein catabolism due to substrate insufficiency develops in CIM.[7] It is argued for the opinion that corticosteroids and neuromuscular blocker agents play significant role in the pathogenesis of thick filament myopathy.[8]

Chawla et al.[9] reported that developing generalized muscle weakness, areflexia and difficulty weaning from the mechanical ventilation is a common clinical presentation for patients particularly with critical illness such as Sepsis, Multiple Organ Failure and Hyperglycemia. Difficult weaning from the mechanical ventilation of our critical patients made us think the polyneuropathy. Deep tendon reflex of our patients was decreased. CIP is related with the use of high dosage non-depolarized muscle blocking medicines and steroids.[2] 4 of 6 treated patients and 7 of 13 untreated patients were given corticosteroid based upon major risk factors. In addition, differential diagnosis is substantial for prediction of diagnosis of the patients remaining in intensive care unit for long periods.[10] We would like to emphasize that because of the identical examination findings of both CIP and CIM, EMG is a golden standard to distinguish those two diseases. Also, high Creatine Kinase level of 50% of patients with CIM which peaks in a few days of the onset illness, is a supporting evidence for the diagnosis. In biopsy results, while atrophy particularly in type 2 muscle fibers and necrosis in muscle fibers was observed, any inflammatory cell was not found. Therefore we diagnosed our patients by performing EMG. During the nerve conduction studies in EMG, low amplitude motor responses were taken as the sensory responses were found normal (sensory responses are decreased in CIP while it is normal in CIM).

Recently there is not any existing intervention or specific treatment reducing the effects CIP/CIM or preventing the beginning of CIP/CIM.[11] Treatment is principally symptomatic and can be performed with the rehabilitation of the medical and surgical situations that cause the sepsis.

It has been indicated that early administration of IVIG in Multiple Organ Failure and gram negative sepsis decrease severe complications. Whether CIP is developed or not, multiple organ failure cases with sepsis 0.3 gr/kg intravenous of IVIG was administered for 3 days as soon as it was diagnosed, 3 cases were administered IVIG treatment after 24 hours and IVIG was not given to 4 sepsis and multiple organ failure cases developed CIP.[12]

Also publications indicating the administration of IVIG treatment are available.[13] Other group with CIP was including 18 patients. IVIG was used for treatment of 6 of the patients; all patients had successful results from the treatment. Guarneri et al.[10] reported that CIM was more prognostic than CIP. Only one of our patients was CIM and well prognosis. However we cannot evaluate because of the insufficient number of our patients.

As a result; we surmised that CIP/CIM can develop in patients who are mechanical ventilation dependent in ICU; those patient should be diagnosed by EMG and IVIG treatment in CIP patients affects the prognosis positively. However we would like to emphasize that number of patients was insufficient and we need multicenter studies.

Reference

1. *Irwin Richard S, Rippe James M. Newly acquired weakness in the intensive care unit: Critical illness myopathy and neuropathy. David A. Chad. Chapter 183. Intencive Care medicine. 6. edition. 2008; 2039-41.*
2. *Pati S, Goodfellow JA, Iyadurai S, Hilton-Jones D. Approach to critical illness polyneuropathy and myopathy. Postgrad Med J 2008; 84: 354-60.*
3. *Latronico N, Guarneri B. Critical illness myopathy and neuropathy. Minerva Anestesiol 2008; 74: 319-23.*
4. *Bolton CF, Gilbert JJ, Hahn AF, Sibbald WJ. Polyneuropathy in critically ill patients. J Neurol Neurosurg Psychiatry 1984; 47: 1223-31.*
5. *Dyck PJ. Hypoxic neuropathy: Does hypoxia plays a role in diabetic neuropathy? The 1988 Wartenberg Lecture. Neurology 1989; 39: 111-8.*
6. *Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Interventions for preventing critical illness polyneuropathy and critical illness myopathy. Cochrane Database Syst Rev. 2009; 21: 238-46.*
7. *Callahan LA, Supinski GS. Sepsis-induced myopathy. Crit Care Med. 2009; 37: 354-67.*
8. *Massa R, Carpenter S, Holland P, Karpati G. Loss and renewal of thick myofilaments in glucocorticoid-treated rat soleus after denervation and reinnervation. Muscle Nerve. 1992; 15: 1290-8.*

9. *Chawla J, Gruener G. Management of critical illness polyneuropathy and myopathy. Neurol Clin. 2010; 28: 961-77.*

10. *Guarneri B, Bertolini G, Latronico N. Long-term outcome in patients with critical illness myopathy or neuropathy: The Italian multicentre CRIMYNE study. J Neurol Neurosurg Psychiatry. 2008; 79: 838-41.*

11. *Nicola D, Colin D.S. Critical illness polyneuromyopathy (CIPNM); rehabilitation during critical illness. Therapeutic options in nursing to promote recovery: A review of the literature. Intensive and Critical Care Nursing, 2010; 26: 353-62.*

12. *Mohr M, Englisch L, Roth A, Burchardi H, Zielmann S.Intensive Care Med. Effects of early treatment with immunoglobulin on critical illness polyneuropathy following multiple organ failure and gram-negative sepsis. 1997; 23: 1144-9.*

13. *Zink W, Kollmar R, Schwab S. Critical illness polyneuropathy and myopathy in the intensive care unit. Nat Rev Neurol. 2009; 5: 372-9.*

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Mortality and related risk factors in *patients* undergoing *maintenance peritoneal dialysis*

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Abstract

Background and Objective: The mortality rates of patients with end-stage renal disease have significantly declined by dialysis. So the aim of this study was to determine the mortality rate and some hematological biomarkers peritoneal dialysis patients.

Material and Methods: In this longitudinal study we enrolled 57 patients with end stage renal disease that receiving maintenance peritoneal dialysis in one referral teaching hospital in Tehran, Iran, for six years. We measured some hematological biomarker regularly and at the time of mortality.

Results: The mortality rate was 31.5% (18/57) and cardiovascular events were mortality cause in 12 patients. The mean of ESR, CRP and ferritin in patients with mortality were significantly higher than other patients ($P<0.05$) and the mean of albumin and hemoglobin in patients with mortality were significantly lower than other ($P<0.05$).

Conclusion: According to these results, cardiovascular disease screening and control of modifiable risk factors and prevention of hypoalbuminemia in these patients were suitable for decline of mortality.

Key words: End stage renal disease, mortality, peritoneal dialysis.

Introduction

Although continuous dialysis leads to reduction of mortality of patients suffering renal failure caused by uremia and its complications, but long term survival and life quality promotion is still an important goal. Type of renal disease and presence of other disease like HTN and situations as infections, malnutrition, inflammatory circumstances and psychosocial disorders that happen in this group of patients affect on long term survival and life quality in dialysis patients (1-3).

Studies show that the commonest reason of death in dialysis patients are cardiovascular disease, infection and disruption of dialysis (4,5) and in more than 50% of cases, coronary artery disease is the reason of mortality in these patients (6). Evaluation of patients specially assessment the risk factors of cardiovascular events and continuous special care in these patients can be suitable (5,7,8), and finding biomarkers that has predicting value for the risk of mortality can be very helpful. Studies has shown that the presence of inflammatory conditions and increased proinflammatory cytokines in dialysis patients induced by infections related to dialysis, uremia, contact with surface of fistula and membranes and contact with solutions that are accompanied with increased of acute phase reactions (9,10) can accelerate atherosclerosis and be effective in cardiovascular events. There are some studies that mentioned the relationship between ESR, CRP, Ferritin and Albumin level with cardiovascular events (11-16). High levels of phosphorus and Ca-P product is a very common disorder that progressively mentioned as a predicting factor of cardiovascular risks in different surveys (17). Inflammatory conditions and infections can lead to malnutrition in dialysis patients (17,18) and malnutrition is accompanied with higher mortality in these patients(19,20). The correlation between albumin, prealbumin, cholesterol level and metabolism of proteins and minerals are effective in life quality and long term survival (21,22).

There exist special factors in peritoneal dialysis rather than hemodialysis that cause exacerbation of inflammation in peritoneal dialysis (23,24) that include: Apparent and hidden episodes of peritonitis related to peritoneal dialysis catheter, permanent connection with dialysis solution that may include biologic adverse materials or endotoxins (23,25) and loss of renal function and fluid overload (23). It has been shown that peritoneal dialysis is accompanied with higher cardiovascu-

lar risk rather than hemodialysis and the reason is unclear (26). Most studies have assessed the relationship of blood markers with the mortality risk in renal failure patients undergoing hemodialysis. In the present study we assessed the relationship of hematologic and biochemical index of blood with mortality in peritoneal dialysis patients.

Methods

The study was a longitudinal study and it has been done in all 57 End stage renal disease (ESRD) patients who underwent continuous Peritoneal Dialysis in Imam Khomeini hospital in Tehran (a teaching referral hospital in capital of Iran) for six years. An informed consent was obtained from each patient. Patients' demographic data including age, sex, etiology of ESRD, duration of the disease and co-morbidities was taken. The blood sample was taken at the first meeting and then monthly. In order to the data sheet, full blood count, Hg, Hct were measured with cell counter and ESR, CRP, ferritin, albumin, iron, TIBC, TG, Cholesterol, LDL, HDL, SGOT, SGPT, and ALP were measured with the auto analyzer Hitachi 902. The ranges of the parameters were defined in order to the handbook of dialysis (27).The results were analyzed with chi-square and T-test.

Results

Of total 57 recruited patients, 28 patients (49.1%) were male and 29 (50.9%) were female. The most common cause of renal failure was diabetes (45.6%), and then hypertension (21.2%). Other causes of renal failure in our patients were glomerulonephritis, kidney stones, polycystic kidney disease, polyarteritis nodosa, SLE and nephrotic syndrome. In 9 patients the cause of renal failure was unknown. Time onset of peritoneal dialysis among studied patients was at least 2 to 27 months during the study period. Mean age of the patients was 54.2 years with a range between 21 to 85 years. Range of patients' BMI was at least 17.1 to maximum 35.1 with a mean of 24.7 respectively. The most common associated diseases among the patients with diabetes were hypertension and cardiovascular disease. Eighteen cases of the 57 patients had been death during the study period and cardiovascular disease was the cause of death in the 12 patients (66.7% of total deaths). Other causes of death were included CVA in two cases, septicemia in two, and one case in each suicidal attempted and hepatitis respectively. Evaluating of the two groups of expired patients and comparing the measured indices of them with patients in alive group (table 1), the mean ESR 96.48 mm/h was significantly higher in dead patients (P=0.000). ESR was abnormal in all dead patients and there was also a significant difference (P = 0.035) with the compared group (Odds ratio = 1.6, CI95%: 1.2-1.9). CRP was also significantly increased in dead patients with a mean of 30.9 gr/dl (P=0.000). The number of abnormal CRP among dead patients was also significantly (P = 0.041) higher than the other group (Odds ratio= 4.05, CI95%: 1-16.33). There were also differences in blood hemoglobin level, such that the hemoglobin level with a mean 9.5 gr/dl in dead patients was significantly lower than the other group (P=0.000). Blood ferritin levels in this group was significantly higher than the average of 1045.9 of dead patients (P=0.002). Abnormal albumin level was significantly (P=0.002) higher in dead patients (Odds ratio=8.8, CI95%: 1.7-44.1). Mean serum albumin level was 2.9 gr/dl in dead group. WBC and PMN counts and mean serum levels of AST, ALT was higher and average HCT and cholesterol levels (177.7 mg/dl) was lower in dead group compared with the other group (187.05 mg/dl). But these differences were not statistically significant. No significant differences were observed in other investigated parameters related to causes of death among studied patients.

Table 1. Comparison of inflammatory markers among the dead patients and the alive patients

Inflammatory markers	Dead patients	Alive patients	P value
Mean of ESR	96.4	32.2	0.000
Abnormal ESR	64%	36%	0.035
Mean of CRP	30.9	11.3	0.000
Abnormal CRP	58%	42%	0.041
Mean of Ferritin	1045.9	395.2	0.002
Mean of Albumin	2.9	3.9	0.002
Mean of Hemoglobin	9.5	11.4	0.000

Discussion

Long-term survival and quality of life in patients undergoing regular dialysis is not desirable. Of 57 patients, 18 patients had been death during the study period. Bloembergen et al. found that cardiovascular and infectious diseases are the most important causes of mortality in dialysis patients (28). Wallen was also outlined cardiac disease as leading causes of death in these patients (29). In our study, the most common cause of death was due to cardiac ischemia (6.66%), septicemia (81.1%) and CVA (11.1%), respectively. Mailloux explained that type of underlying kidney disease could be effective on survival rate of dialysis patient (2). According to this issue, chronic glomerulonephritis and polycystic kidney disease were associated with good survival and hypertension-induced nephropathy associated with moderate survival and diabetic nephropathy was expressed with poor survival. In the present study, the most common cause of renal failure was diabetes in 26 patients (6.45%) and hypertension in 12 cases (2.21%).

In a study by Kalantar-zadeh, increase in CRP and decrease in albumin were introduced as strong factors to estimate the survival of dialysis patients. Other factors such as serum ferritin and platelet, and then TIBC - Iron - WBC and ESR were considered as survival estimators (30). In our study, the number of abnormal CRP as also the mean of CRP was significantly higher in dead patients. The number of abnormal serum albumin level was significantly higher in dead patients, but the mean of serum albumin group was low in dead patients group. Inflammatory processes are active in dialysis patients because of various causes. Sharma believes that decreasing in renal clearance of pre- inflammatory cytokines and bacteria endotoxin play an important role in inflammatory process (31). Another studies pointed to other influencing factors such as uremia, increased exposure to infectious agents, oxidative stress and risk of other diseases (32-34).

Pecoits and Yeun in their studies have noted to the Catheter-related infections, contact with the solution and reduced renal function as other causes of inflammation in patients undergoing peritoneal dialysis (23,25). Langlois introduced the process of inflammation as an accelerated factor of atherosclerosis and coronary artery disease in dialysis patients (35). In several studies, high CRP level and other acute phase proteins were introduced as predictive risk factors for cardiovascular death (17,18,36,37). Also, chronic inflammatory conditions may lead to weight loss, malnutrition and even therapeutic resistance anemia (17,18,38). In our study, the mean levels of CRP in dead patients was significantly higher than in alive patients (P =0.000). Also, the number of abnormal CRP was significantly higher in dead patients. In addition to increasing ESR in inflammatory conditions, it is also useful to determine the risk of morbidity and mortality particularly due to cardiovascular events and for detecting of metastatic malignancies (7,36). Stenvinkel and colleagues expressed ESR as adjunct to CRP and other inflammatory factors is a risk factor for cardiovascular disease and malnutrition (39). In our study, the ESR was abnormal in all patients who died with the mean of 96.48 mm/h that was significantly higher than alive group (P=0.000). Abnormally high levels of ESR and CRP were in close association with mortality of patients in our study. Because the leading cause of death in these patients was due to cardiovascular causes, it can be a reference to the role of the inflammatory processes in the acceleration of atherosclerosis and cardiovascular problems, which is similar to the results of other studies (12,17,18,).

Kalantar-zadeh and colleagues stated in a study that high levels of ferritin as an acute phase proteins is associated with increased risk of mortality in dialysis patients (40). In our study, the mean serum ferritin level of dead patients was significantly higher than the mean serum ferritin level of alive patients (P=0.001). In dialysis patients, although the both existence of inflammatory condition and iron deficiency are influenced on serum ferritin level, it can be used as a valuable factor to evaluate the condition of these patients.

Albumin may also decrease in inflammatory conditions in addition to malnutrition states. Defilippi on hemodialysis patients (20) and Teehan (41) on patients underwent peritoneal dialysis showed an inverse relationship between serum albumin and mortality rate. In this study, the number of cases with abnormal serum albumin levels was significantly higher in dead patients group (P=0.03).

Anemia is existed in dialysis patients because of various causes, including decreased secretion of erythropoietin, chronic inflammatory conditions,

reduction in iron intake or loss of iron, which reduces the oxygen carrying capacity of hemoglobin and cardiovascular vascular stress. In our study, the mean hemoglobin in dead patients was significantly higher than the alive group (p=0.000). Although WBC and PMN counts in dead patients and patients with peritonitis was higher than the other group but the difference was not significant.

Several studies showed that there was an inverse relationship between the serum cholesterol level as an indicator of nutritional status and mortality (22,23). In the present study, the mean serum cholesterol level in dead patients was lower than the mean serum cholesterol in alive patients but this difference was not statistically significant.

Finally, according to these results, cardiovascular disease screening and control of modifiable risk factors and prevention of hypoalbuminemia in these patients were suitable for decline of mortality.

References

1. *Mailloux LU, Napolitano B, Bellucci AG, Mossey RT, Vernace MA, Wilkes BM. The impact of comorbid risk factors at the start of dialysis upon the survival of ESRD patients. ASAIO J 1996; 42: 164-9.*

2. *Mailloux LU, Bellucci AG, Napolitano B, Mossey T, Wilkes BM, Bluestone PA. Survival estimates for 683 patients starting dialysis from 1970 through 1989: Identification of risk factors for survival. Clin Nephrol 1994; 42: 127-35.*

3. *Chung, SH, Lindholm, B, Lee, HB. Influence of initial nutritional status on continuous ambulatory peritoneal dialysis patient survival. Perit Dial Int 2000; 20: 19-26.*

4. *Argain H, Mozaffari S, Zadefatah Y, Rahbani M. Acute phase reactants in hemodialysis and renal transplantion. Transplant Proc 2002; 34: 2420-1.*

5. *Mallamaci F, Tripepi G, Cutrupi S, Malatino LS, Zoccali C. Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction and myocardiopathy in pation with ESRD. Kidney Int 2005; 67: 2330-7.*

6. *Venkatesan J, Henrich WL. Anemia, hypertension, and myocardial dysfunction in end-stage renal disease. Semin Nephrol 1997; 17: 257-69.*

7. *Herzig KA, Purdie DM, Chang W, Brown AM, Hawley CM, Campbell SB, et al. Is C-reactive protein a useful predictor of outcome in peritoneal dialysis patients? J Am Soc Nephrol 2001; 12: 814-21.*

8. *Lacson E Jr, Levin NW. C-reactive protein and end-stage renal disease. Semin Dial 2004; 17: 438-48.*

9. *O'Seaghdha CM, Foley RN. Septicemia, access, cardiovascular disease, and death in dialysis patients. Perit Dial Int 2005; 25: 534-40.*

10. *Garg PP, Frick KD, Diener-West M, Powe NR. Effect of the ownership of dialysis facilities on patients' survival and referral for transplantation. N Engl J Med 1999; 341: 1653-60.*

11. *Horl WH, Cohen JJ, Harrington JT, Madias NE, Zusman CJ. Atherosclerosis and uremic retention solutes. Kidney Int 2004; 66: 1719-31.*

12. *Stenvinkel P, Pecoitsfilho R, Lindholm B. Coronary artery disease in endstage renal disease: No longer a simple plumbing problem. J Am Soc Nephrol 2003; 14: 1927-39.*

13. *Bro S, Bentzon JF, Falk E, Andersen CB, Olgaard K, Nielsen LB. Chronic renal failure accelerates atherogenesis in apolipoprotein edeficient mice. J Am Soc Nephrol 2003; 14: 2466-74.*

14. *Deicher R, Ziai F, Bieglmayer C, Schillinger M, Hörl WH. Low total vitamin C plasma level is a risk factor for cardiovascular morbidity and mortality in hemodialysis patients. J Am Soc Nephrol 2005; 16: 1811-8.*

15. *Boger CA, Gotz A, Stubanus M, Banas B, Deinzer M, Kruger B, et al. C-reactive protein as predictor of death in end-stage diabetic nephropathy: role of peripheral arterial disease. Kidney Int 2005; 68: 217-27.*

16. *Wong JS, Port FK, HulbertShearon TE, Carroll CE, Wolfe RA, Agodoa LY, ,et al. Survival advantage in Asian American endstage renal disease patients. Kidney Int 1999; 55: 2515-23.*

17. *Qureshi AR, Alvestrand A, Divino-Filho JC, Gutierrez A, Heimbürger O, Lindholm B, et al. Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. J Am Soc Nephrol 2002; 13: S28-36.*

18. *Kaysen GA, Dubin JA, Muller HG, Mitch WE, Rosales LM, Levin NW. Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients. Kidney Int 2002; 61: 2240-9.*

19. *Chertow GM, Goldstein-Fuchs DJ, Lazarus JM, Kaysen GA. Prealbumin, mortality, and cause-specific hospitalization in hemodialysis patients. Kidney Int 2005; 68: 2794-800.*

20. *DeFilippi, C, Wasserman, S, Rosanio, S, Tiblier, E. Cardiac troponin T and Creactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing longterm hemodialysis. JAMA 2003; 290: 353-9.*

21. *Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol 2004; 15: 2208-18.*

22. *Kalantar-Zadeh K, Kopple JD. Relative contributions of nutrition and inflammation to clinical outcome in dialysis patients. Am J Kidney Dis. 2001; 38: 1343-50.*

23. *Pecoits-Filho R, Stenvinkel P, Wang AY, Heimbürger O, Lindholm B. Chronic inflammation in peritoneal dialysis: the search for the holy grail? Perit Dial Int 2004; 24: 327-39.*

24. *Avram MM, Fein PA, Rafiq MA, Schloth T, Chattopadhyay J, Mittman N. Malnutrition and inflammation as predictors of mortality in peritoneal dialysis patients. Kidney Int 2007; 70: S4-S7.*

25. *Yeun JY, Kaysen GA. Acute phase proteins and peritoneal dialysate albumin loss are the main determinants of serum Albumin in peritoneal dialysis. Am J Kidney Dis 2000; 30: 923-7.*

26. *Pecoits-Filho R. Managing a peritoneal dialysis patient with high risk for cardiovascular disease. Nephron Clin Pract 2010; 116: c283-8.*

27. *Daugirdas JT, Blake PG, Ing TS. Hand book of Dialysis. 4th ed. Lippincott Williams & Wilkins. 2006. pp 5-12.*

28. *Bloembergen WE, Port FK, Mauger EA, Wolfe RA. Causes of death in dialysis patients: Racial and gender differences. J Am Soc Nephrol 1994; 5: 1231-42.*

29. *Wallen MD, Radhakrishnan J, Appel G, Hodgson ME, Pablos-Mendez A. An analysis of cardiac mortality in patients with new-onset end-stage renal disease in New York State. Clin Nephrol 2001; 55: 101-8.*

30. *Kalantar-Zadeh K. Inflammatory marker mania in chronic kidney disease: pentraxins at the crossroad of universal soldiers of inflammation. Clin J Am Soc Nephrol 2007; 2: 872-5.*

31. *Sharma R, Bolger AP, Li W, Davlouros PA, Volk HD, Poole-Wilson PA, et al. Elevated circulating levels of inflammatory cytokines and bacterial endotoxin in adults with congenital heart disease. Am J Cardiol. 2003; 92: 188-93.*

32. *Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. The importance of diabetic nephropathy in current nephrological practice. Nephrol Dial Transplant. 2003; 18: 1716-25.*

33. *Bergström J, Lindholm B, Lacson E Jr, Owen W Jr, Lowrie EG, Glassock RJ, et al. What are the causes and consequences of the chronic inflammatory state in chronic dialysis patients? Semin Dial 2000; 13: 163-75.*

34. *Panichi V, Migliori M, De Pietro S, Taccola D, Bianchi AM, Norpoth M, et al. C reactive protein in patients with chronic renal diseases. Ren Fail 2001; 23: 551-62.*

35. *Langlois M, Duprez D, Delanghe J, De Buyzere M, Clement DL. Serum vitamin C concentration is low in peripheral arterial disease and is associated with inflammation and severity of atherosclerosis. Circulation 2001; 103: 1863-8.*

36. *Fine A. Relevance of C-reactive protein levels in peritoneal dialysis patients. Kidney Int 2002; 61: 615-20.*

37. *Busch M, Franke S, Müller A, Wolf M, Gerth J, Ott U, et al. Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the Creactive protein. Kidney Int 2004; 66: 338-47.*

38. *Kaysen GA, Dubin JA, Müller HG, Rosales LM, Levin NW. The acute-phase response varies with time and predicts serum albumin levels in hemodialysis patients. The HEMO Study Group. Kidney Int. 2000; 58: 346-52.*

39. *Stenvinkel P, Barany P, Heimbürger O, Pecoits-Filho R, Lindholm B. Mortality, malnutrition, and atherosclerosis in ESRD: what is the role of interleukin-6? Kidney Int Suppl 2002; 80: 103-8.*

40. *KalantarZadeh K, Don BR, Rodriguez RA, Humphreys MH. Serum ferritin is a marker of morbidity and mortality in hemodialysis patients. Am J Kidney Dis 2001; 37: 564-72.*

41. *Teehan BP, Schleifer CR, Brown JM, Sigler MH, Raimondo J. Urea kinetic analysis and clinical outcome on CAPD: A fiveyear longitudinal study. Adv Perit Dial 1990; 6: 181-5.*

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Clinical supervision and peer consultation for Medical Faculty members

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Abstract

Background: Clinical supervision and peer consultation was implemented on faculty members by using an exemplary subject. The aim of this study is to put forward the effect of peer consultation and clinical supervision on the development of the educational competencies of faculty members.

Methods: Our study was made on students who attended the given lecture, one faculty members from department of anatomy.

Results: As a result, in-class performance of the faculty member was found very successful in general.

Conclusion: These findings reinforced clinical supervision and peer consultation, may be used to improve the quality of the in-class activities in medical schools.

Key words: Clinical supervision, peer consultation, faculty member, competence.

Introduction

Formative and summative evaluation is a natural part of professional life for teacher, occurs continuously and emphasizes ongoing growth and development. The supervision is formative evaluation and foremost interested in improving teaching and increasing teacher's personal development.[1] Additionally, supervision has a key role in achieving and monitoring educational quality.[2] Supervision and clinical supervision are interdependent. Clinical supervision refers to face to face contact with teacher with intent of improving instruction and increasing professional growth.[1,3]

Recently, major changes have taken place in educational strategies and practices. Some of these changes are problem-based education, skill development and implementation of faculty development programs. Faculty development programs are applied in many medical faculties throughout the world.[4,5] Qualitative and quantitative feedbacks concerning these applications are received from the students and faculty members. In addition to feedbacks, studies point out to the usefulness of lesson observation in education and in-service training. Lesson observation is also beneficial in determining the requirements of educators. In-class observations help to determine the facts, problems and requirements that can not be revealed with other methods.[7] The benefits from in-class observations lead to the development of clinical and peer evaluation methods. In the final quarter of the 20th century, educational supervision and clinical supervision have been added to the methods in educational sciences, which are used to evaluate the performance of the educators.[1,8,9,10] Clinical supervision is a concept that has been accepted and used in the areas of nursing and psychology.[11] However, to date, this approach has not been used in the evaluation of medical faculty members.

Clinical supervision in education is the sum of planned, co-operative activities to change behavior patterns. Sergiovanni and Staratt[1] defined clinical supervision as refers to face-to-face encounters with teachers about teaching, usually in classrooms, with the double-barreled intent of professional development and improvement of instruction. According to them, clinical supervision is in the center and surrounded by educational supervision while general supervision is located in the perimeter. Clinical supervision cycle is a whole made of the activities including pre-observation conference, observation phase, analysis, post-observation phase and re-planning.[1, 8] The studies showed that clinical supervision resulted in changes in the behavior patterns of the educators. This effect increases cumulatively in concordance with the duration of clinical supervision. Similarly, the productivity of the educator also increases.[12]

In our medical faculty, 214 out of 278 faculty members have attended the faculty development program since 2001.In the framework of this program, the faculty members enrolled in a 5-day-education program and were asked to give a 20-minute-presentation in the last day. The presentations were recorded by video camera. After completion of this program, the educational efficiency of the faculty members was evaluated by student feedbacks. Changes in behavior did not observed in class by course team of the faculty development program. For that reason, we did not know completely how faculty members changed their lecture behavior or program (unpublished data). Therefore the main purpose of this case study is to put forward the effect of peer consultation and clinical supervision on the development of the educational competencies of faculty members by using an exemplary subject.

Methods

Our study was made on students who attended the given lecture, one faculty members from department of anatomy. The faculty member attended to the faculty development program in 2003. The peer consultant and the supervised faculty member were from the same department. The clinical supervisor, on the other hand, was a member of the course team given the faculty development program. The peer consultant evaluated the lesson in terms of knowledge while clinical supervisor's evaluations for both content and educational techniques.

The 101 out of 143 students filled out the feedback forms. 21 students did not filled out the feedback form by different reasons, the rest filled out the form by giving totally 5 or 1. The feedback form consisted of 21 items (Table 1). A five-point scale was used in the evaluation (5 = perfect, 1 = insufficient). The received forms were evaluated and presented on tables (Tables 1-3).

Working process:

1. The evaluation forms for the students, peer consultant, and clinical supervisor were prepared by a faculty member from the faculty of education and a faculty member from the department of medical education.
2. Initially, the study team assembled and reached consensus on what would be done.
3. Before in-class observation, the equipment for video recording was installed in the classroom and preliminary information was given to the students.
4. Lecture was video-recorded without distracting the faculty member. Meanwhile, the clinical supervisor and peer consultant filled out the feedback forms during lecture.
5. The lesson objectives handed out to the students in the beginning of the lesson.
6. At the end of the lesson, evaluation form was distributed to the students and asked for filling out.
7. After the lecture, information regarding the application was obtained via the "in-depth personal interview form".
8. One week later, the video recording was watched together with the faculty member. The feedbacks of the students, peer consultant and clinical supervisor were shared with him.

Results

The alpha reliability coefficient of the feedback form was found as 89.9%. Barttlett's sample coefficient was 0.84%. According to Barttlet's coefficient, the sample size was sufficient.

Distribution of the scores given by students is depicted on Table 1. The item, "influence to the lesson subject" received the highest score. The majority of the students concluded that the faculty member was perfect in all competency areas but three.

The scores given by the peer consultant and the clinical supervisor were classified into nine items that were commonly rated as "perfect" and "good" and five common items, which were judged as requiring improvement.

The opinions of the faculty member about the application reflected through the in-depth personal interview form are presented below:

Before evaluation

The supervised faculty member mentioned that the application excited and motivated him to give his lectures in a better way. He stated that he wished that he could always give his lectures in such an atmosphere of evaluation and realize his weak areas.

Table 1. The distribution of the scores given by the students to the faculty member according to the rated items

	Perfect %	Good %	Neutral %	Acceptable %	Insufficient %
1. Making an appropriate introduction to the lesson	56.4	40.6	3.0		
2. Making the students aware of the aim of the lesson	61.4	36.6	2.0		
3. Making connections with past subjects	53.5	40.6	5.9		
4. Ensuring the active participation of the students during the lesson	19.8	50.5	15.8	10.9	3.0
5. Making use of multiple interactive techniques	54.5	36.6	5.0	1.0	3.0
6. Influence to the lesson subject	79.2	17.8	2.0	1.0	
7. Using time efficiently and effectively	52.5	32.7	9.9	3.0	2.0
8. Using audiovisual tools	62.4	28.7	6.9	1.0	1.0
9. Not only relying on only audiovisual tools but also communicating verbally during presentations	65.3	32.7	2.0		
10. Relating the subject with life	66.3	26.7	5.9		1.0
11. Making connections between the subject and the pre-clinical and clinical experiences	71.3	26.7	1.0		1.0
12. Lecturing according to a plan	62.4	28.7	7.9		1.0
13. Maintaining the interest and attention of the students	56.4	33.7	7.9	1.0	1.0
14. Stressing the crucial points	66.3	21.8	6,9	2.0	
15. Giving the opportunity of taking notes to the listeners	19.8	39.6	28.7	5.9	5.9
16. Responding to the questions and contributions of the students in an appropriate manner	44.6	44.6	5.0	4.0	2.0
17. Making eye contact with the students	50.5	39.6	5.9	1.0	3.0
18. Talking clearly and understandably	58.4	31.7	6.9	2.0	1.0
19. Adjusting tone of voice and talking speed according to the class	50.5	31.7	14.9		3.0
20. Making use of appropriate gestures, facial expressions and body movements	74.3	21.8	1.0	1.0	2.0
21. Summarizing the subject at the end of the lesson	51.5	33.7	11.9	3.0	

Table 2. The common areas that were found sufficient and requiring improvement by the peer consultant and clinical supervisor according to the rated items

Perfect-good	Areas requiring improvement
Making an appropriate introduction to the lesson	Ensuring the active participation of the students during the lesson
Making the students aware of the aim of the lesson	Relating the subject with life
Influence to the lesson subject	Making connections between the subject and the preclinical and clinical experiences
Using tine efficiently and effectively	Maintaining the interest and attention of the students
Using audiovisual tools	Giving the opportunity of taking notes to the listeners
Lecturing according to a plan	
Stressing the crucial points	
Talking clearly and understandably	
Making use of appropriate gestures, facial expressions and body movements	

Table 3. The differences between the evaluations of the peer consultant and the clinical supervisor in terms of the areas found sufficient and those requiring improvement

Perfect-good	Areas requiring improvement
Making connections with past subjects **	Making connections with past subjects *
Making use of multiple interactive techniques **	Making use of multiple interactive techniques *
Responding to the questions and contributions of the students in an appropriate manner *	Responding to the questions and contributions of the students in an appropriate manner **
* Making eye contact with the students	Making eye contact with the students **
Adjusting tone of voice and talking speed according to the class *	Adjusting tone of voice and talking speed according to the class **
Summarizing the subject at the end of the lesson*	Summarizing the subject at the end of the lesson **

**:Clinical supervisor, **:Peer consultant*

After evaluation

The supervised faculty member's answer to the question "was it the result you expected?" was "It was a difficult lesson subject. I expected the students to state that I was going too fast. However, it was far better than I expected. They did not say anything like that".

As for the question, "Did the video recording cause to a stress?", his answer was "No, but actually I feel an anxiety in the beginning. However, I think this anxiety was from "Will I be able to teach such a difficult subject in two hours?". Then he added, "Clinical supervision and peer consultation were not sources of stress for me because residents occasionally join my classes".

His answer to the question "Should this method be widely used in your faculty?" was "It should be applied … We attended the courses on faculty development program. However, it was not followed by supervision and the course content may be forgotten after some time. With this application, the efficiency of the course can be supervised and monitored".

Discussion

This case study aimed to put forward the effect of peer consultation and clinical supervision in the development of the educational competencies of faculty members. The data revealed that both of peer consultation and clinical supervision are important for faculty member.

In the recent years, the importance of faculty development programs has been increased. Nearly a great number of medical faculties implement these programs into their curricula and evaluate them via student feedbacks. The student feedbacks are received at the end of each lesson or at the end of the clerkship period. However, what is done in the classroom, the black box behind closed doors, and whether the things done in the classroom being consistent with the content of the program or not are unknown. On the other hand, clinical supervision and peer consultation allow the evaluation of the teaching-learning activities of the faculty member in all aspects through in-class observation, student evaluation and video recording.[8,1] Thus, it is obvious that the application will offer beneficial results especially in terms of educational quality.

A remarkable finding was that the scores given to the faculty member were identical. The in-class performance of the faculty member was found very successful in general. According to the data of the feedbacks it can be stated that the faculty member was very successful in the competency areas, which had been aimed to develop through the faculty development program. It was concluded that the meeting before lesson and the guidance provided by the peer consultant and clinical supervisor might have resulted to flourish of "good" and "perfect" scores in many competency areas. The competency areas scored as "perfect" and "good" and "the aspects requiring improvement" were identical in the feedbacks of all evaluators, which is an indicator of the objectivity of our study. It was also found that the peer consultant and clinical supervisor had different judgments about certain competency areas. For example, the peer consultant did not find the summary of the lesson satisfactory whereas the clinical supervisor stated that even the presence of a summary was enough by itself. This discrepancy might have resulted from the different expectations of the both evaluators.

During in-depth personal interview, the faculty member mentioned that the application was exciting and arousing motivation for self-development. Similar results had been reported in the previous studies (McMahon and Patton, 2000) performed expect for medical faculty. They suggested that their application were increased the self-confidence of their study group to try new ideas and techniques in education. [13] The results of the peer consultancy studies from the University of Missouri [10] were in concordance with those of ours. Similarly, the faculty members included in their study concluded that the program was beneficial for their educational competencies development and they made changes in their teaching strategies by using the evaluations.

Although clinical supervision and peer consultation has many advantages, the main limitations of clinical supervision are its time-consuming nature and its long preparation phase.

Conclusion

This study was planned to apply on medical faculty members who had previously attended the faculty development program. It is concluded that clinical supervision and peer consultation may be used for improving the quality of the in-class activities in faculties of medicine. We suggest that supervision in medical education has a key role in achieving and monitoring educational quality. Additionally, in-class observation helps to determine weakness and perfect point of faculty members and therefore result to improve quality of the education of the medical faculty.

Acknowledgements

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References

1. *Sergiovanni T, Starratt R. Supervision: A Redefinition (New York, McGraw- Hill) 2002.*
2. *Van Der Linde CH. Clinical supervision in teacher evaluation: a pivotal factor in the quality management of education. Education 1998; 2: 328-335.*
3. *Kilminster S, Cottrell D, Grant J, Jolly B. AMEE Guide No.27: Effective educational and clinical supervision, Medical Teacher 2007; 29: 2-19.*
4. *Garcia-Barbero M. Medical education in the light of the World Health Organization Health for All strategy and the European Union. Medical Education 1995; 29: 3-12.*
5. *Jones R, Higgs R, de Angelis C, Prideaux D. Changing face of medical curricula. The Lancet 2001; 357: 699-703.*
6. *Walton H. Medical education worldwide. A global strategy for medical education: partners in reform. Medical Education 1993; 27: 394-398.*
7. *O'Sullivan M. (2004) The Usefulness of Lesson Observation in a Primary Teachers' INSET Programme in Namibia. Journal of Education for Teaching 2004; 30: 5-25.*
8. *Glickman CD, Gordon SP, Ross-Gordon JM. Supervision of Instruction: A Developmental Approach (Boston, Allyn & Bacon). 1990.*
9. *Goldhammer R, Anderson RH, Krajewski RJ. Clinical supervision: special methods fort he supervision of teachers (New York, Holt, Rinehart and Winston Inc). 1993.*
10. *Happner PP, Johnston JA. Peer Consultation: Faculty and Students Working Together to Improve Teaching. Journal of Counseling & Development 1994; 72: 492-499.*
11. *Falender CA, Shafranske EP. Clinical supervision: A competency-based approach (Washington, DC, American Psychological Assocciation) 2004.*
12. *Nolan J, Hawkes B, Francis P. (1993) Case studies: Windows onto clinical supervision. Educational Leadership 1993; 51: 52-56.*
13. *McMahon M, Patton, W. (2000) Conversations on clinical supervision: benefits perceived by school counsellors. British Journal of Guidance and Counselling 2000; 28: 339-351.*

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Analysis of the dynamics of growth of permanent teeth in intraoral-prefunctional phase

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Abstract

Introduction: Knowing the eruption times and growth dynamics of permanent teeth in intraoral-prefunctional phase has a huge signifance on preventive dentistry.This study aims to establish the dynamics of growth of permanent teeth in intraoral- prefunctional phase of growth.

Methods: The analysis enrolled 1324 examinees, aged 5 to 14 years. The levels of growth of observed permanent teeth, serving to monitor growth dynamics, were verified using numbers I-III: I-a clinically visible portion of dental crown – occlusal surface/incisal edge; II-occlusal surface/ incisal edge at the height of the dental equator; III-occlusal surface/incisal edge reaching the occlusal plane. The obtained statistical data were statistically processed using the probit analysis.

Results: The formation of permanent dentition begins with eruption of the first lower permament molar at the age of 5.59 years, and is completed upon reaching the occlusal plane of the second upper permanent molar at the age of 12.63 years. The duration of growth of permanent teeth in intraoral-prefunctional phase ranges from 1.34 to 3.07 years for upper dentition, and 1.56 to 3.10 for lower dentition. The duration of growth of teeth from level I- II is two times shorter compared to the duration of growth from level II to the level of reaching the occlusal plane. The lowest values for the duration of growth are observed in second permanent molars, and the highest values are observed in first permanent molars.

Conclusion: The period from tooth eruption to the point of reaching the occlusal plane lasts long, depends on the tooth type, matches the time of posteruptive maturation, and represents the most significant period for the institution of preventive-prophylactic and interceptive measures.

Key words: Permanent teeth, tooth eruption, dental growth dynamics.

Introduction

Tooth growth is a complex and dynamic physiologic process, influenced by numerous factors in the fulfillment of its genetically predetermined role. It starts with the formation of dental crown and inception of dental root develoment, with various associated movements leading the tooth towards the mouth cavity. The appearance of teeth in the mouth cavity is termed tooth eruption, representing the first, clinically observable segment of tooth growth (1). In further growth, the tooth enters the posteruptive phase, representing the intraoral (prefunctional and functional) growth phase. The point of reaching the antagonist tooth marks the completion of prefunctional and beginning of functional phase of growth, lasting as long as the tooth is in the mouth cavity (1,2).

Determination of the times of eruption and dynamics of growth of deciduous and permanent teeth in children has been the topic of study of numerous authors (3-6). Tooth growth dynamics depends, above all, on the growth phase; in intraoral phase, it is closely associated with metabolic processes in the periodontal ligament (7). It is thought that the speed of tooth growth in intraoral prefunctional phase is at its highest immediately after eruption, being considerably lower in the phase of approximation to antagonist teeth (8). Precise determination of the time of eruption and growth dynamics of permanent teeth in intraoral – prefunctional phase is very important regarding the use of preventive measures and procedures, since teeth are most sensitive to caryogenic noxae in the period of 2-3 years after eruption – in the phase of posteruptive maturation. Moreover, tooth growth dynamics is essential

in interceptive jaw orthopedics, clinical diagnosis and therapy, as well as in forensics.

This study aimed to establish the dynamics of growth of permanent teeth in intraoral – prefunctional growth phase in examinees aged 5-14 years.

Methods

The study was performed as a part of the project titled „Oral health and required orthodontic treatment in pre-school and school children in the Municipality of Niš", approved by the Ethics Committee of the Faculty of Medicine, University of Niš (No. 01-244-4). The study enrolled the pupils from three elementary schools in Niš, as well as the patients of pre-school and school age of the Dentistry Clinic in Niš, Department of Preventive and Pediatric Dentistry. School principals and parents of the enrolled children gave their written informed consent for the children to participate in the study. The examinees were all healthy, without any diagnosed chronic, systemic diseases. All the examinees were of the Serbian ethnicity, born in the Municipality of Niš territory.

The study was undertaken by a team of researchers, specialists in preventive and pediatric dentistry, with one specialist candidate, in the period January-July 2012. Before the study start, the resarch team members were instructed how to observe and register the data, with clinical demonstration, in order to reduce to the maximum extent the probability of error in data collection and registration.

The study enrolled 1392 examinees, but those on active orthodontic therapy (68 subjects) were deemed ineligible and were excluded from the study. The analysis thus involved 1324 examinees, 669 boys (50.53%) and 655 girls (49.47%), aged 5 to 14 years. The average number of examinees in each of the age groups was 132.4 ± 7.1.

Mouth cavity examination was performed in school dental clinics and dental clinic of the Department of Preventive and Pediatric Dentistry, Dentistry Clinic in Niš, using mouth mirrors and dental picks, under artificial lighting. The following information were obtained: date of birth and examination date, gender, condition of deciduous and permanent teeth, and the level of growth of permanent teeth. The dynamics of growth of permanent teeth, in intraoral-prefunctional growth phase, was evidenced using the levels of growth – a modified method by Ekstrand et al. (9). The levels of growth of permanent teeth were marked using the Roman numbers I-III: I (first level) – clinically visible part of dental crown – occlusal surface/ incisal edge; II (second level) – occlusal surface/ incisal edge at the level of dental equator; III (third level) – occlusal surface/incisal edge reaching the occlusal plane. On examination, all the permanent teeth were registered, regardless of the presence of pathologic changes (caries, caries complications, structural disorders).

Age of the examinees was obtained subtracting the date of birth from the examination date, and expressed decimally (one decimal unit representing a tenth of the year). Statistical data processing was done using the program package SPSS 16.0. The information obtained by statistical analysis were presented as absolute numbers and as percentages. Probit analysis for the 5th, 50th, and 95th percentile of age of the subjects related to all three levels of tooth growth was utilized. The median, i.e. the 50th percentile was equalized to arythmetic mean, used for result interpretation. Extreme cases of early and late eruption of permanent teeth were excluded from our analysis. The time of eruption of permanent teeth was determined based on the level I (first level). Dynamics of growth of permanent teeth in intraoral-prefunctional phase was observed by the analysis of all three levels of growth, for each permanent tooth in the upper and lower jaw, and duration of growth between the levels was determined using the temporal difference between the levels.

Results

The results of this study showed that there was no statistically significant difference in the dynamics of growth of permanent teeth of the left and right side, in the upper and lower jaw ($p < 0.05$), so that the results of intraoral-prefunctional phase of growth were presented for the right side teeth in both jaws.

In the Tables 1, 2, and 3 we presented the results of probit analysis of the dynamics of intraoral-prefunctional phase of growth of permanent teeth, per each tooth, for the I, II, and III level of growth in the examined children.

*Table 1. Dynamics of tooth growth in intraoral-prefunctional phase in children aged 5-14 years – level I **

	Tooth	No. of teeth n	5%	50%	95%
Upper jaw	Central incisor	31	5.93	6.64	7.03
	Lateral incisor	72	5.97	6.82	7.60
	Canine	57	8.91	10.17	10.77
	First premolar	69	7.31	8.94	9.91
	Second premolar	39	7.60	9.88	10.31
	First molar	34	5.34	5.71	6.09
	Second molar	33	10.25	11.29	12.39
Lower jaw	Central incisor	31	5.40	5.68	6.02
	Lateralni incisor	44	5.87	6.67	7.22
	Canine	83	8.50	9.42	9.98
	First premolar	67	8.27	9.26	9.92
	Second premolar	47	7.35	9.23	9.89
	First molar	34	5.25	5.59	5.90
	Second molar	64	10.01	10.85	11.47

**Average duration of growth in years for level I*

*Table 2. Dynamics of tooth growth in intraoral-prefunctional phase in children aged 5-14 years – level II **

	Tooth	No. of teeth n	5%	50%	95%
Upper jaw	Central incisor	116	6.13	7.05	7.73
	Lateralni incisor	173	6.64	7.32	8.58
	Canine	89	9.00	10.46	11.19
	First premolar	146	7.77	9.33	10.20
	Second premolar	67	8.35	10.19	10.76
	First molar	52	5.55	6.02	6.82
	Second molar	79	11.01	11.59	12.59
Lower jaw	Central incisor	98	5.51	6.15	6.73
	Lateralni incisor	87	6.24	7.08	7.73
	Canine	83	8.60	9.72	10.25
	First premolar	130	8.40	9.67	10.10
	Second premolar	83	8.63	9.81	10.52
	First molar	58	5.43	5.91	6.45
	Second molar	105	10.02	11.29	12.16

**Average duration of growth in years for level II*

*Table 3. Dynamics of tooth growth in intraoral-prefunctional phase in children aged 5-14 years – level III**

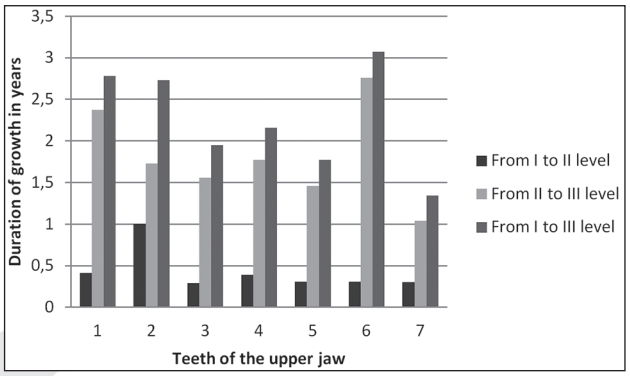
	Tooth	No. of teeth n	5%	50%	95%
Upper jaw	Central incisor	1018	7.14	9.42	10.01
	Lateralni incisor	859	7.85	9.55	10.24
	Canine	435	10.20	12.02	12.82
	First premolar	564	9.56	11.10	12.39
	Second premolar	497	9.88	11.65	12.58
	First molar	1156	6.25	8.78	9.50
	Second molar	277	11.11	12.63	13.21
Lower jaw	Central incisor	1123	6.49	8.65	9.30
	Lateralni incisor	1016	7.29	9.47	10.15
	Canine	562	9.75	11.47	12.46
	First premolar	589	9.43	11.43	12.31
	Second premolar	492	9.81	11.68	12.61
	First molar	1173	6.15	8.69	9.22
	Second molar	329	10.50	12.41	13.07

**Average duration of growth in years for level III*

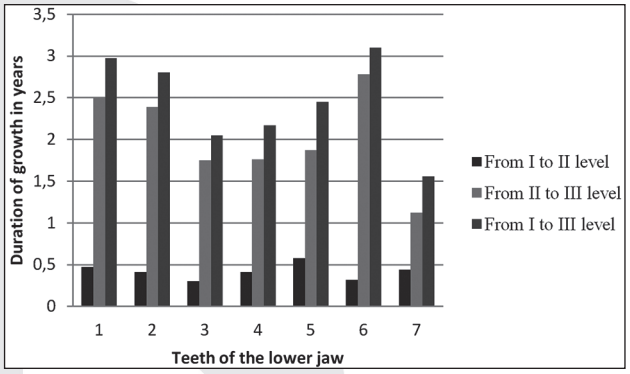
The formation of permanent dentition starts with the eruption of first permanent molar at the age of 5.59 years, and ends upon reaching the occlusal plane of the second upper permanent molar at 12.63 years of age (Tables I and III).

The results indicated earlier eruption of lower jaw teeth compared to the same teeth in the upper jaw, with the exception of first upper premolar which grew earlier than the same tooth in the lower jaw. A slight difference was identified in the time of eruption of first premolar, second premolar, and canines, in both upper and lower jaw (Table 1).

The duration of growth of permanent teeth in intraoral-prefunctional phase (levels I-III) ranged from 1.34 to 3.07 years for upper jaw teeth, and from 1.56 to 3.10 for their counterparts in the lower jaw. The duration of growth of lower teeth in this phase is longer compared to the duration observed for upper jaw teeth. In all examined permanent teeth it was observed that the duration of growth from eruption (level I) to level II was two (or more) times shorter compared to the duration from level II to III, i.e. the point of reaching the occlusal plane. The lowest values of duration of tooth growth in intraoral-prefunctional phase were observed for upper and lower second permanent molars (1.34 and 1.56), and the highest in upper and lower first permanent molars (3.07 and 3.1) (Table 4, Graphs 1 and 2)



Graph 1. Duration of growth of upper teeth in intraoral-prefunctional phase (for 50%)



Graph 2. Duration of growth of lower teeth in intraoral-prefunctional phase (for 50%)

Full (completed) permanent dentition (level III) was observed at the age of 10 (4.47% of examinees), at the earliest; at the age of 14, full permanent dentition was present in 89.15% of exami-

Table 4. Duration of growth of permanent teeth in intraoral-prefunctional phase – 50% of age of the examinees *

		I-II	II-III	I-III
Upper jaw	Central incisor	0.41	2.37	2.78
	Lateralni incisor	0.50	2.23	2.73
	Canine	0.29	1.56	1.95
	First premolar	0.39	1.77	2.16
	Second premolar	0.31	1.46	1.77
	First molar	0.31	2.76	3.07
	Second molar	0.30	1.04	1.34
Lower jaw	Central incisor	0.47	2.50	2.97
	Lateralni incisor	0.41	2.39	2.80
	Canine	0.30	1.75	2.05
	First premolar	0.41	1.76	2.17
	Second premolar	0.58	1.87	2.45
	First molar	0.32	2.78	3.10
	Second molar	0.44	1.12	1.56

**Duration of growth of permanent teeth was presented in years*

nees (Table 5). The remaining 10.85% of examinees, aged 14 years, had mixed dentition (with one or two decuduous teeth).

Table 5. Presence of full permanent dentition by age groups of the examinees

Age group	Full permanent dentition %
10 years	4,47%
11 years	14.10%
12 years	47.95%
13 years	87.41%
14 years	89.15%

Discussion

In the literature, significant differences have been observed in the time of eruption of permanent teeth (level I), which could be explained, among other things, by different methodologic approaches and standards in their determination. Precise time of eruption and dynamics of growth of permanent teeth can be determined using the radiographic method by Demirijen (10) or Nolla (11), but the use of these methods is mostly limited to the monitoring of eruption times of a small number of teeth. Therefore, epidemiologic studies have been introduced, in which the observed permanent teeth were designated as „present" or „absent". This study is a cross-sectional attempt to establish the average duration of growth of permanent teeth in intraoral-prefunctional phase in children aged 5-14 years.

Analyzing the obtained results, it was established that the formation of permanent dentition started with the eruption of lower first permanent molar at 5.59 years of age, and central incisor at 5.68 years, and that full permanent dentition was present at 12.63 years, reaching the occlusal plane of the second upper permanent molar, so that the formation of permanent dentition lasted 7 years on the average. The data of Koch and Kreiborga (12) indicated that the formation of full permanent dentition lasted 6 years on the average.

Studies have indicated earlier eruption of permanent teeth of the lower jaw compared to their upper counterparts, as confirmed in our study as well, with the exception of first upper premolar, which grows earlier compared to the same lower jaw tooth. This could be explained by a high position of upper canine, which does not disturb the growth of first premolar. In addition, a slight temporal difference was observed between the eruption of first and second premolar and canines in the upper and lower jaw.

The dynamics of growth of permanent teeth in intraoral-prefunctional phase has demonstrated a high degree of variability, and depends on the tooth type, time of day, gender, hormonal status. Haavikko established the tooth eruption of 4 mm per 14 weeks in intraoral-prefunctional phase of growth (13).

From the clinical point of view, knowledge of the course and duration of tooth growth in intraoral-prefunctional phase is of huge importance. This study showed that the duration of growth of permanent teeth in intraoral-prefunctional phase, from level I to III, ranged from 1.34 to 3.07 years for upper jaw dentition, and from 1.56 to 3.10 years for lower jaw dentition.

The lowest values of duration of tooth growth in intraoral-prefunctional phase were observed in upper and lower second permanent molars. The main reason for the finding perhaps should have been sought in the crown length of second permanent molar, being shorter than the crown of first permanent molar. Ekstrand et al. (9) pointed out a huge variation in eruption times of second permanent molars, in both girls (from 12 to 44 months) and boys (from 9 to 45 months).

First permanent molar is the tooth that most commonly grows as the first permanent tooth. However, viewed by levels, its gowth phases in intraoral-prefunctional phase are longer compared to other permanent teeth. Difficult growth of this tooth due to insufficiently developed jaws at that age, as well as frequent infections around the crown of a partially grown tooth, may explain this phenomenon. The study by Ekstrand et al. (9) demonstrated that the duration of growth of first permanent molars, from their eruption to the point of reaching the occlusal plane, ranged from 5 to 32 months in girls, and 7-28 months in boys. These authors found no statistically significant correlation between the time of eruption and duration of growth of first permanent molar.

It has been well established that the speed of growth is at its highest immediately after tooth eruption, slowing down as the tooth is getting close to the occlusal plane (8) This study found very intense tooth growth from the eruption to level II, slowing down markedly as the tooth approaches

the occlusal plane, so that the duration of tooth growth from level II to level III was longer two times or more, compared to the growth duration from eruption (level I) to level II. This finding has very wide preventive and clinical implications.

In intraoral growth phase, tooth mineralization is not fully completed, since in the phase of posteruptive maturation, 10-20% of mineral content is being incorporated into a tooth. That is why teeth are most sensitive to caryogenic noxae in the period of 2-3 years after tooth eruption (14), and the above period correspond, in fact, to the period from tooth eruption to the moment of reaching the occlusal plane. Carvalho et al. (15) established that dental biofilm accumulation on the surface of first permanent molars depended on the level of growth, functional use of the tooth in question, and its specific anatomical traits. In the above study, the authors concluded that biofilm accumulation was greater in teeth which did not reach the occlusal plane – being not yet fully functional. In our study, we found that the duration of this period depended on the tooth type, and that in upper jaw teeth this ranged from 1.34 to 3.07, and in lower jaw teeth from 1.56 to 3.10 years. Since it was established in this study that the formation of full permanent dentition lasted, on the average, 7 years, the conclusion could be drawn that this period was most significant regarding the institution of preventive measures and procedures, related primarily to the prevention of caries on all permanent teeth.

The study utilized a cross-sectional analysis of the dynamics of growth of permanent teeth in intraoral-prefunctional phase, and the obtained results indicated that a longitudinal analysis (ongoing, involving the same examinees) could produce even more precise information.

Conclusion

A conclusion may be drawn that this cross-sectional study showed that the duration of growth of permanent teeth in intraoral-prefunctional phase, i.e. the period from tooth eruption to the moment of reaching the occlusal plane lasted considerably long, the shortest period being for the upper second permanent molar (1.34 years) and the longest for lower first permanent molar (3.10 years). This period matches the period of posteruptive dental maturation; this is the most significant period for the institution of preventive-prophylactic measures in the preservation of dental health, since the accumulation of dental biofilm is greater in teeth that did not reach the occlusal plane and its full function. Moreover, the period is of relevance in interceptive orthopedics and choice and planning of orthodontic therapy, in traumatology, clinical diagnosis, treatment of pathologic conditions in young permanent teeth, as well as in forensic science and practice.

References

1. *Avery JK, Chiego DJ. Essentials of Oral Histology and Embryology. A clinical approach. 3rd ed. Belgrade: Data-status; 2011 (Serbian).*
2. *Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. Journal of Dental Research 2008; 87(5):414-434.*
3. *Wedl JS, Danias S, Schmelzle R, Friedrich RE. Eruption times of permanent teeth in children and young adolescents in Athens (Greece). Clinical Oral Investigetion 2005; 9:131-134.*
4. *Wedl JS, Schoder V, Blake FAS, Schmelzle R, Friedrich RE. Eruption times of permanent teeth in teenage boys and girls in Izmir (Turkey). Journal of Clinical Forensic Medicine 2004; 11:299-302.*
5. *Rajić Z, Rajić-Meštrović S, Verzak Ž. Chronology, dynamics and period of permanent tooth eruption in Zagreb children (Part II). Collegium Antropologicum 2000; 24(1):137-148.*
6. *Rajić Z, Rajić-Meštrović S, Verzak Ž. Chronology, dynamics and period of permanent tooth eruption in Zagreb children (Part II). Collegium Antropologicum 2000; 24(1):137-148*
7. *Lee CF, Proffit WR. The daily rhythm of tooth eruption. American Journal of Orthodontics and Dentofacial Orthopedics 1995; 107(1):38-47*
8. *Beloica D i sar. Pediatric dentistry. Belgrade: Elit medica; 2002.*
9. *Ekstrand KR, Christiansen J, Christiansen MEC. Time and duration of eruption of first and sceond permanent molars: a longitudinal investigations. Comunity Dentistry and Oral Epidemiology 2003, 31:344-50.*
10. *Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol 1973; 45(2): 211−27.*

11. *Nolla CM. The development of the permanent teeth. J Dent Child 1960; 27: 254–66.*

12. *Koch G, Kreiborg S, Andreasen JO. Eruption and shedding of teeth. In: Koch G, Poulsen S, editors. Pediatric Dentistry: A Clinical Approach. 2nd ed. Oxford: WileyBlackwell; 2009. p. 197-199.*

13. *Haavikko K. The formation and the alveolar and clinical eruption of the permanent teeth: an orthopantomographic study. Proc Finnish Dent Soc 1970;69:93-98.*

14. *Vojinović J, Vojinović O, Milin J, Tatić E. Biologija zuba. Belgrade :Naučna knjiga; 1986.*

15. *Carvalho JC, Ekstrand KR, Thylstrup A: Dental plaque and caries on occlusal surfaces of first permanent molars in relation to stage of eruption.J Dent Res 1989; 68: 773–779.*

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Upper respiratory system infection frequency is affected by allergic symptoms and cigarette smoking

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Abstract

Aim: The morbidity of infectious diseases due to smoking and allergic symptoms is not widely appreciated by physicians. The purpose of this study is to evaluate the relationship between upper respiratory tract infection frequency in a year period and allergic symptoms and cigarette smoking in a primary care population.

Method: ECRHS (European Community Respiratory Health Survey) questionnaire was applied and cigarette smoking history was evaluated in a primary care population. Patients' history of upper respiratory tract infection in a year was evaluated retrospectively. A median of upper respiratory infection URI (frequency and risks of URI in a year) and Odd's ratios were calculated statistically. All statistical analyzes were performed by using IBM SPSS 20 software. Binary Logistic Regression Analysis was performed and Odd's Ratios were calculated for each group.

Results: Total of 357 adult patients (154 female, 203 male, aged 44.9 ± 15,5 (median± std.dev) years) were included to study. The overall prevalence of current asthma, asthma like symptoms and non-infectious rhinitis were 9.0%, 46.2% and 43.4%, respectively. We found a relationship between URI risk and cigarette smoking and asthma like symptoms. URI risk was found 2.86 (CI (1.78_4.59) p<0.001) fold more in smokers compared with non-smokers. Also URI risk was found 1.63 (CI (1,03_2.57) p=0.035) fold more in the patients with asthma like symptoms.

Discussion: Our findings support the evidence that asthma-like symptoms and cigarette smoking are the risk factors for upper respiratory infections. Physicians should pay attention to allergic symptoms and cigarette smoking. These patients should be followed and appropriate treatments should be given. Cigarette cessation should be advised at every admittance.

Key words: Cigarette smoking, allergy, upper respiratory system infection.

Introduction

Allergic rhinitis (AR) is one of the most common diseases of the respiratory system. Although AR is not a life-threatening disease, because of its significant socio-economic impact on people who are actively working or attending school and its adverse effects on quality of life, is considered to be a global health problem and is thought to affect 40% of world population (1,2). Allergic rhinitis both alone and in association with asthma may adversely affect quality of life (3). Its economic impact cannot be estimated frequently, while effecting social life, sleep, school and work life (4). Although it is low, the prevalence of allergic rhinitis was significantly increased in the last 50 years (5). In a study of European countries, the prevalence of allergic rhinitis is found to be between 17-29% in different countries (6). In a study conducted among university students in Turkey, asthma, wheezing and allergic rhinitis prevalence in males were, 2.1%, 6.9% and 12.7%; in females 2.5%, 7.2% and 14.5%, respectively (7). Despite the prevalence of AR, often become chronic due to inadequate treatment. Chronic nasal inflammation and obstruction develops and leads to the upper and lower respiratory complications. AR is associated with asthma, sinusitis and otitis media with effusion (8). 40% of adults with chronic maxillary sinusitis have any kind of allergy. This ratio increases to 80% in bilateral maxillary sinusitis (9). Studies have shown that there is an increase in respiratory tract infections in allergic cases (8,9,10).

Cigarette smoking causes premature death by leading to cancer, cardiovascular diseases and chronic obstructive pulmonary disease. Smoking is also a risk factor in the respiratory and other systemic infections (11). Increased infection risk due to smoking is linked to suppression of respiratory defense

mechanisms, structural and immunological mechanisms (11,12). Infections caused by smoking are not considered sufficiently by clinicians (11). Smoking is also associated with the presence of asthma and asthma like symptoms (13,14)

In this study, we aimed to reveal the frequency of smoking and allergic symptoms and the relationship between these and the incidence of upper respiratory infections during the last one year in patients presenting to primary care.

Materials and Methods

357 patients over 18 years who applied to Ankara Cankaya Family Health Center were included to this study. The patients were selected by simple random sampling method. A questionnaire consisting of two stages was performed to patients who were accepted to fill out the survey on a voluntary basis. In the first stage of the questionnaire, socio-demographic characteristics, smoking behavior of the patient were recorded and the number of treatment requiring previous upper respiratory tract infections (acute pharyngitis, acute sinusitis etc.) during the last one year was asked retrospectively. In the second stage of the questionnaire, ECHRS (European Community Respiratory Health Survey) questionnaire was used to inquire asthma and asthma-like symptoms. Chi-square test was used to examine "the existence of relationship and univariate analysis" of the data obtained from the questionnaire. "Logistic regression model" was applied for multivariate analyzes (14). All reference groups for categorical variables were determined, the "Odds Ratios (OR) and 95% confidence intervals (95% CI) were calculated. Median value of the annual number of upper respiratory infections was calculated as "2", and ORs were calculated in 2 groups as 0-1/year and ≥2/year. All statistical analyzes were performed by using IBM SPSS 20 software package program.

Findings

A total of 357 patients (154 female, 203 male) were included to study. Mean age ± standard deviation was 44.9 ± 15.6 years. Prevalence was found as 9.0% for current asthma, 46.2% for asthma-like symptoms and 43.4% for non-infectious rhinitis.

Table 1. (European community respiratory health survey) ECHRS questionnaire form distribution of diagnoses

European Community Respiratory Health Survey (ECRHS) Questionnaire	Questionnaire Diagnoses	(n) (%)
Current Asthma: Subjects saying "YES" to Q5 and Q7	With Current Asthma	(32/357) 9,0
	Without Asthma	(325/357) 91,0
Saying "YES" to Q1 and/or Q2 and/or Q3 and/or Q4	With asthma-like symptoms	(165/357) 46,2
	Without asthma like symptom	(192/357) 53,8
Cumulative Asthma: saying "YES" to Q6	With cumulative asthma	(39/357) 10,9
	Without cumulative asthma	(318/357) 89,1
Non-infectious rhinitis: saying "YES" to Q8	With Non-infectious rhinitis	(155/357) 43,4
	Without Non-infectious rhinitis	(202/357) 56,6
Pruritic dermatitis and/or eczema: saying "YES" to Q9	With Pruritic dermatitis and/or eczema	(125/357) 35
	Without Pruritic dermatitis and/or eczema	(232/357) 65

Other results of the questionnaire were given in Table 1. 121 patients participated in the study (33.9%) were regular smokers.

When asthma and asthma-like symptoms were evaluated for some risk factors; we found that presence of smoking and non-infectious rhinitis were associated with risk of waking up with wheezing and cough.

Presence of allergic diseases in the family was found in association with increased waking up with wheezing, cough and shortness of breath. Statistical evaluation of some risk factors for asthma like symptoms is given in Table 2.

Among smoking, current asthma, asthma-like symptoms and non-infectious rhinitis, smoking ($p<0.001$) and asthma-like symptoms ($p<0.05$) has a significant effect in risk of having 2 and more infection in a year (Table 3). When the other two variables (current asthma and non-infectious rhinitis) are excluded smoking and asthma like symptoms have equally significant effect (Table 4).

Discussion

In parallel with the results of other studies, our study revealed that presence of allergic symptoms is a risk factor for upper respiratory infections (15,16). Also allergic rhinitis and chronic upper respiratory infections are associated with inflammation and sometimes some symptoms may

Table 2. Logistic regression assessment of some risk factors of asthma-like symptoms

	Age OR (CI)	Gender OR (CI)	Smoking OR (CI)	Non-infectious rhinitis OR (CI)	Family history OR (CI)
"Wheezing"	1.00 (0.98-1.01)	0.92 (0.59-1.44)	3.11 (1.966-4.93)**	2.50 (1.60-3.91)**	2.10 (1.32-3.35)*
"Wheezing" with shortness of breath	1.01 (0.99-1.03)	0.60 (0.34-1.05)	1.45 (0.83-2.51)	1.87 (1.09-3.20)	2.06 (1.19-3.58)
"Wheezing" without a cold	1.00 (0.98-1.01)	0.93 (0.55-1.57)	1.91 (1.13-3.22)*	1.84 (1.09-3.08)*	2.53 (1.49-4.29) p=0.001
Waking up with tightness and pressure feeling	1.01 (0.99-1.03)	1.11 (0.62-1.99)	1.496 (0.83-2.69)	1.20 (0.67-2.14)	1.50 (0.82-2.73)
Waking up with shortness of breath	1.03 (1.01-1.05)*	0.97 (0.49-1.88)	1.05 (0.53-2.10)	1.20 (0.62-2.32)	2.28 (1.71-4.44)*
Waking up with cough	1.00 (0.98-1.01)	0.71 (0.45-1.12)	1.86 (1.17-2.97)*	1.72 (1.10-2.71)*	1.79 (1.11-2.87)*

**p< 0.05*
***p<0.001*
OR: Odd's ratio; %95 CI: %95 Confidential Interval

Table 3. Binary logistic regression assessment of risk of having 2 and more infection in a year and some risk factors

	Odd's Ratio	%95 CI	p- value
Smoking	2.86	1.78-4.59	<0.001
Current asthma	1.26	0.58-2.76	0.54
Asthma like symptom	1.63	1.03-2.57	0.035
Non-infectious rhinitis	1.09	0.69-1.71	0.70

Table 4. Backward stepwise logistic regression assessment of risk of having 2 and more infection in a year and some risk factors

	Odd's ratio	%95 CI	P value
Smoking	2.80	1.75-4.49	<0.001
Asthma like symptom	1.70	1.09-2.64	0.017

overlap. Presence of allergic symptoms is a facilitating factor for infections. One of the hypotheses in this issue says that increased total and specific IgE and T-cell activation may play a role in the inflammation (17).

One of the most important findings of our study is association between the risks of having 2 and more respiratory infections and smoking. Smoking is seen as a major risk factor for respiratory and other systemic infections. Cigarette smoke consists of more than 4.000 compounds, including more than 50 carcinogens. As a result cigarette smoking leads to chronic respiratory symptoms (18). Increased frequency and severity of respiratory infections is seen in smokers. This is usually due to depressive effects of smoking on respiratory defense mechanism (12). There is an increased risk of infection in both active and passive smokers. Mortality and morbidity from infections caused by smoking are not considered enough by clinicians (11). The sensitivity of the smokers to infections is multifactorial. The studies show that smoking causes peri-bronchial inflammation and fibrosis, increased mucosal permeability and decreased muco-ciliary activity like structural changes leading to changes in adherence and distribution of pathogens on respiratory epithelium (19,20). In addition, cell-mediated and humoral immune responses are also affected by cigarette smoking. Smoking causes decreased circulating immunoglobulin levels and antibody response and inhibits phagocytic activity. Smoking increases the number of respiratory tract infections caused by pneumococcus, legionella and viral pathogens. There is also an increased risk of respiratory tract infection for passive smokers (11). According to our results, there is strong association between asthma like symptoms and smoking, they tend to accompany each other.

In this context, with smoking there is also increased risk of asthma-like symptoms. In one study cigarette smoking, have been associated with the onset of new asthma. According to the same study ex-smokers have increased risk for asthma when compared to non-smokers (13). In another study cigarette smoking was found associated with wheezing and asthma (14).

Except as in allergic responses, stimulation of airway hyper-responsiveness and remodeling, attenuation of allergic airway response by smoking may be effective in the formation of asthma-like symptoms (14, 21,22).

Smoking is considered to be one of the risk factors associated with aggravated respiratory infections and asthma symptoms (23). According to studies cigarette smoking has effects like inhibition of immune response against microbial infections and exacerbation of allergic responses on respiratory defense mechanisms (24,25). There are also studies showing that nicotine found in cigarette smoke has negative effects on the immune system (26,27).

In the family medicine system, which has high patient admittance frequency (28), doctors, in general, take smoking related cancer, atherosclerotic heart disease, chronic obstructive pulmonary disease as a major health problem but ignore tobacco-related infections. In clinical practice smoking related infections are ignored and can be misdiagnosed. In addition, the importance of recommending patients to stop smoking in all visits must be emphasized (11). Furthermore, in recurrent respiratory infections as chronic rhinosinusitis, physicians must research for allergic symptoms and refer these patients for appropriate treatment. Therefore, it is important for physicians to inform patients for acute and chronic complication of smoking and presence of allergic symptoms, and also recommend those smoking patients to quit smoking in every admittance.

References

1. *Costa DJ, Bousquet PJ, Ryan D, Price D, Demoly P, Brozek J, Schünemann HJ, Bousquet J. Guidelines for allergic rhinitis need to be used in primary care. Prim Care Respir J. 2009 Dec; 18(4): 250-7.*

2. *Konno S, Hizawa N, Fukutomi Y, Taniguchi M, Kawagishi Y, Okada C et al.The prevalence of rhinitis and its association with smoking and obesity in a nationwide survey of Japanese adults. Allergy. 2012 May; 67(5): 653-60.*

3. *Leynaert B, Neukirch C, Liard R, Bousquet J, Neukirch F. Quality of life in allergic rhinitis and asthma. A population-based study of young adults. Am J Respir Crit Care Med. 2000 Oct; 162(4 Pt 1): 1391-6.*

4. *Bousquet J, Van Cauwenberge P,Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108(Suppl. 5): S147–334.*

5. *Sears MR, Burrows B, Herbison GP, Holdaway MD, Flannery EM. Atopy in childhood: II. Relationship to airway responsiveness, hay fever and asthma. Clin Exp Allergy 1993; 23: 949–956.*

6. *Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J. 2004 Nov; 24(5): 758-64.*

7. *Elci MA, Elci OC, Odabasi A. Work related asthma-like symptoms among florists. Chest 2004; 125: 2336–2339*

8. *Skoner DP, Complications of allergic rhinitis. Allergy Clin Immunol Vol 105, Number 6, part: 2 p: 605-609*

9. *Slavin RG, Complications of allergic rhinitis: Implications for sinusitis and asthma. Allergy Clin Immunol Vol 101, Number 2, part: 2 p: 357-360*

10. *Ansley PR, Howatson G, Tallent J et al.Prevalence of Allergy and Upper Respiratory Tract Symptoms in Runners of the London Marathon. Med Sci Sports Exerc. 2012 Jun; 44(6): 999-1004.*

11. *Arcavi L, Benowit NLzCigarette Smoking and Infection. Arch Intern Med. 2004; 164: 2206-2216*

12. *Bulbul Y, Ozlu T. Akut Sigara Dumanı Maruziyetinin Bakteri Aderensine Etkisi. Solunum 1999 1: 72-76, 1999*

13. *Polosa R, Knoke JD, Russo CCigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. J Allergy Clin Immunol 2008; 121: 1428-34*

14. *Konno S, Hizawa N, Fukutomi YThe prevalence of rhinitis and its association with smoking and obesity in a nationwide survey of Japanese adults. Allergy 67 (2012) 653–660*

15. *Sacre Hazouri JA. Allergic rhinitis. Coexistent diseases and complications. A review and analysis]. Rev Alerg Mex. 2006 Jan-Feb; 53(1): 9-29.*

16. *Kvaerner KJ, Tambs K, Harris JR et al Otitis media: relationship to tonsillitis, sinusitis and atopic diseases. Int J Pediatr Otorhinolaryngol. 1996 Apr; 35(2): 127-41.*

17. *Lee S, Kundaria S, Ferguson BJ. Practical clinical management strategies for the allergic patient with chronic rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg 2012, 20: 179–187*

18. *Jaakkola MS. Environmental tobacco smoke and health in the elderly. Eur Respir J 2002; 19: 172–181*

19. *Marcy TW, Merrill WW. Cigarette smoking and respiratory tract infection. Clin Chest Med. 1987; 8: 381-391.*

20. *Richardson MA. Upper airway complications of cigarette smoking. J Allergy Clin Immunol. 1988; 81: 1032-1035.*

21. *Moerloose KB, Pauwels RA, Joos GF.Short-term cigarette smoke exposure enhances allergic airway inflammation in mice. Am J Respir Crit Care Med 2005; 172: 168–172.*

22. *Botelho FM, Llop-Guevara A, Trimble NJ, Nikota JK, Bauer CMT, Lambert KN et al. Cigarette smoke differentially impacts eosinophilia and remodeling in a house dust mite asthma model. Am J Respir Cell Mol Biol 2011; 45: 753–760.*

23. *Nouri-Shirazi M, Guinet E. A possible mechanism linking cigarette smoke to higher incidence of respiratory infection and asthma. Immunology Letters 103 (2006) 167–176*

24. *Sopori M. Effects of cigarette smoke on the immune system. Nat Rev Immunol 2002; 2: 372–7.*

25. *Holt PG, Keast D. Environmentally induced changes in immunological function: acute and chronic effects of inhalation of tobacco smoke and other atmospheric contaminants in man and experimental animals. Bacteriol Rev 1977; 41: 205–16.*

26. *Nouri-Shirazi M, Guinet E. Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. Immunology 2003; 109: 365–73.*

27. *Guinet E, Yoshida K, Nouri-Shirazi M. Nicotinic environment affects the differentiation and functional maturation of monocytes derived dendritic cells (DCs). Immunol Lett 2004; 95: 45–55.*

28. *Ustu Y, Ugurlu M, Kasım I, Egici MT. Application Frequencies to Health Institutions after the Health Transformation Project. Konuralp Tıp Dergisi 2012; 4(2): 48-53*

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Effect of the pedicle mucosal (muscle) flap of the lip for primary repair of contralateral introcession

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Abstract

Objective: To observe the clinical effect of mucosal (muscle) pedicle flap of the lip for primary repair of contralateral introcession of soft tissues.

Methods: According to the defective degree of soft tissue of the lip, we designed mucosal (muscle) pedicle flap of 1/2 defective volume in the uninjured side. Based on the line of attachment of fraenum labiorum and vermilion tubercle, the muscle under the mucous membrane was separated as the mucosal (muscle) pedicle flap. We made an incision whose area was smaller than the long axis of area of the bottom of the mucosal (muscle) pedicle flap in the defective side and the tissue of mucous membrane was separated. The lacouna to contain the mucosal (muscle) pedicle flap was made by eye scissors. The tissue of mucous membrane at the junction of two incisions was separated to make a tunnel. The mucosal (muscle) pedicle flap was revolved and moved through the tunnel. This repaired the deformity of the lip.

Results: We carried out the operation in 6 cases. The operations utilized normal tissues of uninjured side of the lip. Incision trace was small and larvaceous. Appearance of the lip was nearly normal and symmetrical to ensure a perfect repair effect.

Conclusion: The mucosal (muscle) pedicle flap of the lip is effective for primary repair of contralateral introcession and deformity, and is applicable in similar circumstances.

Key words: Lip, mucosal (muscle) flap, introcession, deformity, repair.

Introduction

There may be soft tissue introcession and deformity on one side of the lip and asymmetry between the two sides caused by development, diseases or operations and so on in labial part which affect the image and life of patients. Lips have abundant blood circulation which includes well-known arteriae labiales and vena labialis. There are profuse capillary networks between vessels on two sides, forming the arciform vascular net. So, primary repairing contralateral soft tissue introcession and deformity by rotated transplanting mucosal (muscle) pedicle flap of uninjured side with the middle of lip as the axis is feasible. We have repaired 6 patients with labial introcession and deformity with labial mucosa (muscle) flap from uninjured side since 2008, acquiring satisfactory effect.

Clinical data

A total of 6 patients (2 males and 4 female) with ages ranging from 17 to 55 years (mean age 35 years) were included in our group. There were 3 patients with unilateral congenital malformation, 2 patients with introcession after lesion resection and 1 patient with infectious introcession. The defective side of lip accounted for 1/3 to 3/4 of the uninjured side.

The anatomy of lips

The lips have five-layered structure from the outmost to the innermost: skin layer, superficial fascia layer, muscle layer, submucosa layer and mucosa layer. The surface structure of vermilion border is similar to skin and presents semitransparent with no cuticle. There are abundant capillary networks under the papillary layer that is the main cause for the vermilion border showing vermilion. There is no submucosa layer in vermilion border which closely connects with the extrophia part on the edge of deep orbicularis oris. Orbicularis oris has two layers: the deep layer is sphincter, located in the submucosal layer, surrounding the mouth, and mainly origins from alveololabialis. The superficial layer extends to the opposite side from one side of skin and mucous and divides into the

upper and lower beams. The upper beam, nose beam, arises from zygoma, maxilla and nasal bone respectively, while the muscle fibers mainly come from major zygomatic muscle, minor zygomatic muscle, levator labii superioris, levator labii superioris alaeque nasi and nasal transverse muscle. The lower beam is also known as nasolabial beam and its muscle fibers mainly come from musculus caninus and quadrate muscle of lower lip. A part of muscle fibers of buccinator turn over outward along with mucosa, forming the vermilion border. The blood supply of lips is mainly provided by superior labial artery and inferior labial artery, which walk windingly between the muscle and mucosa adjacent to vermilion border. There are rich capillary networks between the left and right sides of vessels to communicate with each other which form arcuate vascular network or vascular arcades. The motor nerve of lip is mainly dominated by buccal branch and marginal mandibular branch of facial nerve. The sensory nerve of upper lip is dominated by the infraorbital nerve while the sensory nerve of lower lip by mental nerve[1, 2].

The design of mucosa (muscle) flap

Determine soft tissue thickness on both sides of lips and design the spindle-shaped mucosa (muscle) flap with the volume as half as defective volume of the defective side on the uninjured side according to the labial introcession levels of the defective side. The design of length, width, height and thickness of mucosa (muscle) flap is calculated on the basis of approximate rhombohedron. First, select 3 sites on both the defective and uninjured sides along the lip curve respectively with equidistant spacing between two sites. Measure lip thickness with pachymete and calculate average thickness of lips and difference values ($\triangle$h) respectively. Then, measure the length from the angle of lip to vermilion tubercle and calculate the approximate volumes and volume differences ($\triangle$v) of lips by the length multiplied with thickness. Third, the depth extending to the deep face of incision on the underside of mucosa (muscle) flap of the uninjured side equals 1/2 of the difference of average thickness of lips between two sides (1/2$\triangle$h), and the volume of mucosa (muscle) flap approximates 1/2$\triangle$v. Forth, design the length and width of incision according to the basal area of mucosa (muscle) flap of the uninjured side. Based on the length of horizontal incision on defective side: that the length of long axis of incision on underside of mucosa (muscle) flap of the uninjured side equals 1: 1.5～2 to design the incision length on defective side.

Surgical procedure

1% lidocaine was used for local infiltration anesthesia. We cut mucosal and submucosal soft tissue with the line of labial frenum and vermilion tubercle as the pedicle and separated submucosal soft tissue with scissors. The volume of fusiform soft tissue separated was approximately 1/2 of defective volume of the defective side. We performed an incision which was slightly smaller than the long axis on the underside of mucosa (muscle) flap of the uninjured side in the centre of defective side, and then slit it and separated toward the deep with scissors, avoiding arteriae labials during the process. Next, we separated soft tissue under the mucosa of junction between the left and right incisions to make it a tunnel. We turned over the mucosa (muscle) flap which passed through the tunnel and entered in the separated lacouna on the defective side (Figure 1D) to repair tissue defects. After completely stopping bleed, we carried out apposition suture of mucosa (muscle) flap and wound edge with nylon line of 3-0 and sutured mucosa with nylon line of 7-0 (Figure 1E). Layered suture was done at donor site carefully. You could refer to Figure 2: Schematic diagram of the surgery for primary repairing contralateral introcession and deformity with mucosal (muscle) pedicle flap of lip.

Results

All patients underwent conventional anti-infection measures after operation. Dressing change was performed at 24h after operation and stitches were removed at 7 days. The wound healed well with concealed and tiny scar. The labial defective side was plump in shape and it was approximately symmetrical between two sides with satisfactory repaired effect. There were no deformation in neighboring parts and other complications and almost no incision marks in an anterior view in 5 patients who were followed up over than 6 months, achieving satisfactory appearance.

Typical case

A female patient aged 28 year-old suffered from right congenital dysplasia of upper lip which had less mucosal and submucosal soft tissue than those of the opposite side with asymmetric two sides (Figure 1A). Surgical process: we turned over the upper lip upward and then designed mucosa (muscle) flap in the left with oval bottom (Figure 1B). Based on the line of attachment of fraenum labiorum and vermilion tubercle, we cut mucosal and submucosal soft tissue and separated submucosal soft tissue with scissors which formed a fusiform mucosa (muscle) flap with the middle of lip as an axis of rotation. The diameter of submucosal soft tissue pedicle was about 3～5mm and the volume of fusiform soft tissue was approximate 1/2 of defective volume on the defective side. We performed an incision which was slightly bigger than the long axis of fusiform mucosa (muscle) flap in the center of right mucosa and separated submucosal soft tissue of junction to make it a tunnel. Then we turned over mucosa (muscle) flap and made it pass through the tunnel and then repaired the right introcession and deformity (Figure 1C). The flap well survived after surgery with unobvious scars, plum appearance and fine shape during the 6 months of follow-up (Figure 1F).

Discussion

The blood supply of labial mucosa (muscle) flap: There is abundant blood in lips where the vessels form networks and have sufficient blood supply. Xia [3] reported that blood supply in upper lip

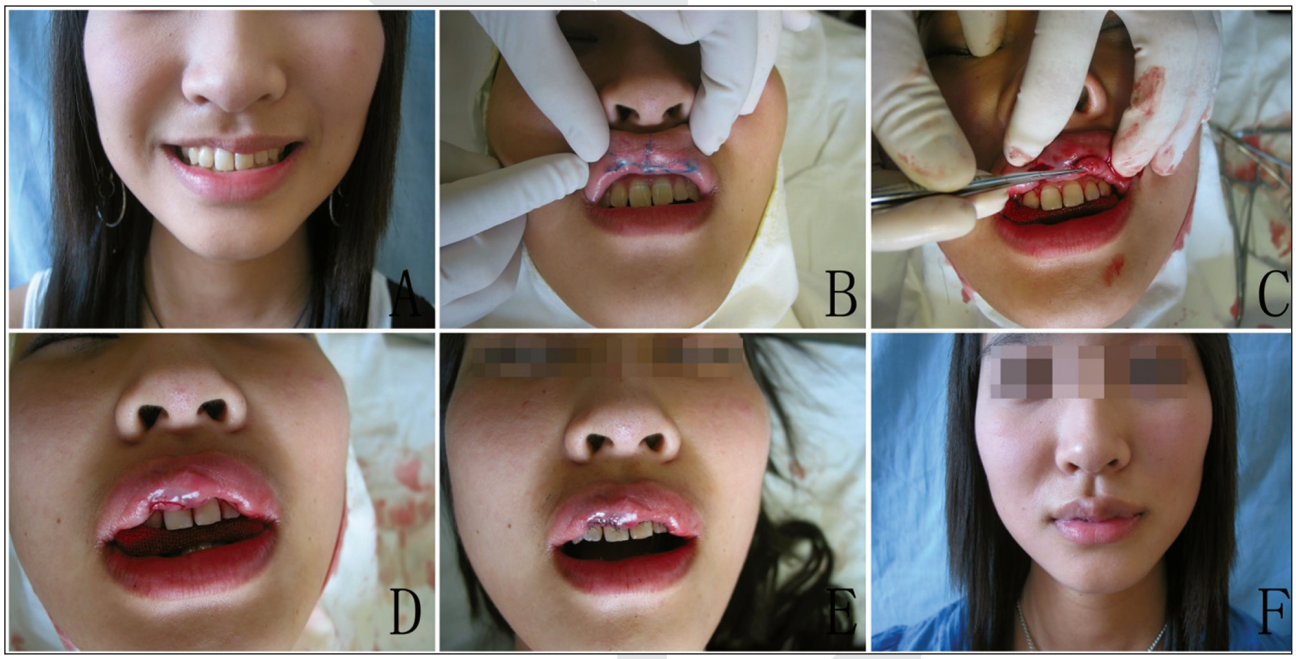

Figure 1. Surgical process for primary repairing contralateral introcession and deformity with mucosal (muscle) pedicle flap of the lips

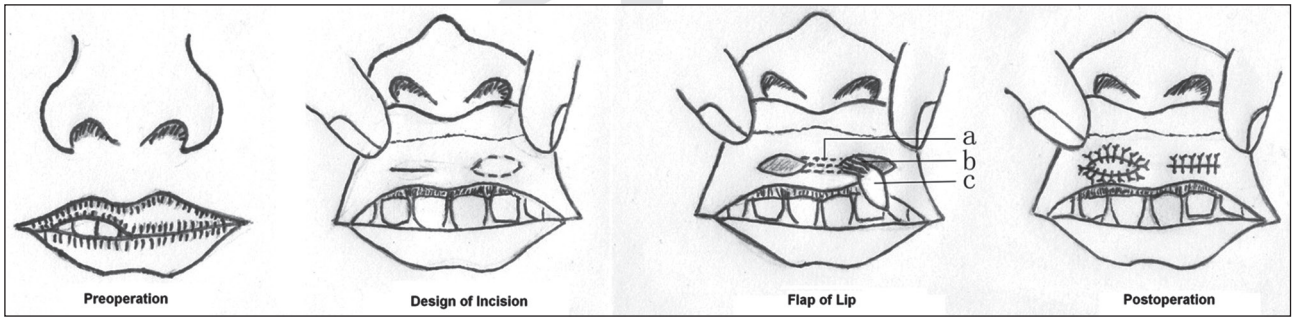

Figure 2. Schematic diagram of the surgery for primary repairing contralateral introcession and deformity with mucosal (muscle) pedicle flap of lip. a. tunnel; b. soft tissue pedicle; c. mucosal (muscle) flap

mainly relied on superior labial artery which passed through the deep face of musculus orbicularis oris, walked windingly between muscles and mucosa near vermilion border and connected to contralateral superior labial artery forming a constant axial arterial arcades, i.e. arterial arcades of upper lip. The coarse branch of superior labial artery develops dense submucosal vascular networks in submucosa of upper lip and the perforating branch which provides muscles and skin as well as mucous with their nutritional needs develops rich subdermal or intradermal vascular networks. Zhong[4] performed angiography and microscopic anatomy on newborn specimens and showed that, in addition to the superior labial artery and its branches, the blood-supply of upper lip also includs the vascular access which is connected by superior labial artery and the branches of lower naso-alar marginal artery, arteria nasalis posterior septi and anterior ethmoidal artery on upper lip. The results we studied on the vascular distribution patterns of lips are generally consistent with previous reports. We believe that transferring labial mucosa (muscle) flap with submucosal soft tissue pedicle at a certain diameter to repair is feasible and the blood circulation also can be guaranteed. Furthermore, Millard [5] reported that although pedicle flaps only contained mucosa and a few muscles without well-know axis artery, mild blood circulation disorder at early stage could be well improved latterly. We also verified from the investigation on clinical efficacy of 6 patients that repairing introcession with mucosal (muscle) pedicle flap of upper lip had well blood supply and were easier to survive without necrosis and other complications.

Another treatment method for local introcession of lip is fat filling which is suitable for deformity and introcession caused by skin, muscle and other soft tissue. It usually absorbs fat from the abdomen. Tumescent fluid contained 0.0125% lidocaine is injected in abdominal subcutaneous tissue followed by absorbing fat by fine needle with a diameter of about 2.0mm. The fat absorbed is injected into labial part after the processing of sedimentation, filtration and so on to make the lip plump. The advantages of this method are smaller wound on lips and easier to be accepted by patients, while the disadvantage is uncertain survival mount of fat graft, thence sometimes it needs for several fillings. Furthermore, most deformity and introcession of lip accompany with scar adhesions of deep tissue or absolute reduction of the volume of mucosa and muscle tissue, so filling with autologous fat or other materials is difficult to reach ideal repair effect. That repair tissue defect of lower lip with mucosa (muscle) flap of upper lip or repair tissue defect of upper lip with mucosa (muscle) flap of lower lip can also acquire reasonable effect. However, this surgical method needs second surgery to cut off pedicle, which has long course of treatment and high cost as well as brings many inconveniences to patients' life.

When designing mucosa (muscle) flap of lip, we should pay attention to accurately measure, only in this way, it can reach symmetry and harmony between the uninjured side and defective side after operation. To ensure postoperative survival quality of labial mucosa (muscle) flap, we should operate carefully during surgery to reduce unnecessary damage. The diameter of soft tissue muscle pedicle maintains around 3～5mm and the tunnel width is suitable to make mucosa (muscle) flap pass through smoothly. The pedicle of soft tissue flap accumulates in the middle of lips, which makes the vermilion tubercle more plum and have well form rather than affects the form of lip. That repairing contralateral introcession and deformity of lip with liable mucosa (muscle) flap on the uninjured side has reliable postoperative effect and symmetrical as well as plum shape after surgery. The form and function of lips occupy a very important position in facial aesthetics, and its complex anatomical structure, physiological function and unique expression function is the focus of emotional concentration and impulse only second to eyes on the face. The introcession or defect of lips may result in function disorders of oral cavity, such as salivation, influences on eating and pronunciation and so forth. The damage or deformity of lip shape will bring the defects of some physiological functions and psychological trauma, accordingly, the reshaping and repairing of lip is assigned more positive and substantial clinical and social significances. That repair contralateral introcession and deformity of lip with mucosa (muscle) pedicle flap fully utilizes normal tissue of uninjured side which has the totally same histology to that of the opposite side, resulting in approximate symmetry between two sides of lips and satisfactory shape. The surgery can be finished one time

that saves time and is convenient as well as reliable. The method is ideal for repairing the introcession and deformity of soft tissue of lips which deserves to be popularized.

References

1. *Kane MA, Lorenc ZP, Lin X, Smith SR. Validation of a lip fullness scale for assessment of lip augmentation. Plast Reconstr Surg. 2012 May; 129(5): 822e-8e.*

2. *Dreyer LN, Brown GC.Oral manifestations of psoriasis. Clinical presentation and management. N Y State Dent J. 2012; 78(3): 14-8.*

3. *Xia YC, Chen XX, Tian L, Xia JL. Relationship between arterial configuration of the lips and blood supply of the labial flap. J Peking Univ (Health Sci), 2001; 33(1): 6-9*

4. *Zhong W, Zhang K, Wang F. Applied anatomical study of blood supply in human nose and lip. Zhonghua Kou Qiang Yi Xue Za Zhi. 2001; 36(2): 136-8.*

5. *Millard DR Jr, McLaughlin CA. Abbe flap on mucosal pedicle. Ann Plast Surg. 1979 Dec; 3(6): 544-8.*

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The effect of adenotonsillectomy on pulmonary function tests

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Abstract

Background: Upper airway obstructions may lead to reversible pulmonary dysfunction in children.

Aim: The aim of the present study was to evaluate the pulmonary function of the children with upper airway obstruction who underwent an operation due to the presence of hypertrophic tonsils and/or adenoids.

Materials and Methods: Twenty-nine patients with upper airway obstruction aged between 4-16 years who were clinically diagnosed with tonsillar and/or adenoid hypertrophy and had the signs of upper airway obstruction and in whom surgery was performed. If suspicious or confirmed asthma history were present subject did not enrolled to the study.Spirometric evaluation was performed prior to surgery and one month after surgery.

Results: The mean age of the patients was 9.52±5.21 years and 14 (48.3%) of the patients were male and 15 (51.7%) were female. No clinical pathology was detected preoperatively regarding the cardiopulmonary system in patients. Results of preoperative and post operative spirometric parameters were as follows respectively forced vital capacity (FVC) (liter); 1.80 ±1.00 and 2.01±0.99, p=0.007 FVC% (of predicted) 84±25 and 93±30, p=0.035, forced expiratory volume in one second (FEV1) (liter); 1.74; ±0.9 and 1.87±0.8, p=0.035, FEV1% (of predicted) 0.92±0.24 and 1.0±0.3, p=0.066, peak pxpiratory flow (liter/sec); 3.87±1.5 and 4.1±1.5, p=0.162.

Conclusion: Pulmonary system function disorders can be early detected by respiratory function tests before the appearance of clinical signs in children having the signs of upper airway obstruction. The mild signs of obstructive disorder determined by the pulmonary function tests preoperatively returned to normal after adenotonsillectomy.

Key words: Adenoid, tonsillar hypertrophy, pulmonary function test, pulmonary system function.

Introduction

Hypertrophy of the adenoid tissue of the nasopharynx is frequently encountered in children. Obstructive adenoid hypertrophy leads to the complaints of hyponasal speech, breathing through the mouth and snoring (1, 2). Clinical data on these symptoms are obtained by questioning the patient (3). The diagnosis is confirmed by anterior and posterior rhinoscopy, direct nasopharyngoscopy, or digital examination. However, there are some difficulties in the physical examination of the children. Owing to the fact that digital examination is a difficult technique, many physicians accept that radiological evaluation of the adenoidal-nasopharyngeal ratio (ANR) is an easy and non-invasive method in the assessment of adenoid size and in the decision of adenoidectomy (3, 4).

Hypertrophy of the tonsillar and adenoid tissue in children may occur due to physiological, allergic or infectious causes. Adenotonsillar hypertrophy occurs during early childhood, continues to increase in size until puberty and after puberty, a slow atrophy and an involution begins (5). The growing adenoid may obstruct the nasopharyngeal airway, especially at nights. Alveolar hypoventilation may occur after prolonged and marked blockage of the nasopharyngeal airway, which may in turn result in pulmonary hypertension and cor pulmonale. Consequent to acute upper airway obstruction, hypoxia, cardiopulmonary arrest, and as a result, death may occur. Providing a sufficient airway opening allow the patients return to their normal conditions (6, 7).

Spirometric evaluation is the most basic method in the assessment of the respiratory functions. It is routinely used in the diagnosis of obstructive and

restrictive pulmonary diseases, in the assessment of disease severity and treatment response, and in the diagnosis, screening, and assessment of disability from occupational diseases. The clinical use of respiratory function tests (RFTs) in which spirometer was used to measure the vital capacity was first utilized by Hutchinson in 1844. With this application, RFT was introduced into practical use and became one of the most important diagnostic tools in pulmonary diseases clinics. Moreover, RFTs have become a gold standard in the diagnosis of subclinical diseases, determination of the types of functional disorders, evaluation of the seriousness of the disease, and follow-up of treatment response. Data obtained from RFTs represent the anatomical or physiological borders. These borders are different in many diseases and usually non-specific. In non-pulmonary diseases, however, rather than assisting in the diagnosis of the primary diseases, RFTs are more useful in the determination of the functional disorders developing secondary to these primary diseases. Spirometric measurement reflects the calm respiration, forced inspiration, forced expiration, and the duration, volume and flow values measured during deep and rapid respiration in a certain time of period (8).

In the present study, the effects of adenotonsillectomy, which is frequently performed for otorhinolaryngologic diseases, on RFTs were investigated.

Material and Method

In the present cross-sectional prospective study, 29 patients aged between 4-16 years who were clinically diagnosed with tonsillar and/or adenoid hypertrophy and had the signs of upper airway obstruction and in whom surgery was planned by otorhinolaryngology clinic of Sivas state hospital between January 2010 and March 2011 were enrolled. Anamnesis was obtained from all patients, and they were questioned regarding nasal obstruction, oral breathing, and nasal and postnasal discharge. Preoperative laboratory tests and chest radiographs were obtained. If the subject was excluded if he or she had a history of asthma broncheiectasis, tuberculosis or cyctic fibrosis.

The signs of upper airway obstruction were defined as difficulties in breathing even causing awake from sleep, snoring, and difficulties in feeding. None of the patients had a history of known heart disease, recurrent cough, wheezing attacks, obstructive sleep apnea-hypopnea and/or hypoventilation, and none of the patients had an upper respiratory tract infection during the study period. All of the patients were examined preoperatively and postoperatively at month one by physical examination, telecardiography, RFTs and complete blood count analysis. Furthermore, the preoperative cardiac and pulmonary functions were assessed Spirometryt was performed at Sivas State Hospital by the same technician at the same room temperature, and appropriate humidity and pressure. Among the spirometric parameters, forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and peak expiratory flow (PEF) were recorded. The parameters were determined as appropriate percentiles according to the age, gender and weight. The values of FVC and FEV1 >80% were accepted as normal.

The statistical analyses of the data were performed using Statistical Package for the Social Sciences version 14.0 (SPSS Inc., Chicago, IL, USA). In the evaluation of the data, paired T-test was used. The p value <0.05 was considered significant.

Results

The mean age of the 29 patients was 9.52±5.21 years (range 4-16). Of these patients, 14 (48.3%) were male. There was a significant difference regarding the values of preoperative and post operative FVC, FVC%, FEV1, and FEV1% ($p<0.05$ for all). No significant difference was present for values of preoperative and post operative FEV1% (of predicted), FEV1/FVC ratio and PEF. The comparison of the pre- and postoperative values of RFT parameters are presented in Table 1.

Discussion

Chronic tonsillitis, adenoid vegetation and tonsillar hypertrophy are very frequently observed health problems in the population. It has been known for many years that the blood oxygen levels in individuals who breathe through the mouth are lower than that of those breathe nasally. Moreover, the subjects who breathe through the mouth

Table 1. The comp arison of the pre- and postoperative values of respiratory function test parameters

Parameter	Preoperative	Postoperative	P value
FVC(liter)	1.80 ±1.00	2.01±0.99	0.007
FVC% (of predicted)	0.84±0.25	0.93±0.30	0.035
FEV_1 (liter)	1.74±0.92	1.87±0.84	0.035
FEV_1% (of predicted)	0.92±0.24	1.01±0.31	0.066
FEV_1/FVC	94.85±6.77	94.60±6.55	0.847
PEF	3.87±1.52	4.10±1.56	0.162

Data are presented as mean±standard deviation. A p value <0.05 are considered significant. FVC, Forced vital capacity; FEV1, Forced expiratory volume in one second; PEF, peak expiratory flow.

are more tired, somnolent and forgetful. Oral breathing may lead to orthodontic problems as well. Some studies have reported a significant correlation between the radiological findings and adenoid weight (9-10). Thus, a lateral radiograph of the nasopharynx should be performed in the detection of adenoid hypertrophy. In the evaluation of patients with upper airway obstruction for whom tonsillectomy and/or adenoidectomy has been planned, Spirometry has practical importance as it is easily applicable, non-invasive and reliable method.

Spirometric measurements are the most commonly used RFTs and provide reliable information regarding the respiratory functions (8). In the present study, the postoperative FVC and FEV1 were significantly higher. This can be attributed to the fact that adenotonsillectomy may increase the lung capacities and as well as the blood oxygen levels. Consequently, an improvement in the complaints of forgetfulness, fatigue and somnolence may be observed.

In childhood, especially between 2-5 years of age, tonsillar and adenoid hypertrophies are the most frequently encountered cause of upper airway obstruction (11, 12). The spirometry provides the diagnosis, follow-up and effective treatment of respiratory system functions (13, 14). In the study of Maruzi et al. (14), it was reported that of the children with adenoid hypertrophy, 65.7% had pulmonary function disorders. In their study, Kavukcu et al. (14) evaluated 45 children with no significant signs of clinical or radiological obstructive manifestations undergoing adenotonsillectomy due to the frequent occurrence of adenotonsillitis preoperatively and postoperatively at month one by RFTs. After the operation, the pre-existing mild signs of obstructive manifestations disappeared in 60% of the children, and the mean values of RFT parameters (FVC, PEF, FEV1, forced expiratory flow between 25% and 75%) increased as well. In that particular study, RFTs were found to be effective to indicate the obstructive effects and surgical indications in adenotonsillar hypertrophy with no accompanying clinical and radiological signs of obstructive manifestations (14). Moreover, they suggested that reflex airway obstruction would have been occurred during an attack of adenotonsillitis in those with prolonged recurrent adenotonsillitis (14).

Conclusion

In the present study, we demonstrated that adenotonsillectomy provided an slight improvement in pulmonary functions at childhood.

Pulmonary function test may help to decide for adenotonsillectomy in patients that the clinician was suspicious about the operation.

References

1. *Wiatrak, B.J., Woolley, A.L.: Pharyngitis and Adenotonsillar disease. Cummings CW, Fredrickson JM, Harker LA, Krause CT, Richardson MA, Schuller DE, editors. Pediatric Otolaryngology-Head and Neck Surgery. St Louis: 3rd. Mosby Year Book; 1998. p. 188-215.*

2. *Brodsky. L.: Tonsillitis, tonsillectomy and adenoidectomy. Bailey BJ, Johnson JT, Kohut RJ, Pillsbury III HC, Tardy ME, editors. Head and neck surgery-Otolaryngology. Philadelphia: J.B. Lippincott; 1993, p.833-847.*

3. *Paradise, J.L., Bernard, B.S., Colborn, D.K., Janosky, J.E.: Assessment of adenoidal obstruction in children: clinical versus roentgenographic findings. Pediatrics 101 (6): 979-86, 1998.*

4. *Kemaloglu, Y.K., Goksu, N., Inal, E. Radiographic evaluation of children with nasopharyngeal obstruction due to the adenoid. Ann Otol Rhinol Laryngol 108 (1): 67-72, 1999.*

5. *Brown OE, Manning SC, Ridenour B. Cor pulmonale secondary to tonsillar and adenoidal hypertrophy: management considerations. Int J Pediatr Otorhinolaryngol 1988; 16: 131-139.*

6. *Ballenger JJ. Orofarinks hastalıkları, In: Otorinolaringoloji Baş ve boyun Cerrahisi (15th ed). Edited by Ballenger JJ, Snow JB. ŞenocakD, çeviri editörü. İstanbul, 1990, s: 236-244.*

7. *McGowan FX, Kenna MA, Fleming JA, O'Connor T. Adenotonsillectomy for upper airway obstruction carries increased risk in children with a history of prematurity. Pediatr Pulmonol 1992; 13: 222-226.*

8. *Chopp GL. Clinics in Chest Medicine; Pulmonary function testing WB Saunders Company Philadelphia, 2001.*

9. *Jeans, W.D., Fernando, D.C., Maw, A.R.: How should adenoidal enlargement be measured? A radiological study based on interobserver agreement. Clin Radiol. 1981; 32 (3): 337-40.*

10. *Hibbert, J., Whitehouse, G.H.: The assessment of adenoidal size by radiological means. Clin Otolaryngol 3 (1): 43-7, 1978.*

11. *Kenna MA. Tonsils and Adenoids. In: Behrman RE, Kliegman RM, Jenson HB, (Eds). Nelson Texbook of Pediatrics. 16th edition. Philadelphia, WB Saunders, 2000; 1267-1268.*

12. *Rosen CL, Haddad GG. Obstructive Sleep Apnea and Hypoventilation in Children. In: Behrman RE, Kliegman RM, Jenson HB, (Eds). Nelson Texbook of Pediatrics. 16th edition. Philadelphia, WB Saunders, 2000; 1268-1271.*

13. *Maurizi M, Paludetti G, Todisco T, Dottorini M, Grassi V. Pulmonary function studies in adenoid hypertrophy. Int J Pediatr Otorhinolaryngol 1980; 2: 243-250.*

14. *Kavukcu S, Coskun S, Cevik N, Kuscu B, Akkoclu A. The importance of pulmonary function tests in adenotonsillectomy indications. Indian J Pediatr 1993; 60: 249- 255.*

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Self-buttressing patch: Alternative technique for surgical closure of Ostium secundum atrial septal defect

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Abstract

Background: Ostium secondum atrial septal defects (ASDs) with large diameters, inadequate rim of tissue surrounding the defect or neighbouring on the adjacent critical structures have unsuitable anatomy for device closure and would increase the likelihood of residual shunt postoperatively. To surgically secure a synthetic patch more efficiently, herein is a report of an alternative technique devised to doubly buttress the defect rims.

Methods: Forty-eight patients with secundum type atrial septal defect who had been discarded for devise closure were enrolled in the current study. Technique: A Dacron patch 10% larger than the actual defect size prepared and tailored up and down-wards from its 3 and 9 o'clock to create four attached strips hinged on its 6 and 12 o'clock. The patch was sat in place using two 4/0 poly propylene mattress sutures on its superior and inferior poles. Each quadrant was sewn by stitching the Dacron strip, the defect rim, and the patch.

Results: 32 patients were operated using the present technique. All patients survived the operation. Follow up duration was six months. The postoperative Doppler echocardiography ruled out any residual shunt immediately after the surgery and one and six months later.

Conclusion: Closing various types of ASD using synthetic self-buttressing patch is a safe and reproducible technique facing various difficult anatomical variants of ASD.

Key words: Atrial septal defect II, surgical closure, self buttressing.

Introduction

Since 1952 when Bailey (1) performed the first successful surgical closure of atrial septal defect, various surgical techniques have been developed to repair ASDs. Since transcatheter methods are used extensively in recent years (2-7), the referred cases for surgery have more unsuitable anatomy than before (the large diameter, inadequate rim of tissue surrounding the defect or neighbouring on the adjacent critical structures), therefore in such situations surgeons need more secure suturing techniques with the least risk of damage to critical adjacent structures and at the same time the lowest residual shunt.

In this study we tried to reinforce the sutureline using a buttressing patch. Using this novel technique a synthetic patch is secured more efficiently on the defect rims.

Methods

Patient selection

The need to repair ASDII defects were decided based on clinical and echocardiographic examination, including transthoracic measurement of *the ASD size and its location*, the content and hemodynamic impact of the left-to-right shunt, and the presence and degree of pulmonary arterial hypertension. Inclusion criteria in our study were the presence of secundum type of ASD, clinically significant shunt, determined when the ratio of pulmonary to systemic blood flow (Qp: Qs) is at least 1.5: 1, or minimal shunt in association with symptoms such as arrhythmias, transient ischemic attacks or right ventricular volume overload and patients who were excluded from transcatheter correction(patients with a defect of 28 mm or **more** in transthoracic echocardiographic, those with inadequate tissue rim surrounding the defect (less than 5 mm), or neighbouring on the adjacent critical structures (a distance of <5 mm from the coronary sinus, atrioventricular valves and right upper pulmonary vein)

After being approved by the Ethics Committee of our research center, all the patients who had been

considered inappropriate for device closure during 12 months in Shaheed Rajai Cardiovascular Medical and Research Center were recruited. From among them, forty-eight patients met the inclusion criteria and were enrolled. A single surgeon operated all the patients in the same hospital. A written informed consent was obtained from all the patients.

Technique

Surgical access was achieved through a standard *median sternotomy.* Following *systemic heparinization*, *cardiopulmonary bypass* was *initiated* with aorto–bicaval cannulation. *The aorta* is then *cross-clamped* and cold crystalloid *cardioplegia* was used for *myocardial protection. A trap*-door opening is made into the right atrium and the remnant tissues of fossa ovalis trimmed away (Figure 1).

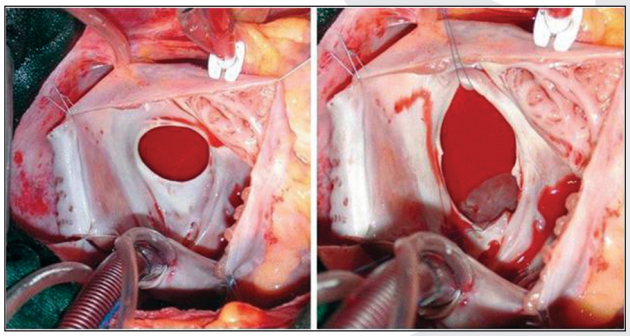

Figure 1. Atrial septal defect. Surgical field image

An oval-shaped Dacron patch made from Bard-Debakey Woven Fabric (C.R. Bard, Inc., Haeverhill, MA, U.S.A.Tempe, AZ, USA**)** 10% larger than the actual defect size was prepared.

The patch was tailored up and down-wards from its 3 and 9 o-clock section to create four attached strips hinged on its 6 and 12o-clock section (Figure 2).

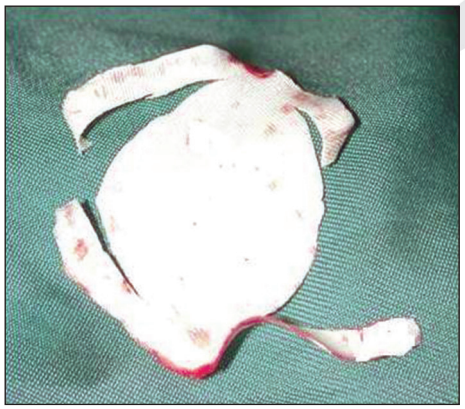

Figure 2. Four attached strips hinged on patch

The patch was then located in place using two 4/0 poly propylen mattress sutures on its superior and inferior poles (Figure 3).

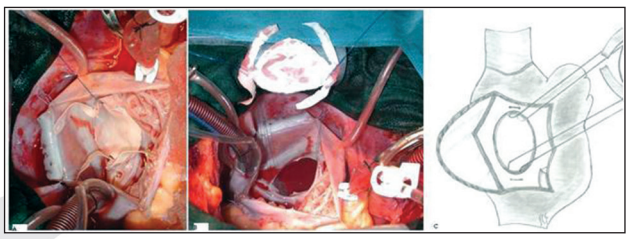

Figure 3. Sutures on superior and inferior poles of the patch. A. Surgical field image, B. The patch were sutured from superior and inferior poles C. Graphic design representing the technique.

Each quadrant was sewn by stitching the Dacron strip, the defect rim, and the patch; doubly buttressing the defect rims (Figures 4, 5).

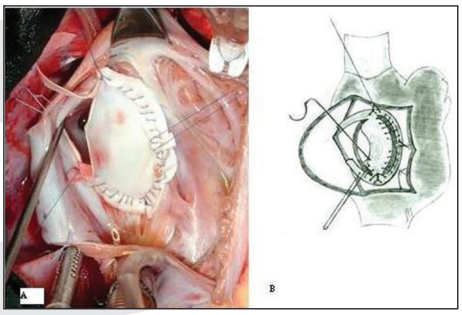

Figure 4. Suturing each quadrant by stitching the Dacron strip, the defect rim, and the patch

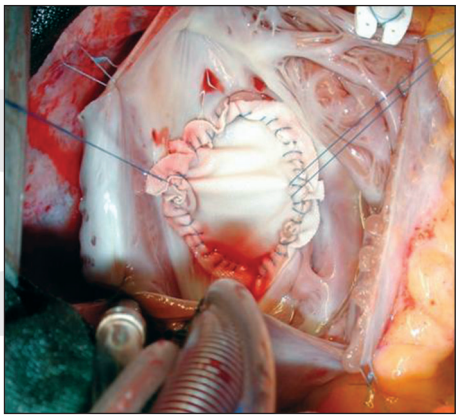

Figure 5. Final outcome, repaired defect

Follow up

Clinical examination, electrocardiography, chest radiography and color Doppler transthoracic echocardiography were performed for all the patients prior to discharge. The patients were monitored for possible recurrence of the shunt by echocardiography within one and six months after the operation.

Results

Forty-eight patients with the mean age of 35.35± 11.76 years, ranging from 14 to56 years, were enrolled. There were 30 females and 18 males among the patients. The clinical characteristics of the patients are shown in Table 1. Nineteen patients had associated anomalies (Table 2); none of them, however, required further treatment.

The cardiopulmonary bypass (CPB) lasted for 46.43 min (min=40, max=60, SD=4.73) and mean aortic cross-clamp time was 23.18 min (min=18, max=30, SD=3.61).

Table 1. Echocardiologic characteristics of ASD patients underwent self buttressing surgical closure

Char	Mean ± (Std)
EF(pre op)	50.73 ± (6.60)
QP/QS	2.83 ±(0 .82)
PA pressure(pre)	38.95 ± (8.50)
ASD size (mm)	22.07 ± (7.68)
Flow	3962.93 ± (472.18)
EF (post op)	51.35 ± (6.62)
PA pressure (post op)	33.8542 ± (.048)

Table 2. Associated anomalies in ASD patients underwent self buttresing surgical closure

Associated anomalies	Number (%)
Anerysm of IAS	4 (8.3)
Mild TR and MR	4 (8.3)
Trivial MR	1 (2)
Mild MR	3 (6.2)
ModerateTR mild MR	1 (2)
Mild to moderate MR&TR	2 (4.1)
Mild TR	1 (2)
Mild MR+aneurysm of IAS	1 (2)
Congenitalabsencent of pericardium	1 (2)
Mild MR &MS	1 (2)

IAS: Inter atrial septum, TR: tricuspid regurgitation, MR: Mitral regurgitation

All the patients had complete closure of the defect immediately after the surgery. No residual shunt was detected by color Doppler transthorasic echocardiography upon discharge and one and six months after the operation.

No deaths were reported during the study period. Five patients, however, developed major complications, and were hospitalized for a longer time to receive the required treatment. A 43-years-old woman experienced large pericardial effusion at right ventricular outflow tract, revealed via sternotomy. The patient arrested due to ventricular tachycardia (VT) in the coronary care unit (*CCU*) and was resuscitated. She was discharged after a 25-days stay in the hospital. Two other patients developed large pericardial effusion (PE) and tamponade, which was drained using the subxyphoid approach. A 53-years-old woman developed pneumonia 4 days after the surgery and therefore needed intravenous antibiotic therapy. A 29-years-old man had atrial flutter and underwent electrophysiologic studies and was discharged while on medical treatment. Minor complications were reported in four patients. Two of them had small PE and one had trivial PE that resolved without treatment. One patient had moderate size hematoma in front of RV (Right ventricle) which still needed no treatment.

The patients were discharged based on their clinical state after a 2.45±1.77days stay in the intensive care unit and a 7.75±4.79 days stay in the ward.

Discussion

Atrial septal defect (ASD) is an anomaly that can be repaired surgically or through interventional catheterization. Although surgical closure results in low morbidity and excellent survival, sternotomy and cardiopulmonary bypass are needed and complications may occur in certain cases. Transcatheter closure is recently applied to prevent such complications; and the technique is thus rapidly replacing the existing surgical treatments in major cardiac centers. Using this method, the length of *hospital stay* after the procedure is *shortened* and the patients could often be discharged on the same day of the intervention (8). In addition, transcatheter intervention reduces the costs and psychological effects of such major surgeries (9). Various catheter-implanted occlusion devices have now been used to close secundum ASD. These devices work best for centrally located secundum defects and unfortunately it is not yet applicable for all types of defects. The morphology and location of the defect is the main factor influencing the selected closure technique. However, more defects could be closed with transcatheter intervention if performed by an experienced surgeon. This

comes while the technique is not applicable for large defects, with an adequate rim of tissue surrounding the defect, or neighbouring on the adjacent critical structures. Therefore open heart surgical treatment in such cases is yet inevitable. On the other hand, we encounter more cases with difficult anatomy than before.

Technique: Anesthesia procedure, sternotomy and commencement of CPB in our series were done according the standard method in conventional technique of ASD repair. The mean CPB time and mean aortic cross-clamp time was respectively 46.43 and 23.18 min in our study which are comparable with previous studies (10). Since most of adverse effects occur due to open heart surgery and CPB, therefore we expect no significant difference in complications between our series and conventional *technique*.

Pastorek et al reported the presence of residual shunt in 7.8% of their patients but did not find any significant difference between primary suturing and the use of Dacron patch (11). Du, et al in a study to compare the outcome of surgical and transcatheter closure, reported no large or moderate residual shunt in the surgical group. Mean ASD size was 14.2 ± 6.3 millimetre in their study (8). Our study similarly showed no residual shunts in either of cases despite the fact that our patients had larger ASDs and unsuitable anatomy. In accordance with previous publications, no deaths were reported during our study (8, 11). Gala et al, however, reported a single case of death secondary to sepsis (0.4% total mortality rate) (11). The complication rate in the Gala study was higher than other studies as only 21.2% of the children and 17.5% of the adults in that study experienced an operation with no complications. Du et al reported major complications in 5.2% of the cases that were mainly the result of large pericardial effusion with tamponade and minor complication in 17.8% of the cases in the surgical group. Pastorek et al reported major complications in 8.1% of the cases, and minor complications in 13.3% of the cases; all of them resolved without any intervention. 1.7% of them developed streptococcus viridans endocarditis that was treated with intravenous antibiotics. In our study, 4 patients (8.3 %) experienced major complications that led to prolonged hospital stay and need for further examination and treatment. Minor morbidities developed in 5 (10.4 %) patients. Two patients had small PE and one had trivial PE that resolved without surgical intervention. One patient had moderate size hematoma in front of RV which needed no treatment. A 29-year-old man (2%) had atrial flutter which was treated medically. No patient in our study had atrial arrhythmia before surgery. In a study by Gatzoulis, 5 patients (2.9%) developed atrial flutter or fibrillation in a short period after the surgery, while none of the studied 174 patients had atrial arrhythmia before the operation (12). Gatzoulis found a direct relation between the age of the patients and the development of atrial arrhythmias; this comes while atrial flutter in our study only occurred in one young patient.

Limitations

We had a limited number of cases and we did not have a control group.

Conclusions

This experience showed that the present method might be used efficiently for ASD repair especially in patients with difficult anatomical ASD variants.

Authors' contributions

FK, suggested the idea, and prepared the patients and participate in preparing manuscript, MHA prepared the patients and participate in preparing manuscript, SA gathered data, prepared the firs draft, revised the final article.

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References

1. *Bailey C, Nichols H, Bolton H, Jamison W, Gomez-Almeida M. Surgical treatment of forty-six interatrial septal defects by atrio-septo-pexy. Annals of Surgery. 1954; 140(6): 805.*

2. *Masura J, Gavora P, Formanek A, Hijazi ZM. Transcatheter closure of secundum atrial septal defects using the new self-centering Amplatzer septal occluder: initial human experience. Catheterization and cardiovascular diagnosis. 1997; 42(4): 388-93.*

3. *Carminati M, Chessa M, Butera G, Bini RM, Giusti S, Festa P, et al. Transcatheter Closure of Atrial Septal Defects with the STARFlex Device: Early Results and Follow-Up. Journal of interventional cardiology. 2001; 14(3): 319-24.*

4. *Rao PS, Sideris EB, Haddad J, Rey C, Hausdorf G, Wilson AD, et al. Transcatheter occlusion of patent ductus arteriosus with adjustable buttoned device. Initial clinical experience. Circulation. 1993; 88(3): 1119-26.*

5. *Das GS, Voss G, Jarvis G, Wyche K, Gunther R, Wilson RF. Experimental atrial septal defect closure with a new, transcatheter, self-centering device. Circulation. 1993; 88(4): 1754-64.*

6. *Rome J, Keane J, Perry S, Spevak P, Lock J. Double-umbrella closure of atrial defects. Initial clinical applications. Circulation. 1990; 82(3): 751-8.*

7. *Pedra C, Pihkala J, Lee K, Boutin C, Nykanen D, McLaughlin P, et al. Transcatheter closure of atrial septal defects using the Cardio-Seal implant. Heart. 2000; 84(3): 320-6.*

8. *Du ZD, Hijazi ZM, Kleinman CS, Silverman NH, Larntz K. Comparison between transcatheter and surgical closure of secundum atrial septal defect in children and adults: results of a multicenter nonrandomized trial. Journal of the American College of Cardiology. 2002; 39(11): 1836.*

9. *Hughes M, Maskell G, Goh T, Wilkinson J. Prospective comparison of costs and short term health outcomes of surgical versus device closure of atrial septal defect in children. Heart. 2002; 88(1): 67-70.*

10. *Galal M, Wobst A, Halees Z, Hatle L, Schmaltz A, Khougeer F, et al. Peri-operative complications following surgical closure of atrial septal defect type II in 232 patients—a baseline study. European heart journal. 1994; 15(10): 1381-4.*

11. *Pastorek JS, Allen HD, Davis JT. Current outcomes of surgical closure of secundum atrial septal defect. The American journal of cardiology. 1994; 74(1): 75-7.*

12. *Gatzoulis MA, Freeman MA, Siu SC, Webb GD, Harris L. Atrial arrhythmia after surgical closure of atrial septal defects in adults. New England Journal of Medicine. 1999; 340(11): 839-46.*

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The susceptibility to staining of orthodontic elastic ligatures caused by food colorants – In vitro study

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Abstract

Introduction: For adolescent patients face aesthetic plays an important role during orthodontic treatment. This population presents a higher expectations regarding the appearance of elements of fixed appliances including elastic ligatures.

Objectives: The aim of the study was an assessment if food dyes affect the color of orthodontic elastic ligatures.

Material and methods: The elastics of four brands: Ortho Organizers, Dentaurum, Ortho Technology and Dentech Corp. were investigated. The 40 samples of the each producer were divided according to the four colors; transparent, white, yellow and silver. The ligatures were stored in solution of food colorant for twenty four hours. The color change for each sample was analyzed by the use of Spectroshade (MHT) spectrophotometer. The complete color change (ΔE) of ligatures was analyzed statistically on the level of p=0,05.

Results: The greatest color change (ΔE) was observed for Ortho Organizers elastics at the mean level of 16,20 points and the lowest for Ortho Technology elastics at the mean level of 12,19 points. No significant differences among the manufacturers were observed. The significant $p \leq 0,05$ color changes were observed in relation to original color of elastics. The highest color change was observed for white at the mean level of 21,24 points and the lowest for silver products at the level of 4,91 points.

Conclusions: The susceptibility to discoloration of elastic ligatures does not depend on producer's brand.

The level of discoloration of orthodontic elastic by food dyes is strongly related to the original color of the material.

Key words: Elastic ligatures, discoloration, orthodontic treatment.

Introduction

Orthodontic treatment with fixed appliances is becoming increasingly common. Corrections of occlusion are performed not only in children and adolescents; also adults choose this form of treatment more and more frequently. For patients who are socially and professionally active, face aesthetic plays an important role also during orthodontic treatment. For this reason there are higher expectations regarding the appearance of elements of fixed appliances and their color, which should match natural dentition. In order to fulfill these requirements, producers of dental materials have expanded their offer with elastic orthodontic brackets, whose color is more similar to natural dentition in contrast with traditional metal brackets. On the market there are also orthodontic arch wires coated with plastics, whose color is a better match for hard tissues of teeth. The purpose of all this is to decrease the visibility of orthodontic appliances within patient's oral cavity.

Treatment with orthodontic appliances takes on average about 24 months, and its extent depends, among other things, on: the degree of occlusal disorders, patient's cooperation and applied method of treatment. Lingual techniques fulfill the aesthetic criteria regarding the appearance of dentition during orthodontic treatment, however they have limitations resulting from a lower effectiveness of such appliances, harder working conditions for dentists, extended time or increased cost of treatment[1].

Most dentists using fixed appliances in their practice, implement labial techniques, which means that elements of appliances, i.e. brackets and arch wires are positioned on labial surfaces of teeth. In order to maintain the arch wire in a bracket's groove, clinicists may apply steel wire ligatures 0.008 or 0.014 inches in diameter[2], elastic ligatures or increasingly more popular self-ligating systems.

Elastic ligatures, which are commonly used in orthodontic treatment with fixed appliances, are made

of elastic polymers usually based on polyurethane. Their exact composition is kept secret by manufacturers. The advantages of elastic ligatures include: ease of fixing and removing from brackets, no irritation of oral mucosal membrane, availability in a large selection of colors. Among the disadvantages are: favoring of dental plaque accumulation, often insufficient maintenance of the arch in the bracket's groove, adsorption of water resulting in change of their physical properties within the oral cavity. Despite the fact that elastic ligatures are not perfect materials, they ensure ease of application, which is why they are preferred by dentists. Young patients readily choose colored elastic ligatures, enabling to individualize the appearance of an orthodontic application thanks to their diverse colors. Adult patients prefer transparent or lightly colored elastomers[3].

Clinical observations indicate that elastomers used in orthodontics do not preserve their original color in clinical conditions. In the water environment of the oral cavity they are exposed to temperature changes within the range from around 0 to 70 degrees Celsius [4]. Food colorants which are present in meals and drinks deposit on elastic ligature surfaces and are absorbed inside the materials due to their porous structure. The color change of orthodontic elastomers in oral cavity is particularly visible in case of transparent and light materials. Therefore it seems important to determine, which elastic ligatures discolor to a higher degree than others, in order to treat patients with materials characterized by relatively stable color.

The discoloration of orthodontic ligatures in clinical conditions during one-month period is presented at Figure 1.

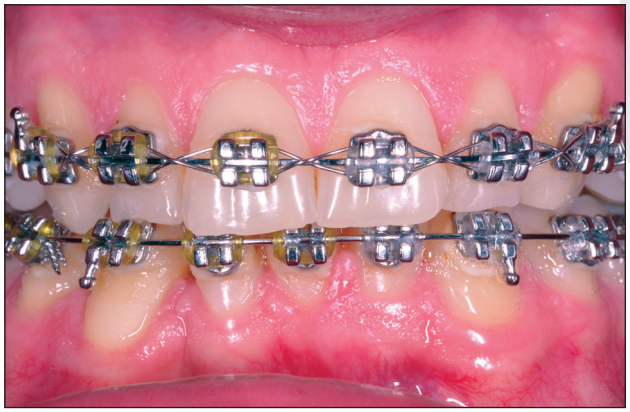

Figure 1. The discoloration of orthodontic ligatures in clinical conditions during one-month period

Objectives

The aim of the study was to determine the impact of food dyes on color change of elastic ligatures in laboratory conditions, depending on the manufacturer and the initial color of elastomers.

Materials and methods

The study included elastic ligatures from four manufacturers: Ortho Organizers (USA), Dentaurum (Germany), Ortho Technology (USA) and Dentech Corp. (South Korea). Materials from each of the above mentioned producers were used to prepare 40 samples 3x3 mm, which were then divided into 4 groups of 10 samples each according to the color of materials offered as: transparent, white, yellow and silver. The initial color of each sample was determined by colorimetric analysis with the use of Spectroshade dental spectrophotometer (MHT, Italy) shown in Figure 2. The 160 studied samples were placed in 5 ml individual silicon containers. Five samples from each group (producer/color) were kept in coffee solution or red wine solution for 24 hours. The samples were stored in an incubator at 37ºC. After incubation the studied materials were removed from containers with solutions of food colorants and rinsed under running water for one minute. Then the elastomers were dried and subjected to

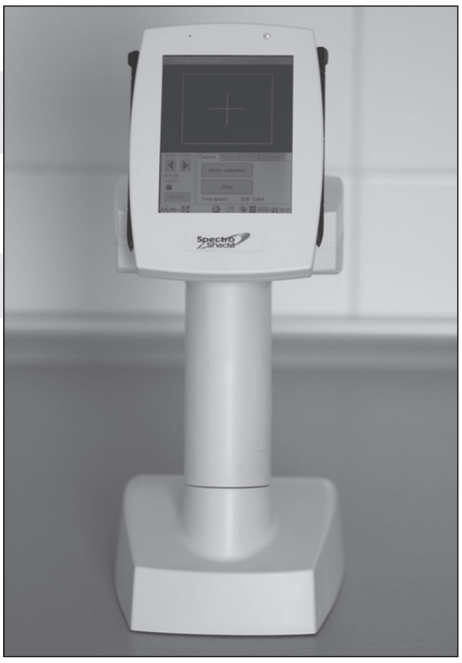

Figure 2. The Spectroshade - dental spectrophotometer

another colorimetric analysis by spectrophotometer. The numeric L*a*b* scale was used to assess the brightness change (ΔL*), green/red saturation (Δa*), blue/yellow saturation (Δb*), and a total color change of samples (ΔE*). The color change was assessed with regard to elastomer's producer, initial color, and applied food colorant. A statistical analysis was performed at the significance level of p≤0.05, with the use of Statictica 6.0 package, as well as the following tests: LSD (least significant differences), Bonferroni and Tukey.

Results

Mean change in brightness of ligatures exposed to food colorants (ΔL)*

Mean decrease in brightness of ligatures exposed to coffee or wine was 5.98 points (p≤0.05). The highest decrease in brightness of 8.88 points on average was observed in elastomers produced by Ortho Organizers, whereas the lowest decrease of 4.28 points on average was observed in products by Dentaurum. The differences were not statistically significant with reference to ligatures' producer.

Storage in coffee solution caused a decrease in brightness of studied ligatures by 3.92 points on average, whereas wine caused a significantly higher (p≤0.05) mean decrease of ligature brightness by 8.04 points.

The most significant (p≤0.05) decrease in brightness was observed in white ligatures (-11.62 points), whereas in case of silver ligatures the brightness increased by 0.64 points. Transparent ligatures changed their brightness by -6.84 points, yellow ligatures by -7.50 points. The difference between the above mentioned color groups was not statistically significant.

Mean change in saturation of red in ligatures exposed to food dyes (Δa)*

Mean increase in red saturation of ligatures subjected to colorimetric tests before and after incubation in food dye solutions was 1.54 points and was not statistically significant.

The highest discoloration within this color range was in products made by Ortho Organizers (increase by 2.60 points), whereas the lowest was in elastomers produced by Ortho Technology (increase by 0.72 points). The observed changes within the red color were not statistically significant depending on manufacturer.

Wine caused a significantly (p≤0.05) higher increase in red saturation (by 2.39 points) than coffee (by 0.56 points).

The highest (by 4.64 points) increase in red saturation was observed in white ligatures, whereas in silver elastomers a decrease in red intensity was observed by 0.46 points. The difference between individual color groups was statistically significant (p≤0.05).

Mean change in saturation of yellow in ligatures exposed to food dyes (Δb)*

Mean increase in yellow saturation of elastic ligatures after incubation in coffee solution or in wine was 6.35 points (p≤0.05).

The highest change within this color range was observed in products made by Dentech (mean increase by 6.47 points), whereas the lowest was in ligatures produced by Dentaurum (mean increase by 3.57 points). No statistically significant difference was observed between products of particular manufacturers.

A significantly higher (p≤0.05) change in yellow saturation was caused by coffee (mean increase by 10.61 points) than by wine (mean increase by 0.43 points).

The highest susceptibility to color change within the yellow color range was observed in transparent ligatures (mean increase by 15.38 points), the lowest susceptibility was observed in silver ligatures (mean increase by 2.34 points).

Mean global change in color of ligatures exposed to food dyes (ΔE)*

Mean global change in color of elastic ligatures exposed to food colorants in the conditions of this study was 13.22 and was statistically significant at the level of p≤0.05.

The color of elastomers produced by Ortho Organizers changed on average by 16.20 points, products of Dentaurum indicated a change of 10.66 points, ligatures from Dentech changed by 13.17 points, and from Ortho Technology by 12.19 points. During the analysis of mean color change at p≤0.05 significance level, no significant differences in susceptibility to discoloration were observed in products of individual manufacturers.

Also, no significant difference was observed between applied food colorants with regard to their impact on color change of studied elastomers. Coffee changed the color of ligatures on average by 14.41 points, whereas wine by 12.03 points.

A significant ($p \leq 0.05$) change in susceptibility to discoloration was observed with reference to the initial color of elastic ligatures. The highest level of color change was observed in white elastomers, whose discoloration was on average 20.70 points, whereas the lowest level was noted in the case of silver ligatures, whose color change equaled 4.91 points. Transparent ligatures changed color to almost the same degree as white ones (change by 17.93 points). No differences were observed between the mentioned groups in susceptibility to discoloration caused by food dyes. Yellow ligatures changed color on average by 11.19 points and their susceptibility to discoloration was at a middle level ($p \leq 0.05$) with reference to white/transparent and silver elastomers.

Mean color changes of elastic ligatures with reference to the manufacturer, applied food dye and ligature color are presented in Table 1.

Discussion

Colorimetric assessment performed with the use of digital optical devices enables an objective and repetitive analysis of color change of dental materials both in oral cavity environment and in laboratory conditions[5,6,7]. The method of spectral assessment, which was applied in this study, allows a numeric determination of color change in dental materials through ascribing certain numeric values to color sensation which is individual for each observer. Color assessment by means of attributed numeric values within a defined scale enables a precise calculation of its changes resulting from environmental conditions.

The analysis of the results in this study indicated that ligatures' susceptibility to discoloration has no significant dependence on their manufacturer. For clinicists it means freedom in the choice of elastomer provider in the context of color stability.

Red wine caused a significantly ($p \leq 0.05$) higher changes in brightness and saturation of the red color in incubated elastic ligatures compared to coffee. However, coffee solution changed the color of studied elastomers within the yellow range to a higher degree than wine. The differences in the impact of food dyes on the red and yellow colors seem justified by the colors of drinks applied in the study. However, it should be noted that both liquids used in the experiment caused a comparable global color change of ligatures on average by 15.08 points for coffee solution and by 12.56 points for red wine.

Color changes of dental materials applied in oral cavity are commonly recognized as noticeable if their values exceed 3 points[8,9,10] in the L*a*b* scale. The results of this study indicate

Table 1. Mean color change of elastic ligatures in relation to producer, kind of used food colorant and basic color of the elastics

Mean color change of elastic ligatures in relation to producer, kind of used food colorant and basic color of the elastics								
	ΔL		**Δa**		**Δb**		**ΔE**	
	avg	**std**	**avg**	**std**	**avg**	**std**	**avg**	**std**
total	**-5,98**	5,82	**1,47**	2,80	**5,52**	11,24	**13,22**	7,82
Ortho Organizers	**-8,88**	6,20	**2,60**	3,65	**5,43**	12,86	**16,20**	8,12
Dentaurum	**-4,28**	4,84	**0,79**	2,50	**3,57**	10,11	**10,66**	6,86
Ortho Technology	**-5,19**	3,40	**0,72**	1,89	**6,12**	11,73	**12,19**	8,18
Dentech	**-5,14**	7,06	**1,61**	2,48	**6,47**	9,99	**13,17**	7,11
cafe	**-3,92**	4,14	**0,56**	2,64	**10,61**	11,71	**14,41**	8,98
wine	**-8,04**	6,53	**2,39**	2,67	**0,43**	8,06	**12,03**	6,29
transparent	**-6,84**	3,97	**0,14**	2,80	**15,38**	7,10	**17,93**	5,90
white	**-11,62**	4,37	**4,64**	2,75	**13,99**	9,31	**20,70**	5,88
yellow	**-7,50**	3,41	**2,37**	1,22	**-7,52**	4,82	**11,19**	5,41
silver	**0,64**	3,94	**-0,46**	0,90	**2,34**	2,28	**4,91**	1,75

that applied food colorants caused color changes in elastomers to the degree which greatly exceeds the limits of aesthetic acceptability. In the experimental conditions a mean color change of more than 3 points in the L*a*b* scale was observed regardless of the initial color of elastomers. However, it should be noted that white and transparent ligatures discolored about 4 times more intensively than silver ones. It seems that this information should encourage orthodontists to apply elastic ligatures with initially darker and more intensive colors in patients whose diet includes large quantities of stimulants, in order to avoid a significant discoloration of elastomers during treatment.

A high susceptibility of elastic ligatures to color change resulting from contact with dyes included in drinks and food was observed by Ardeshna i Vaidyanathan[11]. The authors analyzed elastic ligatures' color change with the use of a Minolta colorimeter, and indicated that transparent elastomers in contact with food dyes change color to a significantly higher degree than initially dark materials. Observations of the authors from the USA quoted above concur with the results obtained in this study.

Color changes of transparent elastic ligatures in experimental conditions were also observed by Kim and Lee [12]. The Korean authors stored 3 different kinds of transparent elastomers in water solution of ethanol, and water solution of methylene blue for a period from 1 to 120 hours. Depending on ligature type, incubation time and environment, they observed color changes of elastomers in the range from 0.6 to 30 points in the L*a*b* scale. The colorimetric analysis was performed with the use of a Nikon D50 digital camera and Adobe Photoshop (ver. 7.0) software package. Unfortunately, the methodology of colorimetric assessment used by the authors Kim and Lee[12], as well as solutions in which they kept elastomers were different from methodology and solutions applied in this research. Therefore any comparison between the two studies is impossible.

Available literature reports about color change of various types of dental materials caused by food dyes present in oral cavity environment, and about a correlation between the type of dye and the degree of material's discoloration. Most frequently they refer to composite materials used for fillings and for making orthodontic brackets[13-17]. There are few reports about the degree of discoloration in elastomers used during orthodontic treatment. We are aware that elastic ligatures change color in the environment of the oral cavity, which is confirmed by observations by both dentists and patients, however our knowledge on the subject is more intuitive than based on proofs. Research on susceptibility of orthodontic elastomers to color change, which is performed by means of objective methods of colorimetric assessment, will allow clinicists to choose materials fulfilling aesthetic demands of patients not only at the moment of application, but also during treatment. Although *in vitro* studies do not reflect the complexity of oral cavity's environment, they significantly help in assessment of dental materials.

Conclusions

In the conditions of the present study, the degree of elastic ligatures' color change caused by the presence of food dyes depends on the initial color of assessed elastomer.

The method of colorimetric assessment by spectral analysis enables an objective assessment of dental materials' color change

References

1. *Russel JS. Current products and practice aesthetic orthodontic brackets. J Orthod. 2005; 32: 146-163.*
2. *Elegdak – Turk S, Ozkalayci N, Isci D, Turk T. Color preferences of patients receiving elastic ligatures. Eur J Dent. 2010; 4: 171-174.*
3. *Taloumis LJ, Smith TM, Hondrum SO, Lorton L. Force decay and deformation of orthodontic elastomeric ligatures. Am J Orthod Dentofacial Orthop. 1997; 111(1): 1-11.*
4. *Barclay CW, Spence D, Laird WRE. Intra-oral temperatures during function. J Oral Rehab. 2005; 12(32): 886-94.*
5. *Cal E, Güneri P, Kose T. Comparison of digital and spectrophotometric measurements of colour shade guides. J Oral Rehab. 2006; 33: 221-228.*
6. *Karamouzos A, Papadopoulos MA, Kolokhitkas G, Athanasiou AE. Precision of on vivo spectrophotometric colour evaluation of natural teeth. J Oral Rehab. 2007; 34: 613-621.*

7. *Braun A, Jepsen S, Krause F. Spectrophotometric and visual evaluation of vital tooth bleaching employing different carbamide peroxide concentrations. Dent Mater. 2007; 23: 165-169.*

8. *Stober T, Gilde H, Lenz P. Color stability of highly filled composite resin materials for facings. Dent Mater. 2001; 17: 87-94.*

9. *Ruyter IE, Nilner K, Moller B. Color stability of dental composite resin materials for crown and bridge veneers. Dent Mater. 1987; 3: 246-251.*

10. *Johnson WM, Kao EC. Assessment of appearance match by visual observation and clinical colourimetry. J Dental Res. 1989; 68: 819-22.*

11. *Ardeshna AP, Vaidyanathan TK. Colour changes of orthodontic elastomeric module material exposed to in vitro dietary media. J Orthod. 2009; 36(3): 17-85.*

12. *Kim SH, Lee YK. Measurement of discolouration of orthodontic elastomeric module with a digital camera. Eur J Orthod. 2009; 31: 556-562.*

13. *Lee Y-K. Changes in the reflected and transmitted color of esthetic brackets after thermal cycling. Am J Orthod Dentofac Orthop. 2008; 133: 641.e1-641.e6.*

14. *Guler AU, Yilmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. J Prosthet Dent. 2005; 94: 118-124.*

15. *Patel SB, Gordan VV, Barrett AA, Shen Ch. The effect of surface finishing and storage solutions on the color stability of resin-based composites. J Am Dent Assoc. 2004; 35: 587-594.*

16. *Faltermeier A, Behr M, Müssig D. In vitro color stability of aesthetic brackets. Eur J Orthod. 2007; 29: 354-358.*

17. *Eldiwany M, Friedl KH, Powers J M. Color stability of light-cured and post-cured composites. Am J Dent. 1995; 8: 179-181.*

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Expression, purification and bioactivity analysis of the human antimicrobial peptide LL-37 in *Escherichia coli*

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Abstract

The cathelicidin derived LL-37 has a broad spectrum of antimicrobial and immunomodulatory activities. Here, a DNA sequence encoding the peptide which was fused to the well characterized 384bp fusion partner was cloned into the pUC18 vector and transformed into Escherichia coli. JM109. After high density fermentation of engineering bacteria, cell disruption, fusion protein isolation and precise chemical cleavage, HPLC purification and lyophilization, the target peptide LL-37 was purified. The antimicrobial bioactivity of LL-37 in vitro was evaluated by bacteria (Escherichia coli., Streptococcus Group B and staphylococcus aureus) incubation and showed high ability in sterilization. Acute toxicity test showed high safety. Together, our analysis indicated that LL-37 is an effective and safe antimicrobial peptide, which can be successfully produced at high levels in the Escherichia coli. All these showed the LL-37 acts as a good potential antimicrobial candidate for clinical applications.

Key words: LL-37, antimicrobial bioactivity, peptide.

Introduction

The increasing resistance of microorganisms toward common antibiotics that have provided safety for the last half a century has become a growing threat for the public health. Antimicrobial peptides(AMPs) play important roles in innate immune defense.[1]More than 1400 AMPs have been identified from the organisms during the past 30 years, among which more than 200 were obtained from frogs skin.[2, 3] As a potential therapeutic agent, antimicrobial peptides have received increased attention in recent years.[4]

LL-37 is the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP18), which is mainly expressed by neutrophils and epithelial cells.[5] LL-37,a 4.5 *kDa*, amphipathic α-helical peptide,[6] is a multifunctional host defense molecule that may mediate various host responses, including bactericidal action, epithelial cell activation, epithelial wound regeneration and activation of chemokine secretion[5, 7, 8]. Various mechanisms have been proposed for the way involved in its bacterial killing. These included the formation of phase separation due to specific peptide–lipid interactions[9, 10], discrete channels that dissipate ion gradients across the membrane[9, 11, 12], detergent-like solubilization of the membrane[13] and disturbance of the lipid bilayer as a result of a carpet or a toroidal-type action[14, 15]. The bacterial membrane is one of the key components for the cell, and the development of bacterial resistance to a membrane-active peptide appears to be very rare[16], therefore AMPs are potentially an effective way to solve the drug-resistance problem of bacteria. LL-37 can also prevent immunostimulatory effects of bacterial wall molecules such as lipopolysaccharide and reduce its lethality in murine models of endotoxemia. Additional reported activities include inhibition of neutrophil apoptosis, NK cell antitumor function and stimulation of angiogenesis, cytokine release (e.g. IL-8), inducing mast cells and monocytes, enableing TLR3 to respond to viral dsRNA and stimulate angiogenesis both in vitro and in vivo.[4, 7, 17-19]

LL-37 is the only human antimicrobial peptide in the cathelicidin was family and it shows a broad spectrum of antimicrobial activities. It is of interest to develop this peptide for pharmaceutical applications. Obtained by a solid phase chemical synthesis, LL-37 should be very expensive. On the other hand,

recombinant methods permit the production of peptides and proteins in microorganisms. Recombinant expression of LL-37 may be cost-effective. However, high-level expression of small peptides with antimicrobial activity is a challenge.[17,20] *Escherichia coli (E. coli)* is the most commonly used host cell because of easy culture, fast growth, and well-established alternative expression systems. There are two major problems in the expression of antimicrobial peptides: the expressed peptides are toxic to bacteria and the high content of positive-charged amino acid residues makes them very sensitive to protease, which are abundant in *E.coli.* cells[21]. Furthermore the potential intrinsic antimicrobial activity of antimicrobial peptides to the host bacteria becomes a question during heterologous expression and then restrain the fermentation.[18, 19] Our strategy is to connect the peptide sequence to well characterized fusion partners(molecular weight: 16k*Da*, designed by our laboratory). After specifically cleaving the fusion protein by cyanogen bromide[22, 23], high yield of recombinant LL-37 was generated. The bioactivity of purified recombinant LL-37 to the common bacteria *(E.Coli, Streptococcus Group B* and *staphylococcus aureus)* was evaluated in vitro.

Materials and methods

The sequence of LL-37

The sequence(GenBank Accession No. Z38026) was as follow, and the C-terminus of the peptide was serine-OH. Leu-Leu-Gly-Asp-Phe-Phe-Arg-Lys-Ser-Lys-Glu-Lys-Ile-Gly-Lys-Glu-Phe-Lys-Arg-Ile-Val-Gln-Arg-Ile-Lys-Asp-Phe-Leu-Arg-Asn-Leu-Val-Pro-Arg-Thr-Glu-Ser-OH

Strains and plasmids

The *E. coli* strain *DH5α* (Invitrogen, USA) was used as host strain for expression of LL-37. The *E. coli* strain *JM109* (Invitrogen, USA) was used for expression of fusion protein. The plasmid *pUC18* (Invitrogen, USA) was used to prepare the gene LL-37 and express the synthesized gene. The well characterized fusion partner was a 128 amino acids peptide (molecular weight: 16k*Da*) designed by our laboratory. Freezer stocks for long-term storage of expression strains are made by adding 0.1ml of 100% (w/w) glycerol to 1 ml of culture in log phase or grown to saturation in non-inducing media, mixing well and placing at -70 °C. Subcultures for working stocks are made by scraping up a small amount of frozen culture with a sterile plastic pipettor tip without melting the rest of the stock and inoculating into non-inducing media. After growth to saturation, working stocks are typically stable for weeks in refrigerator.

Cyanogen bromide, chemical reagents and Growth media

The cyanogen bromide was white crystals (97%purity, Sigma). The culture media LB was prepared as described[24].Yeast extract and polypeptone were obtained from OXOID. All other chemical reagents were made in China with the highest purity of commercially available.

Construction of the expression vector

The DNA sequence expressing LL-37 was designed with *BamH* Ⅰ and a cyanogen bromide site (Methionine) at the N-terminus and the termination codon of *E. coli* and *Sal* Ⅰ site at the C-terminus. The oligonucleotide designed was prepared with five PCR cycles by using the following primers:

Primer 1
ccaaatGGATCCTGCATGCTGCTGGG;
Primer 2 CTTTTACGGAA
GAAATCACCCAGCAGCATGC;
Primer 3 TGATTTCTTCCGTAAAAGCAAA
GAAAAAATCGGTAAAGAA;
Primer 4 CTGAACGATACGTTT
GAATTCTTTACCGATTTTTTCTTTG
Primer 5 TTCAAACGTATCGTTCAGCG
TATCAAAGATTTCCTGCGTA
Primer 6 CGGTACGCGGAACCAGGTTAC
GCAGGAAATCTTTGATACG
Primer 7 ACCTGGTTCCGCGTACC
GAAAGCTAGgtcgacccaaat
Primer 8 ATTTGGGTCGACCTAGCTTT

All primers were synthesized by ShineGene (Shanghai, PR China). The purified PCR product was digested with *Bam H* Ⅰ and *Sal* Ⅰ and ligated into the plasmid *pUC*18- FP plasmid(the recombinant plasmid of *pUC*18 and a fusion partner owned by our laboratory). The resultant recombinant plasmid is referred to M-LL-37/pUC18-FP. plasmid M-LL-37/pUC18-FP was transfor med into *E.coli* DH5α. The positive clone harboring the re-

combination plasmid was confirmed by PCR. The gene encoding LL-37 was confirmed with DNA sequencing by ShineGene. The recombinant plasmids confirmed were ligated and transformed into *E. coli strain JM109* for protein expression.

Expression of LL-37 fusion protein and SDS-PAGE analysis

The transformant was inoculated to LB medium containing 100μg/ml ampicillin. Cells grew at 37 °C with continuous shaking until the optical density at OD600 was 0.9.Then IPTG (isopropyl-beta-D-thiogalactopyranoside) was added to a final concentration of 1mM to initiate the fusion protein expression. Cells grew at 37 °C with continuous shaking for another 4h in the conical flasks before harvest. Before induction and after induction at 37 °C for 4 h in the conical flask, the bacteria were collected, lysed, and then boiled in SDS-PAGE sample buffer. The resulting products were separated by 15% SDS-PAGE.

Purification of the fusion protein

After induction at 37 °C for 4h in the conical flasks, cells were centrifuged for 30min at 5000 rpm. The supernatant was removed and the pallet containing cells were stored at -70 °C overnight. Then, these cells were resuspended in 8M urea and disrupted by homogenizer(IKA T25 Digital Ultra – Turrax, Germany) at 15000 rpm. After centrifuging at 15000rpm for 30min at 4 °C, the soluble fusion protein was in the supernatant. Then the supernatant was diluted at a ratio 1: 25 by dropwise addition to the refolding buffer (IM Tris-HCl, pH7.3, 0.25M sucrose, 2Mm Ethylenediaminetetraacetic acid) (EDTA) under permanent stirring, and finally kept at 20 °C for 24 h. After centrifuging at 15000rpm for 30min at 4 °C, washed five times with 1M Urea followed by 24hr lyophilization, the purified fusion protein was obtained.

Chemical proteolytic cleavage of the fusion protein by cyanogen bromide

Lyophilized fusion protein(30mg) was dissolved in 30ml 80% formic acid under continuous stirring and flushed with N_2 while incubated in room temperature for 20hr. Solutions of CNBr were prepared freshly prior to each experiment by dissolving the appropriate amount of solid in acetonitrile to a final concentration of 0.5g/ml. After solubilization, the fusion protein was incubated with 30mg CNBr (white crystals, a molar ratio of 100: 1 with respect to the Methionine content per molecule) at room temperature under darkness for 24 hr under continuous stirring in the chemical fume hood and followed by addition of 30 ml double distilled water to terminate the reaction. After dialysis(MW 2kDa) in double distilled water overnight for desalting, the solution was lyophilization.

Purification of peptide LL-37 by HPLC and lyophilization

The recombinant peptide LL-37 was further purified by RP-HPLC 1100 series(Agilent, USA) equipped with a C18 column (φ30 X 300mm, Agilent,USA). Processing at a linear gradient of 80 to 20% acetonitrile, containing 0.1% trifluoroacetic acid at 30 ml/min for 30min was performed. Each peak at 220nm was analyzed by small peptide electrophoresis. The samples of target peak were collected. After lyophilization, the recombinant peptide LL-37 was obtained and was identified by electrospray ionization mass spectrometry (ESI-MS)(LCQ Deka XP Plus, Thermo Fenian, USA).

Antimicrobal bioactivity of LL-37 in vitro

E.Coli. Streptococcus Group B and *staphylococcus aureus* were separated from clinic(Huashan hospital, Fudan University, Shanghai, China) (all multiple drug-resistant bacteria).The control bacteria(about 10^9/ml) had been in pre-mixed 37 °C saline water 3hr. The samples were LL-37 50μg/ml mixed with the bacteria in pre-mixed 37 °C saline water 3hr. Then both groups were applied to plates. The plates underwent 37 °C oven overnight and then count the bacterial colony. Bacterio-percentage was calculated and graphed.

Acute toxicity test

The animal experiment was performed according to the instructions of "The Animal Care and Welfare Committee of the Peking Union Medical College (Beijing, China)". Kungming mice (male) 8-week-old, around 25 g (SLAC laboratory animal, Shanghai, China) were used. The animals were housed in an air-conditioned room at 22 ± 2°C with a 12h light/12h dark cycle. Drinking water and a standard rodent maintenance diet were supplied.

Mice were then injected intraperitoneally with LL-37 via three different concentration group (0.1mg, 0.2mg and 1mg) or saline. Each group includes 3 mice. The living percentage was calculated.

Results

The design principle of recombinant LL-37

As there is no Methionine in the peptide sequence of LL-37, we designed the cyanogen bromide site (-Methionine -) at the N-terminus of LL-37 to connect to the fusion partner owned by our lab.

Construction of LL-37 gene

To avoid limiting the supply of the corresponding charged tRNA in the host cell, the gene was designed using the preferential codons of *E. coli*. The *BamH* I site at N-terminus was introduced for subsequent cloning. The termination codon at C-terminus was used for convenience of purification. *The Sal* I site at C-terminus was introduced for subsequent cloning. The constructed plasmid *pUC*18-*FP/M*--LL-37 was confirmed by PCR and DNA sequencing. Here was the DNA sequence of the gene encoding LL-37:

g a a t t c A G A T C T T G C A T G C T - G C T G G G T G A T T T C T T C C G T A - A A A G C A A A G A A A A A A T C G G T A - AAGAATTCAAACGTATCGTTCAGCGTAT-CAAAGATTTCCTGCGTAACCTGGTTCCGC-GTACCGAAAGCTGCATGGGATCCTAGgtcgac

Finally, the confirmed plasmids were used for the transformation of *E. coli* strain *JM109*.

Expression, cleavage and purification of recombinant LL-37 in shake flasks

The expression efficiency of plasmids was tested at a small scale using the transformed *E. coli* strain *JM*109 cell. Lysates of the inducted and non-inducted bacteria were analyzed by 15%SDS-PAGE (Figure 2). High expression was achieved in all cases and most of the fusion protein was soluble in 8M urea. Expression was carried out in 12 shaking flask cultures yielding 18g of wet bacterial biomass/L broth. The frozen cells were then used for extraction with 8M urea and the soluble inclusion bodies purified with refolding buffer. After centrifuged 30min at 15000rpm at 4 °C, washed five times with 1M Urea and then lyophilization, the purified fusion protein was obtained. Lyophilized fusion protein(30mg) was dissolved in 30ml 80% formic acid and flushed with N_2 for 20hr. Solutions of CNBr were prepared freshly by dissolving the appropriate amount of solid in acetonitrile to a final concentration of 5M. After solubilization, the fusion protein was incubated with 30mg CNBr at room temperature under darkness for 24 hr under continuous stirring and followed by addition of 30 ml double distilled water to terminate the reaction. After dialysis(MW 2kDa) against double distilled water overnight for desalting, the solution was lyophilization under vacuum. Then, the powder was further purified by RP-HPLC. After lyophilization, LL-37 was obtained. The purified LL-37 was identified by electrospray ionization mass spectrometry (ESI-MS)(LCQ Deka XP Plus, Thermo Fenian, USA).The peptide peak reflected a mean molecular mass of Da which was consistent with the calculated molecular weight(MW: 4492.7 *Da*). The purity of the peptides was > 95%, and the peptides (8-10 mg peptide per litre) in bacterial culture were obtained (peptide concentration depends on the fermentation conditions).

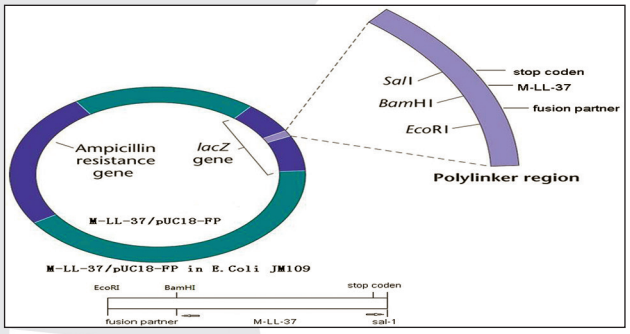

Figure 1. Schematic of pUC/LL-37 construction and transformation into E.Coli JM109

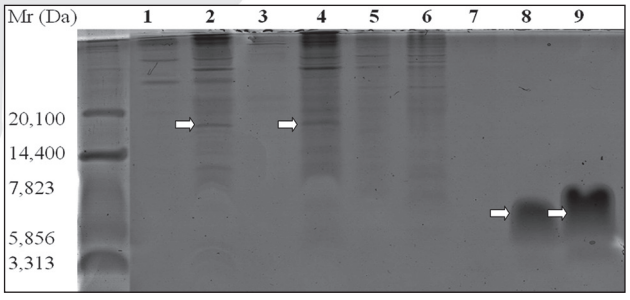

Figure 2. 4, centrifuged deposit after cell disruption; 5,supernatant after refolding; 6,last 1M urea wash; 7,blank; 8,target peptide after purification; 9,target peptide after purification

Table 1. Antimicrobial bioactivity (bacterial colony %)

	E. Coli	*Streptococcus Group B*	*Staphylococcus aureus*
Control	100%	100%	100%
LL-37(50μg/ml)	0	0	0

Bioactivity of LL-37 in vitro

The bacteria(concentration is A_{600nm}=1 O.D., then diluted 1: 10 , approximately 10^9/ml). were applied to plate after being in pre-mixed 37 °C saline water with 50μg/ml LL-37 for 3hr, the bacteria(*E. Coli,Streptococcus Group B* and *staphylococcus aureus*)were 100% killed (Table 1, Figure 3).

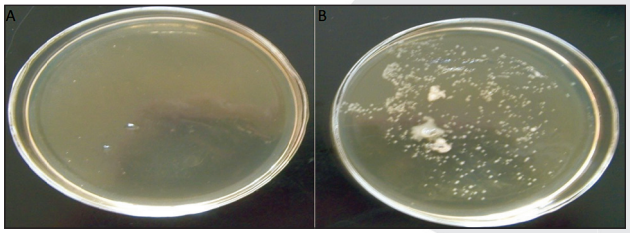

Figure 3. Antimicrobial bioactivity (LL37 50μg/ml, E.Coli. approximately 10^9/ml). A, E.Coli (LL37 50μg/ml); B, E.Coli (control)

Acute Toxicity Test

Kungming mice (male) were injected intraperitoneally with saline or LL-37 via three different concentration group (100μg, 200μg and 1000μg) (Table 2). All mice survived. It proved the safety of LL-37.

Table 2. Acute Toxicity Tests

		survival ratio
Saline	1ml	3/3
LL-37	100μg/ml	3/3
	200μg/ml	3/3
	1000μml	3/3

Discussion

Over the past three decades, antimicrobial peptides (AMPs) have drawn significant attention and some peptides have been prepared successfully by recombinant DNA methods.[18, 19] To date, a few groups have reported the recombinant expression of LL-37 either in the *E. coli* expression system[20, 25-27] or in the Pichia pastoris system.[28] As previous shows *E.Coli* is the most commonly used host cell because of its easy culture, fast growth, and well-established alternative expression systems.[29] For the expressed LL-37 in cells is toxic to bacteria, some people have used Pichia Pastoris yeast in fermentation process.[28] The whole fermentation process took five days, and it was difficult to get the target small peptide in the fermentation solution. Currently, the inclusion body with insolubility was formed to avoid the toxicity and degradation by the genetic engineering bacteria. First, the carrier protein should have both low molecular weight and high expression level. Second, the cleavage of the fusion protein is not so easy. The enzmatic cleavage by thrombin may have wrong incisor point and final peptide was not the same as the target peptide, but GSLL-39(a 39 amino acids, LL-37 analogue).[20]The chemical cleavage by formic acid may have wrong incisor point and final peptide was P-LL-37(a 38 amino acids' LL-37 analogue).[27] As there is no methionine in the peptide sequence of LL-37, we used cyanogen bromide to cleave the methionine in the fusion protein at the N-terminus of LL-37.It is confused that cyanogen bromide should be used to cleave the methionine at the N- terminus of LL-37 but not C-terminus of LL-37 which would produce homoserly or homoserly lactone. It was decided that cyanogen bromide would do better in 80% methanol than 0.2N HCl/8M Urea or 50% trifluoroacetic acid. In order to improve the yield, the cleavage could last for 1-3 days. The reaction was terminated by addition of 30 ml double distilled water. After dialysis and lyophilization, cyanogen bromide was removed. A C18 RP-HPLC was used to obtain the highly pure recombinant LL-37.The purity of the peptides was > 95%, and yields between 8-10 mg peptide/litre bacterial cultures were obtained depending on the fermentation conditions. The downstream of LL-37 was simple without column, so the yields were relatively high.

It was determined in bacterio-experiment that LL-37 should be kept in pre-mixed 37 °C with bacterials for 3hr and then applied plate. The common bacterials (*E.Coli,Streptococcus Group B* and *staphylococcus aureus*) were 100% killed in the sample group (LL-37 50μg/ml mixed with the

bacteria). Most serious infection in clinic are combined or multiple-drug resistant bacteria. To be a superspectrum germicidal agent, LL-37 is suitable. The final metabolic products of LL-37 are amino acids. Acute toxicity tests proved the safety of LL-37. So, LL-37 may be a potential agent in the infection treatment.

Whether human antimicrobial peptide LL-37 can be produced by genetic engineering at a large scale is the key point to be the substance for clinical application.

To increase the expression and avoid cytotoxic effects of antimicrobial peptide LL-37 in the *E. coli* host strain, target peptides were usually fused to cellulose-binding domains or GST,[26] resulting in low yield of target peptide and complicated downstream purification. If the copies of LL-37 gene were linked in tandem with methionine, only 1/n of tandem copies were target peptide while (n-1)/n were peptidyl homoserine lactone in C-terminal after chemical cleavage by cyanogen bromide. Antimicrobial peptide LL-37 was fused to a MW16*kD* carrier protein owned by our laboratory which was neutral efficient system for expression and purification in the *E. coli* host strain. The yield of target peptide was high because of low Molecular Weight of carrier protein. After LL-37 was usually fused to the carrier protein and expressed in *E. coli.,* inclusion bodies were formed without cytotoxic effects to host cells. So the carrier protein may work without neutralization of the positive charges of LL-37 by fusing to an acidic peptide to avoid the lethal effect of the expressed antimicrobial peptide on the host cells.[19]

*pUC*18 was usually applied in cloning vectors but seldom acted as expression vector. *pUC18* was applied to be the expression vector in the *E. coli* host strain to meet the scale-up production according to our experience.[30]

The solubility of the fusion protein was important in chemical efficient cleavage of cyanogen bromide. The reaction was ordinary in 70% formic acid solution or 0.2N HCl/6M guanidine hydrochloride.[23] But the soluble inclusion bodies were better digested with 5M CNBr in acetonitrile in 80% formic acid solution. The reaction may last 1 to 3 days for better yield.

In conclusion, we have developed a useful bacterial expression system to generate biologically active LL-37. This expression system with an cyanogen bromide cleavage site is attractive to be harnessed at a large scale.

In the near future, the availability of purified LL-37 could enable the clinical, structural, biochemical, and biological investigations.

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References

1. *Hurtado P, Peh CA. LL-37 promotes rapid sensing of CpG oligodeoxynucleotides by B lymphocytes and plasmacytoid dendritic cells. J Immunol 2010; 184(3): 1425-35.*

2. *Zasloff M. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci U S A 1987; 84(15): 5449-53.*

3. *Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415(6870): 389-95.*

4. *Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, et al. Antimicrobial natural products: an update on future antibiotic drug candidates. Nat Prod Rep 2010; 27(2): 238-54.*

5. *Bucki R, Leszczyńska K, Namiot A, Sokołowski W. Cathelicidin LL-37: a multitask antimicrobial peptide. Arch Immunol Ther Exp (Warsz) 2010; 58(1): 15-25.*

6. *Bals R, Wilson JM. Cathelicidins--a family of multifunctional antimicrobial peptides. Cell Mol Life Sci 2003; 60(4): 711-20.*

7. *Arrighi RB, Nakamura C, Miyake J, Hurd H, Burgess JG. Design and activity of antimicrobial peptides against sporogonic-stage parasites causing murine malarias. Antimicrob Agents Chemother 2002; 46(7): 2104-10.*

8. *Mendez-Samperio P. The human cathelicidin hCAP18/LL-37: a multifunctional peptide involved in mycobacterial infections. Peptides 2010; 31(9): 1791-8.*

9. Münster C, Spaar A, Bechinger B, Salditt T. Magainin 2 in phospholipid bilayers: peptide orientation and lipid chain ordering studied by X-ray diffraction. Biochim Biophys Acta 2002; 1562(1-2): 37-44.

10. Dathe M, Schumann M, Wieprecht T, et al. Peptide helicity and membrane surface charge modulate the balance of electrostatic and hydrophobic interactions with lipid bilayers and biological membranes. Biochemistry 1996; 35(38): 12612-22.

11. Matsuzaki K. Magainins as paradigm for the mode of action of pore forming polypeptides. Biochim Biophys Acta 1998; 1376(3): 391-400.

12. Ladokhin AS, Selsted ME, White SH. Sizing membrane pores in lipid vesicles by leakage of co-encapsulated markers: pore formation by melittin. Biophys J 1997; 72(4): 1762-6.

13. Ladokhin AS, White SH. 'Detergent-like' permeabilization of anionic lipid vesicles by melittin. Biochim Biophys Acta 2001; 1514(2): 253-60.

14. Chen Z, Wang D, Cong Y, et al. Recombinant antimicrobial peptide hPAB-beta expressed in Pichia pastoris, a potential agent active against methicillin-resistant Staphylococcus aureus. Appl Microbiol Biotechnol 2011; 89(2): 281-91.

15. Ramamoorthy A, Thennarasu S, Lee DK, Tan A, Maloy L. Solid-state NMR investigation of the membrane-disrupting mechanism of antimicrobial peptides MSI-78 and MSI-594 derived from magainin 2 and melittin. Biophys J 2006; 91(1): 206-16.

16. Hancock RE. Concerns regarding resistance to self-proteins. Microbiology 2003; 149(Pt 12): 3343-4; discussion 3344-5.

17. Büchau AS, Morizane S, Trowbridge J, Schauber J, Kotol P, Bui JD, et al. The host defense peptide cathelicidin is required for NK cell-mediated suppression of tumor growth. J Immunol 2010; 184(1): 369-78.

18. Lee JH, Kim JH, Hwang SW, Lee WJ, Yoon HK, Lee HS, et al. High-level expression of antimicrobial peptide mediated by a fusion partner reinforcing formation of inclusion bodies. Biochem Biophys Res Commun 2000; 277(3): 575-80.

19. Lee JH, Minn I, Park CB, Kim SC. Acidic peptide-mediated expression of the antimicrobial peptide buforin II as tandem repeats in Escherichia coli. Protein Expr Purif 1998; 12(1): 53-60.

20. Yang YH, Zheng GG, Li G, Zhang XJ, Cao ZY, et al. Expression of bioactive recombinant GSLL-39, a variant of human antimicrobial peptide LL-37, in Escherichia coli. Protein Expr Purif 2004; 37(1): 229-35.

21. Thomas-Virnig CL, Centanni JM, Johnston CE, He LK, Schlosser SJ, Van Winkle KF, et al. Inhibition of multidrug-resistant Acinetobacter baumannii by nonviral expression of hCAP-18 in a bioengineered human skin tissue. Mol Ther 2009; 17(3): 562-9.

22. Sánchez D, Moussaoui M, Carreras E, Torrent M, Nogués V, Boix E. Mapping the eosinophil cationic protein antimicrobial activity by chemical and enzymatic cleavage. Biochimie 2011; 93(2): 331-8.

23. Andreev YA, Kozlov SA, Vassilevski AA, Grishin EV. Cyanogen bromide cleavage of proteins in salt and buffer solutions. Anal Biochem 2010; 407(1): 144-6.

24. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. third ed 2001. 1595-1597.

25. Ramos R, Domingues L, Gama M. Escherichia coli expression and purification of LL37 fused to a family III carbohydrate-binding module from Clostridium thermocellum. Protein Expr Purif 2010; 71(1): 1-7.

26. Moon JY, Henzler-Wildman KA, Ramamoorthy A. Expression and purification of a recombinant LL-37 from Escherichia coli. Biochim Biophys Acta 2006; 1758(9): 1351-8.

27. Li Y, Li X, Wang G. Cloning, expression, isotope labeling, and purification of human antimicrobial peptide LL-37 in Escherichia coli for NMR studies. Protein Expr Purif 2006; 47(2): 498-505.

28. Hong IP, Lee SJ, Kim YS, Choi SG. Recombinant expression of human cathelicidin (hCAP18/LL-37) in Pichia pastoris. Biotechnol Lett 2007; 29(1): 73-8.

29. Huang L, Wang J, Zhong Z, Peng L, Chen H, Xu Z, et al. Production of bioactive human beta-defensin-3 in Escherichia coli by soluble fusion expression. Biotechnol Lett 2006; 28(9): 627-32.

30. Wu X, Lu Y, Zhao H, Hu R. EW, a recombinant analogue of Exendin-4 expressed in Escherichia coli. Sci Res Essays 2011; 6(14): 2941-2949.

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Investigation of free-living Amoebae and respiratory bacterial pathogens in water samples taken from recreational fountains and ornamental pools in Ankara, Turkey

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Abstract

Introduction: Free-living amoebae (FLA), besides being agents of human infections, may also serve as hosts for other microorganisms including respiratory bacterial pathogens (RBPs). Recreational fountains and ornamental pools (RF-OPs) spread aerosolized water droplets in the air which can easily be inhaled.

Objective: The aim of this study was to investigate the presence of FLA and RBPs in water samples taken from RF-OPs and to determine if RBPs existed in FLA that were cultured from the initial samples.

Methods: Ninety-eight water samples collected during 2010 and 2011 summer months were cultured xenically by using non-nutrient agar for FLA. DNA was extracted from both water samples and FLA-positive culture material by a standard phenol-chloroform extraction method. FLA identification was performed by multiplex-PCR. Presence of respiratory bacterial pathogens (*S. pneumonia, H. influenzae, C. pneumoniae, L. pneumophila, B. pertussis, M. pneumoniae*) and *Mycobacterium* spp. in water samples and the cultured FLA were investigated by a commercial multiplex-PCR and a *Mycobacterium* specific PCR, respectively.

Results: FLA were detected in 39 (39.8%) of the total water samples by culture, 32 of which were also identified by PCR. Nine (23.1%) of these cultured FLA were also RBP-positive. FLA and RBPs were identified by PCR in 78 (79.6%) and 32 (32.7%) of water samples, respectively.

Conclusion: The FLA and RBPs can constitute biofilms on water surface and the possibility of RBPs also living as endosymbionts should be considered. In either situation, RF-OPs can be sources of FLA and RBPs, thus may pose a public health risk.

Key words: Free-living amoebae, respiratory bacterial pathogens, pool, fountain, water.

Introduction

Free-living amoebae (FLA) such as *Acanthamoeba, Naegleria, Balamuthia, Sappinia, Vahlkampfia* and *Hartmannella* species are ubiquitous organisms found in various environmental reservoirs such as air, soil, sand, dust, fresh and salty waters, as well as man-made water systems like recreational and domestic water sources, cooling towers, air-conditioning units, humidifiers, tap water, or drinking water production plants (1-6). Among these FLA, *Naegleria fowleri, Acanthamoeba* spp. and *Balamuthia mandillaris* are pathogenic to humans causing central nervous system, cornea, lung, and skin infections in susceptible individuals (6).

FLA trophozoites feed on bacteria including RBPs, some of which are able to survive phagocytosis (amoeba-resisting bacteria, ARB), and thus use the FLA as hosts (7). Besides being etiological agents of human infections, FLA serve not only as reservoirs but also as amplification and transport vehicles for water-based pathogenic ARB such as *Legionella* spp., *Mycobacterium avium,* and *Chlamydia pneumoniae* (7-10). Some of the advantages FLA offer ARB are: supplying critical nutrients and growth factors; protecting against biological, chemical and physical inhibitors especially in the cystic form; helping to survive and spread in water and environment; and increasing the virulence, infectivity and resistance of bacteria (1, 5, 9-14). FLA

protect its hidden resistant microorganism from the first line of human defenses and the strategies developed by ARB for surviving inside the amoeba help them to survive in human macrophages. As a result, the FLA/ARB couple synergically produce pathogens to which humans are frequently exposed, that are highly resistant to decontamination, and able to resist destruction by human macrophages (7). Human infection can occur via inhalation of aerosolized water containing free bacteria and/or FLA containing infective ARB (10).

Recreational fountains and ornamental pools (RF-OPs) affect people by spreading aerosols which can be easily inhaled especially in spring and summer months. Aerosols allow the transmission of possibly existing FLA and bacteria over considerable distances (15). The aim of this study was to investigate the existence of FLA and respiratory bacterial pathogens (RBPs) separately or as endosymbionts in RF-OP water that may pose a potential health risk for humans via respiratory route.

Methods

Water Samples

Ninety-eight water samples were collected from different outdoor (n=91) and indoor (n=7) RF-OPs located at different regions of Ankara city center, Turkey, during the summer months (June-August) of 2010 and 2011. The source of the water was municipal water system. The one water sample per RF-OP were collected from the surface of the water in 50 ml sterile centrifuge tubes, and transported to the laboratory. These samples were used for both FLA culture and total DNA extraction for PCR amplification.

FLA culture

FLA were cultured xenically by using non-nutrient agar. For this purpose, water samples (50 ml) were centrifuged at 250xg for 10 min. The supernatant was pipetted out and discarded leaving 1 ml of the sediment in the tube. This sediment was resuspended by pipetting and used for FLA culture and DNA extraction. FLA culture was performed by placing one drop of suspension in the center of a non-nutrient agar plate that had been streaked with *Escherichia coli* (ATCC 25922) in an X configuration, which promoted the multiplication and accumulation of amoeba in a defined area along the line of bacteria. The plate was sealed with parafilm and incubated at room temperature. The plates were examined at x400 magnification with an inverted microscope for the presence of amoebae daily for one week (16). For DNA extraction, the grown FLA were harvested in 1 ml sterile distilled water by scraping the surface of the agar.

DNA extraction

DNA was extracted both from the initial water samples and cultured FLA by a previously published extraction method with additional phenol-chloroform extraction steps (17). The additional steps were as follows: Following the inactivation of Proteinase K, the sample was mixed with 1:1 phenol-chloroform-Isoamyl alcohol solution (25:24:1), vortexed for 10 min, chilled on ice for 10 min, and centrifuged at 14.000xg for five min at +4°C. The upper phase was collected into a new tube, 1:1 chloroform was added, and the same steps were applied as mentioned above. 500 µl of 95% ethanol was added to the upper phase which was collected to a new tube. Following vortexing and centrifugation at 14.000xg for five min at 4°C, the pellet was washed with 70% ethanol. Supernatant was removed, the pellet was air-dried, and the extracted DNA was re-suspended in 200 µl sterile distilled water. DNA samples were stored at 4°C until PCR amplifications were performed.

PCR Amplification of FLA

FLA identification was done by the PCR protocol described by Tsvetkova et al. (6). This PCR provided identification of *Hartmannella vermiformis* (800 bp), *Naegleria fowleri* (900 bp), *Vanella spp./ Vahlkampfia ovis* (950 bp), *Acanthamoeba castellanii/ polyphaga/ lenticulata/ hatchetti* (1080 bp), *A. comandoni* (1350 bp), and *A. astronyxis* (1500 bp) according to the amplified product size.

PCR Amplification of RBPs

The presence of RBPs (*Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia pneumoniae, Legionella pneumophila, Bordetella pertussis,* and *Mycoplasma pneumoniae*) in initial water and culture-positive FLA samples were investigated by using a multiplex-PCR kit (Seeplex Pneumobacter Detection Kit, Seegene, Korea) following

manufacturer's instructions. *Mycobacterium* genus specific PCR was performed by using hsp65 gene directed primers as described previously (18). All PCR products were analysed by agarose gel electrophoresis and stained with ethidium bromide.

Results

FLA Culture

Microscopic evaluation of the cultures on non-nutrient agar plates showed that 39 (39.8 %) out of 98 water samples yielded amoeba growth, thus they were considered as culture-positive.

FLA-PCR of Culture-Grown Amoeba

In 32 (82.1%) of the 39 culture-positive water samples, FLA could be identified by PCR. *H. vermiformis* was detected in 29 (74.4%), *N. fowleri* in 6 (15.4%), *Acanthamoeba* spp. in 4 (10.3%), and *Vanella* spp./*V. ovis* in 2 (5.1%) of the FLA-PCR positive culture samples. Eight of these samples contained more than one amoeba species: *H. vermiformis* + *N. fowleri* (n=3), *H. vermiformis* + *Acanthamoeba* spp. (n=3), *H. vermiformis* + *Vanella* spp./*V. ovis* (n=1), *H. vermiformis* + *N. fowleri* + *Vanella* spp./*V. ovis* (n=1). In seven (17.9%) of the 39 culture positive material, FLA-PCR was negative although amoebae were observed microscopically, suggesting that they may contain FLA species for which PCR was not directed for detection.

FLA-PCR of Water Samples

The evaluation of water samples (n=98) revealed 78 (79.6%) positive samples. 76 (77.6%) were positive for *H. vermiformis*, 19 (19.4%) for *Acanthamoeba* spp., 7 (7.1%) for *N. fowleri* and 2 (2%) for *Vanella* spp./*V. ovis*. In 25 of the water samples, more than one amoeba species were detected: *H. vermiformis* + *Acanthamoeba* spp. (n=18), *H. vermiformis* + *N. fowleri* (n=5), *H. vermiformis* + *Vanella* spp./*V. ovis* (n=1), *H. vermiformis* + *N. fowleri* + *Vanella* spp./*V. ovis* (n=1) (Figure 1). Twenty (20.4%) water samples were FLA-PCR negative. Eight (8.2%) of these samples were culture-positive, though only one yielded *H. vermiformis* after performing FLA-PCR of culture samples.

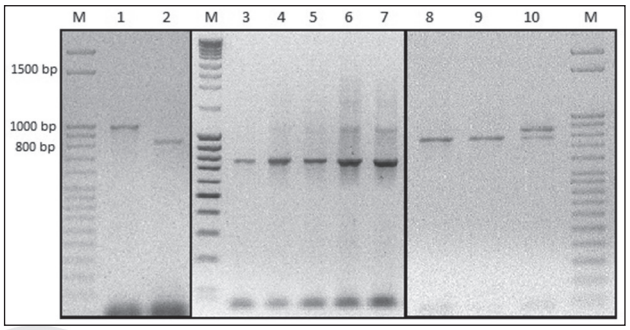

Figure 1. Representative FLA-PCR products. M represents 100 bp molecular size marker (Mass Ruler, Fermentas, Lithuania). 1: Acanthamoeba spp. positive sample, 2-5, 8,9: H. vermiformis positive samples, 6,7: H. vermiformis+ Acanthamoeba spp. positive samples, 10: H. vermiformis+ N. fowleri positive sample

RBP-PCR of Culture-Grown Amoebae

RBPs were found in nine (23.1%) of the 39 culture material. *S. pneumoniae* was detected in five, *Mycobacterium* spp. in four, *C. pneumoniae* in two, and *B. pertussis* in one. In three cultures, more than one RBP existed: *S. pneumoniae* + *Mycobacterium* spp. (n=2), and *S. pneumoniae* + *C. pneumonia* (n=1). The amoebae coexisting with these RBPs are shown in Table 1.

RBP-PCR of Water Samples

Of the water samples evaluated, 32 (32.7%) were positive for RBPs. *S. pneumoniae* DNA was detected in 25 (78.1%), *Mycobacterium* spp. in 10 (31.3%), *H. influenzae* in six (18.8%), *C. pneumoniae* in two (6.3%), and *B. pertussis* in one (3.1%) water sample. Twelve water samples contained more than one RBP: *S. pneumonia*e + *Mycobacterium* spp (n=5), *S. pneumoniae*+*H. influenzae* (n=4), *S. pneumoniae*+*B. pertussis* (n=1), *S. pneumoniae*+*C. pneumoniae* (n=1), and *C. pneumoniae*+*Mycobacterium* spp. (n=1) (Figure 2). Only three of the RBP-positive water samples were FLA-negative both by culture and PCR. In six RBP-positive water samples there were culture-grown amoebae, but they could not be identified by FLA-PCR, suggesting that they may belong to other genera than the studied ones. The amoebae detected in the remaining 23 RBP-positive samples are shown in Table 1.

Table 1. Co-existening amoeba and RBP in culture and/or water samples

Water Sample No	FLA Culture	PCR Results of Culture Material		PCR Results of Water Samples	
		FLA-PCR	RBP-PCR	FLA-PCR	RBP-PCR
2	(+)	*N. fowleri*	*S. pneumoniae, Mycobacterium* spp.	*N. fowleri*	(-)
15	(+)	(-)	(-)	(-)	*S. pneumoniae H. influenzae*
18	(+)	(-)	(-)	(-)	*H. influenzae*
20	(-)	(-)	NP*	*H. vermiformis*	*S. pneumoniae*
21	(-)	(-)	NP	*H. vermiformis Acanthamoeba* spp.	*S. pneumoniae*
22	(+)	*H. vermiformis*	*S. pneumoniae*	*H. vermiformis*	(-)
24	(+)	*H. vermiformis*	*S. pneumoniae*	*H. vermiformis*	(-)
26	(+)	*H. vermiformis*	*Mycobacterium* spp.	*H. vermiformis*	(-)
28	(+)	(-)	(-)	(-)	*S. pneumoniae*
31	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*S. pneumoniae*
36	(-)	(-)	NP	*H. vermiformis*	*S. pneumoniae*
38	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*S. pneumoniae*
43	(+)	*H. vermiformis*	*S. pneumoniae Mycobacterium* spp.	*H. vermiformis*	(-)
44	(+)	(-)	(-)	(-)	*S. pneumoniae*
45	(+)	(-)	(-)	(-)	*S. pneumoniae*
51	(+)	(-)	(-)	(-)	*S. pneumoniae H. influenzae*
52	(+)	*N. fowleri*	*B. pertussis*	*H. vermiformis N. fowleri*	(-)
54	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*S. pneumoniae*
55	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*S. pneumoniae Mycobacterium* spp.
57	(-)	(-)	NP	*H. vermiformis*	*Mycobacterium* spp.
59	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*S. pneumoniae Mycobacterium* spp.
61	(-)	(-)	NP	*H. vermiformis*	*Mycobacterium* spp.
65	(+)	*H. vermiformis*	*S. pneumoniae C. pneumoniae*	*H. vermiformis*	(-)
70	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*Mycobacterium* spp.
72	(+)	*H. vermiformis N. fowleri*	*Mycobacterium* spp.	*H. vermiformis N. fowleri*	(-)
73	(-)	(-)	NP	*H. vermiformis Acanthamoeba* spp.	*S. pneumoniae Mycobacterium* spp.
74	(+)	*H. vermiformis*	(-)	*H. vermiformis*	*Mycobacterium* spp.
75	(-)	(-)	NP	*H. vermiformis*	*S. pneumoniae B. pertussis*
77	(-)	(-)	NP	*H. vermiformis*	*S. pneumoniae*
80	(-)	(-)	NP	*H. vermiformis Acanthamoeba* spp.	*S. pneumoniae Mycobacterium* spp.
86	(-)	(-)	NP	*H. vermiformis Acanthamoeba* spp.	*C. pneumoniae Mycobacterium* spp.

91	**(-)**	**(-)**	NP	*H. vermiformis* *Acanthamoeba* spp.	*S. pneumoniae*
92	**(-)**	**(-)**	NP	*H. vermiformis* *Acanthamoeba* spp.	*S. pneumoniae*
93	**(-)**	**(-)**	NP	*H. vermiformis*	*S. pneumoniae*
95	**(-)**	**(-)**	NP	*H. vermiformis*	*S. pneumoniae* *Mycobacterium* spp.
97	**(-)**	**(-)**	NP	*H. vermiformis*	*S. pneumoniae* *H. influenzae*
98	**(+)**	*H. vermiformis*	*C. pneumoniae*	*H. vermiformis*	*S. pneumoniae* *C. pneumoniae*

NP: Not performed;* **(-)*: negative,* **(+)***: positive*

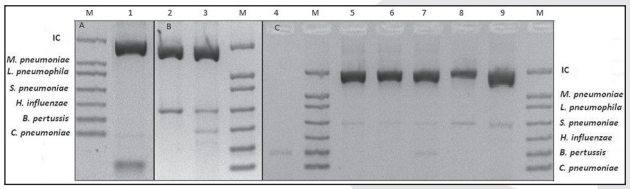

Figure 2. Representative RBP-PCR products. M represents molecular size marker corresponding to the given pathogens on each side. IC represents internal control. 1: C. pneumoniae positive sample, 2, 5,6,8,9: S. pneumoniae positive samples. 3: S. pneumoniae + H.influenzae positive sample, 4: B. pertussis positive sample, 7: S. pneumoniae + B. pertussis positive sample

Discussion

Ankara is the capital city of Turkey, with about 4.750.000 inhabitants. During the past ten years, RF-OPs have been increasingly constructed for esthetic purposes in the open-air areas such as parks, divided highways, traffic junctions and also in indoor areas like shopping malls located at Ankara city center. These RF-OPs are filled from the city water network, but water is recycled throughout spring and summer months. These RF-OPs spread out aerosols, which come into contact with people even with summer breeze.

In low nutrient environments such as freshwater, the association of bacteria with FLA is an adaptive survival mechanism of ARB that promotes their persistence and dissemination. ARB infected FLA genera identified by laboratory research to date are; *Acanthamoeba, Dictyostelium, Echinamoeba, Hartmannella, Naegleria* and *Vahlkampfia* (10). In Bulgaria, 61.1% of the environmental soil and water sources were reported to contain FLA (6). The frequency of FLA in various environmental sources was reported to be between 17.2-31% in Turkey by culture. They were identified as *Hartmannella* spp., *Naegleria* spp., *Acanthamoeba* spp., and *Vahlkampfiidae* by PCR (19, 20). In our study 86 (87.8%) of the water samples obtained from RF-OPs were found to contain FLA by culture (n=12) or PCR alone (n=47), or by both (n=27).

Direct PCR evaluation of water samples yielded twice as much amoebae than culture (79.6% vs 39.8%) in our study. Culture-based methods can underestimate both FLA diversity and density in water (10). Culturing water is a laborious procedure, and it is often difficult to achieve appropriate enrichment. Moreover, FLA concentrations in water may be too low for cultural detection but still be high enough to cause infection. Therefore the use of highly-sensitive and specific molecular detection methods based on the detection of specific segments on the pathogens's genome are needed for the evaluation of water-borne pathogens (21). Presence of microorganisms in water samples can be directly demonstrated by PCR without prior culture isolation (22). On the other hand, PCR can only detect the targeted genomes, thus any FLA which is not covered for detection can not be identified by that specific PCR. In our study, all but one (*H.* vermiformis) of the cultured amoebae could also be identified by direct PCR of the water samples. For one culture whose PCR yielded the presence of *N. fowleri,* PCR of the water sample detected both *N. fowleri* and *H. vermiformis.* On the other hand, we could not identify amoebae from seven (7.2%) culture-positive water samples. These FLA may belong to other genera such as *Sappinia, Dictyostelium* or *Echinamoeba* which were out of the range of the PCR method used.

The most frequently encountered FLA in tap water are; *Acanthamoeba, Hartmannella, Vahlkampfia,* and *Vanella* (10). Corsaro et al. have isolated *Naegleria* spp. (44.4%), *Acanthamoeba* spp. (25%), and *H. vermiformis* (13.8%) from the samples obtained from water treatment plants in Spain (2). FLA density and diversity in tap water show seasonal variation, and increases during the summer months due to warming of water (10, 15). *Acanthamoeba* is more prevalent in spring, while *Vahlkampfia* and *Naegleria* are frequently detected in autumn (9). In our study, water samples were collected during the summer months, and the most frequently encountered FLA were *H. vermiformis* (78.6%), followed by *Acanthamoeba* spp. (19.4%), *N. fowleri* (7.1%), and *Vanella* spp./*V. ovis* (2%). Similarly, in the study of Tsvetkova et al., *Acanthamoeba* and *Hartmannella*, but not *Naegleria*, were found to be abundant in water and soil samples from Bulgaria (6).

Bacterial endosymbionts of FLA were microscopically observed nearly 30 years ago, but due to the inability to culture these bacteria outside their eukaryotic hosts, their identification and analysis delayed until the introduction of molecular techniques. Using PCR for the investigation of FLA and RBPs in water samples may have some drawbacks, despite its speed, ease to perform, high sensitivity, and specificity. Prolonged exposure to water may lead the bacterial pathogens to enter a viable, but non-culturable state, and these bacteria may not be detected by culture, although they retain their infective potential. Using PCR, these bacteria can be detected, but PCR cannot distinguish between viable and non-viable organisms. The standardization and validation of protocols is very important for the implementation of molecular techniques either in the environmental or clinical field. Direct PCR amplification of some bacterial pathogens from water samples may be difficult due to the presence of low numbers of bacteria in environmental sources, and the presence of inhibitors in the samples may lead to false-negative results (21).

Bacterial endosymbionts belong to five lineages: (i) the *Alphaproteobacteria*, (ii) the *Betaproteobacteria*, (iii) the *Gamaproteobacteria*, (iv) the *Bacteroidetes*, and (v) the *Chlamydiales* (4).

Legionella pneumophila is the first human pathogen shown to multiply and persist in amoebae (23). FLA are necessary for the multiplication of *Legionella* in water, and humans are probably infected by inhaling the amoeba filled with *Legionella* (23, 24). Growth in amoebae has been shown to enhance the entry, intracellular survival and replication of *Legionella pneumophila* in macrophages. *Legionella* grown in amoeba has higher virulence and infectivity (25). *L. pneumophila* can infect and multiply within *Hartmannella, Acanthamoeba, Vahlkampfia, Echinamoeba* and *Naegleria* spp (1, 26). We didn't detect *L. pneumophila* in either cultured FLA or water samples. This finding is consistent with the study of Pagnier et al. who also could not detect any *Legionella* in the environmental water samples (27).

C. pneumoniae is the second example of respiratory pathogens which can use the amoebae as hosts (12, 28). *C. pneumoniae* also benefits from the amoebal adaptation process for infection of animal cells and macrophages (4, 12). In humans, mixed infections due to *Acanthamoeba* and *Chlamydia* have been reported (29). We detected the presence of *C. pneumoniae* in three water samples by either culture (n=1) or PCR (n=1), or both (n=1). All three contained *H. vermiformis,* and one was also positive for *Acanthamoeba spp.* (Table 1).

Mycobacterium species are widely distributed in the environment. They can infect and multiply in FLA, and use them as training grounds to adapt and survive within human macrophages (2, 5). To date, many mycobacterial species such as *M. tuberculosis*, *M. bovis* and BCG strains, *M. avium* complex (MAC)*, M. marinum, M. ulcerans, M. simiae, M. habane, M. smegmatis, M. fortuitum, M. phlei, M. gordonae, M. kansasii,* and *M. xenopi* are shown to survive within FLA (7, 27, 30-32). Besides, MAC, *M. marinum, M. kansasii, M. scrofulaceum, M. chelonae, M. xenopi, M. abscessus, M. bolettii, M. massiliense* and *M. fortuitum* are shown to be the free living mycobacterial species associated with contaminated water supplies (30, 31). In our study, four of the FLA-positive culture samples, and ten of the water samples containing FLA were also positive for *Mycobacterium* spp (Table 1).

Legionella, Chlamydia and *Mycobacterium* species are among the most frequently investigated RBPs capable to survive in FLA. With amoebal co-culture, Corsaro et al. have isolated *Legionella* spp., *Chlamydia*-like bacteria., and rapid growing

Mycobacterium spp. in 29.6%, 10.4%, and 19.2% of the water treatment plant samples, respectively (2). Thomas et al. investigated hospital network for the presence of FLA and bacterial endosymbionts by amoeba co-culture (5). They isolated *H. vermiformis* and *A. polyphaga* in 6.5% and 0.5% of the samples, respectively. The samples containing bacterial isolates (45.5%) revealed *Mycobacterium* spp. and *Legionella* spp. but they couldn't isolate any *Chlamydia*-related organisms. Although we identified the presence of *Mycobacterium* spp. in 10.3%, and *C. pneumoniae* in 3.1% of the water samples, we did not detect *L. pneumophila* (Table 1). *C. pneumoniae* has not been detected from natural or artificial water sources before. Our findings may have resulted from a cross-reaction between *Chlamydia* and *Chlamydia*-like bacteria.

In the present study, *S. pneumoniae* DNA was present in 25 (25.5%) water samples, 17 of which were also containing FLA-DNA (Table 1). We detected *S. pneumoniae* DNA in one *N. fowleri* and four *H. vermiformis* positive culture samples. *S. pneumoniae* was reported to be very common in ground and sea water (33), although only one study reported *S. pneumoniae* as an ARB until today (3). Our findings may support the evidence that *S. pneumoniae* can either be found as endosymbionts of FLA or free-living forms in water.

One interesting finding of our study which needs further verification was the determination of *B. pertussis* in two samples; one, *N. fowleri positive* culture sample and the other, an initial water sample simultaneously harboring *S. pneumoniae, H. vermiformis* and *Acanthamoeba* spp. *Bordetella* spp. belong to the family *Alcaligenaceae* which also comprises the genera *Alcaligenes* and *Achromobacter* (34, 35). *B. pertussis* is an obligate pathogen for humans, whereas *Achromobacter* and *Alcaligenes* are environmental species. *B. bronchiseptica, B. petrii,* and *B. ansorpii* are shown to live environmentally (35, 36). *B. petrii* can cause human infections, and has a 97.9-98.6% sequence similarity with the members of the genus *Bordetella* (35). *Achromobacter xylosoxidans* and *Alcaligenes* species can infect FLA (13, 27, 37). The Seeplex Pneumobacter Detection kit used to detect RBPs in this study is a highly sensitive, and specific kit validated for respiratory pathogens. İn the kit's manual, only *B. parapertussis* and *B. bronchiseptica* are excluded for cross-reaction with *B. pertussis*. Thus, its detection may also reflect either a cross-reaction with other species, or human contamination of water samples. Whether it may be an endosymbiont of FLA or not, needs further confirmation.

The interactions between FLA and ARB are generally species-specific (4, 10), but coinfection of amoebae by more than one bacterial species may occur (2). In the culture material, we detected the presence of two bacterial species (*S. pneumoniae* and *Mycobacterium* spp) along with *H. vermiformis* (n=1) and *N. fowleri* (n=1). Seven water samples with *H. vermiformis* also yielded two bacteria: *S. pneumoniae* and *Mycobacterium* spp. (n=4), *S. pneumoniae* and *B. pertussis* (n=1), *S. pneumoniae* and *H. influenzae* (n=1), and *C. pneumoniae* and *Mycobacterium* spp. (n=1).

The presence of bacterial DNA along with FLA-DNA in water samples suggest that either these bacteria are endosymbionts of FLA, or they live together in biofilms, most of which are composed of multiple eukaryotic and prokaryotic species (1, 26). Amoebae establish and stimulate the productivity of biofilms by adhering to surfaces, secreting metabolic substances, and enhancing bacterial productivity (38).

As the water samples in this study were taken from RF-OPs located in the crowded parks and shopping centers at the city center, they could be contaminated by human excretions containing RBPs. Survival time of certain RBPs in the external environment are; 244.3 days for *M. tuberculosis*, 28.6 days for *S. pneumoniae*, 11.6 days for *B. pertussis* and 1.3 days for *H. influenzae*. There is insufficient information about the survival of *C. pneumoniae* in the external environment (39). *H. influenzae* is a human respiratory pathogen, which has not been previously shown to infect amoebae, but is hypothesized to produce biofilms (40). Its presence, as well as other RBPs in RF-OP water samples may also indicate contamination from human respiratory excretions. As PCR cannot discriminate between dead and alive bacteria we cannot speculate on the infectivity of the pathogens, but due to their ability to survive in the environment for a matter of days, their presence in RF-OPs may pose a public health threat.

In our study, 32.6% and 16.3% of 86 FLA containing water also contained *S. pneumoniae,* and *Mycobacterium* spp., respectively. Of the 12

FLA-negative water samples, only three (25%) contained RBPs (*S. pneumoniae* and/or *H. influenzae*). The difference between the presence of RBP in water samples with or without amoebae was not statistically significant ($p>0.05$). None of the FLA-negative water samples contained *Mycobacterium* spp. Some studies reported that there was no difference between presence of *Mycobacterium* in water samples with and without amoeba (41), while other studies showed that FLA containing water samples contained statistically significantly more *Mycobacterium* spp. (2, 42). These differences were attributed to the presence of biofilms, population density, complexity of ecosystems, biocide treatments, and sampling bias (2).

Although we have no proof of RBPs being endosymbionts of FLA in water samples in this study, their coexistence makes it a possibility. These RBPs are among the leading causes of community acquired pneumoniae in both adults and children (43, 44). The presence of FLA and RBPs in RFOPs may pose a public health problem especially for the ones with underlying predisposing factors.

Acknowledgement

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References

1. *Barker J, Brown, M.R.W, 1994. Trojan horses of the microbial World: protozoa and the survival of bacterial pathogens in the environment. Microbiology 140, 1253-1259.*

2. *Corsaro D., Pages, G.S., Catalan, V., Loret, J-F., Greub, G., 2010. Biodiversity of amoeba and amoeba-associated bacteria in water treatment plants. Int. J. Hyg. Environ. Health 213, 158-166.*

3. *Evstigneeva A., Raoult, D., Karpachevskiy, L., La Scola, B., 2009. Amoeba co-culture of soil specimens recovered 33 different bacteria, including four new species and Streptococcus pneumoniae. Microbiology 155, 657-664.*

4. *Molmeret M., Horn, M., Wagner, M., Santic, M., Kwaik, Y.A., 2005. Amoebae as training grounds for intracellular bacterial pathogens. Appl. Environ. Microbiol. 71, 20-28.*

5. *Thomas V., Herrera-Rimann, K., Blanc, D.S., Greub, G., 2006. Biodiversity of amoebae and amoeba-resisting bacteria in hospital water network. Appl. Environ. Microbiol. 72, 2428-2438.*

6. *Tsvetkova N., Schild, M., Panaiotov, S., Kurdova-Mintcheva, R., Gottstein, B., Walochnik, J., Aspöck, H., Lucas, M.S., Müller, N., 2004. The identification of free-living environmental isolates of amoebae from Bulgaria. Parasitol. Res. 92, 405-413.*

7. *Greub G., Raoult, D., 2004. Microorganisms resistant to free-living amoebae. Clin. Microbiol. Rev. 17, 413-433.*

8. *Fritsche T.R., Horn, M., Wagner, M., Herwig, R.P., Schleifer, K.H., Gautom, R.K., 2000. Phylogenetic diversity among geographically dispersed Chlaymdiales endosymbionts recovered from clinical and environmental isolates of Acanthamoeba spp. Appl. Environ. Microbiol. 66, 2613-2619.*

9. *Marciano-Cabral F., Jamerson, M., Kaneshiro, E.S., 2010. Free-living amoebae, Legionella and Mycobacterium in tap water supplied by a municipal drinking water utility in the USA. J. Water Health 8, 71-82.*

10. *Thomas J.M., Ashbolt, N.J., 2011. Do free-living amoebae in treated drinking water systems present an emerging health risk? Environ. Sci. Technol. 45, 860-869.*

11. *Aksozek A., McClellan, K., Kniederkorn, J.Y.H., Alizadeh, H., 2002. Resistance of Acanthamoeba castellanii cysts to physical, chemical and radiological conditions. J. Parasitol. 88, 621-623.*

12. *Essig A., Heinemann, M., Simnacher, U., Marre, R., 1997. Infection of Acanthamoeba castellanii by Chlamydia pneumoniae. Appl. Environ. Microbiol. 63, 1396-1399.*

13. *Greub G., La Scola, B., Raoult, D., 2004. Amoebae-resisting bacteria isolated from human nasal swabs by amoebal coculture. Emerg. Infect. Dis. 10, 470-477.*

14. *Tomov A., Kassovsky, V., Chorbadjiska, L., Tsvetkova, E., Tasnev, N., Vancheva, Z., 1982. Lytic activity of Bdellovibrio bacteriovorous against bacteria of teh family Legionellaceae. Zentralb. Bakteriol. Mikrobiol. Hyg. Ser. A 252, 96-100.*

15. *Wotton R.S., Preston, T.M., 2005. Surface films: Areas of water bodies that are often overlooked. BioScience 55, 137-145.*

16. *Newsome A.L., Scott, T.M., Benson, R.F., Fields, B.S., 1998. Isolation of an amoeba naturally harbouring a distinctive Legionella species. Appl. Environ. Microbiol. 64, 1688-1693.*

17. *Da Silva A., Schwartz, D.A., Visvesvara, G.S., de Moura, H., Slemenda, S.B., Pieniazek, N.J., 1996. Sensitive PCR diagnosis of infections by Enterocytozoon bieneusi (Microsporidia) using primers based on the region coding for small-subunit rRNA. J. Clin. Microbiol. 34, 986-987.*

18. *Telenti A., Marchesi, F., Balz, M., Bally, F., Böttger, E.C., Bodmer, T., 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. 31, 175-178.*

19. *Burak D.M., Zeybek Z., 2011. Investigation of Legionella pneumophila and free-living amoebas in the domestic hot water systems in İstanbul. Turk. J. Biol. 3, 679-685.*

20. *Saygı G., Akın, Z., 2000. Sivas'ta toprak ve termal su örneklerinden Acanthamoeba ve Naegleria türlerinin soyutulması. Turkiye Parazitol. Derg. 24, 237-242.*

21. *Girones R., Ferrus, M.A., Alonso, J.L., Rodriguez-Manzano, J., Calgua, B., Correa, A.A., Hundesa, A., Carratala, A., Bofill-Mas, S., 2010. Molecular detection of pathogens in water-The pros and cons of molecular techniques. Water Res. 44, 4325-4339.*

22. *Kilvington S., Beeching, J., 1995. Development of a PCR for identification of Naegleria fowleri from the environment. Appl. Environ. Microbiol. 61, 3764-3767.*

23. *Rowbotham T.J., 1986. Current views on the relationships between amoebae, Legionella, and man. Isr. J. Med. Sci. 22, 678-689.*

24. *Murga R., Forster, T.S., Brown, E., Pruckler, J.M., Fields, B.S., Donlan, R.M., 2001. Role of biofilms in the survival of Legionella pneumophila in a model potable-water system. Microbiol. 147, 3121-3126.*

25. *Cirillo J.D., Cirillo, S.L.G., Yan, L., Bermudez, L.E., Falkow, S., Tompkins, L.S., 1999. Intracellular growth in Acanthamoeba castellanii affects monocyte entry mechanisms and enhances virulence of Legionella pneumophila. Infect. Immun. 67, 4427-4434.*

26. *Snelling W.J., Moore, J.E., McKenna, J.P., Lecky, D.M., Dooley, J.S., 2006. Bacterial-protozoa interactions; an update on the role these phenomena play towards human illness. Microbes Infect. 8, 578-587.*

27. *Pagnier I., Raoult, D., La Scola, B., 2008. Isolation and identification of amoeba-resisting bacteria from water in human environment by using an Acanthamoeba polyphaga co-culture procedure. Environ. Microbiol. 10, 1135-1144.*

28. *Amann R., Springer, N., Schönhuber, W., Ludwig, W., Schmid, E.N., Müller K.D., Michel, R., 1997. Obligate intracellular bacterial parasites of Acanthamoeba related to Chlamydia species. Appl. Environ. Microbiol. 63: 115-121.*

29. *Reddy V.M., Pepose, J.S., Lubniewski, A.J., Gans, L.A., Smith, M.E., 1991. Concurrent Chlamydial and Acanthamoeba keratoconjunctivitis. Am. J. Ophtalmol. 112, 466-468.*

30. *Cirillo J.D., Falkow, S., Tompkins, L.S., Bermudez, L.E., 1997. Interaction of Mycobacterium avium with environmental amoebae enhances virulence. Infect. Immun. 65, 3759-3767.*

31. *Medie F.M., Salah, I.B., Henrissat, B., Raoult, D., Drancourt, M., 2011. Mycobacterium tuberculosis complex mycobacteria as amoeba-resistant organisms. PLoS One 6, e20499.*

32. *Salah I.B., Ghigo, E., Drancourt, M., 2009. Free-living amoebae, a training field for macrophage resistance of mycobacteria. Clin. Microbiol. Infect. 15, 894-905.*

33. *Harakeh S., Yassine, H., El-Fadel, M., 2006. Antimicrobial-resistance of Streptococcus pneumoniae isolated from the Lebanese environment. Mar. Environ. Res. 62,181-193.*

34. *Gerlach G., von Wintzingerode, F., Middendorf, B., Gross, R., 2001. Evolutionary trends in the genus Bordetella. Microbes Infect. 3, 61-72.*

35. *Von Wintzingerode F., Schattke, A., Siddiqui, R.A., Rösick, U., Göbel, U.B., Gross R., 2001. Bordetella petrii sp. nov., isolated from an anaerobic bioreac-*

tor, and emended description of the genus Bordetella. Int. J. Syst. Evol. Microbiol. 51, 1257-1265.

36. *Bastian F, Alabouvette, C., Saiz-Jimenez, C., 2009. Bacteria and free-living amoeba in the Lascaux cave. Res. Microbiol. 160, 38-41.*

37. *Tyndall R.L., Ironside, K.S., Little, C.D., Katz, D.S., Kennedy, J.R., 1991. Free-living amoeba used to isolate consortia capable of degrading trichlorethylene. Appl. Biochem. Biotechnol. 28/29, 917-925.*

38. *Hoffmann R., Michel, R., 2001. Distribution of free-living amoebae (FLA) during preparation and supply of drinking water. Int. J. Hyg. Environ. Health 203, 215-219.*

39. *Moxon E.R., Sweetman, W.A., Deadman, M.E., Ferguson, D.J.P., Hood, D.W., 2008. Haemophilus influenzae biofilms: hypothesis or fact? Trends Microbiol. 16, 95-100.*

40. *Walther B.A., Ewald, P.W., 2004. Pathogen survival in the external environment and the evolution of virulence. Biol. Rev. 79, 849-869.*

41. *Eddyani M., DeJonckheere, M., Dunez, J.F., Suykerbuyk, L., Leirs, P., Portaels, F., 2008. Occurence of free-living amoebae in Southern Benin communities of low and high endemicity of Buruli ulcer. Appl. Environ. Microbiol. 74, 6547-6553.*

42. *Berk S.G., Gunderson, J.H., Newsome, A.L., Farone, A.L., Hayes, B.J., Redding, K.S., Uddin, N., Williams, E.L., Johnson, R.A., Farsia, M., Reid, A., Skimmyhorn, J., Farone, M.B., 2006. Occurrence of infected amoebae in cooling towers compared with natural aquatic environments: Implications for emerging pathogens. Environ. Sci. Technol. 40, 7440-7444.*

43. *Woodhead M., 2002. Community acquired pneumoniae in Europe: causative pathogens and resistance patterns. Eur. Respir. J. 20 Suppl 36, 20s-27s.*

44. *Harris M., Clark, J., Coote, N., Fletcher, P., Harnden, A., McKean, M., Thomson, A., on behalf of the British Thorasic Society Standards of Core Committee, 2011. British Thorasic Society guidelines for the management of community acquired pneumonia in children: Update. Thorax 66, ii1-23.*

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Molecular typing of Staphylococcus aureus isolated from patients and healthy carriers on the basis of coagulase gene polymorphism

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Abstract

Coagulase gene, an important characteristic of *Staphylococcus aureus*, has polymorphic repeat region that can be used for typing of *S.aureus* isolates. Determination of the Coa gene variation among *S.aureus* isolated from healthy carriers with clinical isolates, and *MRSA* with *MSSA* strains was the objective of this research. In this study Coa gene variation among 170 isolates of *S. aureus*; 95(56%) cases from patients and75 (44%) cases from healthy carriers were studied by PCR RFLP method. For this purpose the repeated units encoding hyper variable regions of *S.aureus* coagulase gene were amplified by the PCR method, followed by *AluI* restriction enzyme digestion of PCR product. The isolates revealed 7 types of Coa gene products between 570-1200 ±20 bp and 17 distinct RFLP Patterns were obtained with *AluI* digests of PCR products. Majority of isolates belong to the band class 800±20 bp and PCR-RFLP pattern P8 with one restriction site (405-324bp). Some PCR-RFLP patterns had specific for *S.aureus* isolated from carriers (p3,p7,p15 types), did not observe in patients, The differences in types is significant between two groups of carriers and patients (P <0.05) but counter to this difference is not statistically meaningful among *MRSA* and *MSSA* strains. The method investigated in this study, with high discriminatory index value (*D:* 0.9), can be useful, rapid and efficient for typing of *S.aureus* isolated from clinical samples or healthy carriers. This typing procedure could be used to analyze large number of strains within a short period of time and thus useful for epidemiological investigation.

Key words: Coagulase gene, polymorphism, *Staphylococcus aureus.*

Introduction

Staphylococcus aureus is a common pathogen associated with serious community and hospital acquired diseases and has for a long time been considered as a major problem of Public Health (1). The drug resistant strains are arising rapidly and thus making the treatment difficult (2). Rapid identification of infected patients and interruption of strain transmission is very crucial in controlling the spread of infection. Rapid and accurate typing of *S. aureus* is important to understand the transmission of this infections organism (3). Staphylocoagulase (SC) that causes coagulation of plasma is one of the extracellular virulence factors produced by *S. aureus* strains, and is regarded as a marker for discriminating *S. aureus* from other less pathogenic staphylococci called as coagulase-negative staphylococci. SC binds to prothrombin and the complex of SC and prothrombin induces plasma coagulation by converting fibrinogen in to fibrin. Variation in SC have been noticed as the differences in the antigenicity (4).

The coagulase gene consists of three distinct regions: (i) the N-terminus containing the prothrombin-binding site (ii) a central region which is highly conserved, and (iii) a C-terminal region composed of 81-bp tandem repeat units, which differ among *S. aureus* isolates, both in their number of tandem repeat and in the location of *AluI* restriction sites (5). It is known that phenotypically and genotypically different many strains of *S. aureus* exist; however, there is not enough information about distribution of the types of the pathogen in our geographic locations, Gorgan, Northern of Iran. In this research, the diversity of Coa gene in *S. aureus* isolated from patients and healthy carriers in this region was established and their diversity among *MRSA* and *MSSA* isolates were compared.

Material and Methods

In this study 170 *S. aureus* strains were used; 95clinical isolates were collected from different clinical specimens, such as pus, blood, sputum, urine and wound of patients and 75 cases were obtained from health care workers in 3 educational hospitals in Gorgan, north of Iran during 2010-2011. The primary diagnosis of *S. aureus* were based on bacterial growth on Manitol Salt Agar media, Gram Staining, Catalase, slide or tube coagulase and Dnase tests (6). It confirmed by specific glutamate synthetase gene primer and Sensitivity of resistance of *S. aureus* isolates to methicillin was determined on the base of presence of *mecA* gene, using specific primers were shown in table 1 (7, 8 and 9). According to this method we found that from 170 *S. aureus* isolates in different clinical specimens and health care workers, 51(30%) and 119 (70%) were *MRSA* and *MSSA*, respectively.

Polymerase chain reaction (PCR)

The DNA extraction was done using the Lysostaphin, Phenol-chloroform(10) and PCR amplification was performed using DNA and primers of 3-end hyper variable region containing 81bp tandems repeats. The sequence of primer used for amplification was shown in table in table 1(11). PCR conditions used were as follows:

94°C for 2 min, followed by 30 cycles of 94°Cfor 30 seconds, 55°C for 2 min and 72°C for4 min. Followed by final extension of 72°C for 7 min. PCR products were electrophoresis on 1.5% agarose gel (12).

Restriction enzyme digestion of Coa amplicon

We used *AluI* restriction endonuclease, which recognizes AT/GC sequences, for RFLP typing. Depending on the number of 81bp repeats a strain Analysis of restriction fragment length polymorphism of PCR products performed with *AluI* (Fermentas, India). Amplicons (10µl) were digested over night with 2µl of *AluI*, 2µl of enzyme buffer and 18µl nuclease free water. Digested products were electrophoresed in agarose gel 2% according to standard procedures (11, 13). In this study COL strain was used as a positive and distilled water as negative control. Data were entered in SPSS software version 16 and analyzed with chi square tests and $P<0.05$ was considered as significant.

Determination of numerical index of discrimination

The discriminatory power of coagulase gene PCR-RFLP was determined according to the numerical index described by Hunter and Gaston (1998) (14). The D-value indicates the probability that two unrelated isolates sampled from the test population will be placed in to different typing group's (12). The following formula was used as Eq1 (14):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} x_j(x_j - 1) \quad \text{..... Eq1}$$

Result

Amplification of coagulase gene

The PCR amplification of coagulase gene of 170 *S. aureus* isolates distinguished 7 PCR products of approximately 570-1200±20 bp (Table2 and Figure1). Pattern Analysis, it was showed that the type 800±20 bp (42.9%), 730±20 bp (28.2%) were the most common types. The distribution of different Coa genotypes in *MRSA* and *MSSA* was similar and there wasn't significant difference among them. The most Common Coa types in *S. aureus* isolated from patients were 730±20 (37.9%) and 800±20 bp (37.9%) but in healthy carrier isolates was 800±20 (49.3%), 730±20 and 650±20 bp (16%), this difference was statistically meaningful ($P<0.05$), (Table 2).

Table 1. The genes and related primers used in this study

Gene	Primers	Amplicon size	Reference
sa442	F 5-CGTAATGAGATTTCAGTAGATAATACAACA-3 R 5-AATCTTTGTCGGTACACGATATTCTTCACG-3	bp 108	(6)
mec A	F 5-AAAATCGATGGTAAAGGTTGGC-3 R 5-AGTTCTGCAGTACCGGATTTGC-3	bp 533	(7,8)
Coa	F 5-GCAGACCAAGATTCAACAAG R 5-AAAGAAAACCACTCACATCA	bp 600-1000	(11)

The variability of amplification products reflects the variation in the Coa gene lengths of *S. aureus* isolates. The differences in average length of Coa gene were not significant between two groups of patients (791bp) and carriers (788bp). Also we found that no discriminative bands or banding patterns were obtained that can distinguish between healthy carrier and patients.

The numerical index of discrimination based on PCR product sizes was 0.70, which is less for typing based on PCR product size. PCR amplification of the Coa gene which clearly emphasizes the use of molecular methods in detecting *S. aureus***.**

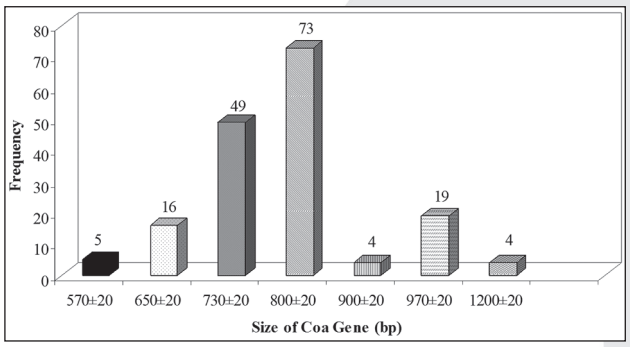

Figure 1. Type of Coa gene frequency

AluI Restriction enzyme digestion

Restriction digestion of the PCR product with *AluI* gave one to three bands in each isolates and their sizes varied from 81- 972 bp (Table 3), it means our *S. aureus* isolates Coa gene have maximum 2 enzyme restriction sites, but in 40 cases PCR products did not show any restriction cut with *AluI*; most of them were observed in *MSSA* stains (30%) and healthy carriers (32%) (Table 3). The differences in types is significant between two group of carriers and patients (P<0.05)but this difference is not statistically meaningful among *MRSA* and *MSSA* strains. 17 distinct PCR-RFLP patterns were observed among the 170 isolates examined after *AluI* digestion, the pattern 324-405 bp (28.2%) was found to be dominant type (Table 3). Amplicons of 570, 730, 900 and 1200±20 bp produced only one pattern of p10, p8, p11, p2 respectively, Amplicons of 970±20bp produced three patterns of p15, p6, p3, Amplicons of 650±20 bp produced 4 patterns of p17, p13, p9, p5 and amplicons of 800±20bp produced 6 PCR RFLP patterns of p16, p14, p12, p7, p4 and p1 (Table 4). In this study we realized that 16 types (from 17 types) were presented in healthy carrier isolates but only 13 types were found in patients isolates. Types p17, p15, p7, p3 were not in healthy carrier isolates. In the other hand, the variety of patterns in *MSSA* strains were more than *MRSA* strains and the Coa types p9, p11, p17 were found only in *MSSA* strains (Table 3). We couldn't found any relation between special PCR RFLP Coa types and the type of infection and all Coa gene types were present in different source of *S. aureus* in patients. Noticeable calculation of the discriminatory power of the *AluI* typing method yield high power (D: 0.90) and can be interpreted with confidence is desirable.

Discussion

Molecular strain typing of microorganisms is now recognized as an essential component of infection control program (15). Meanwhile the coagulase gene amplification has been considered a simple and accurate method for typing *S. aureus*. This method is found to be technically simple with a good reproducibility and discriminatory power. Comparisons between large numbers of bacterial strains can be made within a short time; present

Table 2. Size and type of Coa bands frequency in Healthy Carriers, patients and MRSA, MSSA isolates

Band Class of Coa gene	Size of Coa gene (bp)	Healthy Carriers	Patients	*MSSA*	*MRSA*	Total
1	570±20	2(2.7%)	3(3.2%)	2(1.7%)	3(5.9%)	5(2.9%)
2	650±20	12(16%)	5(5.3%)	14(11.8%)	3(5.9%)	17(10%)
3	730±20	12(16%)	37(37.9%)	31(26.1%)	17(33.3%)	48 (28.2%)
4	800±20	37(49.3%)	36(37.9%)	54(45.4%)	19(37.3%)	73(42.9%)
5	900±20	1(1.3%)	2(2.1%)	3(2.5%)	0(0%)	3(1.8%)
6	970±20	10(13.3%)	10(10.5%)	14(11.8%)	6(11.8%)	20(11.8s %)
7	1200±20	1(1.3%)s	3(3.2%)	1(.8%)	3(5.9%)	4(2.4%)
	P value	0.01	0.01	0.1	0.1	

Table 3. Band patterns by AluI Restriction enzyme digestion of PCR-Products

Pattern Code	Band Pattern – AluI (approx bp)	Number and Percent of isolates				
		Healthy carrier	Patients	*MSSA*	*MRSA*	Total
P1	405-324-81	5(6.7%)	3(3.2%)	4(3.4%)	4(7.8%)	8(4.7%)
P2	405-324-486	1(1.3%)	(3.2%)3	1(0.8%)	3(5.9%)	4(2.4%)
P3	405-324-243	2(2.7%)	0	1(0.8%)	1(2%)	2(1.2%)
P4	405-243-162	12(16%)	(11.6%)11	16(13.4%)	7(13.7%)	23(13.5%)
P5	405-162-81	2(2.7%)	3(3.2%)	3(2.5%)	2(3.9%)	5(2.9%)
P6	486-324-162	3(4%)	(10.5%)10	9(7.6%)	4(7.8%)	13(7.6%)
P7	486-243-81	2(2.7%)	0	1(0.8%)	1(2%)	2(1.2%)
P8	405-324	12(16%)	36(37.9%)	31(26.1%)	17(33.3%)	48(28.2%)
P9	405-243	5(6.7%)	1(1.1%)	6(5%)	0	6(3.5%)
P10	405-162	2(2.7%)	3(3.2%)	2(1.7%)	3(5.9%)	5(2.9%)
P11	486-405	1(1.3%)	2(2.1%)	3 (2.5%)	0	3(1.8%)
P12	486-324	2(2.7%)	3(3.2%)	2(1.7%)	3(5.9%)	5(2.9%)
P13	486-162	2(2.7%)	1(1.1%)	2(1.7%)	1(2%)	3(1.8%)
P14	648-162	0	3(3.2%)	2(1.7%)	1(2%)	3(1.8%)
P15	972	5(6.7%)	0	4(3.4%)	1(2%)	5(2.9%)
P16	810	16(21.3%)	16(16.8%)	29(24.4%)	3(5.9%)	32(18.8%)
P17	648	3(4%)	0	3(2.5%)	0	3(1.8%)
P value		0.005	0.005	0.1	0.1	

Table 4. Distribution of AluI PCR RFLP patterns among Coa gene bands

Band Class of Coa gene	Size of Coa band	AluI Pattern code	Number
4	20 ± 800	P16	32
		P14	3
		P12	5
		P7	2
		P4	23
		P1	8
2	20 ±650	P17	3
		P13	3
		P9	6
		P5	5
6	970 ± 20	P15	5
		P6	13
		P3	2
7	1200 ± 20	P2	4
5	900 ± 20	P11	3
3	730 ± 20	P8	48
1	570 ± 20	P10	5

method is suitable for general clinical setting in the hospital and for epidemiological investigations of *S. aureus* (11). Some researchers carried out similar studies on Coa gene products and RFLP patterns using *AluI* for *S .aureuas* isolates and demonstrated the utility of RFLP typing in epidemiological investigation of *S. aureus* infection in hospital (4, 5, 11, 16, 17, 18and19). In our study among 170 *S. aureus* isolates distinguished 7 PCR products of approximately 570-1200±20 bp. Since, the numerical index of discrimination based on PCR product sizes were 0.70, which is less for

typing based on PCR product size, can't be used alone. Also we found that no discriminative bands or specific type for diagnosis of healthy carrier or patient. This point was observed in *MSSA* and *MRSA* strains. These PCR products were further subjected to RFLP analysis with *AluI* digestion. The only remarkable item observation is that the Coa gene type II (650±20 bp) is significantly present in *S. aureus* isolated from healthy carriers more than the patients, also it was observed that the type III Coa gene (730±20) in strains isolated from patients in particular are more than carriers. Also 170 *S. aureus* strains were sub typed by PCR-RFLP patterns, as a whole the results obtained from this study indicated that 17 different types were present in our investigation. Types 8-14 showing two bands RFLP pattern (with one restriction site) accounted for 74 (43.5%) of total population; types 1-7 with three bands RFLP pattern (with 2 different restriction sites) 56(33. %); types 15-17 with single band RFLP patterns accounted for 41(23.5%) probably indicates the absence of *AluI* restriction sites amongst these isolates. These results explain a considerable heterogeneity in the Coa gene of the strains isolated in Gorgan, north of Iran. With this method can clearly classify *S.aureus* types consisting of either patients or healthy carrier and there is statistically meaningful difference between the PCR-RFLP pattern among *S.aureus* isolated from patients or healthy carrier ($P< 0.05$).

In a similar study Goh *et al*. (1992) classified 19 distinct groups out of 69 *S. aureus* isolates on the basis of coagulase PCR amplified gene products and unique *AluI* RFLP profiles (20). Studies by Tiwari *et al*. (2008) and Himabindu *et al*. (2009) from Indian indicated greater variability of RFLP models of Coa gene (33 and 31types*) S. aureus* isolated from patients (11, 13). This variation is relevant to the bacterial pool, hard management and environmental conditions in each geographical region. It was also concluded coagulase genotype 4 and PCR-RFLP pattern 8 were predominant in patients and healthy carriers. Band class 2 and its *AluI* subtypes of the already mention, it became clear that: subtypes 17 and 9 significantly were higher in carrier isolates than patients and none of them were not existed in *MRSA* strains. Considering type17 and type9 in class 2, raises the possibility that some *S. aureus* isolates are despite the host's body but they can't intervene in the disease or eliminated by the body quickly. Result stable 4showsthattype4 Coa is probably the oldest type which is available in this area, because this latter type is divided 6differenttyping, but the type 3 which is the second most common type in the area. This is a newer type in evolution and all isolates of this type has one subtype. Our result showed that type distribution in carriers and *MSSA* isolates were more than other groups.

Conclusion

This method proved to be useful, rapid and efficient for typing *S. aureus* strains isolated from clinical samples. There with this method could clearly classify *S. aureus* types consisting of either patients or healthy carrier. The results of this study showed that Coa marker and *AluI* subtypes could also be used to determine the evolutionary pattern of *S. aureus*. However, it should be noted that the carboxyl end of the coagulase encoded by this repetitive region did not appear to be required for its prothrombin–activating function. Also the information generated in this study could be useful to develop an efficient control measure.

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References

1. *Pesavento G, Ducci B, Comodo N, Nostro AL. Antimicrobial resistance profile of Staphylococcus aureus isolated from raw meat: a research for methicillin resistant Staphylococcus aureus (MRSA). Food Control 2007; 18: 196–200.*

2. *Skov R, Smyth R, Larsen AR, Bolmstrom A, Karlsson A. Phenotypic detection of Methicillin Resistance in staphylococcus aureus by disk diffusion testing and Etest on Muller Hinton agar . Journal of clinical microbiology 2006; 44: 4395-4399.*

3. *Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S. Comparison of traditional and molecular methods of typing isolates of Staphylococcus aureus. Journal of clinical microbiology 1994; 32: 407-415.*

4. *Watanabe S, Ito T, Sasaki T, Li S, Uchiyama I, Kishi K, Kikuchi K, Skov RL, Hiramatso K. Genetic diversity of Staphylocoagulase Genes(Coa):Insight in to the Evolution of Variable Chromosomal Virulence Factors in Staphylococcus aureus . Ploseone 2009; 4(5): 1-11.*

5. *Janwithayanuchit I, Ngam-Ululert S, Paungmoung P, Rangsipanurant W. Epidemiological study of Methicilin – Resistant Staphylococcus aureus by coagulase gene polymorphism. Science Asia 2006; 32: 127-132.*

6. *Rahimi Alang S, Amini A, Cheraghali F, Tabbaraei A, Ghaemi EA. The Frequency of MRSA carriers in Health care workers in Gorgan, North of Iran. HealthMED 2011; 5(6): 1885-1890.*

7. *Samadi N, Alvandi M, Fazeli MR, Azizi E, Mehrgan H, Naseri M. PCR-based detection of low levels of Staphylococcus aureus contamination in pharmaceutical preparations. Journal of Biological Sciences 2007; 7(2): 359–363.*

8. *Louie L, Good Fellow J, Mathieu P, Glatt A, Louie M, Simor AE. Rapid detection of Methicillin-Resistant staphylococci from blood culture bottles by using a multiplex PCR assay. Journal of Clinical Microbiology 2002; 40(8): 2786–2790.*

9. *Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of Methicillin Resistance in Staphylococcus aureus. Journal of Clinical Microbiology 2000; 38(6): 2170–2173.*

10. *Vaez H, Tabaraei A, Moradi A, Ghaemi EA. Evaluation of methicillin resistance Staphylococcus aureus isolated from patients in Golestan province-North of Iran. African Journal of Microbiology Research 2011; 5(4): pp. 432-436,*

11. *Himabindu M, Sugapthamiriya Muthamilselvan D, Verma S. Molecular analysis of coagulase gene polymorphism in clinical isolates of Methicillin Resistant staphylococcus aureus by restriction fragment length polymorphism based genotyping. American Journal Of Infectious Diseases 2009; 5(2): 170-176.*

12. *Ram Undo O, Dighton M, Capstick J, Gerraty N. Molecular typing of staphylococcus aureus of bovine origin by polymorphisms of the coagulase gene. Vet Microbiol 1999; 66: 275-284.*

13. *Tiwari HK, Sapkota D, Gaur A, Mathuria JP, Singh A, Sen MR. Molecular typing of clinical staphylococcus aureus isolates from Northern India using coagulase gene PCR-RFLP. Southeast Asian J Trop Med Public Health 2008; 39: 467-473.*

14. *Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpsons index of diversity. journal of clinical microbiology 1988; 26: 2465-2466.*

15. *Laplana LM, Cepero MA, Ruiz J, Zolezzi PC, Calvo MA, Erazo MC. Molecular typing of Staphylococcus aureus clinical isolates by pulsed-field gel electrophoresis, Staphylococcal cassette chromosome mec type determination and dissemination of antibiotic resistance genes. Int J Antimicrob Agents 2007; 30: 505-513.*

16. *Hookey JV, Richardson JF, Cookson BD. Molecular typing of Staphylococcus aureus based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. Journal of clinical microbiology 1998; 36: 1083-1089.*

17. *Shopsin B, Gomez M, Montgomery S, Smith Waddington D, Kreiswirth N, Riehmann M. Use of coagulase gene (Coa) Repeat Region Nucleotide Sequence for typing of Methicillin–Resistant Staphylococcus aureus Strains. journal of clinical microbiology 2000; 38(9): 3556-3563.*

18. *Udo EE, AL-Sweih N, Mohana Krishnan S, West PWJ. Antibacterial resistance and molecular typing of Methicillin-Resistan Staphylococcus aureus in a Kuwaiti general hospital. Med PrincPract 2006; 15: 39-45.*

19. *Sanjiv K, Kataria AK, Sharma R, Singh G. Epidemiological typing of Staphylococcus aureus by DNA Restriction Fragment Length Polymorphism. Vet. arhiv 2008; 78(1): 31-38.*

20. *Goh SH, Byrne SK, Zhang JL, Chow AW. Molecular Typing of Staphylococcus aureus on the Basis of coagulase Gene Polymorphism. Journal of clinical microbiology 1992; 30(7): 1642-1645.*

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Advanced adenoid cystic carcinoma of the Parotid gland treated with surgery, chemotherapy and targeted therapy: A case report and literature review

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Abstract

Adenoid cystic carcinoma (ACC) is a type of highly aggressive malignant tumor which often occurs in the salivary glands. Recurrence of the tumor will generally be accompanied by metastases to major organs such as the lungs. Chemotherapy or local radiotherapy is often used as the main type of treatment for advanced ACC. In recent years, targeted therapy has emerged as a promising treatment for this disease, utilizing medication that can effectively inhibit the proliferation, infiltration and metastasis of the tumor by blocking abnormal signal transduction pathways in cells. In this paper, we review a case study of the treatment for advanced ACC of the parotid gland and suggest an alternative treatment plan based on these results as well as from reviews of recent literature.

Key words: Adenoid cystic carcinoma, parotid gland, targeted therapy, epidermal growth factor receptor, tumor angiogenesis.

Introduction

Adenoid cystic carcinoma (ACC) is a type of malignant tumor that typically occurs in the head and neck, particularly in the salivary glands. This type of carcinoma is highly aggressive and spreads along neurovascular bundles, thereby giving cancer cells access to distant organs such as the lungs, liver, brain or bones via bloodstream. The most common symptoms of ACC are the presence of slow-growing masses and pain (H Spiro R, 1997). At present, the standard treatment for ACC is surgical resection followed by adjuvant radiotherapy. For recurrence or metastasis of advanced ACC of the salivary gland, current effective treatments are lacking and the efficacy of chemotherapy molecular targeted therapy has not been confirmed (Khafif A et al, 2005; Laurie SA et al, 2011).

In this paper, we present the detailed clinical data of one case of ACC of the parotid gland. Recurrence was present post-surgery and the patient showed no response to combination chemotherapy. Targeted therapy with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) and anti-angiogenesis played a palliative role. Symptomatic and supportive treatments were also provided. The clinical data of this case indicate that with effective treatments, patients with advanced ACC can achieve longer survival and higher quality of life for a certain period.

Case report

The patient was a 46 year-old male who underwent surgery for a malignant parotid tumor at Zhejiang Provincial People's Hospital in December 2006. The tumor was first discovered as a mass in the left parotid gland. The postoperative pathological analysis revealed "Malignant tumor of the left parotid gland, with the size of 1.2 cm × 1 cm × 0.8 cm; Adenoid cystic carcinoma of the left parotid gland; Nerve invasion exists, no vessel invasion, the surrounding parotid gland tissue has been infiltrated by the tumor cells, and no lymph node involvement." In February 2008, the patient was diagnosed with multiple pulmonary metastases from adenoid cystic carcinoma at Zhejiang Cancer Hospital and was given two cycles of docetaxel (DTX), cisplatin (DDP), 5-Fluorouracil (5-FU)-based chemotherapy and 6 cycles of chemotherapy with Gemcitabine and Nedaplatin. The lung masses noted on the chest radiograph did not significantly reduce after chemotherapy. The patient experienced worsening tachypnea and dull pain in the chest and back. Between May 2009 and February 2010, the patient received targeted therapy by daily oral administration of 0.25 g gefitinib tablets. The follow-up examination

at 3 months after the start of this targeted therapy showed that the pulmonary lesions remained stable (Figure 1). During the gefitinib administration period, the symptoms, including tachypnea and cough, were relieved and the pulmonary lesions were stable. In February 2010, the patient suffered from worsening chest pain accompanied by tachypnea and leg swelling. Results of a CT scan demonstrated significant progression of the pulmonary lesions. Therefore, gefitinib was withdrawn. Following this, an alternative antitumor medicine sunitinib was administered orally beginning in February 2010. During this treatment period, symptoms like tachypnea, fatigue and dyspnea were slightly relieved, but the patient's blood pressure was significantly increased. The highest recorded blood pressure was 200/110 mmHg. Therefore, the combination treatment with losartan potassium and hydrochlorothiazide tablets and nifedipine extended-release tablets was provided to control blood pressure. The patient experienced a gradual worsening of the disease and needed continuous oxygen inhalation. At this point, the fentanyl transdermal system (skin patches) was used for cancer-related pain relief. In July 2010, the patient died due to multiple organ failure as a result of disease progression. Before the introduction of targeted therapy, detection of the mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene showed an EGFR exon 19 deletion (2235-2250Del). The serum level of vascular endothelial growth factor (VEGF) suggested that VEGF expression was low (251.18pg/ml).

Discussion

Currently, a standard treatment regimen for progressive advanced adenoid cystic carcinoma (ACC) has not been established. At present, systemic chemotherapy treatment and targeted therapy are used as alternative methods to treat ACC. However, since ACC is a neoplasm with a low malignant potential, there has not been an agreement as to whether patients with ACC benefit from chemotherapy. The literature shows varying degrees of efficacy of chemotherapy with different drugs or different combinations to treat ACC. Anthracyclines

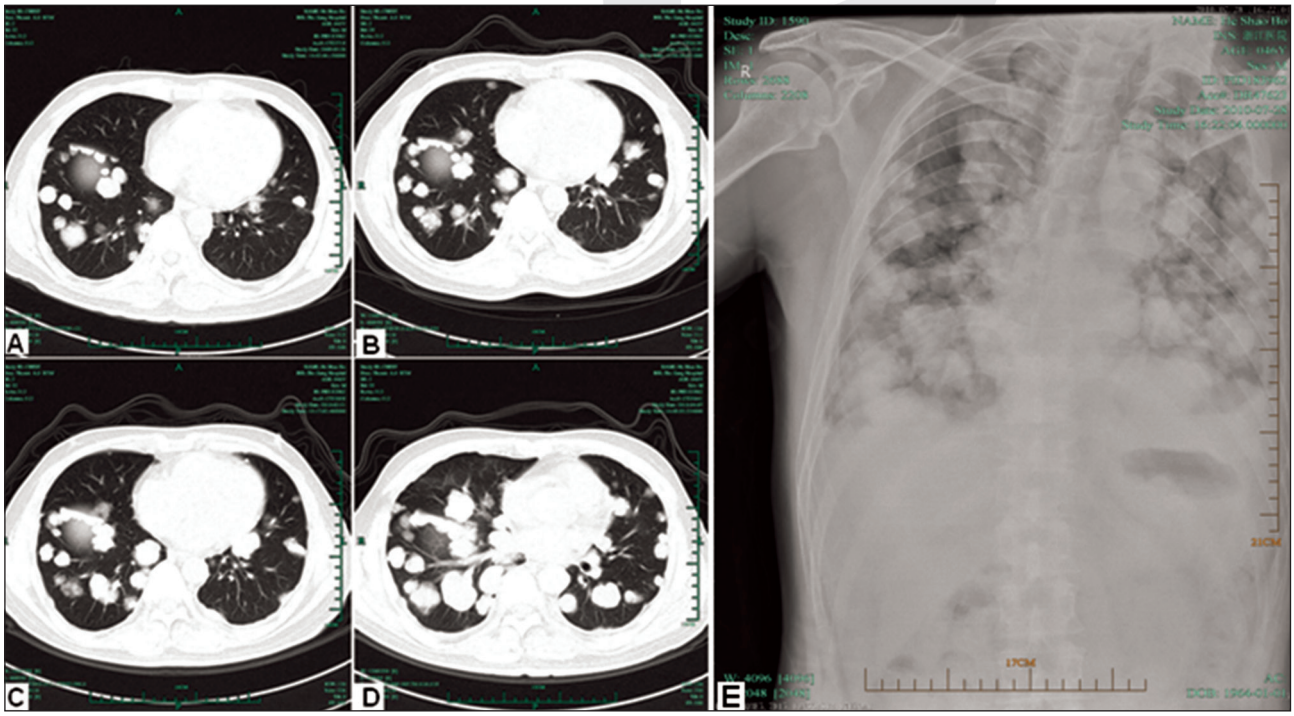

Figure 1. The chest CT scan and radiograph of the patient with advanced ACC. A. Chest CT scan performed in May 2009, prior to gefitinib administration, showing multiple pulmonary metastases. B. Chest CT scan in December 2009, at 7 months after the start of gefitinib administration. C. Chest CT scan in February 2010, at 10 months after the start of gefitinib administration. D. Chest CT scan in April 2010, at 2 months after the start of sunitinib administration. E. Chest radiograph in July 2010, at 5 months after the start of sunitinib administration, showing progression of the pulmonary lesions

including cisplatin and 5-FU rank among the most effective antitumor drugs, with the efficacy rate of combination therapies vary from 25% to 30%(Ross PJ et al,2009). Till et al. (Till BG et al,2009) reported that paclitaxel (Taxol) was effective in the treatment of 2 consecutive patients suffering from ACC with pulmonary metastasis. Some researchers have also reported that gemcitabine monotherapy was well tolerated by patients with ACC, but the efficacy was not significant (van Herpen CM et al,2008). Overall, there is a lot of variation regarding the efficacy of different drug combinations and therapies to treat ACC and currently there is no standard procedure or treatment plan.

One study demonstrated that overexpression of EGFR and ErbB2 is to some extent associated with the aggressiveness and poor prognosis of ACC (Bell D et al,2010). Due to this association, some researchers recommended that EGFR and ErbB2 be used as specific molecular targets. When EGFR and ErbB2 overexpression is inhibited by administration of drugs, the growth and metastasis of ACC will be also inhibited.

In some patients with non-small cell lung cancer (NSCLC), mutations have been found in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene. Somatic mutations in the EGFR kinase domain are found in NSCLC, particularly in lung adenocarcinomas and are associated with sensitivity to TKI (point mutations G719A/C in exon 18, L858R and L861Q in exon 21 and in-frame deletions at position 745 of exon 19). This suggests that EGFR tyrosine kinase inhibitors (EGFR-TKI) may significantly improve treatment efficacy in these patients. However, some researchers have reported that the frequency of EGFR-TK mutations is low in patients with ACC. In one study, allele-specific PCR was used to detect the mutations in EGFR exons19-21 in 25 malignant salivary tumor samples and found that mutations in the TK domain of the EGFR gene were not common. Only two samples showed exon 19 deletions (c.2235_2249del15), one of which was an ACC sample. No mutations at other genetic loci were detected in that study(Dahse R et a, 2008).

The effectiveness of EGFR-TKI in the treatment of NSCLC can be attributed to the activating mutations at corresponding genetic loci, while the mechanism of action for the parotid gland tumor is quite different. In one study, a total of 65 parotid gland tumor samples with confirmed histopathological types were used for the detection of common EGFR mutations. Comprehensive sequence analysis indicated that the frequency of EGFR gene mutations was low. EGFR exon 19 mutations were only detected in one mucoepidermoid carcinoma of the parotid gland samples and one ACC of the parotid gland samples, while no other EGFR-related mutations were detected(Dahse R et a,2009). In this study, genetic mutation detection of the tumor samples also demonstrated that EGFR exon 19 mutations occurred. In this case, the patient received gefitinib, an EGFR-TKI, for one year, at which point the pulmonary lesions and symptoms of tachypnea and cough were stable. During this treatment period, the patient achieved stable disease status in an efficacy assessment.

In gastrointestinal stromal tumors (GIST), a mutated form of KIT protein is generally expressed. KIT is a c-kit pro-oncogene protein and is a transmembrane receptor tyrosine kinase (RTK), playing a role in the control of cell growth, differentiation and migration during tumor development. Researchers have reported that the expression rate of KIT protein reached up to 89% in ACC. Imatinib mesylate can inhibit c-kit RTK activity and has been considered a promising drug in the treatment of ACC. Alcedo et al. reported two cases of ACC that were sensitive to imatinib therapy, however, two subsequent Phase II clinical trials on the effect of imatinib in the management of advanced ACC demonstrated no efficacy of imatinib on the progression of the tumor. Therefore, further studies are needed to determine if imatinib is an appropriate pharmacological option for the treatment of ACC(Alcedo JC et al,2004).

Tumor angiogenesis is associated with the growth, invasion and metastasis of tumors. Many studies have reported that vascular endothelial growth factor (VEGF) plays an important role in the invasion and metastasis of tumors. The association between VEGF expression and tumor angiogenesis has been clearly defined. Vascular endothelial growth factor can increase microvascular permeability, stimulate the division and proliferation of endothelial cell of different sources, promote angiogenesis, and induce endothelial cell migration(Younes MN et al,2006). Adenoid cystic carcinoma is considered a slow-grow-

ing tumor which tends to recur locally and spread along neurovascular bundles with a significant propensity for distant metastasis. Sunitinib is a VEGF inhibitor with multiple targets that has been shown to be an active agent for the treatment of multiple tumors including kidney cancer and GIST(Demetri GD et al,2006; Motzer RJ et al,2006). During treatment of ACC, sunitinib exhibited antitumor activity to some extent in this case. Reports have shown that low levels of serum VEGF would contribute to the antitumor activity of sunitinib(Porta C et al,2010). In this case, in order to predict the treatment outcome, serum levels of VEGF were detected before administration, with low levels of serum VEGF suggesting the possibility of treatment efficacy.

For advanced ACC of the parotid gland with pulmonary metastasis after surgical management and either chemotherapy or radiotherapy, there are few options for further treatment. In addition to the metastasis-related targets of the tumor cells such as EGFR, c-kit and VEGF as the potential targets for therapy, other signal pathway targets on the genetic mutations present in advanced ACC could serve as possible promising targets for ACC therapy in the future.

The use improved therapeutic targeting agents in sequential or combined treatment paradigms would enhance tumor control and overall survival. This would not only improve the patient outcome, but would make the treatment more efficient for all stakeholders involved in the patients care. Based on the successful outcomes in this case, we feel that identifying prediction of efficacy before treatment, including sequential treatment with EGFR-TKI and the use of multi-targeting of anti-angiogenic agents will enhance the treatment of advanced AAC.

References

1. *H Spiro R. Distant metastasis in adenoid cystic carcinoma of salivary origin. The American journal of surgery. 1997; 174: 495-498.*
2. *Khafif A, Anavi Y, Haviv J et al. Adenoid cystic carcinoma of the salivary glands: a 20-year review with long-term follow-up. Ear Nose Throat J. 2005; 84: 662, 664-667.*
3. *Laurie SA, Ho AL, Fury MG et al. Systemic therapy in the management of metastatic or locally recurrent adenoid cystic carcinoma of the salivary glands: a systematic review. Lancet Oncol. 2011; 12: 815-824.*
4. *Ross PJ, Teoh EM, A'Hern R P et al. Epirubicin, cisplatin and protracted venous infusion 5-Fluorouracil chemotherapy for advanced salivary adenoid cystic carcinoma. Clin Oncol (R Coll Radiol). 2009; 21: 311-314.*
5. *Till BG, Martins RG. Response to paclitaxel in adenoid cystic carcinoma of the salivary glands. Head Neck. 2009; 30: 810-814.*
6. *Van Herpen CM, Locati LD, Buter J et al. Phase II study on gemcitabine in recurrent and/or metastatic adenoid cystic carcinoma of the head and neck (EORTC 24982). Eur J Cancer. 2008; 44: 2542-2545.*
7. *Bell D, Roberts D, Kies M et al. Cell type-dependent biomarker expression in adenoid cystic carcinoma: biologic and therapeutic implications. Cancer. 2010; 116: 5749-5756.*
8. *Dahse R, Kosmehl H. Detection of drug-sensitizing EGFR exon 19 deletion mutations in salivary gland carcinoma. Br J Cancer. 2008; 99: 90-92.*
9. *Dahse R, Driemel O, Schwarz S et al. Epidermal growth factor receptor kinase domain mutations are rare in salivary gland carcinomas. Br J Cancer. 2009; 100: 623-625.*
10. *Alcedo JC, Fabrega JM, Arosemena JR et al. Imatinib mesylate as treatment for adenoid cystic carcinoma of the salivary glands: report of two successfully treated cases. Head Neck. 2004; 26: 829-831.*
11. *Younes MN, Park YW, Yazici YD et al. Concomitant inhibition of epidermal growth factor and vascular endothelial growth factor receptor tyrosine kinases reduces growth and metastasis of human salivary adenoid cystic carcinoma in an orthotopic nude mouse model. Mol Cancer Ther. 2006; 5: 2696-2705.*
12. *Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastroint. 2006; 368: 1329-1338.*
13. *Motzer RJ, Michaelson MD, Redman BG et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006; 24: 16-24.*
14. *Porta C, Paglino C, De Amici M et al. Predictive value of baseline serum vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in advanced kidney cancer patients receiving sunitinib. Kidney Int. 2010; 77: 809-815.*

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Concomitance of acute pancreatitis and thrombocytopenia due to valproic acid intoxication: A rare case report

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Abstract

Background: Acute intoxication of VPA, which is a antiepileptic drug, may be a life-threatening condition that can result in progressive coma, acute pancreatitis, pancytopenia caused by bone marrow depression. Although a few cases have been reported about thrombocytopenia relation with VPA intoxication, there are no case report about coexistence of acute pancreatitis and thrombocytopenia.

Case report: We report a 24 year-old female patient with acute pancreatitis and thrombocytopenia induced by VPA overdose who presented with coma. Serum valproate concentration was 701 μg/ml. The laboratory abnormalities showed thrombocytopenia and increased amylase. Hyperhydration and alkalinization for enhanced renal clearance were started. To control cerebral edema, she was given carnitine 60 mg/kg. Then, hemodialysis was performed to the patients. The patient's clinical and laboratory findings completely recovered at follow-up.

Conclusions: Pancreatitis is a serious complication of VPA that should be suspected in a patients using VPA, presenting with coma.

Key words: Valproic acid, intoxication, thrombocytopenia, acute pancreatitis, hemodialysis, carnitine.

Introduction

Valproic acid (VPA), a antiepileptic drug, is increasingly used in bipolar affective disorders, schizoaffective disorders, schizophrenia and migraine prophylaxis (1). It is usually well tolerated. However, VPA intoxication with suicide attempt is a relatively serious clinical problems that can result in progressive coma, respiratory failure, renal failure, acute pancreatitis, hepatotoxicity, pancytopenia caused by bone marrow depression, hemodynamic instability and death (2). Toxic effects of VPA are usually associated with daily doses above 1800 mg and blood levels above 100 μg/ml (3).

VPA, which is metabolised in the liver, is excreted by the kidneys. Therapeutic serum levels of VPA is 50 to 100 μg/ml . It has been shown that VPA has a protein binding of 90%–95% at therapeutic levels (4). Thus, at the therapeutic serum levels, drug removal obtained through hemodialysis is negligible. However, it has been suggested that hemodialysis can be effective as protein binding may become saturated at high VPA levels. Indeed, some studies mention successful treatment with hemodialysis (5). In VPA intoxication with suicide attempt, the preferred treatment is carnitine supplementation (6), gastric lavage, administration of laxatives and multiple-dose activated charcoal (7), hyperhydration, forced diuresis and extracorporeal removal (5).

Only a few cases have been reported about acute pancreatitis association with VPA intoxication in a few adults and in some children (8-10). Most of the cases report previously described presented with acute pancreatitis in the initial stage. However, the clinical course of our patient, was different from those previously described, suggesting the presence of coma related to VPA. In addition, to our knowledge, only a few cases have been reported about thrombocytopenia associated with VPA (11,12). However, there are no case report about coexistence of acute pancreatitis and thrombocytopenia due to VPA intoxication in the medical literature.

Therefore, we want to case report a 24 year-old female patient with acute pancreatitis along with thrombocytopenia induced by VPA overdose who presented with coma to emergency department of our hospital. The patient was successfully treated with hemodialysis.

Case report

A 24-year-old female patient with a history of epilepsy was admitted to the emergency room department of our hospital 3 hr after ingestion 20000 mg enteric-coated VPA tablets. She had sore throat, fever and headache 3 days before admission. She had a history of well controlled epilepsy, which was treated with 1 g of VPA once daily.

On pyhsical examination, she was stuporous (Glasgow coma score 3) with isocoric pupils and a sluggish light reflex. Immediately after admission, she was intubated for airway protection and connected to mechanical ventilation. At that time, the patient's blood pressure was 120/70 mm Hg, pulse rate 78/min, respiratory rate 26 per minute, and body temperature 36.8°C. A Levin tube was inserted, activated charcoal to reduce absorbsion and to intercept the enterohepatic cycle was administered at a dose of 1 g/kg. The patient was transferred to the intensive care unit for further treatment.

Arterial blood gases in room air showed pH: 7.19, PaCO2: 23.0 mm Hg, PaO2: 76 mm Hg, base excess: 19 mmol/L, HCO3: 8.2 mmol/L, and SpO2: 92.0%. Her blood ammonia level was 30.92 μg/ml on admission (normal range: 20–85 μg/dl). The serum VPA concentration was 701 μg/ml on admission (therapeutic range 50 to 100 μg/ml).

The laboratory investigations showed a hemoglobin of 14.2 mmol/l (normal: 8.5–11.0 mmol/l), white blood cell count $21.5x10^3$ (neutrophil 83.8%) (normal: $3.5–11.0x10^9$/l) and thrombocytes $100x10^9$/l (normal: $150–400x10^9$/l). Serum amylase level was 286 U/l (28–100 U/l). The serum lipase level was 22.6 U/l. The C-reactive protein level was 12.9 mg/dl. Prothrombin time was 16.5 seconds.

Blood urea levels (73.9 mg/dL; normal range: 0-50 mg/dL), creatinine (2.41 mg/dL; normal range: 0.7-1.3 mg/dL), aspartate aminotransferase (86,9 I/U; normal range: 0-31 I/U), alanine aminotransferase (55,3 I/U; normal range: 0-41 I/U), sodium (152 mmol/L; normal range 136-145 mmol/L) levels were increased. Serum glucose, total bilirubin, total protein, albümin, potasium and phosphorus levels were normal ranges.

An abdominal ultrasound showed an enlarged pancreas with a homogeneous aspect. She had no history of pancreatitis. Based on clinical presentation, abdominal ultrasound and elevated serum lipase, the diagnosis pancreatitis was made.

Hyperhydration and alkalinization for enhanced renal clearance were started. To control cerebral edema, she was given L-carnitine 60 mg/kg per day intravenously in 3 doses and a lactulose enema every 3 hr. Then, hemodialysis was started to the patients.

Hemodialysis was started 3 hr after admission and was performed for 4 hr. During this hemodialysis session, the blood flow rate was 300 ml/min, and dialysate flow rate was 500 ml/min. Nine hours after admission, her mental status improved to drowsy. After one session of hemodialysis, her serum VPA level (reference range, 50-100 μg/mL), which was 701 μg/mL on admission, had fallen to 398 μg/mL. After a second round of hemodialysis on the second day, her serum VPA level was 84.1 μg/mL. On the third day of hospitalization, her serum VPA level was 38.9 μg/mL.

Clearance of VPA with hemodialysis is more effective than with hemoperfusion, but hemoperfusion is generally better tolerated in hemodynamically unstable patients (13). However, the hemoperfusion cartridge is not readily available in our institution, so we applied hemodialysis.

The patients was comatose for 2 days and extubated in 3th day. The patient's clinical and laboratory findings began to improve at follow-up. The patient's general medical condition had improved by the 5th day of hospitalization. After normalization of blood tests for acute pancreatitis, the patient had a full clinical recovery at follow-up. In addition, the patient remained hemodynamically stable. The patient was discharged from the internal medicine service without sequelae 10 days after admission.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989. The patient was informed about the study protocol and the written consent was obtained from patients.

Discussion

This is the first reported case coexistence of acute pancreatitis and thrombocytopenia after severe VPA intoxication, which fully recovered after normalization of VPA levels.

VPA is commonly prescribed as an anticonvulsive. Also, VPA has widely been used in the treatment of bipolar affective disorders, schizoaffective disorders, schizophrenia, and migraine prophylaxis. The drug is well tolerated by most people. The most common side effects associated with VPA therapy are transient nausea, vomiting, abdominal cramps, and diarrhea. Acute VPA intoxication is a life-threatening clinical problem. More serious adverse reactions can occur in multiple organ systems, including the liver, kidneys, brain, heart and bone marrow. Common adverse reactions include leucopenia or thrombocytopenia caused by bone marrow depression (14). To our knowledge, only a few cases have been reported about thrombocytopenia associated with VPA (11, 12). In an study, Thanacoody (11) reported the case of thrombocytopenia in a 76-year-old woman patients receiving chronic VPA. In addition, Auinger et al. (12) reported the case of pancytopenia associated with the VPA overdose in a 19-year-old man patient. Hovewer, in these case report, there was no evidence of acute pancreatitis associated with the VPA intoxication. In our patient, thrombocytopenia along with acute pancreatitis associated with the VPA overdose was observed. The patient's thrombocyt count began to improve at follow-up.

Drug-induced disorders may be observed in any organ such as liver and pancreas. Acute pancreatitis has been related to a number of drugs, including VPA. To our knowledge, only a few cases have been reported about acute pancreatitis association with VPA intoxication in the literature (10-12). Most patients treated with VPA had normal or slightly elevated serum amylase values (8). Serum lipase is mainly secreted by the pancreas. It has been considered serum lipase may to be more specific for pancreatitis than amylase and remains elevated for a longer period (15). Routine measurements of serum amylase and lipase during VPA use are not recommended (8). Our patients had clinical findings of acute pancreatitis induced by VPA overdose who presented with coma. We detected increased serum amylase levels along with increased serum lipase levels in our patients. After normalization of blood tests for acute pancreatitis, the patient had a full clinical recovery at follow-up.

The link between administering VPA and pancreatitis has been well documented (10-12). However, the mechanism of pancreatitis associated with VPA therapy is not clearly understood. One possible cause is that the drug has a direct toxic effect due to the free radicals (8). Recent studies deal with pancreatitis have focused on the role of oxidative stress as an etiological mechanism . Moreover, it was recently shown that free radical-mediated capillary endothelial injury contributes to the early stage of acute pancreatitis. It has been suggested that the depletion of carnitine due to VPA may also have played an important part in pancreatic injury . Drugs clearly can be a source of such oxidative stress. VPA specifically has been suggested to reduce free radical scavenger enzyme activity (16). Besides, acute pancreatitis induced by VPA may be due to some form of an idiosyncratic complication. In addition, it was shown that the protein binding of VPA may alter due to the patient's age, dose of treatment and concomitant medication (5,8).

Management of acute VPA intoxication is mainly supportive care and close attention to the airway. There is no specific antidote or guidelines for managing VPA toxicity. Some reports suggest the efficacy of L-carnitine, but the studies are insufficient to make strong conclusions. L-Carnitine is recommended for the hyperammonemia caused by VPA toxicity. Besides, it has been suggested that carnitine supplementation could hasten resolution of coma, prevent development of hepatic dysfunction (17). Various extracorporeal techniques for managing VPA toxicity have been described, but none has prevailed as standard therapy . At higher concentrations, protein-binding sites become saturated and there is more free VPA available for removal by extracorporeal means (18). Some studies have shown succesful extracorporeal removal of VPA by hemodialysis (5). Minville et al. (19) reported a case of severe VPA overdose in a 36-year-old man. They suggested that hemodialysis and L-carnitine therapy were decreased the high serum VPA concentration. Hyperhydration and alkalinization for enhanced renal clearance were started to our patients. To control cerebral edema, she was given carnitine 60 mg/kg. Finally, hemodialysis was performed to the patients.

Symptoms of VPA intoxication are related to the VPA plasma concentration. Plasma concentrations of less than 450 mg/l produce limited toxicity, such as mild central nervous system depression, with be-

nign outcomes. Severe intoxication (concentration >850 mg/l) is at greater risk of serious or life-threatening clinical effects such as respiratory depression, hypotension, metabolic acidosis, and also coma (8). In addition, cerebral edema can develop in patients after 48–72 hr ingestion.

The patient's VPA serum level was 701 μg/ml on admission and dropped to 38,9 μg/ml the following day. In our patient, there were no factors involved in the onset of pancreatitis other than the administration of VPA. The patient's clinical and laboratory findings began to improve at follow-up. The patient's general medical condition had improved by the 5th day of hospitalization. Then, the patient was discharged from the hospital without complication 10 days after admission.

Acute pancreatitis is a serious complication of VPA therapy that should be suspected in a patients using VPA, presenting with coma. In addition, hemodialysis in VPA intoxication a sensible therapeutic option with increasing efficiency when plasma concentration is high.

References

1. *Garnier R, Boudignat O, Fournier PE. Valproate poisoning. Lancet 1982; 2: 97.*
2. *Peces R, Fernandez EJ, Sanchez RJ, et al. Hemoperfusion in the treatment of acute valproic acid intoxication. Nefrologia 2007; 27(3): 370-3.*
3. *Tank JE, Palmer BF. Simultaneous "in series" hemodialysis and hemoperfusion in the management of valproic acid overdose. Am J Kidney Dis 1993; 22: 341-4.*
4. *Pinkston R, Walker LA. Multiorgan system failure caused by valproic acid toxicity. Am J Emerg Med 1997; 15: 504-6.*
5. *Franssen EJ, van Essen GG, Portman AT, et al. Valproic acid toxicokinetics: serial hemodialysis and hemoperfusion. Ther Drug Monit 1999; 21(3): 289-92.*
6. *Murakami K, Sugimoto T, Woo M, et al. Effect of L-carnitine supplementatin on acute valproate intoxication. Epilepsia 1996; 37: 687–9.*
7. *Farrar HC, Herold DA, Reed MD. Acute valproic acid intoxication: enhanced drug clearance with oral-activated charcoal. Crit Care Med 1993; 21: 299–301*
8. *Pellock JM, Wilder BJ, Deaton R, et al. Acute pancreatitis coincident with valproate use: A critical review. Epilepsia 2002; 43: 1421-4.*
9. *Norgaard M, Jacobsen J, Ratanajamit C, et al. Valproic acid and risk of acute pancreatitis: A population-based case-control study. Am J Ther 2006; 13: 113-7.*
10. *Taira N, Nishi H, Mano M, et al. Pancreatitis induced by valproic acid: report of a case. Surg Today. 2001; 31(11): 1027-31.*
11. *Thanacoody HK. Chronic valproic acid intoxication: reversal by naloxone. Emerg Med J. 2007; 24(9): 677-8.*
12. *Auinger K, Müller V, Rudiger A, et al. Valproic acid intoxication imitating brain death. Am J Emerg Med. 2009 ; 27(9): 1177.e5-6.*
13. *Hicks LK, McFarlane PA. Valproic acid overdose and haemodialysis. Nephrol Dial Transplant 2001; 16: 1483-1486.*
14. *Smith FR, Boots M. Sodium valproate and bone marrow suppression. Ann Neurol 1980; 8: 197-9.*
15. *Grauso-Eby NL, Goldfarb O, Feldman-Winter LB, et al. Acute pancreatitis in children from Valproic acid: case series and review. Pediatr Neurol 2003; 28(2): 145–8.*
16. *Uden S, Acheson DW, Reeves J, et al. Antioxidants, enzyme induction, and chronic pancreatitis: a reappraisal following studies in patients on anticonvulsants. Eur J Clin Nutr 1988; 42(7): 561-9*
17. *Ishikura H, Matsuo N, Matsubara M, et al. Valproic acid overdose and L-carnitine therapy. J Anal Toxicol 1996; 20: 55-8.*
18. *Al Aly Z, Yalamonchilli P, Gonzales E. Extracorporeal management of valproic acid toxicity: A case report and review of the literature. Semin Dialysis. 2005; 18: 62–66.*
19. *Minville V, Roche Tissot C, Samii K. Haemodialysis, L-carnitine therapy and valproic acid overdose. Ann Fr Anesth Reanim 2004; 23: 357-60.*

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Exposure of students to passive inhalation of tobacco smoke

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Abstract

Introduction: The aim of this study was to determine the impact of exposure to passive smoke on the students of medical branch faculties as well as the differences in exposure between students smokers and non-smokers.

Method: The study was carried out by the methodology of the World Health Organization, Centers for Disease Control in Atlanta and The American Public Health Association (CPHA) who designed a special questionnaire to survey the prevalence of smoking among medical branch students of related faculties, i.e. Faculty of Medicine, Dentistry, Nursing and Pharmacy (Global Health Professions Student Survey - GHPS). The obtained results of the survey were analyzed using statistical methods: descriptive and nonparametric analysis method for testing the significance of differences.

Results: There is a statistically significant difference in exposure to tobacco smoke between smokers and nonsmokers in their own home, due to other people's smoking. Smokers are significantly more exposed to the impact of passive smoking in their homes. The exposure to the influence of tobacco smoke in students of faculties of medical branches who are non-smokers is higher outdoors than indoors. In smokers, the exposure outside the home is less frequent than at home and at the same time there was a statistically significant difference in the exposure to the influence of tobacco smoke between smokers and nonsmokers. Those who smoked were statistically more likely to respond that a policy of prohibiting tobacco use at their faculties does not comply or is completely absent. In addition, the respondents were generally more likely to respond that the policy of prohibiting the use of tobacco products is not being applied or is absent in comparison to those who answered that the banning policy is being implemented on their faculties.

Conclusions: Students smokers are significantly more exposed to the impact of passive smoking at home and outside. Most students said that at their faculties the policy of restriction of the use of tobacco products does not apply or is completely absent.

Key words: Medical branch faculty students, passive smoking, health risks.

Introduction

The risk of passive smoking is a very often neglected determinant of health, although the harmful effects of tobacco on the body were proved many times by a large number of research scientists in their researches(1). The association between involuntary inhalation of cigarettes and increased number of various respiratory and other diseases, either in children or in adults, is now clearly established (2). The new study puts passive smokers at more than double risk of developing coronary heart disease than earlier estimates, which were based only on studies done on non-smokers living with a partner who smokes. Very frequent, but also very severe consequences of passive smoking are various malignant diseases, which often end in death or severe complications. The negative impact of passive smoking on health is especially recognized in young people (3). When the air is tainted with cigarette smoke, young, developing lungs receive a higher concentration of inhaled toxins than do older lungs (4). Negative effect on the organism is associated with active, but significantly with passive smoking (5).

Psycho-socio-cultural specifics of the students' population (stress during the preparation of examinations, psycho-emotional problems, separation from home, the desire for group affiliation and for confirmation, the existing cultural models) are the reasons for 'taking risks' such as smoking tobacco (6). However, the target group - medical students were made the focus of this survey because they constitute a group of young adults which has shown

an increasing prevalence for smoking in the West, and they are also potential health professionals and their attitudes and views could affect future policies . Awareness and knowledge of students at medical branch faculties about the harmful effects of tobacco smoke should be determining their behavior. It is quite understandable that a population group of medical branch students avoids smoking as a form of conduct, and residence in the company of smokers as well, in order to avoid the negative effects of passive tobacco smoke consumption. The behavior, knowledge and skills of medical branch students should represent an example of healthy behavior (7).

Health Professionals are in an excellent position that allows them to have a prominent role on tobacco control (8). They are in a position to participate in the processes of disease prevention of smoking, treatment of the disease, rehab and treatment of complications caused by smoking, and they are able to work on increasing the levels of knowledge about the negative effects of tobacco on health through all levels of health care . They can intervene in order to prevent the consumption of tobacco and demand reduction measures concerning tobacco dependence and cessation as they reach a high percentage of the population through education, communication, informational campaigns that raise awareness regarding effects of tobacco on health. They have the opportunity to help people change their behavior and can give advice, guidance and answers to questions related to the consequences of tobacco use.

Another very important measure in restricting the use of tobacco products is their price as well, because the use of tobacco products correlates significantly with their availability. Apart from that, the number of cases of lung cancers, chronic obstructive pulmonary diseases and myocardial infarction are increasing since the sale of cigarettes is rising (9).The aim of this study was to determine the effects of exposure of students at medical branch faculties to secondhand tobacco smoke, as well as the differences in exposure between students smokers and non-smokers. In addition, the aim was to determine the policy of the faculties, which determines the possibilities of avoiding the exposure of students to secondhand tobacco smoke effects on the university they attend.

Working Method

The survey was conducted in accordance with the methodology of World Health Organization, Center for Disease Control in Atlanta and The Canadian Public Health Association (CPHA) who designed the special questionnaire to survey the prevalence of smoking among the students at medical branch faculties (Faculty of Medicine, Dentistry, Pharmacy and Nursing (Global Health Professions Student Survey - GHPS). The questionnaire was answered anonymously, with no identification data about examinees, and answers were entered on a special form. In Montenegro the survey was conducted between October 2010 and March 2011.

The research sample included all students from all health care faculties (Medical, Nursing, Dentistry and Pharmacy faculty). Since the lectures are mandatory the survey was done in class by years of study and at faculties. Faculty response rate was 100%, while the student response rate was 97%, because some students from a variety of reasons, were absent from classes in the days when the research was done.

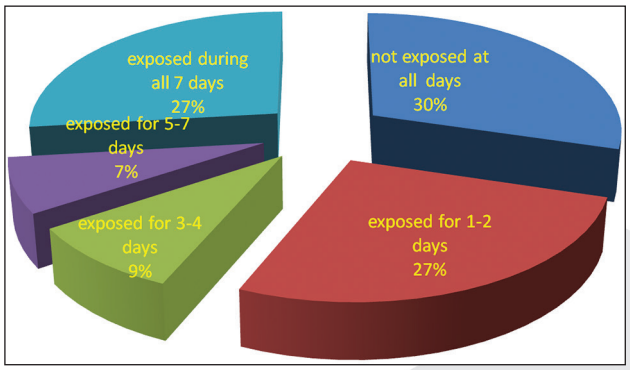
Pilot survey of group that consisted of 20 medical students was conducted before the complete survey started. It was conducted in order to test the time necessary to enter answers and the accuracy of answers. Testing and pretesting were supervised by specially educated coordinators from the Institute of Public Health.

The obtained results of survey were analyzed using statistical methods: descriptive and nonparametric analysis method for testing significance of differences, used to examine the significance of differences in attitudes and behavior generally among the examined students smokers and non-smokers, and their exposure to secondhand smoke.

Results

From a total of 822 examined students, one third of them said that during the last seven days not once were they exposed to tobacco smoke in their own home due to people smoking in their environment. One fifth of the total number of subjects were exposed to this influence for one to two days, and nearly a third of respondents were exposed to tobacco smoke during all seven days,

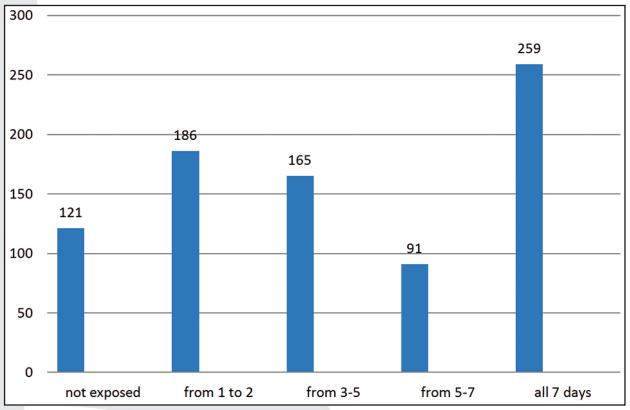
due to smoking of other people in their environment (*Graphic1*).



Graphic 1. The exposure to the effects of tobacco smoke in their own home due to other people smoking

From the following table 1 it can be concluded that there was a statistically significant difference (X 2 = 92 547, p <0.001) in exposure of smokers and non-smokers to tobacco smoke in their own homes, because other people are smoking. Smokers were significantly more exposed to passive smoking at home than non-smokers.

In places outside their own home one third of respondents were exposed to tobacco smoke, seven days a week. Exposure to passive inhalation of tobacco smoke outside their own homes lasted one to two days a week in more than one fifth of respondents, as well as those who were exposed for three to four days, while the exposure to tobacco smoke for five to seven days, in places outside their own home, was recorded in the lowest number of respondents (11.1%) (Graphic 2).



Graphic 2. Exposure to tobacco smoke outside home

Table 1. Subject's exposure to the influence of tobacco smoke at their own homes

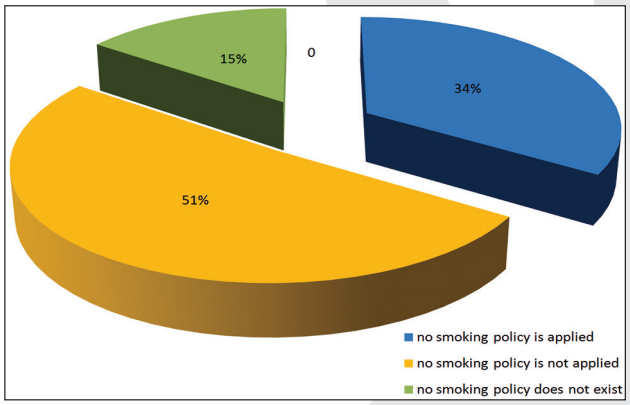
Number of days of the last week when subjects were around smokers	Subjects non smokers		Subjects smokers		All subjects	
	No.	%	No.	%	No.	%
0 days	256	98,5%	4	1,5%	260	100,0%
1-2 days	165	97,1%	5	2,9%	170	100,0%
3-4 days	84	97,7%	2	2,3%	86	100,0%
5-7 days	58	90.6%	6	9,4%	64	100,0%
All seven days	185	76,4%	57	23,6%	242	100,0%
Total	748	91,0%	74	9,0%	822	100,0%
Significance of the difference	Value		df		Asymp. Sig. (2-sided)	
Person Chi-Square	92,547a		4		,000	

Table 2. The exposure of subjects to the effects of tobacco smoke outside their own home

The number of days of the last week during which the subjects were around smokers in their own homes	Subjects non smokers		Subjects smokers		All subjects	
	No.	%	No.	%	No.	%
0 days	121	99,2%	1	0,8%	122	100,0%
1-2 days	182	97,8%	4	2,2%	186	100,0%
3-4 days	158	95,8%	7	4,2%	165	100,0%
5-7 days	84	92,3%	7	7,7%	91	100,0%
All seven days	204	78,8%	55	21,2%	259	100,0%
Total	748	91,0%	74	9,0%	822	100,0%
Significance of the difference	Value		df		Asymp. Sig. (2-sided)	
Person Chi-Square	72,601[a]		4		,000	

The exposure to the influence of tobacco smoke in students of medical faculties that are non-smokers is higher outside the home than at home (Table 2). For smokers, the exposure outside the home is somewhat rarer than at home and at the same time there was a statistically significant difference in the exposure to tobacco smoke between smokers and non-smokers (X2 = 72.601, p <0.001).

A third of respondents stated that the official policy of prohibiting the use of tobacco products is being implemented in their higher education institution (faculty), just over half of them considered that this policy does not apply in their institution, while about 15% of respondents stated that on their faculty an official policy of prohibiting the use of tobacco products does not exist *(Graphic 3)*.



Graphic 3. The application of the policy of prohibition of smoking at the faculties

Those who smoked have significantly more often responded that a policy of prohibiting the use of tobacco products at their faculties is not being respected, or does not exist at all (X 2 = 6644, p <0.05). All respondents were more likely to respond that the policy of prohibiting the use of tobacco products is not being implemented at their faculties or that such a policy does not exist at all (Table 3).

Discussion

Health Professionals are in an excellent position that allows them to have a prominent role on tobacco control (10). They can intervene to prevent and demand reduction measures concerning tobacco dependence and cessation, as well as the effects of exposure to tobacco smoke. They have the opportunity to help people change their behavior and can give advice, guidance and answers to questions related to the consequences of tobacco use. It is therefore particularly important that health workers are non-smokers.

The research has shown that the exposure to the effects of passive smoking is evident in students at medical branch faculties to significantly higher level among those who themselves are smokers. Approximately 35% of all respondents were exposed to the effects of tobacco smoke every day, which is slightly less than the students of these faculties in Saudi Arabia, where the prevalence was 38.2% of the total number of students tested (11). Every fourth respondent in Montenegro who does not smoke and more than three-quarters of those who smoked were exposed to tobacco smoke every day at their homes. The exposure to tobacco smoke for students non smokers is bigger outside the home than at home, while in smokers this exposure is less frequent. Similar results were obtained in the researches conducted by the Center for Chronic Disease Prevention and Health Promotion and the CDC (Centers for Disease Control and Prevention) among high school students. Even though exposure has gone down over the last decade, 22.8% of students who did not smoke reported that they had breathed in environmental tobacco smoke during the previous seven days - 75.3% of smoking students had done so too (12).

Table 3. Ban on use of tobacco products at the faculties

The application of official policy of prohibiting the use of tobacco products at the faculty	**Subjects non smokers**		**Subjects smokers**		**All subjects**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Yes, it is being implemented	244	87,5%	35	12,5%	279	100,0%
No, it is not implemented	391	93,18%	29	6,9%	420	100,0%
The university does not have an official policy	113	91,9,8%	10	9,1%	123	100,0%
Total	748	91,0%	74	9,0%	822	100,0%
Significance of the difference	Value		df		Asymp. Sig. (2-sided)	
Pearson Chi-Square	6,644[a]		2		,036	

The causes of exposure to tobacco smoke in students are often related to smoking status of their parents and friends (13). It is therefore understandable why students smokers are more exposed to tobacco smoke at home and outside, since they usually reside in the company of smokers. It is evident that there is an association between smoking children and their parents, which is also the result of the research carried out in Croatia. In fact, every second smoking student has a parent smoker, while among students non-smokers only every third student has a parent who smokes (14).

The restriction of use of tobacco products influences the prevalence of smoking in the population. It is significant to point out that two thirds of tested students in Montenegro stated that the policy of prohibiting the use of tobacco products is not being applied at their faculties or does not exist at all. The research done on similar populations in Serbia showed that a small part of the surveyed students saw the importance of the prohibition of advertising tobacco products for the prevalence of use of tobacco products. This fact is one of the most powerful weapons of the tobacco industry that is being used to recruit 2.5 million of new smokers every year, most of whom are young people (15).

References

1. *Biasco F, Hartnett JP. Colleges students' attitudes toward smoking. College Student Journal, 2002; 36: 442-447*

2. *Bravo MJ. Exposure to environmental tobacco smoke at home increases the need for medical attention for respiratory diseases in childhood. An Pediatr (Barc) 2007; 66: 475-80.*

3. *Omair A, Kazmi T, Alam SE . Smoking prevalence and awareness about tobacco related diseases among medical students of Ziauddin Medical University. J Pak Med Assoc 2002; 52: 389-92*

4. *Moreira MA, Moraes MR, Silva DG, Pinheiro TF, Vasconcelos Jr HM, Maia LF, et al. Comparative study of respiratory symptoms and lung function alterations in patients with chronic obstructive pulmonary disease related to the exposure to wood and tobacco smoke. J Bras Pneumol 2008; 34: 667-74.*

5. *Zil-a-Rubab, Ata-ur-Rahman M. Passive smoking status of students and employees of a private medical university. Pak J Med Sci 2007; 23: 425-8.*

6. *Kear ME. Psychosocial determinants of cigarette smoking among college students. Community Health Nurs 2002; 19(4): 245-57.*

7. *Khan FM, Husain SJ, Laeeq A, Awais A, Hussain SF, Khan JA. Smoking prevalence, knowledge and attitudes among medical students in Karachi, Pakistan. East Mediterr Health J 2005; 11: 952-8.*

8. *World Health Organization. Tobacco Free Initiative (TFI) Website. (Online) (Cited 2009 Oct). Available from URL: http://www.who.int/tobacco/.*

9. *Zil-a-Rubab, Ata-ur-Rahman M. Passive smoking status of students and employees of a private medical university. Pak J Med Sci 2007; 23: 425-8.*

10. *World Health Organization. Tobacco Free Initiative (TFI) Website. (Online) (Cited 2009 Oct). Available from URL: http://www.who.int/tobacco/. Pattenden S, Antova T, Neuberger M, Nikiforov B, De Sario M, Grize L, et al.*

11. *Yousef A. Al-Turki, Smoking habits among medical students in Central Saudi Arabia. Saudi Med J 2006; Vol. 27(5): 700-703.*

12. *Teen Secondhand Smoke Exposure Down, But Not Enough http://www.medicalnewstoday.com/articles/241228.php Article Date: 06 Feb 2012 - 9:00 PST*

13. *Parental smoking and children's respiratory health: independent effects of prenatal and postnatal exposure. Tob Control 2006; 15: 294-301.*

14. *Sović S, Musil V, Majer M, Božikov J, Jureša V. Učestalost pušenja u studenata medicine // Hrvatski kongres preventivne medicine i unaprjeđenja zdravlja s međunarodnim sudjelovanjem Knjiga Sažetaka/ Šogorić, Selma; Štimac, Danijela (ur.). Zagreb, 2010; 130-131.*

15. *Mackay J, Eriksen M. The Tobacco atlas. WHO; Geneva 27, Switzerland, 2002; 30-32.*

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Late presenters and significance of screening tests in early diagnosis of HIV infection in Istanbul

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Abstract

Introduction: Delayed diagnosis of HIV infection is a common problem leading to an increase both in HIV related morbidity and mortality, and rate of HIV transmission. Patients presenting with a CD4 lymphocyte count below 350/mm³ or presenting with an AIDS defining event are defined as late presenters. HIV/AIDS patients can be diagnosed earlier through screening tests. The scope of this study was to determine the frequency of late HIV presenters and the potential of screening tests for an early diagnosis of HIV infection in Istanbul.

Method: Demographic characteristics, stage of presentations stratified by reasons for HIV testing at the time of diagnosis were analyzed retrospectively at the outpatient clinic of Department of Infectious Diseases and Clinical Microbiology at Haseki Research and Training Hospital between January 2006 and March 2011.

Results: In total, 209 individuals were tested HIV positive, 172 (82%) were males and a mean age was 37 years (range 20-78 years). Overall, 137 (66%) were late presenters and 58% of these were presented with advanced HIV. As 76 (37%) patients presented with a clinical manifestation, the diagnosis of HIV was made based on screening tests in 133 individuals. 90.3% of none-late presenter, 67.2% of late presenters, and 30.3% of patients with advanced HIV were diagnosed by screening test ($p<0.001$).

Discussion: The fraction of late presenters was high. A substantial number of asymptomatic HIV positive individuals were identified by screening test, while the diagnosis of late presentation of the disease was overall mostly based on clinical symptoms.

Key words: Early diagnosis, HIV, screening tests.

Introduction

Early diagnosis and treatment is of great importance in HIV infection. After the highly active antiretroviral therapy (HAART) has been in use, its enormous impact on clinical improvement of late diagnosed patients was observed [1,2].

It has been demonstrated that starting HAART in asymptomatic period and in patients with CD4+ lymphocyte counts > 200/mm³ results in a decrease in morbidity and mortality [2,3,4]. However, in Europe still one-third of HIV infected individuals are not diagnosed until later stages of the disease, and this is an important public health issue, which indicates that new strategies for prevention and control of HIV/AIDS should be developed [5,6,7].

The incidence of HIV/AIDS in Turkey is estimated to be low but the exact number is not known. The notification rate has increased during the last decade. According to Ministry of Health, in 2000 there were 157 new cases, but in 2011 the number increased to 699. However, the official estimates are not reflecting the real size of the problem due to poor reporting systems, and lack of focus on this area from society and the authorities. We recently described the epidemiological characteristics of our HIV cohort and poor level of education was remarkable feature [22].

Routine testing for HIV is essential to diagnose and control the infection in the asymptomatic early stage. The Centers for Disease Control and Prevention recommends opt-out testing in health care settings where the prevalence of undiagnosed infection is 0.1% or higher [8].

In this trial, we aimed to investigate the proportion of late presentation of HIV infected individuals and potential of screening tests in the early diagnosis.

Materials and Methods

We included all adult HIV-1 positive individuals diagnosed and followed up at outpatient clinic of the Department of Infectious Diseases and Clinical Microbiology of training and research hospital between January 2006 and March 2011. The hospital is one of the largest training and research hospital in Istanbul, which is the biggest cosmopolitan city in Turkey.

Age, gender, stage of presentation and baseline CD4+ lymphocyte counts upon diagnosis were evaluated from the patients' records retrospectively. The relationship between reason of first clinical contact and baseline CD4+ lymphocyte counts were investigated.

The European definitions for late presentation and advanced HIV disease were used in this study14. Late presentation was defined as presenting for care with a CD4+ lymphocyte counts below 350 cells/mm3 or presenting with an AIDS-defining event, regardless of the CD4 cellcount. Presentation with advanced HIV disease was defined as presenting for care with a CD4+ lymphocyte count below 200 cells/mm3 or presenting with an AIDS-defining event, regardless of the CD4 cell count.

Statistical analyses were performed with Graphpad Prism 5.0 software. Numerical data are presented as the mean and range, and categorical data are presented as ratios. Fisher's exact test was used to calculate between-group differences of independent groups for categorical data. The level of statistical significance was considered as $p< 0.05$.

Results

During the 5-years follow up period, 209 HIV-1 positive cases were registered, 172 (82%) cases were male and 37 (18%) were female with the mean age of 37 years (range 20-78 years). Most common route of transmission was heterosexual intercourse (66%) followed by men having sex with men (MSM) (33%) and IV drug use (1%). Education levels were low in the majority of cases. Only 21 (10%) patients were university graduates. General characteristics of patients between different stages of HIV infection are shown in Table 1. There was no significant association between age, gender, education level, transmission route and stage of HIV infection. 137 cases (66%) were late presenters. 90.3% of none-late presenters, 67.2% late presenters and 30.3% of patients with advanced HIV were diagnosed by screening tests. The association between early diagnosis and screening tests was statistically significant ($p<0.001$). CD4+ lymphocyte counts were over 50 cells/mm³ in all 133 (63.6%) patients diagnosed by screening tests, and none of these patients suffered from any symptoms or had clinical finding at the time of diagnosis.

Distribution of screening tests leading to diagnosis of 133 asymptomatic patients is presented in Table 2. Most frequent reasons were routine check-up, blood donation and preoperative screening.

All patients reported that they have changed their high-risk sexual behavior and began to use condom in their sexual intercourses after they became aware of the diagnosis.

Table 1. Characteristics of patients and stage of HIV infection

Characteristics	None late presenters n=72 (34%)	Late presentation n=58 (28%)	Advanced HIV n=79 (38%)	p-value
Age, mean ±SD	36.0± 9.5	38.4±12.1	39.7±10.5	0.080
Male, n (%)	60 (83.3)	49 (84.5)	62 (78.5)	0.545
Education levels Primary school, n(%) High school, n(%) University, n(%)	 32 (44.4) 33 (45.8) 7 (9.7)	 29 (50) 23 (39.7) 6 (10.3)	 49 (62.8) 22 (27.8) 8 (10.3)	0.155
Transmission routes Heterosexual, n(%) MSM, n(%) IV drug use, n(%)	 44 (61.1) 27 (37.5) 1 (1.3)	 39 (67.2) 19 (32.9) 0 (0)	 54 (68.4) 23 (29.1) 2 (2.5)	0.570
Diagnosed by screening tests, n (%)	65 (90.3)	39 (67.2)	24 (30.3)	*<0.0001*

Table 2. Number of patients diagnosed with HIV-1 by screening test and the reasons for screening

Screening tests	Number (n=133)	%
Check-up	29	21.8
Blood donation	27	20.3
Preoperative	26	19.5
After suspected sexual intercourse	22	16.5
Job application	12	9
Marriage	12	9
Military service	3	2.2
Pregnancy	2	1.5

Baseline CD4+ lymphocyte counts of all patients were grouped as <200/mm³, 200-350/mm³, 350-500/mm³ and >500/mm³. The proportions of reasons to HIV-1 test stratified by CD4+ lymphocyte counts are presented in Figure 1.

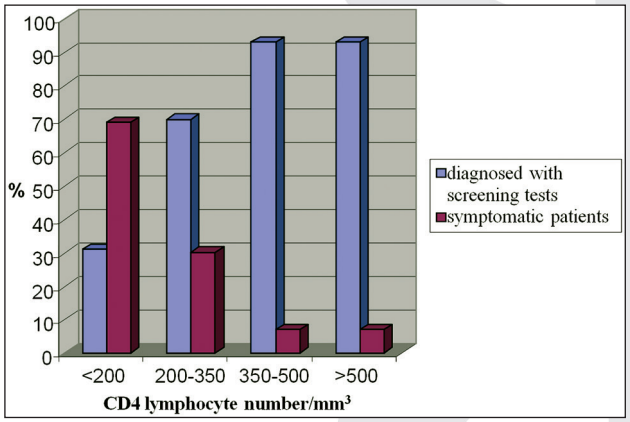

Figure 1. Relationship between reasons of the HIV test and CD4+ lymphocyte numbers of cases

CD4+ lymphocyte counts were below 350 cells/mm³ in 137 patients at the time of the diagnosis and HAART started immediately. CD4+ lymphocyte counts in 54% of patients were <200 cells/mm³ and they received prophylactic trimethoprim-sulphametoxazol

(TMP/SMX) in addition to the treatment. Furthermore, 16% of patients had CD4+ count <50 cells/mm³ and received prophylactic clarithromycine as well.

Discussion

HIV/AIDS disease progresses asymptomatically averagely 8-10 years after the infection [9,10]. Since the individuals who are unaware of their infection, can continue their risky behaviors, the treatment is delayed and all of these contribute into the spread of HIV.

Delayed diagnosis in HIV/AIDS disease is directly related to the disease spread, and Marks et al estimated 3.5 folds higher HIV transmission rate from a HIV positive unaware group then HIV positive aware group [11]. Marks et al. reported in their meta-analysis study that HIV positive individuals decreased their high risk sexual behaviors prominently after the diagnosis [12]. In our study, all patients reported that they have changed their high risk sexual behaviors after the diagnosis of HIV/AIDS. Late diagnosed patients are under great risk in clinical progression, their responses to the treatment are lower and development of opportunistic infections are higher in these patients, when compared to the early diagnosed ones [2,14].

Approximately 25% of HIV mortality has been detected to be related to delayed diagnosis (or advance disease [2,15,16]. Treatments of late diagnosed individuals are generally more complex and, depending on this, have higher treatment costs [17]. 66% of our population was late presenters which is considerably higher compared to 49% late presenters in the European Union and European Economic area in 2010 [23]. There is a need for focus on population based HIV education programmes and screening in Istanbul. There is free access to HIV care in Turkey. All late presenters received antiretroviral treatment, and both with prophylactic agents and directed at concomitant diseases.

Late presentation of HIV infection has serious health consequences for the individual and poses a high financial burden on society. Krentz and Gill found significantly higher costs for late presenters, especially inpatient costs, during the first year after accessing care, and that costs remained signif-

icantly higher (>50% higher) during subsequent years for HIV-infected patients who present with a CD4 count <350 cells/mm3 compared to early presenters [21].

HIV screening tests are an important strategy to decrease incidence of HIV infection among sexually active adolescents. WHO stated in the progress report in 2011 that testing and counseling for HIV and sexually transmitted infections was one of the key approaches for prevention. In 2006, routine HIV infection screening in all individuals between ages 13-64 years, who have given consents, was placed within the CDC recommendations [18]. *The Society for Adolescent Medicine* also recommends routine screening tests in sexually active adolescents, especially those who live in regions with high HIV prevalence [19]. Moreover, American College of Obstetricians and Gynecologists recommends routine screening in sexually active females above 19 years of age [20]. In Turkey, compulsory screening tests are performed in special conditions like marriage procedures, job applications and for the military service. Furthermore, all blood donors are screened for HIV infection. Occasionally HIV tests may be performed in people, who have applied to a healthcare unit or in patients, who are admitted to hospital without informing them and receiving consents. Although the ethical background of these situations are debatable, these may have positive impacts on patients since diagnosis at an early phase can have positive impacts on the disease progression. Nevertheless, if individuals are informed about the importance of early diagnosis for themselves and the society, then it is a fact that these tests will be performed on voluntary basis rather than a compulsory one.

In conclusion, performing screening tests and supporting individuals with high risks to have HIV tests voluntarily can increase the numbers of early diagnosed patients. Thus, spread of the disease and treatment costs can be decreased by an important extent.

References

1. *Palella F, Delaney K, Moorman A, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998; 338(13): 853-60.*

2. *Palella FJ, Deloria-Knoll M, Chimel JS, Moorman AC, Wood KC, Greenberg AE, et al. Survival benefits of initiating antiretroviral therapy in HIV-infected persons in different CD4$^+$ cell strata. Ann Intern Med 2003; 138(8): 620-6.*

3. *Sabin C, Smith C, Gumley H, Murphy G, Lamphe FC, Phillips AN, et al. Late presenters in the era of highly active antiretroviral therapy: uptake of and responses to antiretroviral therapy. AIDS 2004; 18(16): 2145-51.*

4. *Centers for Disease Control and Prevention (CDC). Late versus early testing of-16 sites, United States, 2000-2003. MMWR Morb Mortal Wkly Rep 2003; 52: 581-86.*

5. *Fisher M. Late diagnosis of HIV infection: major consequences and missed opportunities. Curr Opin Infect Dis 2008; 21: 1–3.*

6. *Adler A, Mounier-Jack S, Coker RJ. Late diagnosis of HIV in Europe: definitional and public health challenges. AIDS Care 2009; 21: 284–293.*

7. *Antinori A, Coenen T, Costagiola D, Dedes N, Ellefson M, Gatell J, et al.Late presentation of HIV infection: a consensus definition.*

8. *Centers for Disease Control and Prevention (CDC). Person Tested for HIV—United States, 2006. MMWR Morb Mortal Wkly Rep 2008 Aug 8; 57(31): 845-49.*

9. *Centers for Disease Control and Prevention (CDC). Projections of the number of persons diagnosed with AIDS and the number of immunosupressed HIV-infected persons-United States, 1992-1994.MMWR Morb Mortal Wkly Rep 1992; 41(RR-18): 1-29.*

10. *Rutherford GW, Lifson AR, Hessol NA, Darrow WW, O'Malley PM, Buchbinder SP, et al. Course of HIV-1 infection in a cohort of homosexual and bisexual men: An 11 year follow up study. BMJ 1990; 301(6762): 1183-88.*

11. *Marks G, Crepaz N, Janssen RS. Estimating sexual transmission of HIV from persons aware and unaware they are infected with the virus in the USA. AIDS 2006; 20(10): 1447-50.*

12. *Marks G, Crepaz N, Senterfitt JW, Janssen RS. Meta-analysis of high-risk sexual behavior in persons*

aware in persons aware and unaware they are infected with HIV in the United States: Implications for HIV prevention programs. J Acquir Immune Defic Syndr 2005; 39(4): 446-53.

13. *Centers for Disease Control and Prevention. HIV/AIDS Surveillance Report, 2005. Vol. 17. Rev ed. Atlanta: U.S Department of Health and Human Services, Centers for Disease Control and Prevention; 2007: 5-9.*

14. *Antinori A, Coenen T, Costagiola D, Dedes N, Ellefson M, Gatell J. Late presentation of HIV infection: a consensus definition. HIV Med 2011; 12: 61-4*

15. *Losina E, Figueroa P, Duncan J, Divi N, Wolf LL, Hirschhorn LR, et al. HIV morbidity and mortality in Jamaica: analysis of national surveillance data, 1993--2005. Int J Infect Dis 2008; 12(2): 132-38.*

16. *Chadborn TR, Delpech VC, Sabin CA, Sinka K, Evans BG. The late diagnosis and consequent short-term mortality of HIV-infected heterosexuals (England and Wales, 2000-2004). AIDS 2006; 20(18): 2371-79.*

17. *Krentz HB, Auld MC, Gill MJ. The high cost of medical care for patients who present late (CD4 <200 cell/microL) with HIV infection. HIV Med 2004; 5(2): 93-98.*

18. *Centers for Disease Control and Prevention. Revised recommedations for HIV testing of adults, adolescents, and pregnant women in health care settings. MMWR Morb Mortal Wkly Rep 2006; 55: 1-17.*

19. *D'Angelo LJ, Samples C, Rogers AS, Peralta L, Friedman L. HIV infection and AIDS in adolescents: an update of the position of the Society for Adolescent Medicine. J Adolesc Health 2006; 38(1): 88-91.*

20. *ACOG Commitee Opinion. Routine human immunodeficiency virus screening. Obstet Gynecol 2008; 112(2 Pt 1): 401-3.*

21. *Krentz HB, Gill MJ. The Direct Medical Costs of Late Presentation (<350/mm^3) of HIV Infection over a 15-Year Period. AIDS research and treatment 2012; 2012: 757135.*

22. *Karaosmanoglu HK, Aydin OA, Nazlican O. Profile of HIV/AIDS patients in a tertiary hospital in Istanbul, Turkey. HIV Clin Trials. 2011; 12(2): 104-8*

23. *Likatavicius G, van de Laar MJ. HIV infection and AIDS in the European Union and European Economic Area, 2010 Euro Surveill. 2011; 16(48): pii=20030. Available online: http: //www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20030*

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Relationship between STAT3 signal pathway and recurrent spontaneous abortion

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Abstract

It has been considered that STAT3 plays an important role in early embryonic development, but the relationship between STAT3 and recurrent spontaneous abortion and its possible mechanisms have not been fully elucidated. In the present study, we evaluated the expressions of STAT3 signal pathway associated proteins in the allogeneic mouse models of low abortions (CBA/J*Balb/c) and abortion prone (CBA/J*DBA/2J). Results showed, in contrast to the control group, the IL-6 was decreased and STAT3 signal pathway associated proteins were down-regulated in the abortion-prone mating on gestation day 7.5. Our results suggest that STAT3 signal pathway may be associated with recurrent abortion. Decreased IL-6 may block the STAT3 activation and thus lead to abortion.

Key words: Recurrent spontaneous abortion, STAT3, signaling pathway, relationship.

Introduction

Recurrent spontaneous abortion is a common complication of pregnancy and also one of the refractory diseases(1). It has been ascribed to a variety of causal factors. Although many studies have been carried to investigate the recurrent spontaneous abortion, the pathogenesis of recurrent spontaneous abortion is still largely unknown, which poses difficulty to its treatment (2). The pathway involving STAT transcription factor was initially discovered by researchers studying the transcriptional activation of genes through cytokines and growth factors in mammalian cells. It soon becomes clear that this pathway is conserved in many species and plays a central role in the regulation of numerous biological processes (3-4). STAT3, one of the members of the STAT family, is a key mediator in growth regulation and its crucial effects have also been observed in many tissues. There is evidence showing that STAT3 is important for the early embryonic development. Therefore, we speculate that STAT3 signaling pathway may be related to the recurrent spontaneous abortion.

STAT3 has been shown to be activated by various cytokines and growth factors especially the interleukin-6 (IL-6) (5). IL-6 is a multifunctional cytokine regulating immune response, hematopoiesis, and acute phase reaction (6). IL-6 exhibits its biological function through its receptor. The complex of IL-6 and soluble IL-6R can generate the IL-6-mediated signal transduction. Furthermore, the binding of IL-6 to its receptor triggers the association of IL-6R with a second membrane glycoprotein with the molecular weight of 130 kD (gpl30).

STATs are potential transcription factors involved in the normal cellular response to the stimulation of cytokines or growth factors. They are activated by the members of Janus kinase (JAK). p-STAT, the active form of STAT, forms hetero and/or homodimers, then translocate to the nucleus and bind the conserved DNA sites such as the high affinity sis-inducible element, which, together with other transcription factors, regulate the expression of specific effector genes (7-9). STAT3 is one of the members of STAT family. Gene deletion assay, microarray, proteomics and chromatin immunoprecipitation assay have revealed STAT3 related target genes with a broad range of functions. Vascular endothelial growth factor (VEGF) is an endothelial cell specific mitogen and is a disulphide-linked homodimer of 34~42 kD that shares structural homology with the platelet derived growth factor (PDGF)-family (10). It can regulate the proliferation, differentiation and survival of endothelial cells, act as a chemoattractant for monocytes, mast cells and pericytes and enhance the vascular permeability. VEGF is considered as one of the major modulators of angiogenesis in a variety of physiological processes, including wound healing and embryonic development, and plays a key role in the pathogenesis of diseases including diabetic retinopathy, psoriasis and tumor growth.

Survivin is a member of the inhibitor of apoptosis (IAP) family containing a single baculovirus IAP repeat (BIR) domain and an extended -COOH terminal α-helical coiled coil (11). Survivin can regulate both cell proliferation and apoptosis. Initially, survivin expression was thought to be confined to the developing fetus, as it is largely undetectable in normal adult tissues with the exception of placenta, testis and thymus. However, survivin is also expressed in the growth hormone-stimulated hematopoetic progenitor cells as well as the proliferating blood vessels (12). Moreover, survivin expression is also found in the chromaffin cells and renal tubule epithelial cells (13). At present, it has been considered to increase the angiogenesis and carcinogenesis (14, 15).

In the present study we explored the relationship of STAT3 signal pathway and the recurrent spontaneous abortion. The CBA/J * BALB/c mice served as controls and CBA/J * DBA/2 mice with abortion prone as the experimental group were investigated. On the gestation day 7.5, serum IL-6 was determined by ELISA. The expressions of IL-6R, GP130, STAT3, P-STAT3, VEGF and survivin were evaluated by western blot assay and immunohistochemistry. Results suggest that STAT3 signal pathway may be involved in the pregnancy loss.

Materials and methods

Animals

Mice were purchased from the Institute of Experimental Animals of the Chinese Academy of Medical Sciences and housed in an animal facility with a 12-h light/dark cycle. Animal care and experimental procedures were followed according to institutional guidelines and conformed to requirements of the state authority for animal research. CBA/J females were caged with DBA/2J or Balb/c males overnight and examined by vaginal smear in the morning every day. The presence of plug determined the first day of pregnancy. At this time, mice were segregated and pregnant females were sacrificed on the gestation day 7.5. Uteri and placenta were removed and cut into pieces. A fraction of tissues were used for immunochemistry and the remaining tissues were used for western blot assay.

Detection of serum IL-6 by ELISA

After preparation and dilution, standard wells, testing sample wells and bank wells were set. Diluted standard 50μl was added to the standard well; Sample dilution 25μl was added to the testing sample well, then testing sample 25μl was added. Nothing was added in blank well. Taking blank well as zero, the optical densit(OD) was measured at 450 nm after adding stop solution and within 15 min.

Western blot assay

Tissues were lysed with the Total Protein Extraction Kit for 30 min at 4℃. After clarification, equal amounts of protein were separated by electrophoresis on a SDS-polyacrylamide gel and transferred onto a PVDF membrane using a Bio-Rad semidry transfer cell. The membrane was blocked in 5% non-fat milk containing 0.1% Tween 20 at room temperature for 1 h. The membrane was then incubated with corresponding primary antibodies at 4℃ overnight followed by washing and incubation with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1: 5000). β-actin served as an internal reference. After washes, visualization was performed. The optical density (OD) of each band was quantified using GIS gel analysis software.

Immunohistochemistry

Cryostat sections (10 mm) were treated with 3% H_2O_2 for 25 min to inactivate the endogenous peroxidase. After incubation, sections were rinsed in PBS thrice for 5 min each. Sections were then incubated with normal goat serum (50 μl/section) for 30 min. The monoclonal rabbit anti-mouse antibodies (50 μl/section) were applied to treat these sections for 1 h. Then, sections were rinsed in PBS thrice for 5 min each followed by incubation with peroxidase-conjugated goat anti-rabbit secondary antibody for 30 min. After rinsing in PBS thrice for 5 min each, the visualization was done by incubating sections with diaminobenzidine (DAB), followed by counterstaining with Mayer's hematoxylin. Slides were examined under a light microscope (Zeiss Axioscope). Analysis was performed using a digital image analysis system.

Results

Results from ELISA

According to the concentration of the standards and the corresponding OD values, the linear regression equation of standard curve was y=1244.1x-57.548, R^2=0.9962. The IL-6 was then calculated. The IL-6 was 2.41 ± 72.820 ng/ml in the abortion group and 2.24 ± 62.01ng/ml in the control group, showing statistically significant difference (P<0.01).

Results from Western blot assay

The expressions of STAT3 signal pathway associated proteins including GP130, STAT3, P-STAT3, survivin and VEGF were determined by western blot assay. Because the gestational day 7.5 is crucial with respect to the decidualisation, we focused on this particular day. Actin expression served as an internal control. Results showed that the CBA/J *DBA/2J pregnant females exhibited the significant down-regulation of these proteins as compared to low abortion mating (Figure 1).

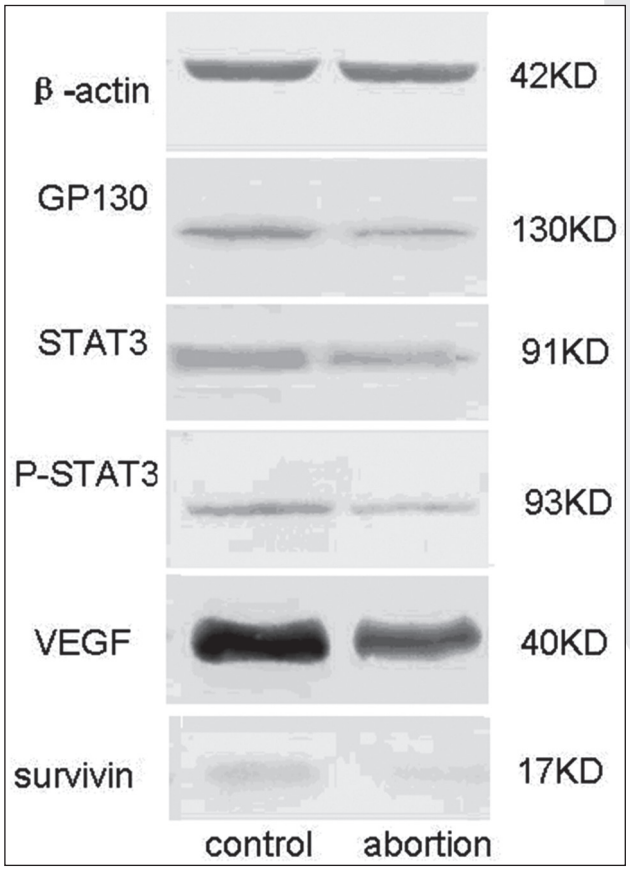

*Figure 1. Protein expressions of GP130, STAT3, P-STAT3, Survivin and VEGF by western blot assay (**P< 0.01 and *P < 0.05, n=6)*

Results from immunohistochemistry

The STAT3 signal pathway associated proteins were mainly expressed in the cytoplasm except for the p-STAT3. Immunohistochemistry revealed higher expression of those proteins in the CBA/J *Balb/c mice than in the CBA/J*DBA/2J mice (Figure 2).

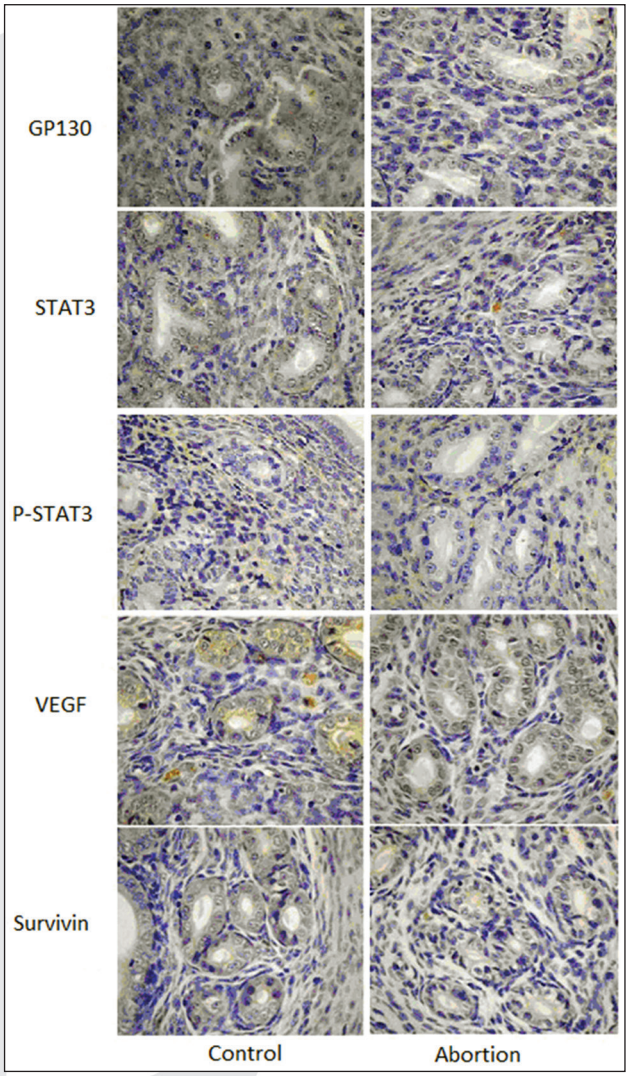

Figure 2. Protein expression of GP130, STAT3, P-STAT3, VEGF and Survivin by immunohistochemistry (×400)

Discussion

The mechanisms underlying the gestation failure are not fully elucidated, and now attributed to some immunological factors. We studied the expressions of STAT3 signal pathway associated proteins in the allogeneic mouse models with low abortions (CBA/J*Balb/c) and abortion prone (CBA/J*DBA/2J). Our results showed that there

were significant differences in the expressions of these proteins in the decidua between two groups, implying that there is a close relationship between STAT3 signaling pathway and recurrent spontaneous abortion.

In this study, the blood was collected and serum IL-6 detected by ELISA on the pregnant day 7.5. Results showed that the IL-6 level was lower than that in the control group ($P<0.01$). Thus, we speculate that IL-6 plays an important role in the early embryonic stage and the low serum IL-6 level may be involved in the pathogenesis of recurrent spontaneous abortion.

IL-6 exerts the biological function through its receptor system. The complex of IL-6 and soluble IL-6R can generate the IL-6-mediated signal transduction. Furthermore, the binding of IL-6 to its receptor triggers the association of IL-6R with a second membrane glycoprotein, gp130. High expression of GP130 mRNA has been found in the endometrium of pregnancy (16). In our study, results showed the GP130 was down-regulated in abortion pregnancies, suggesting a role of GP130 in the maintenance of mouse pregnancy. On the other hand, low IL-6 serum level influences the association of GP130 with the complex of IL-6 and IL-6R.

STAT3 is the center of the signalling pathway. Thus, the role of STAT-3 and p-STAT3 was investigated in the pregnancy. Our results showed that both proteins were down-regulated in the abortion matings, CBA/J*DBA/2J. We speculate that the STAT-3 activity is involved in the maintenance of pregnancy. Decidualization was a prevented reason for down-regulation of STAT3. Furthermore, the embryos and the interaction contact with maternal tissues were also blocked.

Two effector proteins, STAT3, VEGF and Survivin, were determined in the present study. The expressions of both proteins showed similar findings to previous studies. VEGF is one of the major modulators of angiogenesis. Low VEGF level may pose difficulty in the decidual angiogenesis, and thus embryo is lost due to lacking of blood supply. Survivin regulates both cell proliferation and apoptosis. Down-regulation of Survivin leads to the apoptosis of decidual cells, and thus the embryos lose the support and fall off the endometrium. These findings suggest the expressions of these proteins are necessary to maintain the pregnancy.

The binding of IL-6 to its receptor triggers the association of IL-6R with the gpl30. STAT3 is activated by the members of the JAK family of tyrosine kinases complexed with the cytoplasmicdomain of ligand-bound cytokine receptors by GP130. p-STAT3 can form hetero and/or homodimers, then translocate to the nucleus and bind the conserved DNA sites, which together with other transcription factors regulate the expressions of VEGF and Survivin. Due to the down-regulation of serum IL-6, STAT3 signaling pathway is inhibited. The development of embryo is stopped or even lost. In conclusion, our data points that IL-6/GP130/STAT3 signaling pathway plays an important role in the embryonic development. Regulating these proteins may improve the survival of embryos, which may be a novel strategy for the treatment of recurrent spontaneous abortion.

References

1. *Abou-Nassar K, Karsh J, Giulivi A and Allan D. Successful prevention of thrombotic thrombocytopenic purpura (TTP) relapse using monthly prophylactic plasma exchanges throughout pregnancy in a patient with systemic lupus erythematosus and a prior history of refractory TTP and recurrent fetal loss. Transfus Apher Sci. 2010; 43(1): 29-31*

2. *Venkatesh S, Thilagavathi J, Kumar K, Deka D, Talwar P, Dada R. Cytogenetic, Y chromosome microdeletion, sperm chromatin and oxidative stress analysis in male partners of couples experiencing recurrent spontaneous abortions. Arch Gynecol Obstet. 2011; 284(6): 1577-84*

3. *Wittig I and Groner B. Signal transducer and activator of transcription 5 (STAT5), a crucial regulator of immune and cancer cells. Curr Drug Targets Immune Endocr Metabol Disord 2005; 5: 449-63*

4. *Valentino L and Pierre J. JAK/STAT signal transduction: regulators and implication in hematological malignancies. Biochem Pharmacol 2006; 71: 713–21*

5. *Liao W, Yu C, Wen J, et al. Adiponectin induces interleukin-6 production and activates STAT3 in adult mouse cardiac fibroblasts. Biol Cell 2009; 101(5): 263-72*

6. *Kishimoto T. IL-6: from its discovery to clinical applications. Int Immunol 2010; 22(5): 347-52*

7. *Schaefer TS, Sanders LK and Nathans D. Cooperative transcriptional activity of Jun and Stat3 beta, a short form of Stat3. Proc Natl Acad Sci U S A. 1995; 92: 9097-101*

8. *Wagner BJ, Hayes TE, Hoban CJ and Cochran BH. The SIF binding element confers sis/PDGF inducibility onto the c-fos promoter. EMBO J1990; 9: 4477-84*

9. *Zhang X, Wrzeszczynska MH, Horvath CM and Darnell JE: Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. Mol Cell Biol1999; 19: 7138-46*

10. *Conn G, Bayne ML, Sodermann DD et al. Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-dervived growth factor. Proc Natl Acad Sci USA 1990; 87: 26 28-32*

11. *Altieri DC: Molecular circuits of apoptosis regulation and cell division control: The survivin paradigm. J Cell Biochem 2004; 92: 656-663*

12. *Ambrosini G, Adida C and Altieri DC. A novel anti-apoptosis gene, Survivin, expressed in cancer and lymphoma. Nat Med 1997; 3: 917–921*

13. *Koch CA, Vortmeyer AO, Diallo R, et al. Survivin: a novel neuroendocrine marker for pheochromocytoma. Eur J Endocrinol 2002; 146: 381-388*

14. *Koch CA, Vortmeyer AO, Diallo R, et al. Survivin: a novel neuroendocrine marker for pheochromocytoma.Eur Endocrinol 2002; 146: 381-388*

15. *Graaf AO, de Witte T and Jansen JH. Inhibitor of apoptosis proteins: new therapeutic targets in hematological cancer?. Leukemia2004; 18: 1751-1759*

16. *Lam SP, Luk JM, Man K, et al. Activation of interleukin-6-induced glycoprotein 130/signal transducer and activator of transcription 3 pathway in mesenchymal stem cells enhances hepatic differentiation, proliferation, and liver regeneration. Liver Transpl 2010; 16: 1195-206*

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Use of intermittent hypobaric pressure in the treatment of inoperable peripheral arterial occlusive disease (PAOD) in elderly patients

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Abstract

Objective: The goal of this study was to assess the value of intermittent hypobaric pressure (the Greensac® kit) in the treatment of patients with inoperable peripheral arterial occlusive disease (PAOD).

Methods: The prospective study included 68 patients with pathology of lower extremities. Effects of the Greensac® kit on the claudicating distance prolongation and/or wound healing in critical leg ischemia were analyzed. Results were analyzed using standard statistical methods.

Results: The obtained data showed a significant claudicating distance prolongation in patients with arteriosclerosis lesions predominantly in early stages of the disease. In the group with critical leg ischemia, the claudicating distance improvement was much lower, compared to the group of patients with an early stage of the disease.

Conclusion: Treatment using the Greensac® kit may decrease the claudicating pain more in the earlier phases of the disease. In order to assess the value of the Greensac® kit in the treatment of ischemic foot ulcers in critical leg ischemia, a greater number of patients are necessary.

Key words: Arterial occlusive disease, therapy, leg, blood supply, aged.

Introduction

It is believed that over 5% of adult population today suffers from some kind of peripheral arterial occlusive disease (PAOD) of lower extremities. A majority of these patients are inoperable; either they need no surgery, or due to advanced disease, adequate revascularization cannot be performed (1, 2).

The incidence of PAOD tends to shift toward the younger age, mainly because of the prevalence of risk factors (smoking, dyslipidemia, obesity), physical inactivity, inadequate diet and so on. That is why it is reasonable to expect a significant increase in the number of patients with PAOD in the future, and therefore a rise in the cost of treatment, so each new method that may be used in its treatment deserves attention of clinicians (2).

In recent years, a new type of conservative treatment, by intermittent hypobaric pressure, has appeared in our environment, and literature data show that it significantly improves circulation and increases lymphatic drainage (3–8). Its basic physiopathology mechanism includes increasing blood and lymph movement in the subcutaneous tissues and manipulation with this relatively small quantity of fluids. That is why circulation through the tissues is better and peripheral oxygenation is improved. Improvement of peripheral perfusion may lead to a reduction of symptoms and helps wound healing (4, 9-11). This study was designed to investigate the value of intermittent hypobaric pressure in the treatment of patients with PAOD of the lower extremities. The objective of this paper was to analyze effects of intermittent hypobaric pressure on the following:

- Elimination of claudication symptoms during a 6-minute walk on flat ground;
- Indicators of peripheral circulation improvement - **ankle-brachial index (ABI)** and skin temperature;
- Pain in patients with critical limb ischemia;
- Wound healing in patients with critical limb ischemia.

Methods

The study included patients aged 65 and over with clinical symptoms of PAOD: 68 patients had a verified PAOD, but were inoperable (for general and/or local reasons) and could not undergo endovascular revascularization.

The patients were submitted to intermittent hypobaric pressure using the Greensack® kit (manufactured by "Iskra-Medical" from Slovenia) during 2 sessions, each of 10 treatments lasting 20 minutes, 3 times a week. There was a pause of 14 – 21 days between sessions. All patients underwent a standard diagnostic algorithm, which included clinical examinations, noninvasive diagnosis, and invasive arteriography. Angiography was not performed in patients with stage II of the disease (Fontaine staging).

Inclusion criteria are provided in Table 1.

Table 1. Inclusion criteria

Inclusion criteria	
1.	Age 65 and over
2.	Symptoms of claudication and/or trophic foot ischemia
3.	Established PAOD with contraindicated revascularization: • Morbus cruris • Subcritical ischemia (Fontaine stage IIa) • Comorbidity (recent myocardial infarction, chronic renal failure, malignant diseases, cerebrovascular insult

All patients filled out a form with information related to PAOD and possibly existing critical foot ischemia. Important data measured the claudication distance before and after treatment and one month after therapy, as well as ulcer surface before treatment and one month after therapy. The subjective feeling of pain was also evaluated on a scale of 1 to 10 (after the abovementioned time intervals). These parameters were gathered and correlated with factors and comorbidity. Two groups of patients were formed:

Group A included 36 patients with moderate symptoms and subcritical ischemia. These patients were not referred for surgery due to the clinical stage of the disease (IIa stage after Fontaine), previous insult with paralysis, or malignant diseases. The claudication distance was measured before and after treatment with result correlation.

Group B included 32 patients with critical limb ischemia. These patients presented with advanced crural lesions, with trophic changes and/or pain at rest. Foot wound healing was also measured. In patients with toe gangrene, the speed of recovery and demarcation were measured, and in those with trophic ulcers, the ulcer surface reduction was measured in relation to the time of healing. The wound healing progress was evaluated from 1 to 5 on each weekly control, and results were summarized at the end of the treatment.

The treatment outcome was evaluated after the second treatment and one month later. All data were statistically analyzed using standard statistical methods and the statistical program SPSS 7.0. The gathered data are presented in tables and figures.

Results

Group A

This group included 36 patients with symptoms of subcritical ischemia and claudication during a 6-minute walk. One month after treatment, 14 patients reported that they were claudication free.

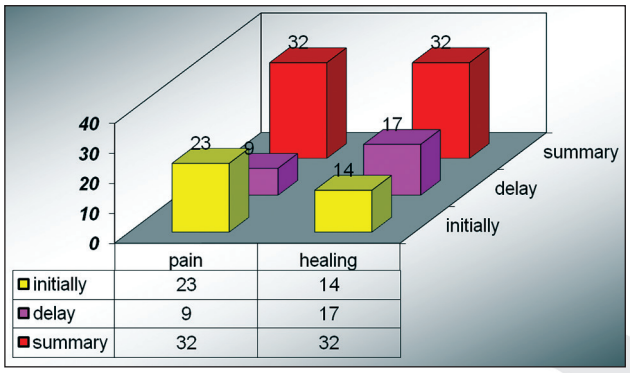
Table 2. Claudication before and after therapy

Group A	Before	After	T-test results
Number of pts	36	22	$p<0.05$

Group B

This group included patients with critical limb ischemia and they were evaluated related to their subjective feeling of pain in the legs (from 1 to 10) and volume of wound healing during the examination period – before and after therapy and one month after treatment.

The group included 32 patients with pain at rest and trophic changes, ischemic ulcers, dry toe or toes gangrene and/or "wet" gangrene. The first group of data was related to the subjective feeling of pain at rest in patients with critical limb ischemia**.** A significant improvement and pain relief was reported by 23 patients (71.9%) in the initial phase, after the second treatment. Out of this number, reduced symptoms remained after one month in 9 patients (28.1%), indicating relatively short-term effects of the therapy. Regarding wound healing, fast effects occurred in 14 patients (43.8%), whereas delayed improvement occurred in 17 patients (53.1%) that are presented in Graph 1.



Graph 1. Treatment outcome related to pain relief and wound healing progress

Discussion

The ideal course of treatment for patients with PAOD is revascularization. However, feasibility of revascularization depends on the general condition and operability of patients, as well as existence of comorbid factors such as advanced cardiac insufficiency, recent acute myocardial infarction, chronic renal failure, recent cerebrovascular insult, advanced malignant disease, condition after cerebrovascular accident with advanced organic psychosyndrome. Also, patients with an ischemic or paralyzed limb, characteristic for the state after insult with contractures which cannot be corrected, monoplegia, hemiplegia, paraplegia, dementia and so on, are not feasible for revascularization.

Conservative therapy of symptomatic occlusive disease is still insufficient and reserved mostly for patients who may not undergo some form of revascularization, but a significant number of patients with PAOD are inoperable for various reasons. In such cases treatment with intermittent hypobaric pressure, like Greensack® is available.

Effects of Greensack® kit in Group A (36 patients with moderate symptoms and subcritical lower limb ischemia) was satisfactory, with a statistically significant decrease of patients with claudication symptoms.

Group B included 32 patients with critical limb ischemia. In this group, intermittent hypobaric pressure was used for pain relief and improvement of wound healing.

Pain is a subjective category and its interpretation depends on objective circumstances (stage of ischemia), but also on subjective conditions. In 71.9% of patients, Greensack® caused initial relief of pain at rest. In the further course of therapy, reduction of its effects on pain occurred, and 28.1% of patients experienced satisfactory effects of therapy. An explanation for this could be sought in a more favorable effect of therapy in the treatment of diabetic neuropathy due to evacuation of xylitol and acid products in the skin and subcutaneous tissue that occurs when affecting fluids in the feet and lower leg (7-11).

Wound healing was significantly affected by the fact that this group of patients mostly presented with absolute prevalence of diabetic etiology (diabetes mellitus type IIb was registered in 27 out of 32 patients) and with accompanying crural diabetic arteriopathy and low systolic pressure in the ankle. This indicates to a small amount of blood flow and fluid retention in the lower leg. Diabetic arteriopathy is characterized by extreme rigidity and calcification of blood vessels as well as microangiopathy. Under these conditions, the effect of nutritive flow is restricted so that the exchange of fluids through the skin is also limited. According to the findings of this study, 43.8% of patients reported a remarkable immediate effect on wound healing, while a delayed effect occurred in 53.1% of patients. Given that these patients were exposed to other conservative methods of treatment as well, the findings point to the synergy of the therapy using intermittent hypobaric pressure with other methods of treatment. Taking into consideration the total number of patients, we believe that a larger sample is necessary to obtain relevant conclusions. This analysis requires a special investigation (3, 4, 9).

Conclusion

Based on the obtained findings, the following conclusions can be made:

1. Intermittent hypobaric pressure therapy using the Greensack® kit leads to significant reduction of the claudication symptoms.
2. Improved wound healing in patients with critical limb ischemia occurs after the end of treatment, but due to a relatively small number of subjects, it cannot be fully contributed to Greensack® therapy.
3. Pain relief is more pronounced immediately after completion of treatment, but after

a month as well; more than a quarter of patients feels significantly less pain after therapy.

Our general conclusion is that the Greensack® kit deserves its place in the treatment of inoperable peripheral arterial disease, but for final evaluation in patients with critical limb ischemia, further studies are necessary with more patients and a greater number of tested parameters.

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References

1. *Hirsh AT, Criqui MH, Treat-Jacobson D. Peripheral arterial disease detection, awareness, and treatment in primary care.JAMA 2001; 286: 1317-24*

2. *Damjanović Ž. Fizikalna terapija periferne obliterativne arteriosklerotične bolesti. Timočki Medicinski Glasnik. 2004; (29)2: 97-102*

3. *Himmelstrup H, Himmelstrup B, Mehlsen J, Trap-Jensen J.: Effects of vacusac in intermittent claudication: a controlled cross-over study. Clin Physiol. 1991 May; 11(3): 263-9.*

4. *Bostrom K, Fors B.: "What I do is not dangerous and no hocus pocus". Vacusac therapist fights for recognition. Interview by Elisabet Forslind. Vardfacket. 1997 Dec 8; 21(11): 16-9.*

5. *Mehlsen J, Himmelstrup H, Himmelstrup B, Winther K, Trap-Jensen J.: Beneficial effects of intermittent suction and pressure treatment in intermittent claudication. Angiology. 1993 Jan; 44(1): 16-20.*

6. *Himmelstrup H, Himmelstrup B, Mehlsen J, Bonde J, Trap-Jensen J. The effect of natural medicine and vacuum therapy (Vacusac) in patients with stable intermittent claudication Ugeskr Laeger. 1987 Mar 23; 149(13): 845-8. Danish.*

7. *Delis KT, Nicolaides AN, Wolfe JH, Stansby G.: Improving walking ability and ankle brachial pressure indices in symptomatic peripheral vascular disease with intermittent pneumatic foot compression: a prospective controlled study with one-year follow-up.J Vasc Surg. 2000 Apr; 31(4): 650-61*

8. *Mehlsen J, Himmelstrup H, Himmelstrup B, Winther Hansen KF. Positive effect of intermittent overpressure and underpressure (Vacusac) in intermittent claudication Ugeskr Laeger. 1994 Jan 10; 156(2): 169-71*

9. *Ramaswami G, D'Ayala M, Hollier LH, Deutsch R, McElhinney AJ.: Rapid foot and calf compression increases walking distance in patients with intermittent claudication: results of a randomized study.J Vasc Surg. 2005 May; 41(5): 794-801.*

10. *Devečerski G, Teofilovski M, Miličević B, Novaković B, Popović V, Radaković N. Uticaj terapije intermitentnog negativnog pritiska na poboljšanje mirkocirkulacije u bolesnika sa dijabetesom – prikaz slučaja. Med Pregl 2006; 59 (Suppl): 63-5.*

11. *Devečerski G, Teofilovski M, Miličević B, Konstantinović Lj, Lazović M, Radaković N, Mujović N, Krstin A, Jelenc J. Effects of Greensack® on lower limb functional status. Europa Medicophysica 2006; 42 (Suppl): 145-6.*

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Styloid process elongation in end stage renal disease patients with peritoneal dialysis: is there any role for ectopic calcification?

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Abstract

Objectives: The aim of this study is to determine the presence of styloid process elongation (SPE) detected on panoramic radiographs in end-stage renal disease (ESRD) patients with peritoneal dialysis (PD) and to investigate the role of Ca and P metabolism (ectopic calcification) for SPE by evaluating the PD period and the correlation between the styloid process length and this period.

Study design: One hundred and ten patients with ESRD and PD were enrolled in the present study. The PRs of 5 patients who have questionable SP and the other 5 patients who have co-disorders (3 thyroid gland and 2 parathyroid gland operations) were excluded. Therefore, 100 ESRD patients with PD were included.

Results: Eleven (11%) subjects demonstrated SPE at least one side. The PD period was not statistically different between the patients with SPE and the patients without SPE. There was not a significant correlation between the PD period and styloid process length in these patients.

Conclusions: According to our knowledge, this is the first study investigating the role of ectopic calcification in the elongation of styloid process in patients with ESRD. In conclusion, instead of the high prevalence, ectopic calcification or the abnormality in Ca and P metabolism may not have a role in this elongation.

Key words: Ectopic calcification, styloid process elongation, end-stage renal disease, panoramic radiography.

Introduction

The styloid process (SP) is a cylindrical, long cartilaginous bone located on the temporal bone. There are many vessels such as carotid arteries and nerves adjacent to the SP.[1-3] The normal length of the SP is approximately 20-30 mm.[4-9] The styloid process elongation (SPE) can be assumed if either the SP or the adjacent stylohyoid ligament ossification shows an overall length in excess of 30 mm.[4,6,8-17] SPE resulted in facial and neck pain is known as Eagle's syndrome.[6,7,11,13,18-20] More uncommonly, symptoms such as dysphagia, tinnitus, and otalgia may occur in patients with this syndrome. All these symptoms may be due to SPE causing the compression of the nerves such as glossopharyngeal, vagus, etc., carotid arteries compression and other reasons. Therefore, it may also cause stroke due to this compression.[4,21,22]

The exact cause of the elongated SP due to calcified and ossified bone and ligament is not clear. It was suggested that local chronic irritations, surgical trauma, endocrine disorders in female at menopause, persistence of mesenchymal elements, growth of the osseous tissue and mechanical stress or trauma during development of SP could result in calcified hyperplasia of the SP.[2,4,13,23,24]

Extraskeletal (ectopic) calcification (deposition calcium phosphate crystals) or ossification (true bone formation) may have a role for the elongation of SP. Ectopic calcification (EC) in nonosseous soft tissue may be due to three mechanisms; metastatic calcification due to disorders causing abnormal serum Ca and phosphate P levels, dystrophic calcification due to mineral deposition into metabolically impaired or dead tissue despite

normal serum levels of Ca and P, and ectopic ossification. In patients with end-stage renal disease (ESRD) have risks for EC or ossification due to disorders (renal failure, dialysis, secondary hyperparathyroidism) causing metastatic calcification. [25] The increase of iPTH over the recommended levels, associated with hypercalcemia and hyperphosphataemia, can induce extraosseous calcifications and cardiovascular events. [26, 27] Extraosseous, especially vascular calcifications are an important complication for the patients treated with PD.[28,29]

In the present study, our aim is to investigate the prevalence of SPE- the role of EC-in ESRD patients with peritoneal dialysis (PD) and to analyze the correlation between SP length and PD period. According to the literature, this the first study investigating the role of Ca and P metabolism in the elongation of SP.

Materials and methods

One hundred and ten ESRD patients with PD are investigated for SPE by panoramic radiography (PR). All the PRs were taken at the Erciyes University Faculty of Dentistry, Department of Oral Diagnosis and Radiology. The PRs of the 110 ESRD patients with PD had originally been taken for routine examination. It was not necessary to seek ethical approval as the PRs were essential for the routine clinical evaluation of the patients. The PRs who have questionable SP were excluded in the present study. Patients had disorders causing dystrophic calcification (scleroderma, dermatomyositis, systemic lupus erythematosis, trauma-induced) and ectopic ossification (post surgery, burns, neurologic injury, myositis ossificans) were not included. A detailed differential diagnosis was done for the disorders causing–metastatic calcification-abnormal serum Ca and P levels. Except renal failure, disease causing metastatic calcification such as sarcoidosis, tumoral calcinosis, primary hyperthyroidism, milk alkali syndrome etc. were not enrolled.[25] PD periods of all the patients were noted.

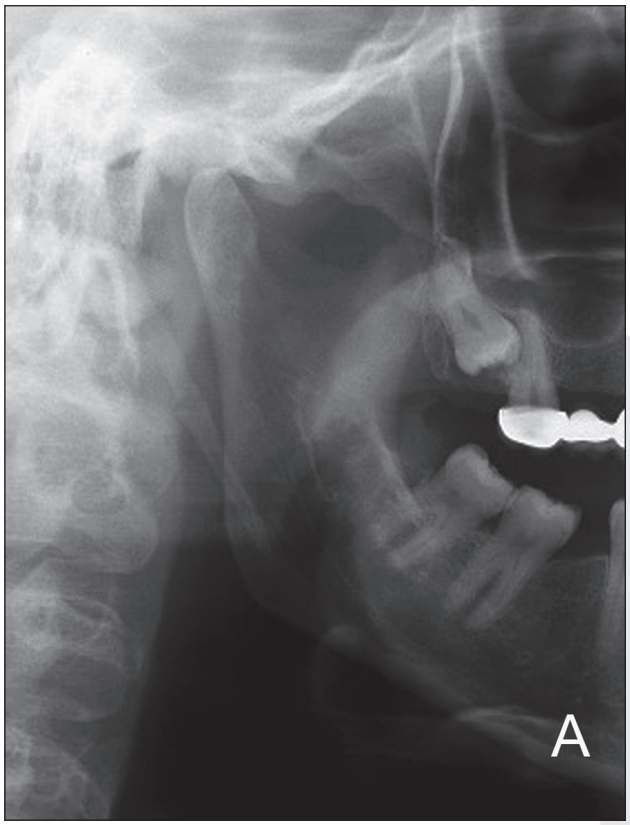
All the panoramic radiographs were done and evaluated in the same fashion (Orthopantomography® OP100, Tuusula, Finland). The PRs were processed in relation to the manufacturer's recommendations in an automatic film processor. For mineralized stylohyoid complexes and lengths of bilateral SPs were evaluated by using the measurement method of Jung et al'study.[8] In brief, the measurements were taken on the temporal bone's frontal side. A thin transparent line is usually imagined between the SP shadows and the tympanic bone in this area on the PRs. This transparent line corresponds to the cleft between the SP and the temporal bone's tympanic plate [8,30] (Figure 1 A,B,C). The tip of the SP is its bony end including calcified parts of the ligament. All the PRs were viewed in subdued ambient light using transmitted light from a standard viewbox. The lengths of the SPE variants were measured using a true-toscale radiometric ruler (magnification factor: 1.4). The radiographs were investigated and the measurements were performed by the same author (Y.S). To check the intraobserver variations, measurements were repeated after some weeks on a subset of 50 PRs. The length of SP and/or stylohyoid ligament, which are longer than 30 mm were considered to be SPE.[4,6,8-13]

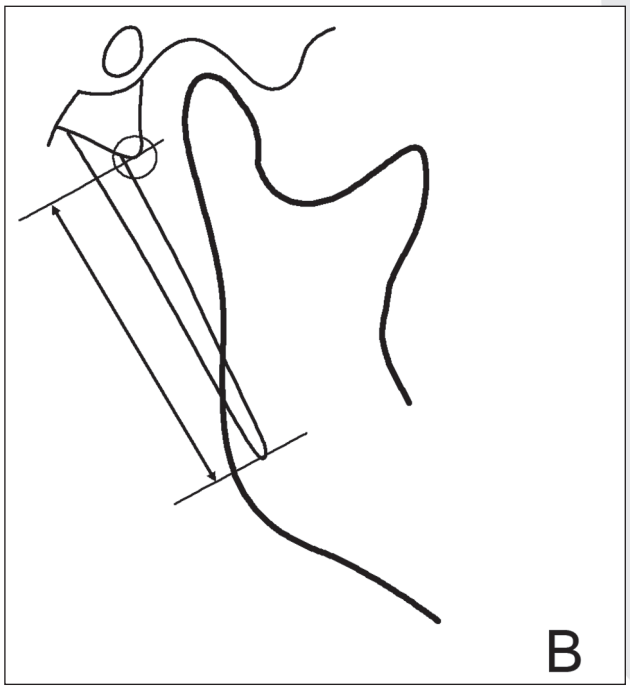
The observed results were analyzed with SPSS 10.0 (Statistical package for social science Inc., Chicago, Illinois, USA). *t*-test analysis was used to compare PD periods in patients with SPE and without SPE. A correlation analysis was performed between the SP length and PD period. P values less than 0.05 were accepted as statistically significant.

Results

One hundred and ten patients with ESRD and PD were enrolled in the present study. The PRs of 5 patients who have questionable SP and the other 5 patients who have co-disorders (3 thyroid gland and 2 parathyroid gland operations) were excluded. Therefore, 100 ESRD patients with PD were included. The mean age of these patients was 46.4 ± 13.4 years. On all the 100 PRs, the length of the SP could be measured. No significant difference in mean age between the males (48.7 ± 12.2 years) and the females (44.1 ± 14.3 years) was detected.

Eleven (11%) subjects demonstrated SPE at least one side. This abnormality (elongation) was present in both genders. The majority of these abnormalities 8 (8%) were unilateral with 6 (6%) occurring on the right and 2 (2%) on the left side. The remainder (n = 3) were bilateral. There was a greater tendency for the abnormality in male population (n = 9).





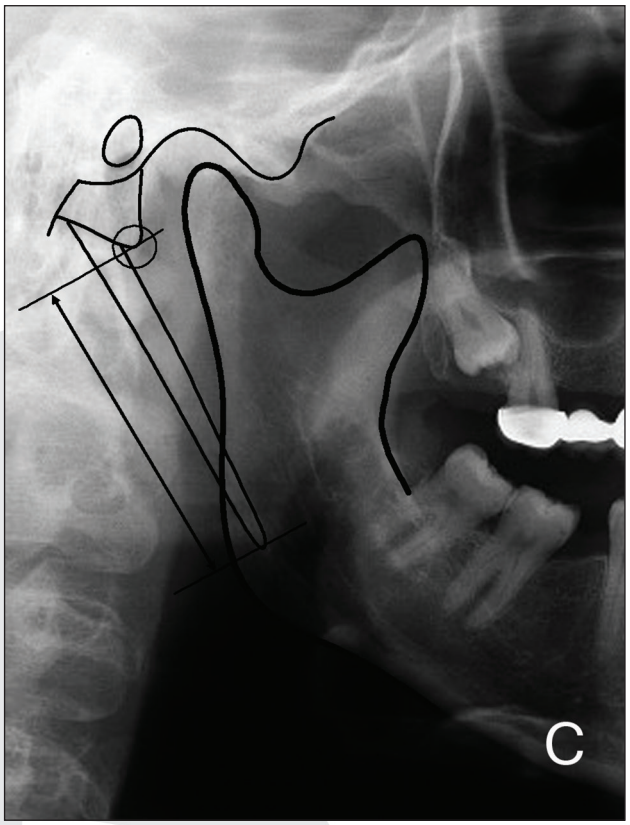


Figure 1. (A,B,C). The styloid process length was measured by using anatomical landmarks on the panoramic radiographs in patients with end-stage renal disease. (Based on Jung et al[8] and Sisman et al.[17])

The mean PD period and SP length of these patients were 4.0 ± 3.3 years (range: 1-17 years) and 21.5 ± 8.1 mm (range: 7-47 mm), respectively. No significant difference in mean age between males (48.7 ± 12.2 years) and females (44.1 ± 14.3 years) was detected. There was not a statistical difference for the PD period between the patients with SPE (5.2 ± 3.4 years) and the patients without SPE (3.8 ± 3.3 years) (Table 1). There was also not a significant correlation between the PD period and SP length in these patients (Figure 2).

Table 1. PD periods in ESRD patients

Patients	PD period (years)
ESRD patients with SPE	5.2±3.4
ESRD patients without SPE	3.8±3.3
p	>0.05

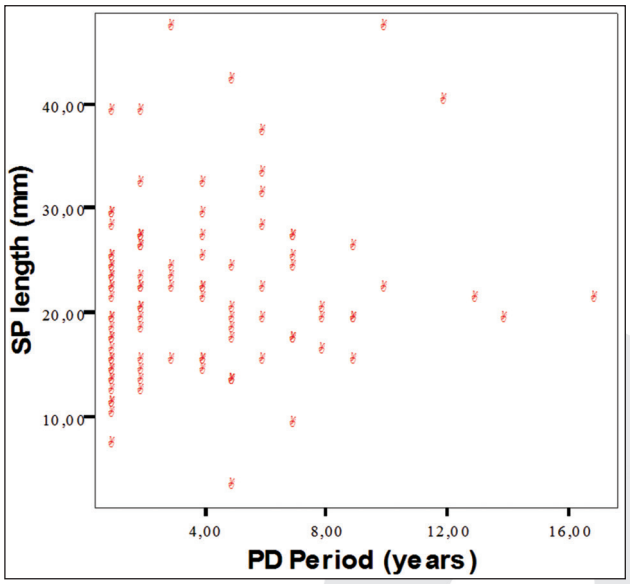


Figure 2. A correlation graphic between the PD period and SP length in patients with ESRD

Discussion

The elongated SP and structural changes in stylohyoid ligament with its clinical symptoms were first described by Eagle. So, it is also known as the Eagle's syndrome.[18,31] The symptoms and signs with this syndrome are due to the anatomic relationship between the SP and its surrounding structures.[1,32] The symptoms due to this syndrome can be confused with some disorders including a wide variety of facial neuralgias, oral, dental and, temporomandibular diseases. Therefore, a detailed differential diagnosis for SPE should be done.[20]

Although there are many suggested hypotheses, the definite etiology of calcified and ossified SPE is unknown.[2,4,13,23,24] However, according to our knowledge, there is no study investigating the role of Ca and P metabolism in patients with elongated SP. EC-metastatic calcification- in nonosseous soft tissue due to abnormal serum Ca and P levels (abnormality in Ca and P metabolism) is very common in patients with ESRD.[25] Therefore, this disease is a good model for the investigation of the EC in development of SPE.

There are limited Turkish reports investigating the SPE prevalence in normal population. Ilguy et al evaluated the PRs of 860 subjects in terms of SPE. Of these patients, 32 patients (3.7%) had SPEs.[6] In another study, the SPE prevalence on PRs was investigated for the 900 adult patients with dental problems and the prevalence was 1.1%.[33] In our previous study, based on 689 PRs consecutively retrieved from the archival records of our faculty, we aimed to investigate the prevalence of SPE by PR and to analyze this prevalence in relation to gender and age subgroups in a Turkish population living in the same region. The mean age of these 698 patients (285 male; 413 female) was 34.9±14.1 years and fifty four (7.7%) subjects demonstrated SPE at least one side.[14]

As in our study, the length of SP and/or stylohyoid ligament, which are longer than 30 mm were considered to be SPE in these Turkish reports. According to our knowledge, the present study is the first report investigating the SPE prevalence on PRs in ESRD patients with PD. In the present study, the prevalence was 11% in these patients and the findings were relatively high comparing to our previous results if we consider as a control cohort. This high prevalence in comparison to the other Turkish reports may be due to underlying disorder, but the sample of the study may not be enough for this comment.

The extent of EC increases with the dialysis period and age.[34] EC due to the abnormality in calcium and phosphate metabolism which is related to the duration of dialysis is also very important for this calcification and it is very common in patients with ESRD.[25, 34] This is the first study evaluating the duration of the peritoneal dialysis between the patients with SPEs and the patients without SPE and analyzing the correlation between SPE and PD period in terms of EC. It was found that the PD period was not significantly different in the patients with SPEs than in the patients without SPEs. Also, there was not a significant correlation between the SP length and the PD period in the patients with ESRD.

As a result, SPEs found as incidental findings on standard PRs may be important clinically in not only patients with ESRD, but also normal population. Instead of many hypotheses and studies, the exact etiology of elongated SP and the role of ectopic calcification are unknown. SPEs in the present study were detected by PRs in 11% of the ESRD patients with PD. The PD period of the patients with SPEs was not significantly different than the patients without SPEs. Also, there was not a significant correlation between the length of SP and PD period in these patients. According to our knowledge, this is the first study done in ESRD patients

with PD. We also firstly investigated the role of Ca and P metabolism in the development of the SPEs. In conclusion, instead of the high prevalence, ectopic calcification or the abnormality in Ca and P metabolism may not have a role in the elongation of SP. However, further studies and large samples are needed to clarify the etiology of this disorder.

References

1. *Gözil R, Yener N, Calgüner E, Araç M, Tunç E, Bahcelioğlu M. Morphological characteristics of styloid process evaluated by computerized axial tomography. Ann Anat. 2001; 183: 527-35.*

2. *Krennmair G, Piehslinger E. Variants of ossification in the stylohyoid chain. Cranio. 2003; 21: 31-7.*

3. *Camarda AJ, Deschamps C, Forest D. II. Stylohyoid chain ossification: a discussion of etiology. Oral Surg Oral Med Oral Pathol. 1989; 67: 515-20.*

4. *Prasad KC, Kamath MP, Reddy KJ, Raju K, Agarwal S. Elongated styloid process (Eagle's syndrome): a clinical study. J Oral Maxillofac Surg. 2002; 60: 171-5.*

5. *Monsour PA, Young WG. Variability of the styloid process and stylohyoid ligament in panoramic radiographs. Oral Surg Oral Med Oral Pathol. 1986; 61: 522-6.*

6. *Ilgüy M, Ilgüy D, Güler N, Bayirli G. Incidence of the type and calcification patterns in patients with elongated styloid process. J Int Med Res. 2005; 33: 96-102.*

7. *Kursoglu P, Unalan F, Erdem T. Radiological evaluation of the styloid process in young adults resident in Turkey's Yeditepe University faculty of dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod. 2005; 100: 491-4.*

8. *Jung T, Tschernitschek H, Hippen H, Schneider B, Borchers L. Elongated styloid process: when is it really elongated? Dentomaxillofac Radiol. 2004; 33: 119-24.*

9. *Zaki HS, Greco CM, Rudy TE, Kubinski JA. Elongated styloid process in a temporomandibular disorder sample: prevalence and treatment outcome. J Prosthet Dent. 1996; 75: 399-405.*

10. *Ferreira de Albuquerque R Jr, Müller K, Hotta TH, Gonçalves M. Temporomandibular disorder or Eagle's syndrome? A clinical report. J Prosthet Dent. 2003; 90: 317-20.*

11. *Lee S, Hillel A. Three-dimensional computed tomography imaging of Eagle's syndrome. Am J Otolaryngol. 2004; 25: 109.*

12. *Keur JJ, Campbell JP, McCarthy JF, Ralph WJ. The clinical significance of the elongated styloid process. Oral Surg Oral Med Oral Pathol. 1986; 61: 399-404.*

13. *Murtagh RD, Caracciolo JT, Fernandez G. CT findings associated with Eagle syndrome. Am J Neuroradiol. 2001; 22: 401-1402.*

14. *Gokce C, Sisman Y, Tarim Ertas E, Akgunlu F, Ozturk A. Prevalence of styloid process elongation on panoramic radiography in the Turkey population from Cappodocia region. Eur J Dent. 2008; 2: 18-22.*

15. *Gokce C, Sisman Y, Sipahioglu M. Styloid process elongation or Eagle's syndrome: is there any role for ectopic calcification? Eur J Dent. 2008; 2: 224-8.*

16. *Sisman Y, Gokce C, Sipahioglu M. Bilateral elongated styloid process in an end-stage renal disease patient with peritoneal dialysis: is there any role for ectopic calcification? Eur J Dent. 2009; 3: 155-7.*

17. *Sisman Y, Gokce C, Tarim Ertas E, Sipahioglu M, Akgunlu F. Investigation of elongated styloid process prevalence in patients with torus palatinus. Clin Oral Investig. 2009; 13: 269-72.*

18. *Eagle WW. Elongated styloid process. Report of two cases. Arch Otolaryngol. 1937; 25: 548-87.*

19. *Cernea CR, Hojaij FC, De Carlucci D Jr, Plopper C, Vanderley F, Guerreiro CA., et al. First-bite syndrome after resection of the styloid process. Laryngoscope. 2007; 117: 181-2.*

20. *Aral IL, Karaca I, Güngör N. Eagle's syndrome masquerading as pain of dental origin. Case report. Aust Dent J. 1997; 42: 18-9.*

21. *Chuang WC, Short JH, McKinney AM, Anker L, Knoll B, McKinney ZJ. Reversible left hemispheric ischemia secondary to carotid compression in Eagle syndrome: surgical and CT angiographic correlation. Am J Neuroradiol. 2007; 28: 143-5.*

22. *Strauss M, Zohar Y, Laurian N. Elongated styloid process syndrome: intraoral versus external approach for styloid surgery. Laryngoscope. 1985; 95: 976-9.*

23. *Balbuena L Jr, Hayes D, Ramirez SG, Johnson R. Eagle's syndrome (elongated styloid process). South Med J. 1997; 90: 331-4.*

24. *Fini G, Gasparini G, Filippini F, Becelli R, Marcotullio D. The long styloid process syndrome or Eagle's syndrome. J Craniomaxillofac Surg. 2000; 28: 123-7.*

25. *Favus MJ, Vokes TJ. Paget disease and other dysplasias of bone. In: Jameson JL, ed. Harrison's endocrinology. USA: McGraw-Hill Companies, 2006: 485–98.*

26. *Rusu CC, Moldovan D, Valea A, Parvu L, Kacso I, Bondor C, Patiu IM, Racasan S, Gherman-Caprioara M. The calcium phosphorus product is a better indicator for survival than immunoreactive Parathormone in chronic hemodialysis patients with renal failure. Possible role of serum albumin level. Acta Endocrinologica (Buc). 2009; vol. V, no. 3, p. 349-60.*

27. *Kacso I, Rusu A, Racasan S, Patiu I. M., Orasan R. , Rogojan A, Georgescu C, Airizer M, Moldovan D,Gherman-Caprioara M. Calcific uremic arteriolopathy related to hyperparathyroidism secondary to chronic renal failure. A case-control study. Acta Endocrinologica (Buc) 2008; vol. IV, no. 4, p. 391 – 400.*

28. *Moldovan D, Rusu C, Patiu I, Racasan S, Orasan R, Kacso I, Brumboiu I, Bondor C, Gherman-Caprioara M. Could the serum parathormone be a predictive marker for peripheral vascular calcifications in chronic dialysis patients? Experience of a single Center in Transylvania. Acta Endocrinologica (Buc). 2010; vol. VI, no. 1; 43-55.*

29. *Mircescu G. Oxidative stress of chronic kidney disease. Acta Endocrinologica (Buc) 2008; vol. IV, no. 4, p. 433 – 46.*

30. *Okabe S, Morimoto Y, Ansai T, Yamada K, Tanaka T, Awano S, et al.. Clinical significance and variation of the advanced calcified stylohyoid complex detected by panoramic radiographs among 80-year-old subjects. Dentomaxillofac Radiol. 2006; 35: 191-9.*

31. *Eagle WW. Elongated styloid process, further observations and a new syndrome. Arch Otolaryngol. 1948; 47: 630-40.*

32. *Bafaqeeh SA. Eagle syndrome: classic and carotid artery types. J Otolaryngol. 2000; 29: 88-94.*

33. *Erol B. Radiological assessment of elongated styloid process and ossified stylohyoid ligament. Journal of Marmara University Dental Faculty. 1996; 2: 554-6.*

34. *Guérin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant. 2000; 15: 1014-21.*

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Efficacy of Linezolid on gram-positive bacterial infection in elderly patients and the risk factors associated with thrombocytopenia

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Abstract

Background: Linezolid is active against drug-resistant gram-positive bacteria. However, the efficacy and safety of linezolid in the treatment of gram-positive bacterial infection in the elderly have not been well characterized.

Methods: This was a retrospective analysis of 50 elderly patients who were treated with intravenous linezolid for gram-positive bacterial infection. Clinical data and bacteriological responses were assessed. Risk factors associated with thrombocytopenia in elderly patients were analyzed.

Results: The overall clinical cure rate of linezolid was 74%, and the bacteriological eradication rate was 69%. Thrombocytopenia occurred in 24 patients, and thrombocytopenia was associated with both the duration of treatment ($P = 0.005$) and the baseline platelet count ($P = 0.042$). Based on a logistic regression analysis, the baseline platelet count $<200\times10^9$/L (OR = 0.244; 95% CI=0.068-0.874; $P = 0.030$) was identified as the only significant risk factor for linezolid-associated thrombocytopenia in elderly patients. The mean platelet count decreased significantly from the 7th day of treatment, and decreased to the lowest value 1-2 days after the end of therapy.

Conclusions: Linezolid is effective and safe for the elderly with gram-positive bacterial infections. Adverse effects such as thrombocytopenia are of greater concern. Platelet counts should be monitored in patients who are treated with linezolid and that measures should be taken in advance to avoid hemorrhagic tendencies.

Key words: Linezolid, risk faktors, thrombocytopenia

Introduction

Gram-positive bacteria are important pathogens in community and nosocomial infections. In recent years, antibiotic-resistant gram-positive bacteria have caused a variety of diseases and deaths, among which methicillin-resistant staphylococcus aureus (MRSA) is a major pathogen [1]. Linezolid was the first oxazolidinone to be developed and approved for clinical use in the USA in April 2000, and it is active against drug resistant gram-positive bacteria [2]. Cross-resistance between linezolid and other protein synthesis inhibitors is really uncommon, and linezolid seldom induces drug resistance of bacteria in vitro [3,4]. Therefore, linezolid is predominantly used in clinical treatment of bacterial infection. However, linezolid has been associated with hematologic adverse effects such as thrombocytopenia [5]. At present, there are few studies that reported the efficacy and safety of linezolid in the elderly. The purpose of this study was to evaluate the efficacy of linezolid in the treatment of the elderly with gram-positive bacterial infection and to investigate the risk factors associated with the development of thrombocytopenia in these patients.

Methods

Patients and study design

This was a retrospective study that enrolled 50 patients with a mean age of 81 years (range, 60–96 years) who were treated with linezolid at 600 mg IV BID (q12h) between Jan 2008 and Oct 2010 at Jiangsu Province Hospital in Nanjing, China. Data were extracted from the electronic medical records obtained from the central database at the hospital. These patients were treated with linezolid as part of their primary antibiotic management of a suspected or proven Gram-positive infection.

The medical records of the study population were analyzed retrospectively. For each patient the following data were collected: demographics; length of hospital stay; intensive care unit (ICU) admission; type of infection and microbiological data; co-morbidities; previous and concomitant antimicrobial treatments; duration of linezolid therapy; outcome of linezolid treatment. The following laboratory findings before, during and after treatment were collected: hematologic properties (white blood cell count, hemoglobin, platelet count); routine biochemical tests; C-reactive protein (CRP); hepatic and renal function. The results of bacterial culture, smear, susceptibility tests and correlative imaging examinations were collected as well.

Evaluation of the outcomes

According to the response to treatment, patients were classified as cured, failed or indeterminate. Clinical cure was defined as the resolution of the baseline signs and symptoms of infection with improvement or lack of progression of radiographic findings. Failure was defined as the persistence or progression of the signs and symptoms of infection after at least 7 days of therapy, administration of a potentially effective other antibiotics during the treatment because of lack of efficacy, or the absence of clinical assessments at the end of therapy. Indeterminate was defined as the inability to assign classification to one of these two categories.

Microbiological outcomes were classified as eradication, presumed eradication, persistence, eradication with reinfection. Microbiological response rate was defined as the number of patients with eradication or presumed eradication divided by the total number of patients in the analysis.

Analysis of hematologic properties

Hematologic properties (white blood cell count, hemoglobin, platelet count) before, during and after the treatment were extracted from electronic medical records. All the data were statistically analyzed.

Risk factors for thrombocytopenia

Thrombocytopenia was defined a decrease in platelet count of ≥25% and a final count of <100 × 10^9/L. To investigate the risk factors associated with the development of thrombocytopenia in elderly patients who received linezolid therapy, the patients were divided into 2 groups according to whether thrombocytopenia occurred, and the following clinical characteristics were collected and compared in the thrombocytopenia group and the non-thrombocytopenia group: age, sex, length of hospital stay, baseline alanine aminotransferase (ALT) and creatinine clearance rate(CCr), baseline hemoglobin(HGB) concentration and platelet count, duration of linezolid therapy and lactic acid dehydrogenase(LDH) before the treatment.

Statistical analysis

Results were expressed as mean values (±SD) unless otherwise specified. The unpaired Student's *t* test was used to analyze continuous data, and either X^2 analysis or Fisher's exact test was used to analyze categorical data. Risk factors associated with thrombocytopenia were identified via logistic regression analysis. Statistical analyses were performed with SPSS for Windows, version 13.0 (SPSS). A p value of <0.05 was considered to be statistically significant.

Results

Patient demographic and clinical characteristics

In total, 50 patients (36 men and 14 women, age≥60 years, mean age 81±10 years) were included in the study. Linezolid at the dose of 600 mg was administered by IV infusion q12h. Mean duration of treatment was 13±2 days (range 6-21 days), average hospital stay was 49±26 days. The clinical characteristics were shown in Table 1.

Mean planet count, hemoglobin concentration, liver and renal function and creatinine clearance rate of all patients were analyzed (Table 2). Based on t-test, a significant difference was observed only in the platelet count (P=0.000), whereas hemoglobin concentration, liver and renal function showed no significant differences before and after the treatment. In the 17 patients with renal dysfunction, renal function did not get worse after linezolid was used, and renal function of some patients improved with the improvement of their primary diseases.

A microbiologically documented diagnosis was made in 26 patients (52%). The most commonly isolated pathogen was *Staphylococcus au-*

Table 1. Clinical characteristics of the study population

Clinical condition	n	percentage %
Types of infection		
Pneumonia	33	66
Pneumonia concomitant with bloodstream infection	11	22
Pneumonia concomitant with urinary infection	1	2
Bloodstream infection	4	8
Empyema	1	2
Characteristics associated with infection		
Mechanical ventilation	30	60
Hematological disease	2	4
Renal inadequacy	17	34
Age >70 years old	43	86
Length of stay >30 days	38	76
Poor malnourished condition	24	48
Vessel in deep vein and urinary canal remaining	37	74
ICU admission	43	86

Table 2. Comparison of laboratory findings in the study population before and after treatment

Indices	Mean value before treatment	Mean value after treatment	*P*
PLT($\times 10^9$/L)	239±114	146±87	0.000
HGB(g/L)	102±18	94±16	0.052
ALT(U/L)	32±34	24±13	0.303
AST(U/L)	40±37	40±25	0.984
Cr(μmol/L)	122±84	130±135	0.780
CCr(ml/min)	54±40	50±31	0.668

PLT, platelet; HGB, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatine; CCr, creatinine clearance.

reus (34%, of which 88% were MRSA), followed by *Staphylococcus epidermidis* (28%, of which 64% were MRSE), *Enterococcus faecium* (10%), *Enterococcus avium* (8%), *Staphylococcus lugdunensis* (4%), *Enterococcus faecalis* (4%), *Staphylococcus haemolyticus* (4%), *Staphylococcus capitis* **(4%),** *Staphylococcus hominis* **(2%),** *Staphylococcus sciuri* **(2%). All isolates were susceptible to linezolid.**

Clinical and microbiological outcomes

Out of the 50 treated patients, 37 patients (74%) were considered to be cured. 5 patients were dead because of severe physical illnesses accompanied by respiratory and circulation failure. In the cured patients，the mean time to a decrease in temperature was 6±2 days, and the mean time to a decrease in white blood cell counts was 5±2 days. The bacteriological eradication rate was 69% (18/26).

Analysis of hematologic properties

Thrombocytopenia occurred in 24 patients, indicating the incidence of 48% (24/50). The platelet counts in all the patients in the thrombocytopenia group decreased since the beginning of linezolid therapy and decreased maximally at about day 15, then began to increase gradually (Figure 1). Among them, 4 patients accepted platelet transfusion because of the lower platelet counts and hemorrhagic tendencies, which happened on day 13, day 14, day 15 and day 17 of treatment, respectively. The platelet counts of most patients increased to near normal values 7 days after the end of therapy. The patterns of the changes in the platelet counts were demonstrated in Figure 2 of the patients who were treated with linezolid for 14 days, excluding those who accepted platelet transfusion. Simultaneously, the variations in white blood cell count and hemoglobin concentration in these patients were recorded (Figure 3).

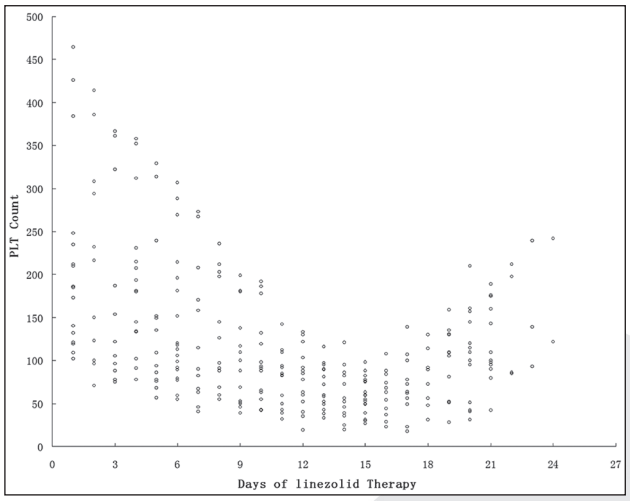

Figure 1. Platelet count (open circles, ×10⁹/L) in patients of the thrombocytopenia group

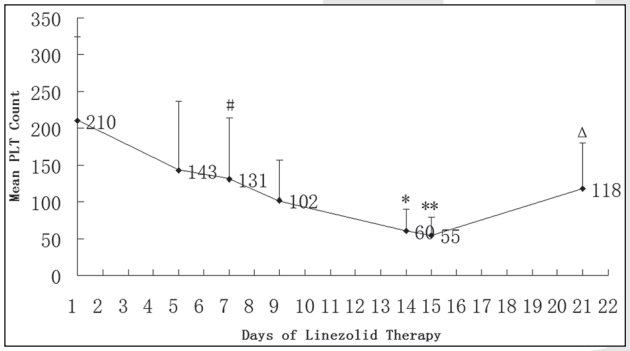

Figure 2. Mean platelet count in patients of the thrombocytopenia group

*Planet count was expressed as (x 10⁹/L, n=18) #P<0.05, * P<0.01, **P<0.01 compared with mean PLT count before treatment Δ P<0.01 compared with mean PLT count(d15)*

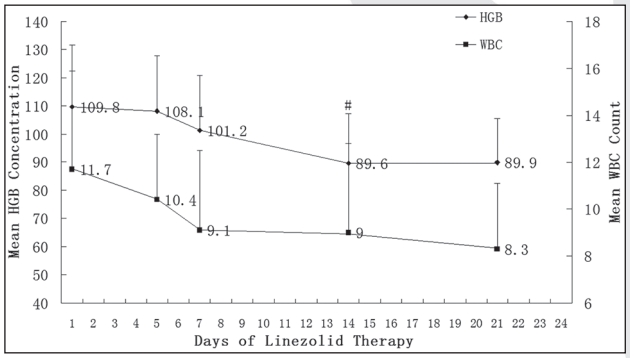

Figure 3. Mean white blood cell count (×10⁹/L, n=18) and hemoglobin concentration (g/L, n=18) in patients of the thrombocytopenia group

#P<0.01 compared with mean hemoglobin concentration before treatment

Further statistical analysis showed that the mean platelet count of the patients was as follows: 210±114×10⁹/L (before treatment), 131±83×10⁹/L (d7), 102±55×10⁹/L (d9), 60±30×10⁹/L (d14), 55±24×10⁹/L (d15, 1 day after the end of therapy), and 118±62×10⁹/L (d21, 7 days after the end of therapy). We found a significant difference in platelet count between the time before treatment and 7 days after the therapy (d7, *P*=0.048). The difference became more significant at the end of therapy (d14, *P*=0.000), and the lowest mean platelet count were observed 1 day after the end of therapy (d15, *P*=0.000). On the other hand, the mean white blood cell count and hemoglobin concentration both decreased with the duration of linezolid therapy, and the hemoglobin concentration was significantly higher at the end of treatment than before the treatment (*P*=0.009).

In addition, we observed that in half of the 24 patients who developed thrombocytopenia, the platelet count decreased to below normal level when linezolid was used for one week (Figure 4).

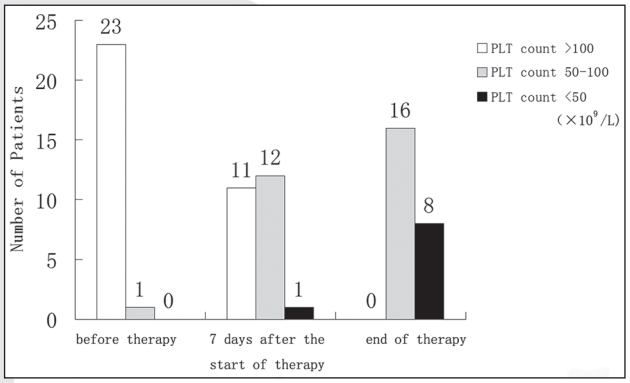

Figure 4. The population distribution classified by platelet counts in patients of thrombocytopenia group during treatment

Risk analysis of thrombocytopenia

To investigate the risk factors associated with the development of thrombocytopenia in elderly patients who received linezolid therapy, the characteristics of patients were compared between those with and those without thrombocytopenia. As shown in Table 3, the mean (SD) treatment duration of linezolid was significantly longer in patients with thrombocytopenia than in those without thrombocytopenia (*P*=0.005). In addition, the mean baseline platelet count was significantly lower in patients with thrombocytopenia than in those without thrombocytopenia (*P* = 0.042). Other risk factors such as age, sex, length of hospital stay, baseline alanine aminotransferase, creatinine

clearancerate，hemoglobin concentration, lactic acid dehydrogenase were not significantly different between the 2 groups. However, based on a logistic regression analysis, the baseline platelet count <200×10^9/L (OR = 0.244; 95% CI=0.068- 0.874; *P* =0.030) was a significant risk for linezolid-associated thrombocytopenia in elderly patients (Table 4).

Discussion

Gram-positive bacteria, particularly multidrug-resistant *Staphylococcus aureus*, increasingly become the common causes of nosocomial and community-acquired infection [6]. In USA，the rate of MRSA infection increased to 50%-60% according to data from the National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention [7]. Similarily, bacterial resistance monitoring in 9 major hospitals in China in 2006 showed that MRSA accounted for 58. 4% of infection by *Staphylococcus aureus* [8]. In addition, the appearance of vancomycin resistant enterococcus (VRE), vancomycin intermediate S aureus (VISA) and vancomycin resistant S aureus (VRSA) brings difficulties to curing infectious diseases. Consistent with previous data, in this study we found that

Table 3. Comparison of patient characteristics between those with thrombocytopenia and those without thrombocytopenia who received intravenous linezolid therapy

	Patients With Thrombocytopenia (n=24)	**Patients Without Thrombocytopenia (n=26)**	***P***
Age (years)	83±9	80±11	0.344
Sex(no) Male Female	 17 7	 19 7	 0.861
Length of stay (days)	47±29	51±32	0.658
Baseline ALT (U/L)	44±42	23±14	0.159
Baseline CCr (ml/min)	46±36	60±43	0.290
Baseline HGB (g/L)	104±21	99±16	0.358
Baseline PLT (×10^9/L)	204±118	272±101	0.042
Treatment duration (days)	14±2	12±2	0.005
Baseline LDH (U/L)	289±182	272±168	0.815

Table 4. Risk factors associated with the development of thrombocytopenia in elderly patients who received intravenous linezolid therapy (n = 50)

	OR	**95% CI**	***P***
Age(>80 years)	1.091	0.300- 3.960	0.895
ALT(>50U/L)	0.420	0.079- 2.218	0.307
CCr(<30μmol/L)	1.064	0.220- 5.148	0.938
HGB(<90g/L)	1.444	0.309-6.734	0.640
PLT(<200×10^9/L)	0.244	0.068- 0.874	0.030
Treatment duration (>14 days)	0.299	0.022-4.128	0.368

OR=odds ratio

Staphylococci including MRSA and MRSE was the major pathogens isolated in the patients.

Infection by drug resistant gram-positive bacteria such as MRSA could increase mortality rates and hospital charges and prolong the length of hospital stay. In 2003，Cosgrove et al. comprehensively analyzed previous studies and concluded that the fatality rate of MRSA infection was 20 times that of MSSA infection [9]. Glycopeptide antibiotics are thought to be the drugs of choice in the treatment of MRSA infection, but failures in the treatment of pulmonary infections have occurred recently. The main causes of these failures are the poor lung tissue penetration of glycopeptide antibiotics and the drift of minimal inhibitory concentrations. In addition, glycopeptide antibiotics have a narrow therapeutic range and require therapeutic drug monitoring, particularly in patients with renal dysfunction [10]. Potential adverse effects of vancomycin include renal toxicity and 'red-man' syndrome.

As the first oxazolidinone to be developed and approved for clinical use, linezolid blocks the first step of protein synthesis-initiation, unlike most other protein synthesis inhibitors which inhibit elongation. Due to this unique mechanism of action, cross-resistance between linezolid and other protein synthesis inhibitors is rare [11]. With high penetration in tissues and fast efficacy for pulmonary infections, linezolid has become the recommended choice of treatment for vancomycin-resistant enterococcus infections, hospital- and community-acquired pneumonia, tuberculosis and meningitis [12,13]. A two double-blind studies of patients with MRSA nosocomial pneumonia showed that initial therapy with linezolid was associated with significantly better survival and clinical cure rates than therapy with vancomycin [14]. Similar clinical trial in China confirmed that the efficacy rate of linezolid was 78.6% in pneumonia patients [15]. At present, the use of linezolid in the elderly remains limited and is mainly based on incidental case reports, with not enough information regarding its efficacy and tolerability in old patients. In the present study, the clinical data concerning the use of linezolid in 50 elderly patients were retrospectively analyzed. We found that linezolid was effective in the treatment of the elderly with gram-positive infection and the total clinical efficacy was 74%, similar to previous reports. In addition, the effective cases included not only bacterial culture positive patients but also smear positive patients, implying that linezolid can be used empirically in the following conditions: lack of bacterial culture; infection not controlled after initial antibiotic therapy; pathogenic bacteria in nubibus. Linezolid could be especially useful as appropriate empiric therapy for elderly patients with pulmonary gram-positive bacterial infection.

In our study, the main adverse reaction thrombocytopenia was reversible, in agreement with previous study [16]. However, the incidence of thrombocytopenia was 48%, higher than what reported previously [17-19]. The possible reason was that the selected patients in our study were the elderly, and the hematogenesis function of the elderly might be lower than in younger patients.

To investigate the risk factors associated with the development of thrombocytopenia, the patients were divided into 2 groups based on the occurrence of thrombocytopenia. In the thrombocytopenia group, we found that the platelet count decreased significantly on the 7^{th} day of treatment, and decreased to the lowest value 1-2 days after the end of therapy, then increased significantly 1 week later. In addition, thrombocytopenia was often accompanied by anemia. The pattern of changes in platelet count we observed is useful for monitoring of platelet counts when patients are treated with linezolid. The mechanisms underlying linezolid induced hematologic toxicity remain to be clarified, although bone marrow suppression may be involved. In our study, we observed the decrease of hemoglobin and white blood cell counts accompanying the thrombocytopenia, but we could not confirm the existence of bone marrow suppression due to the complicated influencing factors and the deficiency of the marrow smears. Based on a univariate analysis, we found that the mean treatment duration of linezolid was significantly longer and the mean baseline platelet count was significantly lower in patients with thrombocytopenia than in those without thrombocytopenia. However, based on a logistic regression analysis, only the baseline platelet count $<200\times10^9$/L was a significant risk for linezolid-associated thrombocytopenia in elderly patients. It has been reported that thrombocytopenia occurred when the time of treatment exceeded 14 days，implying that this

side effect was associated with the accumulation of drug or metabolite [20]. In the present study, the duration of linezolid treatment (>14 days) was not a significant risk for thrombocytopenia based on multivariate analysis. The difference in the patient population and relatively short duration may explain the inconsistent result. Because the activity of LDH in blood serum usually increased when platelet and red blood cells were destroyed, we collected the data of LDH levels before and after treatment and did not find evidence for the destruction of platelets. In addition, by statistical analyses, we found that thrombocytopenia was not associated with age, sex, length of stay, liver and renal function before treatment or baseline hemoglobin concentration, although it was reported that the incidence of linezolid-associated thrombocytopenia was higher in patients with renal dysfunction than in those with normal renal function [21]. In our study, we did not find correlated factors except for the pretreatment PLT count and the duration of treatment. Future studies involving larger samples may help identify and confirm the correlated risk factors. During treatment with linezolid, we found that although platelet counts decreased below normal level, hemorrhagic tendency did not always occur, and platelet counts could increase gradually after the end of therapy. This proved that thrombocytopenia was a reversible adverse effect although platelet transfusion is necessary when hemorrhagic symptoms appear.

In conclusion，linezolid is effective and safe for the elderly with gram-positive bacterial infection. Linezolid causes minimal damage to the liver and renal function of the aged, but adverse effects such as thrombocytopenia should be monitored. Thrombocytopenia was associated with baseline PLT count and duration of treatment, and the baseline platelet count $<200\times10^9$/L was a significant risk for linezolid-associated thrombocytopenia in elderly patients. These findings suggest that platelet counts should be monitored in patients who are treated with linezolid and that measures should be taken in advance to avoid hemorrhagic tendencies.

Acknowledgements

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References

1. *Backx M, Healy B: Serious staphylococcal infections. Clin Med 2008; 8(5): 535-8.*
2. *Taylor JJ, Wilson JW, Estes LL: Linezolid and serotonergic drug interactions: a retrospective survey. Clin Infect Dis 2006; 43(2): 180-7.*
3. *Diekema DJ, Jones RN: Oxazolidinone antibiotics. Lancet 2001; 358(9297): 1975-82.*
4. *Rodriguez JC, Ethlvarez JM, Escribano I, Royo G: In vitro activity of linezolid against Staphylococcus aureus: a population study. Chemotherapy 2005; 51(2-3): 86-8.*
5. *Hiraki Y, Tsuji Y, Hiraike M, Misumi N, Matsumoto K, Morita K, Kamimura H, Karube Y. Correlation between serum linezolid concentration and the development of thrombocytopenia. Scand J Infect Dis. 2012; 44(1): 60-4.*
6. *Cercenado E, Garcia-Garrote F, Bouza E: In vitro activity of linezolid against multiply resistant Gram-positive clinical isolates. J Antimicrob Chemother 2001; 47(1): 77-81.*
7. *Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R: Changes in the epidemiology of methicillin-resistant Staphylococcus aureus in intensive care units in US hospitals, 1992-2003. Clin Infect Dis 2006; 42(3): 389-91.*
8. *WangFu: CHINET 2006 surveillance of bacterial resistance in China. Chinese Journal of Infection and Chemotherapy 2008; 8 (1) : 1-9.*
9. *Cosgrove SE, Carmeli Y: The impact of antimicrobial resistance on health and economic outcomes. Clin Infect Dis 2003; 36(11): 1433-7.*
10. *Goldstein FW, Kitzis MD: Vancomycin-resistant Staphylococcus aureus: no apocalypse now. Clin Microbiol Infect 2003; 9(8): 761-5.*
11. *Honeybourne D, Tobin C, Jevons G, Andrews J, Wise R: Intrapulmonary penetration of linezolid. J Antimicrob Chemother 2003; 51(6): 1431-4.*

12. *Sipahi OR, Bardak S, Turhan T, Arda B, Pullukcu H, Ruksen M, et al: Linezolid in the treatment of methicillin-resistant staphylococcal post-neurosurgical meningitis: A series of 17 cases. Scand J Infect Dis. 2011 Jun 15. [Epub ahead of print]*

13. *Kjöllerström P, Brito MJ, Gouveia C, Ferreira G, Varandas L. Linezolid in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis in paediatric patients: experience of a paediatric infectious diseases unit. Scand J Infect Dis. 2011 Jul; 43(6-7): 556-9*

14. *Wunderink RG, Rello J, Cammarata SK, Croos-Dabrera RV, Kollef MH: Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant Staphylococcus aureus nosocomial pneumonia. Chest 2003; 124(5): 1789-97.*

15. *Lin DF, Wu JF, Zhang YY: A randomized,double-blinded,controlled,multicenter clinical trial of linezolid versus vancomycin in the treatment of gram positive bacterial infection. Chinese Journal of Infection and Chemotherapy 2009; 9(1): 10-17*

16. *Bishop E, Melvani S, Howden BP, Charles PG, Grayson ML: Good clinical outcomes but high rates of adverse reactions during linezolid therapy for serious infections: a proposed protocol for monitoring therapy in complex patients. Antimicrob Agents Chemother 2006; 50(4): 1599-602.*

17. *French G. Safety and tolerability of linezolid. J Antimicrob Chemother.2003; 51(Suppl 2): ii45–ii53.*

18. *Lin YH, Wu VC, Tsai IJ, et al. High frequency of linezolid-associated thrombocytopenia among patients with renal insufficiency. Int J Antimi-crob Agents. 2006; 28: 345–351.*

19. *Rao N, Hamilton CW. Efficacy and safety of linezolid for Gram-positive orthopedic infections: A prospective case series. Diagn Microbiol Infect Dis. 2007; 59: 173–179*

20. *Gerson SL, Kaplan SL, Bruss JB, et al. Hematologic effects of linezolid: Summary of clinical experience.Antimicrob Agents Chemother. 2002; 46: 2723–2726.*

21. *Soriano A, Ortega M, Garcia S, Penarroja G, Bove A, Marcos M: Comparative study of the effects of pyridoxine, rifampin, and renal function on hematological adverse events induced by linezolid. Antimicrob Agents Chemother 2007; 51(7): 2559-63.*

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Efficacy and safety of use of the combination of Ketamine and Propofol ("Ketofol") in procedural sedation and analgesia in children

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Abstract

Objective: This study evaluated the doses, effectiveness, recovery time and safety of intravenous Ketamine-Propofol („Ketofol") combination for procedural sedation and analgesia (PSA) in children.

Methods: Prospective data were collected in the period of one year in the trauma receiving hospital. Patients receiving intravenously1: 1 mixture Ketofol (10mg/ml Ketamine and 10mg/ml Propofol) a single-syringe.

Results: Ketofol was performed in 31 patients, with a median age 10,4 years. Mean values of Ketofol doses was 0.645 mg / kg. Apnea which occurred only in one patient (3.2%). Alpha

Other side effects were not observed. Median recovery time was 7,84min (range 4 to 20 minutes).

Conclusion: "Ketofol" procedural sedation and analgesia is highly effective. Ketofol achieving adequate sedation, analgesia, respiratory and hemodynamic stability and faster awakening and postoperative recovery of patients.

Key words: Ketamine, Propofol, analgesia

Introduction

Procedural sedation (PS) is a technique that provides a safe sedation and analgesia. This technique involves an adequate level of sedation, elimination of analgesia and fear, amnesia, controlling the level of consciousness, and maintaining the stability of the respiratory and cardiovascular system. The ideal pharmacological agent for this type of procedure should be easy to use, with fast onset and cessation of action, safe for all age categories and that is economically acceptable. It is clear that it is difficult to expect to find a single drug that meets all the requirements, so the combination of two ore more drugs is often used, mainly in various doses, as well as in different combinations and means of application. The use of combination of Ketamine and Propofol, under the popular name "Ketofol" is quite popular in procedural sedation, especially in emergency departments (1,2).

Ketamine is a derivative, actually a precursor of phenylcyclidin that is due to the effects that is posesses described as a "dissociative anesthetic". It is the blockator of NMDA (N methyl D aspartate) receptors and leads to analgesia and amnesia. After the application of this anesthetic the swallowing reflex and spontaneous respiration are preserved, depending on the dose, with the stimulation of the cardiovascular system (sympatomimetic effect), and preservation of muscle tone. The use of this anesthetic as monoanestetic, is limited by the longer awakening, agitation, unpleasant dreams, nausea and vomiting, especially in adults.

Propofol is nonopioid, nonbarbiturate, sedative-hypnotic agent with rapid dissolution of action and short action and rapid awakening. The side effects of propofol are bradycardia and cardiovascular and respiratory depression, which are dose dependent. It has antiemetic and amnestic but no analgesic effect. Its use and application locally, even leads to pain because it is liposolubile. Use of Ketamine and Propofol in combination is documented in a small number of clinical studies, including the most common gynecological, ophthalmic, cardiovascular and hematological procedures, both for adults and children. Given the physical and chemical compatibility of these two agents, which is very important, their application is possible both individually and in a single syringe, protected from light (3,4). The name "Ketofol" is settled for the combination of these anesthetics in a single syringe, it has a proven efficacy, both in operating rooms, ambulatory surgery, and in the emergency department, which is of a great impor-

tance. Most often these agents are combined in a 1: 1 ratio, 10mg/ml and 10mg/ml Ketamine with Propofol, in a syringe 10 or 20 ml. This results in the concentration of 5mg/ml of each anesthetic. Application of Ketofol is described in a variety of procedures: heart catheterization (5,6), MRI (7,8), endoscopic procedures of GIT (9), burns dressing changes (10), orthopedic fracture (11,12,13), procedures in oncology (14,15,16,17,18) and procedural sedation in some cases of regional or local anesthesia (19,20). It is usually administered at intervals of several minutes to achieve a satisfactory level of sedation and analgesia, the stability of the cardiovascular and respiratory systems, rapid awakening and safety and patient satisfaction. This is a relatively new possibility of application of these anesthetics in procedural sedation and analgesia.

Methodology

Prospective study, which included pediatric patients aged from 1 to 17 years in the period of one year on the Clinic for paediatric surgery at the Institute of Healthcare of Children and Youth from Vojvodina in Novi Sad. From the study were excluded patients with a history of sensitization or allergic reaction to Propofol, Ketamine, eggs, soy products, patients in whom there is hemodynamic instability, data on seizures, increased intracranial or intraocular pressure and patients with head injuries. All patients included in the study were classified by the ASA I and ASA II.

Procedural sedation and analgesia was carried out with a previously inserted peripheral venous line, complete equipment, and with obligatory standard monitoring of the cardiovascular and respiratory system. Patients were premedicated intravenously (IV) with Midazolam or with intramuscular (IM) Midazolam and Atropine. Ketofol was prepared in a syringe of 10 or 20 ml, in a ratio of Ketamine: Propofol of 1: 1, 10mg/ml of Propofol and 10mg/ml Ketamine.

Procedural sedation was achieved with injection of drugs intravenously for about one minute, and then repeated in boluses of Ketofol every 3-5min. If the intervention lasted longer and the procedure was painful, opioid analgesics were applied, usually Fentanyl. The dosage of Ketofol was adjusted individually, depending on the type of premedication of the patient, duration of the procedure and the application of analgesics during the intervention. All patients breathed spontaneously during the procedure and received supplemental oxygen supply, through face masks. Recovery was monitored under the surveillance of trained staff in the recovery room. We monitored the following parameters: age, body weight, diagnosis and types of surgical procedures, drug doses, duration of procedure, a period of awakening, vital signs and side effects. Monitoring of vital parameters during procedural sedation was continuous, and all the complications were recorded. The following complications were documented: drop in SaO2 desaturation, apnea, hypotension, nausea and vomiting and anxiety.

Desaturation is a condition of hypoxemia, and it is a decrease in saturation below 90% or 10% of the initial value in less than 30 seconds. Hypotension is defined as a decrease in systolic blood pressure values of TA in more than 20% compared with baseline.

Results

In one year, in the Department of Pediatric Surgery in Novi Sad, a total of 31 patients were analyzed, aged from 1 to 17 years, male and female, ASA classification I and II. There were 10 female (32.3%) and 21 males (67.7%). In the ASA I classification were 27 patients (87.1%), and 4 patients of ASA II (12.9%). Age of patients ranged from 1 to 17, the mean value was 10.4 years (Table 1).

Table 1. The caracteristics of patients

	N	Min. (yrs)	Max. (yrs)	Mean
Age	31	1,67	17,00	10,43
Sex				%
M	21			67,7
F	10			32,3
ASA I	27			87,1
ASA II	4			12,9

A total of 7 patients (22.6%) who received Ketofol during the intervention, received also opiate analgesia (Fentanyl). Mean values of Ketofol doses was 0.645 mg / kg. There was no significant hemodynamic instability or other complications, except for apnea, which occurred only in one patient (3.2%), and it was transient with no significant desaturation.

Other side effects were not observed. Median recovery time was 7,84min (range 4 to 20 minutes). The most common diagnoses are shown in Table 2. In our study, most interventions were related to changing of dressing in patients with burns, and other interventions were: revision of wounds, orthopedic interventions, scleroembolisation and drainage (Table 3).

Table 2. Diagnoses

Abscessus, Phlegmona	3	9,7
Amputatio traumatica	1	3,2
Combustio	16	51,6
Conquasatio manus	3	9,7
Decolment	2	6,5
Epiphyseolisis femoris	1	3,2
Fractura radii	1	3,2
Osteoepiphiseolysis	1	3,2
Lymphangioma	1	3,2
VLC	2	6,5
TOTAL	31	100%

Table 3. Interventions

Drainage	1	3,2
Extensio	2	6,5
Incisio	1	3,2
Repositio	1	3,2
Revisio vulneris	3	9,7
Scleroembolisatio	1	3,2
Dressing burns	22	71,0
TOTAL	31	100%

Table 4. Duration of intervention and awakening

	Minimum (min)	**Max (min)**	**Mean (min)**
Duration	15	75	35
Awakening	4	20	7,84

Discussion

The clinical experience in application of Ketamine and Propofol as a single drug during procedural sedation indicated a possibility for their adequate use in "one syringe" in a 1: 1 ratio, which is discussed in a great number of clinical studies (3, 4,12). In this way effective deep sedation and analgesia is achieved, with a short recovery time and awakening, and a small incidence of side effects and complications, and significant respiratory and hemodynamic stability of patients.

There are not many studies on this subject, especially in the pediatric age group. Most often it comes to analyzing a small number of patients for various procedures, from diagnostic to therapeutic procedures, especially in pediatric hematology (14). A large clinical trial in adults was published by Andolfatto, with a total of 728 patients in the emergency department for a period of 4-5 years (4). A small number of patients was documented in literature, simlarily to our study. Perhaps one reason is that the use of drug combinations such as Ketofol is relatively new as an idea and is still little literature data of the results on a great number of patients.

A different ratio of Ketamine and Propofol (0.5 mg / kg ketamine and 1.0 mg / kg Propofol) is also described. Use of Ketamine and Propofol in combination requires a lower dose of each drug, when compared to the dose when they are used as a single agent. Moreover, in this way many adverse events are avoided or reduced to a minimum. In a study in which different concentrations of Ketofol was used (ratio Propofol: Ketamine, 1: 1, 2: 1, 3: 1), indicated that the ratio of 1: 1 drug leads to the lowest rate of respiratory depression, to the most adequate hemodynamic stability and to significantly shorter duration of sedation.

Erden has compared Propofol 0.5 mg / kg Ketamine 0.5 mg / kg compared to Propofol 0.5 mg / kg Ketamine 0.25 mg / kg (ratio 2: 1) and found that this combination of drugs showed no significant difference between the two groups in relation to hemodynamic parameters, oxygen saturation and adverse events (21). The combination of these two drugs is also interesting because of their different mechanisms of action and duration. The analgesic effect of Ketamine in subdissociative doses is well known, and on the other a sedative effect of Propofol is significant, which is directly dependent on the dose.

All these elements indicate that in the use of these two drugs in combination provides significant analgesia and hemodynamic stability which is primarily due to Ketamine, and that lower initial doses of Propofol significantly contribute to reduced incidence of hypoxia, apnea, and postoperative nausea. In our study, Ketamine and Propofol ratio was 1: 1, and the observed side effects were: hypotension, bradycardia, vomiting or laryngospasm.

Hypoxia was avoided by using a facial mask oxygenation, although respiratory depression and apnea were recorded in only one patient (3.2%). Roback notes in his study a respiratory depression in 19% of the cases of procedural sedation, but for the combination of Fentanyl and Midazolam (22), and Green descried cases of apnea and hypoxia in cases where the drug was administered intravenously too rapidly. Muscle rigidity, which is one of the side effects of Ketamine, has been avoided just by using Ketofol (23, 24).

In our study, most interventions were related to changing of dressing in patients with burns, with the additional application of opioids (Fentanyl). Of the total number of patients, 7 (22.6%), received additional opioid analgesia. Other interventions were: revision of wounds, orthopedic interventions, scleroembolisation and drainage.

A large number of clinicians give opioids before induction with Propofol, because of the pain caused by Propofol during its application. This certainly adds to appearance of respiratory depression. However, the combination of drugs in the form of Ketofol painful application or the pain is kept to a minimum due to the significant analgesic effects of Ketamine. (25). Ketofol has a significantly smaller emetic effect and emergence agitation when compared with Ketamine, especially in adults, so its use is limited in this age group (12).

The median time for emergence after Ketofol is significantly shorter than that of Ketamine, but it is longer than Propofol (8, 24). Data on the median time of awakening from Ketamine varies: from 32-103 min. (17, 18, 22, 25). In procedures in which Propofol was used median emergence time ranged from 8-93 minutes (26, 27, 28, 29, 30). The average time of awakening in our study was 7.8 minutes.

Awakening in the period of time up to 20 min was present in all patients. In a study concerning the use of the Ketofol during orthopedic interventions, 90% of patients had an awakening in time up to 20 min, and the others were awake in 30 minutes. Mean dose of drug was 0.8 mg / kg, and in 2 patients (2.2%) hypoxia was recorded (3). Probably a smaller dose per kg (0.645 mg) is the reason why the patients awakening time is shorter, and apnea and hypoxia is less common. To confirm this assertion it is certainly necessary to conduct a study on a much larger number of patients.

Conclusion

Drugs which are used in anaesthesia, are often combined in order to favor their preferred therapeutic effects and side effects and complications reduce to a minimum. The combination of Ketamine and Propofol, "Ketofol" favores sedative and antiemetic effects of Propofol with the analgesic effect and simpatomimetic effect of Ketamine, achieving adequate sedation, analgesia, respiratory and hemodynamic stability and faster awakening and postoperative recovery of patients.

References

1. *Sanjay Arora. Combining ketamine and Propofol („Ketofol") for Emergency Department Procedural Sedation and Analgesia: A Review. Western Journal of Emergency Medicine, Vol IX, No 1: 2008: 20-23.*
2. *E. V. Willman, G. Andolfatto. A prospective Evaluation of „Ketofol" (Ketamine/ Propofol Combination) for Procedural sedation and Analgesia in the Emergency Department. Annals of Emergency Medicine, 2007. Vol 49, No1: 23-30.*
3. *Gary Andolfatto, Elaine Willman. A Prospective Case series of Pediatric Procedural sedation and Analgesia in the Emergency department using Single-syringe Ketamine-Propofol Combination (Ketofol). Acad Emerg Medicine, 2010, Vol 17, No 2. 194-201. www.aemj.org*
4. *Gary Andolfatto, Elaine Willman. A Prospective Case series of Single syringe Ketamine-Propofol (Ketofol) for Emergency Department Procedural sedation and Analgesia in Adults. Acad Emerg Medicine 2011, Vol 18, No3. 237-245. www.aemj.org*
5. *Akin A, Esmaoglu A, Guler G, Demircioglu R, Narin N, Boyaci A. Propofol and Propofol-Ketamine in pediatric patients undergoing cardiac catheterization. Pediatr Cardiol. 2005; 26: 553-7.*
6. *Kogan A, Efrat R, Katz J, Vidne B.A. Propofol-Ketamine mixture for anesthesia in pediatric patients undergoing cardiac catheterization. J. Cardiothorac Vasc. Anesth. 2003; 17: 691-3.*
7. *Tomatir E, Atalay H, Gurses E, Erbay H, Bozkurt P. Effects of low dose ketamine before induction on propofol anesthesia for pediatric magnetic resonance imaging. Pediatr Anaesth 2004; 14: 845-50.*
8. *MachataAm, Willschke H, Kabon B, Kettner Sc, Marhofer P. Propofol-based sedation regimen for infants and children undergoing ambulatory magnetic resonance imaging. Br J Anaesth 2008; 101: 239-43.*

9. *Tosun Z, Aksu R, Guler G.et al. Propofol-ketamine vs propofol-fentanyl for sedation during pediatric upper gastrointestinal endoscopy. Pediatr Anaesth 2007; 17: 983-8.*

10. *Tosun Z, Esmaoglu A, Coruh A. Propofol-ketamine vs propofol-fentanyl combination for deep sedation and analgesia in pediatric patients undergoing burn dressing changes. Pediatr Anaesth 2008; 18: 43-7.*

11. *Sharieff GQ, Trocinski DR, Kanegaye JT, Fisher B, Harley JR. Ketamine-propofol combination sedation for fracture reduction in the pediatric emergency department. Pediatr Emerg Care 2007, 23: 881-4.*

12. *Saeed E. Ketofol infusion as a Procedural sedation and Analgesia Modality for Minor Orthopedic Surgeries: Evaluation of Dose-Outcome Relation. Ain Shams Journal of Anesthesiology 2011; 4: 63-74.*

13. *Roback MG, Wathen JE, Mackenzie T, Bajaj L. A randomized contolled trial of i.v. versus i.m ketamine for sedation of pediatric patients receiving emergency department orthopedic procedures. Ann Emerg Med 2006; 48: 605-12.*

14. *Paulo Sergio Lucas da Silva,V. E de Aguiar, D.R. Waisberg, R.M.A. Passos and M.V. Flor Park. Use of Ketofol for procedural sedation and analgesia in children with hematological diseases. Pediatrics international, 2011, 53: 62-67.*

15. *Chiaretti A, Ruggiero A, Barone G, et al. Propofol-alfentanil and propofol-ketamine procedural sedation in children with acute lymphoblastic leukaemia: safety, efficacy and their correlation with pain neuromediator expression. Eur J Cancer Care (Engl) 2009; 21: 212.20.*

16. *Aouad MT, Moussa AR, Dagher CM et al. Addition of ketamine to propofol for initiation of procedural anaesthesia in children reduces propofol consumption and preserves hemodynamic stability. Acta Anaesthesiol Scand 2008; 52: 561-5.*

17. *Evans D, Turnham L, Barbour K et al.Intravenous ketamine sedation for painful oncology procedures. Paediatr Anaesth 2005; 15: 131-8.*

18. *Mason KP, Padua H, Fontaine PJ, Zurakowski D. Radiologist-supervised ketamine sedation for solid organ biopsies in children and adolescents. AJR Am J Roentgenol 2009; 192: 1261-5.*

19. *Santiveri X, Molto L, Rodriguez C, Sandin F, Vilaplana J, Castillo J. Sedation and analgesia with propofol plus low-dose ketamine for retrobulbar block. Rev Esp Anestesiol Reanim 2006; 53: 545-9.*

20. *Singh R, Batra YK, Bharti N, Panda NB. Comparasion of propofol versus propofol-ketamine combination for sedation during spinal anesthesia in children: randomized clinical trial of efficacy and safety. Paediatr Anesthesia 2010; 20: 439-44.*

21. *Erden IA, Pamuk AG, Akinci SB, Koseoglu A, Aypar U. Comparasion of two ketamine-propofol dosing regiments for sedation during interventional radiology procedures. Minerva Anestesiol 2010; 76: 260-5.*

22. *Roback MG, Wathen JE, Bajaj L et al. Adverse events associated with procedural sedation and analgesia in a pediatric emergency department: a comparasion of common parenteral drugs. Acad Emerg Med.2005; 12: 508-513*

23. *Green SM, Ketamine sedation for pediatric procedures: part 2, review and implications. Ann Emerg Med 1990; 19: 1033-1046.*

24. *Hasan RA, Reddy R. Sedation with propofol for flexibile bronchoscopy in children. Pediatr Pulmonol 2009; 44: 373-8.*

25. *Mc Queen A, Wright RO, Kido MM, Kaye E, Krauss B. Procedural sedation and analgesia outcomes in children after discharge from the emergency department: Ketamine versus fentanyl/midazolam. Ann Emerg Med.2009; 54: 191-7*

26. *Zed PJ, Abu-Laban RB, Chan WW, Harrison DW. Efficacy, safety and patient satisfaction of propofol for procedural sedation and analgesia in the emergency department: a prospective study. Can J Emerg Med 2007; 9: 421-7.*

27. *Bell A, Treston G, Cardwell R, Schabort WJ, Chand D. Optimisation of propofol dose shortens procedural sedation time, prevents resedation and removes the requirement for post-procedure physiologic monitoring. Emerg Med Australas. 2007; 19: 411-7.*

28. *Bassett KE, Anderson JL, Pribble CG, Guenther. Propofol for procedural sedation in children in the emergency department. Ann. Emerg. Med. 2003; 42: 773-82.*

29. *M.Daabiss, M. Elsherbiny, R. Alotibi. Assessment of diferent concentration of Ketofol in procedural operation. BJMP 2009; 2: 27-31.*

30. *H.Aboeldahab, R.Samir, H.Hosny, A.Omar. Comparative study between Propofol, Ketamine and their combination (Ketofol) as an induction agent. Egyptian Journal of Anaesthesia 2011; 27: 145-150.*

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Laparoscopic versus open appendectomy

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Abstract

Background and Aims: Our aim in this study is retrospective evaluation of patients with a diagnosis of acute appendicitis that underwent laparoscopic or open appendectomy and determination of the significance of laparoscopic appendectomy.

Methods: In this study, the patients' data with a diagnosis of acute appendicitis that were operated by same surgeon in same centers were retrospectively analyzed between March 2010 and September 2011. Forty-two laparoscopic, 41 open appendectomies were performed. Both groups were compared with respect to the use of drains, surgical time, hospital stays and complication rates.

Results: In terms of patient characteristics between the two groups did not differ statistically. Operative times, complication rates, hospital stay and the use of drains were similar in both groups.

Conclusion: Laparoscopic appendectomy could not be the quality of gold standard as Laparoscopic cholecystectomy and Nissen fundoplication recently. However, suspected cases should be done by laparoscopy for diagnosis and treatment. Diagnostic laparoscopy is especially may be preferred because of good vision providing of the abdomen and utility of an emergency diagnosis in pathology.

Key words: Acute appendicitis, laparoscopic appendectomy, treatment method, hospital stay, postoperative complications, operative time.

Introduction

Acute appendicitis is the most common cause of acute abdomen in all age groups and appendectomy is the most common emergent operative procedure performed worldwide (1). Since the first description of open appendectomy (OA) by McBurney, it has been the gold standard for the treatment of acute appendicitis (2).

Although appendectomy is the first surgical procedure for most surgeons, its diagnosis may be difficult for even experienced surgeons. In today's modern surgery, the treatment of many diseases are remodelling with rapid advance of the medical technology. One of the most important advances in the medical technology is laparoscopic surgery (3). Laparoscopic appendectomy was first described in 1983 by a German gynecologist, Kurt Semm. Despite the many studies done about laparoscopic appendectomy, it is not yet considered as a gold standard (4,5). In this study, we aimed to review our experience with appendicitis to compare OA and LA techniques.

Methods

We retrospectively reviewed 83 patients who underwent appendectomy for acute appendicitis from March 2010 to September 2011. The patients were divided into two consequtive groups as open appendectomy and laparoscopic appendectomy. [Group I (n=42) LA and Group II (n=41) OA] All these patients were under single consultant and surgery was performed by same consultant surgeon. Data regarding age, gender, duration of operation, usage of a drain, perforation of the apendix, hospital stay, and postoperative complications were recorded.

The diagnosis of appendicitis was made preoperatively on the basis of clinical signs, physical examination findings, serum levels of leukocyte and radiological findings. Patients diagnosed as appendicitis were operated within 12 hours. The choice of operative procedure as open or laparoscopic was related to the patient and surgeon's preference. Operation time was defined as the the time between induction of anesthesia and extubation. All patients received a standard perioperative antibiotic regimen of intravenous second-generation cephalosporin.

Laparoscopy was converted to open appendectomy in the case of technical difficulties, uncertain anatomy or bleeding. For the laparoscopic approach, after a nasogastric tube and bladder catheter insertion, pneumoperitoneum was achieved with veress needle and a 10 mm trocar was placed to umbilicus. Two additional ports 5mm and 10mm were placed in the left iliac fossa and hypogastrium just below pubic hair line respectively. A 10 mm bipolar vessel sealer (Liga-Sure ™ Valley lab, Tyco, USA) was used to divide the appendix and mesoappendix. The specimens were retrieved through the 10 mm port in the left iliac fossa. If appendix was expected not to fit into the port, then a part of a surgical glove or a retrieval bag was used. The base of the appendix was ligated with 2-0 silk. Before completion of the operation, the abdominal cavity was thoroughly inspected in order to exclude other intraabdominal or pelvic pathology. In the open approach, we used a traditional oblique or transverse incision over McBurney's point. A surgical drain was inserted in the perforated and suspected cases. All specimens were sent for histopathologic investigation. In the postoperative period, patients with complete nutrition, normal physical examination and vital findings were discharged without a problem. While in the perforated cases daily wound care was performed with oral antibiotics, in other cases patients were checked when skin sutures were removed or patient have a complaint.

Statistical analysis was performed using MedCalc Software for Windows (MedCalc, Mariakerke, Belgium). In case of normal distribution t-test, in case on abnormal distribution Mann-Whitney U tests were performed. Compliance of data with normal distribution was assessed by Kolmogorov-Smirnov test. The groups were compared by using Chi-square test and Fisher's exact test for categorical variables. The *P* value of <0.05 was considered as significant.

Results

Mean age of patients was 33,97±16,00 (16-77) years in group I and 35,17±17,31 (16-87) years in group II (p = 0,7449). There were 21 males and 21 females in group I with male/female ratio (1: 1); 19 males and 22 females (male/female ratio 0.86: 1). The mean operative time of 41,86±19,57 (20-121) minutes for the laparoscopic group was similiar with the mean operative time of 41,19±8,96 (28-75) minutes for open appendectomy (p=0,5448) (Table 1).

Laparoscopic cholecystectomy was performed simultaneously in 2 (4.76 %) patients due to symptomatic cholelithiasis in group I. Conversion to open surgery was needed for 3 (7.2 %) patients. Appendectomy didn't performed in 1 (2.38%) patient in whom uterus was hyperemic and edematous in exploration. The median postoperative hospital stay was 1 day (1-20 days) in LA compared with 2 days (1-12 days) in OA, which was statistically not significant (p=0.1662). A drain was used in 7 (16.7 %) patients in group 1 and 6 patients in group 2 (14.6 %) (p=0,9623). In exploration, perforation was observed in 6 (14.6 %) patients in group 1 and 7 (17.1 %) patients in group 2 (p=0.9623).

There was no mortality in the study. Postoperative complications were compared and there was no statistically significant difference between the two groups. A total of 2 (4.8%) complications occurred in the group I, while 5 (12,2%) complications occurred in the group II (p=0,15). In group I, wound site infection developed in one patient and intra-abdominal abscess in the other. This latter patient was treated with ultrasound guided drain-

Table 1. Demographic and Medical Characteristics

	Group 1 (laparoscopic group)	Grup 2 (open group)	P*
Mean age (year)	33.97±16.00 (16-77)	35.17±17.31 (16-87)	0.7449
Gender	21 (50 %) male 21 (50 %) female	19 (46.3 %) male 22 (53.7 %) female	0.9094
Mean operation time (minute)	41.86±19.57 (20-121)	41,19±8,96 (28-75)	0.5448
Complication	2 patients (4.76 %)	5 patients (12.2 %)	0.15
Median hospital stay	1 day (1-20)	2 days (1-12)	0.1662
Drain usage	7 patients (16.67 %)	6 patients (14,63 %)	0.9623
Perforation	6 patients (14.63 %)	7 patients (16.67 %)	0.9623

age and antibiotics. In the open group, complication occured in 5 (12.2%) patients. Pre-peritoneal hematoma developed in one patient. Ultrasonography-guided drainage was performed but as it relapsed for the third time, the patient necesseriated laparotomy. Subhepatic abscess and wound infection developed in one patient who responded conservative treatment. The third patient who developed early ileus responded to stopping oral intake and nasogastric decompression. The complication of the last two patients was wound infection which healed with conservative treatment (Table 2).

Table 2. The distribution of complications according to the groups

Complication	Group I	Group II
Wound infection	1 (2.38 %)	3 (7.31 %)
Intra-abdominal abscess	1 (2.38 %)	1 (2.43 %)
Ileus	-	1 (2.43 %)
Preperitoneal hematom	-	1 (2.43 %)

(Note: Wound infection and intra-abdominal abscess was developed in one same patient in group II)

Discussion

Appendetomy is the most common cause of emergency abdominal surgery. Open appendectomy is applied as a gold standard in many clinics. Considered its easy applicability and results, laparoscopic appendectomy is a reliable method alternative to open appendectomy. On the other hand, laparoscopy is also a diagnostic tool alongside to therapeutic procedure in the patients with acute abdominal pain and suspected clinic (6).

Laparoscopy has been the subject of many discussions since the first day being used. Many studies suggest that diagnostic laparoscopy significantly reduces the negative appendectomy rate, especially in reproductive-age women. The highest negative appendectomy rate is in young adulth women. The rate of normal appendices unnecessarily removed varies between 32 to 45 % in appendectomies of the women aged between 15-45 years. One of the most important advantage of laparoscopic appendectomy is possibility of abdominal exploration. The diagnosis and treatment of some diseases considered in the differential diagnosis of acute appendicitis in woman may be possible with laparoscopy. On the other hand, when a surgeon encounters a normal appendix during open appendectomy, it may be difficult to search for other pathologies from the same incission. (7,8). Therefore, fertile women suspected with appendicitis have the most probability of benefiting from diagnostic laparoscopy. In this study, the patient whose operation was terminated without appendectomy was a 22 years old female. The patient was diagnosed as pelvic inflammatory disease and thus laparoscopy prevented unnecessary appendectomy.

It is another controversial issue whether to remove the normal appendix or not. We often remove laparoscopically normal appearing appendices in our clinic. In this retrospective review, only one patient with normal appendix did not undergo appendectomy after laparoscopic exploration. In this point, one can ask why this number is so low. We think that it is because most surgeons prefer to apply appendectomy for even normal appearing appendices of patients with clinical signs of acute abdomen. When we asked whether they prefer to carry out appendectomy to all patients uderwent Mcburney incission or not, 4 of 5 surgeons in our clinic replied that they prerefered to carry out appendectomy even to patients with normal appearing appendicitis. Likewise, 3 of 5 surgeons stated that they prefered to remove normal appearing appedices by laparoscopic exploration in patients whit unclear picture. According to studies pathologic findings were revealed by hystopathologic examination in 19 % of normal-appearing appendices (9). The probability of endoluminal appendicitis is the main justification for the surgeons who prefer to remove the normal appearing appendix. On the other hand, other surgeons suggest to remove the normal appearing appendix to avoid future confusion as to whether it was removed or for prevention of future appendicitis. But still leaving the normal appendix in situ when another cause for the pain was found in laparoscopic exploration seems to be a safe approach (10).

The long duration of surgery is a disadvantage of laparoscopic appendectomy. The learning curve is generally thought to be responsible for that. In a meta-analysis, while the duration of open surgery was 25 to 87 minutes, laparoscopic surgery took 35 to 102 minutes (11). In the same study, the duration of operation is shorter for open appendectomies than laparoscopic appendectomies in all analyzed studies. In addition, studies in which the average

operative time was longer than one hour were older in terms of publication date. In our study, the mean operative times for both groups did not contradict with the literature. The duration of open surgery was close to that of laparoscopic surgery. This may be because our hospital is a training clinic and open cases are performed by junior surgeons accompanied by former surgeons. On the other hand, Tzovaras et all (12) suggested an alternative explanation for the longer duration of surgery in the laparoscopic group; it could be because of the relatively high number of converted cases. In this study, there was no statistically significant difference in operative time between the open and laparoscopic groups after exclusion of the converted cases.

Consequently, in our opinion, laparoscopic appendectomy can not be a gold standart in the treatment of acute appendicitis as laparoscopic cholecystectomy in cholelithiasis and laparoscopic Nissen funduplication in gastroesophageal reflux disease. It may be useful in diagnosis and treatment of suspected cases. Otherwise, because there is no apparent advantage or disadvantage of laparoscopic appendectomy, the surgeon will decide the appropriate method according to his or her experience.

References

1. *Addiss DG, Shaffer N, Fowler BS, Tauxe RV. The epidemiology of appendicitis and appendectomy in the United States. Am J Epidemiol. 1990; 132: 910–25.*

2. *McBurney C. The incision made in the abdominal wall in case of appendicitis with a description of a new method of operating. Ann Surg. 1894; 20: 38–43.*

3. *Yong JL, Law WL, Lo CY, Lam CM. A comparative study of routine laparoscopic versus open appendectomy. Apr-Jun, 2006; 10: 188-92.*

4. *Semm K. Endoscopic appendectomy. Endoscopy, 1983; 15: 59-64.*

5. *Song JY, Yordan E, Rotman C. Incidental appendectomy during endoscopic surgery. 2009 Jul-Sep; 13: 376-83.*

6. *Frazee RC, Roberts JW, Symmonds RE, Snyder SK, Hendricks JC, Smith RW, Custer MD, Harrison JB. A prospective randomized trial comparing open versus laparoscopic appendectomy. Ann Surg. 1994 ; 219: 725-8.*

7. *Snyder TE, Selanders JR. Incidental Appendectomy--Yes or No? A Retrospective Case Study and Review of the Literature. Infectious Diseases in Obstetrics and Gynecology 1998; 6: 30-37.*

8. *Flum DR, Koepsell T. The clinical and economic correlates of misdiagnosed appendicitis. Arch Surg. 2002; 137: 799-804.*

9. *Grabham JA, Sutton C, Nicholson ML. A case for the removal of the 'normal' appendix at laparoscopy for suspected acute appendicitis. Ann R Coll Surg Engl 1999; 81: 279-280.*

10. *Turner EJH, Lightwood R. Management of the 'Normal' Appendix during Laparoscopy for Right Iliac Fossa Pain. gement of the 'Normal' Appendix during Laparoscopy for Right Iliac Fossa.*

11. *Golub R, Siddiqui F, Pohl D. Laparoscopic versus open appendectomy: A metaanalysis. J Am Coll Surg 1998; 186(5): 545-553.*

12. *Tzovaras G, Baloyiannis I, Kouritas V, Symeonidis D, Spyridakis M, Poultsidi A, Tepetes K, Zacharoulis D. Laparoscopic versus open appendectomy in men: A prospective randomized trial. Surg Endosc 2010 Dec; 24(12): 2987-92.*

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Effects of intermittent negative pressure treatment on vascular and platelet component of hemostasis system in patients with lymphoedema of lower limbs

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Abstract

Objective: After discovering the fact that applying intermittent hipobaric pressure causes a long-time improvement of microcirculation, we investigated the functionality of the hemostatic system in patients with lymphoedema of lower limbs.

Matherials and methods: The total of 86 patients with lymphoedema have been treated with intermittent hipobaric pressure using hypobaric sack "Greensac" manufactured by "Iskra-Medical" from Slovenia. The functionality of the hemostatic system was tested by using a number of laboratory tests which included vascular and platelet components of the hemostatic system.

Results: On the basis of the gathered results, the degree of the platelet reactivity was moderately increased-with the addition of ADP 80%, adrenaline 72% and collagen 78%. However, two weeks after the treatment had finished, a significant normalization in the platelet reactivity degree-bellow the upper limit of the physiological range of 65%.

Conclusion: Based on the data we acquired, we can conclude that the treatments with hipobaric intermittent pressure had a positive impact on the degree of thrombocytic reactivity. Although the group of patients we examined is not large, these starting results give us the hope that, combined with hipobaric treatments, the dosage of antithrombocytic drugs could be lowered.

Key words: Intermittent pneumatic compression devices, microcirculation, platelet aggregation.

Introduction

Lymphoedema emerge as a consequent of balance disorder between utilization and draining capacity of lymphatic system. Slower blood circulation and hypoxia do compromise functionality of hemostasis mechanism which becomes more suitable for creation of thrombosis masses and additionally pressurize blood circulation. Based on earlier reports of favorable effects of intermittent negative pressure treatment (INP) on blood circulation [1-2] and our own researches [3-6] we assumed that INP treatment can also improve functionality of hemostasis system. In this article we put focus on vascular and platelet component through determination of aggregability of platelets.

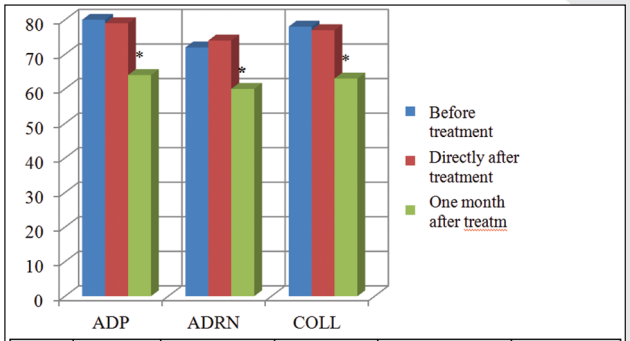
Materials and methods

Patients with lymphedema, 86 of them, average age of 59.4 years were submitted to INP treatment in hypobaric sack produced by »Iskra-Medical« from Slovenia, model „Greensack®". The following protocol was used: 10 treatments in hypobaric sack, 3 times per week, 20 minute each treatment. Before treatment, right after treatment and one month after treatment, aggregability of platelets test and hemostasis system screening were performed together with CBC and CRP. Aggregability of platelets test was performed with addition of ADP, Adrenaline and Collagen, and was examined with two-channel aggregometer device Chrono-log (USA) with reagents produced by Chrono-par (USA). As antagonists were used Adrenaline in concentration of 10 mmol/L, ADP in concentration of 10 mmol/L and Collagen in concentration of 2 mg/mL. Results were processed with statistical software SPSS 7.0.

Results

Typical lab tests results were in referent values scope. Degree of platelets reactivity measured with aggregometer before treatment was slightly enhan-

ced: with addition of ADP 80±4,7%, Adrenaline 72±5,2% and Collagen 78±4,9%. Right after treatment result was almost unmodified: with addition of ADP 79±4,8%, Adrenaline 74±5,0% and Collagen 77±4,9%. However, one month after completion of treatment degree of platelet aggregability was below upper limit of physiological scope of 65%: with addition of ADP 64±4,4%, Adrenaline 60±4,6% and Collagen 63±4,7%. (Chart 1)



	Before treatment	Directly after treatment	Significance	One month after treatment	Significance *
ADP	80±4,7	79±4,8	N.S.	64±4,4	p<0,005
ADRN	72'±'5,2	74±5,0	N.S.	60±4,6	p<0,005
COLL	78'±'4,9	77±4,9	N.S>	63±4,7	p<0,005

Chart 1. Percent of platelet reactivity with addition of ADP, adrenaline and collagen
*ADP = Adenosine diphosphate; ADRN = Adrenaline; COLL = Collagen; *=Significance*
Number of patient's = n = 86

Discussion and conclusion

We can conclude that use of hypobaric sack has had positive effect in relation of platelets reactivity degree causing reduction of thrombosis potential of activated platelets. We can assume that hypobaric therapy with its effect on endothelial cell can cause greater disengagement of tissue plasminogen activator and shift dynamic balance of hemostasis system in direction of greater fibrinolysys activity, which is a status of lower risk for origination of thrombosis complications. We conclude that use of intermittent negative pressure treatment with vacuum sack significantly and long-lasting decreases degree of platelet reactivity in patients with lymphoedema. On this way a risk of origination of thrombus and occlusion of blood vessel of diseased limbs is reduced and also a preserved and improved permability of blood vessels has positive effect on final results of treatment.

Acknowledgment

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References

1. *Himmelstrup H, Himmelstrup B, Mehlsen J, Trap-Jensen J. Effects of vacusac in intermittent claudication: a controlled cross-over study. Clin Physiol 1991 May 11: 3 263-9*

2. *Mehlsen J, Himmelstrup H, Himmelstrup B, Winther Hansen KF. Positive effect of intermittent overpressure and underpressure (Vacusac) in intermittent claudication. Ugeskr Laeger 1994 Jan 10 156: 2 169-71*

3. *Devecerski G, Teofilovski M, Milicevic B, Novakovic B, Popovic V, Radakovic N. Effects of intermittent negative pressure therapy on improvement of microcirculation in patients with diabetes - Case study. Med Pregl 2006; 59 (Suppl): 63-5.*

4. *Devecerski G, Teofilovski M, Milicevic B, Konstantinovic Lj, Lazović M, Radaković N, Mujovic N, Krstin A, Jelenc J. Effects of Greensack® on lower limb functional status. Europa Medicophysica 2006; 42 (Suppl): 145-6.*

5. *Devecerski G, Teofilovski M, Milicevic B, Radakovic N, Lazović M. Improvement of lower limb joint mobility using Greensac® in a diabetes patient – case report. Abstract Book 6th Mediterranean Congress of PRM, Algarve, Portugal, 2006; P079*

6. *Popovic V, Devecerski G, Radakovic N, Milicevic B, Jelenc J. Use value of Greensac® device in treatment of non-operable periphery arterial obliterative disease in older patients. Med Pregl 2007; 60 (Suppl 1): 49-53*

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Ethyl pyruvate attenuation of hepatocyte injury by ATP-related mechanism under hypoxia/reoxygenation in vitro

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Abstract

Background: The present study aims to investigate the effect of ethyl pyruvate (EP) on L-02 hepatocytes under hypoxia/reoxygenation condition in vitro and to elucidate the underlying mechanism.

Methods: L-02 hepatocytes under hypoxia/reoxygenation condition were generated as an in vitro injure model by using a Billups–Rothenberg device. Protective reagents, such as Lactate Ringer's (LR group), ethyl pyruvate (EP group), and solvent control (Con group) were added to the media when the cells are under hypoxia condition. After reoxygenation, the levels of Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were measured to determine the degrees of cell damage. Cell apoptosis and necrosis were measured using the Hoechs 33342 Propidium Iodide double staining, flow cytometry, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling methods. Moreover, the hepatocyte expression levels of Caspase9, Caspase3, and Poly(ADP-ribose) polymerase (PARP) were measured using Western blot analysis. Furthermore, the Adenosine Triphosphate (ATP) levels in hepatocytes were measured using the luciferase luminescence method.

Results: The concentrations of AST and ALT in the EP group were significantly lower than those in the Con and LR groups ($p < 0.05$). The percentage of viable cells in the EP group was the highest among the three groups ($p < 0.05$), whereas the percentage of necrotic cells in this group was the lowest ($p < 0.05$). Moreover, the percentage of apoptotic cells in the EP group was the highest compared with the Con and LR groups. Western blot analysis also showed that the cleaved Caspase9, Caspase3, and PARP protein levels in the EP group were higher than those in the Con and LR groups. The concentration of ATP in the EP group was considerably higher than those in the Con and LR groups (3.476 μmol/g ± 0.5 μmol/g vs. 1.78 μmol/g ± 0.3 μmol/g and 1.73 μmol/g ± 0.4 μmol/g, $p < 0.05$).

Conclusions: In the proposed in vitro model, we validated the protective function of EP for hepatic L02 cells under hypoxia/reoxygenation. The protective effect of EP may be attributed to the activation of the switch from necrosis to apoptosis and to the upregulation of intracellular ATP levels.

Key words: Hypoxia/reoxygenation; ethyl pyruvate; hepatocyte; apoptosis; necrosis

Introduction

Liver ischemia-reperfusion injury (IRI) is a common pathophysiological and life-threatening condition for seriously ill patients, such as those undergoing liver surgery to reduce bleeding in a partial local block, those who have undergone transplantation surgery or tissue resections, and those under conditions of hemorrhagic shock. IRI causes severe liver damage, including hepatocyte swelling, vacuolization, endothelial cell injury, neutrophil infiltration, and eventually, liver apoptosis and necrosis (1-4). The prevention and treatment of liver IRI has long been considered an important clinical issue. Previous studies have shown that the progression of IRI might be involved in the activation of Kupffer and endothelial cells; enrichment of oxygen free radicals; calcium overload; inflammatory mediators, such as doctrine; and mitochondrial dysfunction (5-12). Some experimental interventions for IRI prevention have been reported, including the utilization of free radical scavengers, heme oxygenase and

heat shock protein, and ischemia pre/post-conditioning. However, efficient cellular protective medications for clinical prevention remain lacking, and the underlying mechanisms of IRI have never been precisely and clearly investigated.

Pyruvate, an important energy metabolite during glucose metabolism, is the end-product of glycolysis and is the starting substrate of the citric acid cycle (tricarboxylic acid cycle, TCA). The α-keto acid structure of pyruvate can neutralize intracellular oxygen free radicals [reactive oxygen species (ROS)] derived from H_2O_2, and the OH^- effect (13, 14). However, sodium pyruvate solution is extremely unstable and is generated via pyruvate (parapyruvate), a TCA reaction inhibitor, thus hindering its clinical application(15). The esterification of pyruvate, called ethyl pyruvate (EP), has a similar but significantly more stabilized antioxidant effect compared with the pyruvate solution. Thus, EP has been widely investigated as a cell protective substance.

EP can protect various types of cells after such injuries as hemorrhagic shock (16, 17), sepsis (18, 19), burns (20), and acute pancreatitis (21, 22). EP has also been demonstrated to have anti-inflammatory and anti-oxidative properties in vivo and in vitro (23-26). The potential therapeutic role of EP in ischemic-induced hepatic damage has not yet been addressed. Therefore, EP is considered to be a very promising cell protective substance for clinical applications. In a number of Western countries, EP has been used in pre-clinical studies. However, a number of studies were unable to verify the protective effect of EP. For instance, in a clinical phase II study, EP did not show a clear protective effect for myocardial cells in patients who have undergone cardiopulmonary bypass surgeries (27). Kenneth et al. reported that the mechanism of the inconsistently protective effect of EP in clinical applications remains to be clarified, given that its clinically protective effect varies dramatically when given at different timings (28).

Recent studies suggest that EP has a protective effect on organ IRI, as well as in cells cultured in vitro. However, the underlying mechanism remains unclear. Thus, elucidating the mechanism of the protective role of EP in IRI would supply guidance for future preclinical or clinical studies on EP. In this study, we used L-02 liver cells under hypoxia/reoxygenation as an in vitro injury model to investigate the protective effect of EP against hepatic IRI. We aim to investigate the protective effects of EP on ischemic-induced hepatic damage and to identify its underlying mechanisms.

Materials and Methods

Materials

RPMI-1640 medium was purchased from GibcoBRL Inc. USA (Cat. No. 12430-054). A total of two-thirds volume of the glucose medium was diluted with glucose-free medium four times, configured as low-glucose medium (< 1 mM). Fetal calf serum (FCS) was purchased from Hyclone Inc. USA (Cat. No. SH30070.03). Trypsin (0.25% Trypsin- Ethylene diaminete traacetic acid) was purchased from GibcoBRL Inc. USA (Cat. No. 25200-056). HMGB1, as well as Caspase3, 9, and Poly(ADP-ribose) polymerase (PARP) antibodies were purchased from Chemicon Corporation. EP was purchased from Sigma, St. Louis, MO. Hoechst 33342 was purchased from Invitrogen Corporation. A total of 10 mg Hoechst 33342 was diluted in 2 mL phosphate buffered saline solution, followed by filtration using a disposable filter. The aliquots were then stored at 4 °C. The Billups–Rothenberg hypoxia device (Del Mar, San Diego, CA), "95% N_2 + 5% CO_2" mixed gases, and "95% O_2 + 5% CO_2" mixed gas were purchased from the Shanghai Institute of Organic Chemistry. The terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end-labeling (TUNEL) kit S7100 was purchased from Chemicon, USA. Nuclear-specific dye 4'-6-Diamidino-2-phenylindole (DAPI) and propidium iodide (PI) were from Sigma. The Adenosine Triphosphate (ATP) detection kit and Alanine Aminotransferase (ALT)/Aspartate Aminotransferase (AST) assay kit were purchased from Nanjing Jiancheng Biological Engineering Institute.

Cell culture and treatment

Human hepatic L02 cells were provided by the Institute of Liver Cancer, Fudan University. L02 cells were cultured at 37 °C with 5% CO_2 in an RPMI-1640 medium containing 10% FCS.

Hypoxia/reoxygenation model: The media were replaced by a serum-free and low-glucose RPMI-

1640 medium, and the cells were placed in the Billups–Rothenberg hypoxia device (Del Mar, San Diego, CA). The "95% N_2 + 5% CO_2" gas mixture was inputted into the device. After 5 min, the input and output pipes were sealed, and the oxygen concentration inside the device was less than 0.5%. After 60 min of hypoxia treatment at 37 °C, the media were replaced by normal RPMI-1640 medium with serum and glucose, and the "95% O_2 + 5% CO_2" gas mixture was inputted into the device at 5 L/min for 60 min as reoxygenation treatment.

Groups: The Lactate Ringer's (LR), EP, and solvent control (Con) groups were set as triplicates.

Con group: The cell medium was replaced by serum-free RPMI-1640 medium with low glucose, followed by 60 min hypoxia, then by normal RPMI-1640 medium with serum and glucose, followed by 60 min reoxygenation.

LR group: The cell medium was replaced by serum-free RPMI-1640 medium with low glucose and LR at equivalent EP volume, followed by 60 min hypoxia, then by normal RPMI-1640 medium with serum and glucose, followed by 60 min reoxygenation.

EP group: The cell medium was replaced by serum-free RPMI-1640 medium with low glucose and EP at a final concentration of 2 mmol/L, followed by 60 min hypoxia, then by normal RPMI-1640 medium with serum and glucose, followed by 60 min reoxygenation.

After hypoxia/reoxygenation, the cells were used for a series of assays.

Measurement of ALT and AST concentrations

A total of 1 mL cell-conditioned medium was centrifuged at 1000 rpm for 10 min, and 50 μL supernatant was used to measure the serum ALT and AST concentrations by using a HITACHI 7020 automated analyzer.

Hoechs 33342 Propidium Iodide (HO/PI) staining

HO/PI double fluorescence labeling was used to stain apoptosis and necrosis cells. Cells from different groups were collected, and 20 μL Hoechst 33342 dye (1 mg/mL) was added to the cells at a final concentration of 10 μg/mL, followed by 10 min reaction at 37 °C. Then, 20 μL PI staining solution (1 mg/mL) was added at the final concentration of 10 μg/mL, followed by 20 min reaction at 4 °C. Cell suspension was dropped on the glass slide, and the spreading cells were observed and photographed under the fluorescence microscope. Hoechst 33342 was excited by krypton UV laser at a 352 nm wavelength, and the emission wavelength was from 400 nm to 500 nm, thus resulting in blue fluorescence. PI with argon ion laser induced fluorescence at an excitation wavelength of 488 nm, and the emission wave is over 630 nm, thus resulting in red fluorescence. The Fluorescent microscope (SMZ800) was a product of the Japanese Nikon Company, whereas the Fluorescent microscope (BX-40) was a product of the Japanese Olympus Company.

TUNEL assay of apoptotic cells

Apoptosis was detected using DNA in situ TUNEL staining according to the manufacturer's protocol (Roche Co Ltd). In brief, adherent L02 cell smears and cytospin were prepared, fixed, and then permeated. After equilibration, the cells were end labeled with digoxigenin-11-dUTP by TdT enzyme in a buffer for 1 h at 37 °C in a humidifying chamber. After treatment with stop-wash buffer, the slides were incubated with DAPI for 30 min and then observed under a fluorescence microscope. The apoptotic cell population was counted in 10 random fields (100×).

Western blot analysis of Casapase3, Caspase9, and PARP protein expression

In brief, the cells were lysed in 1× sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% SDS, 100 μg/mL phenylmethylsulfonyl fluoride). Protein concentration was determined by a BCA kit (Bio-colour, Shanghai, China). Equal amounts of protein were separated by SDS- PolyAcrylamide Gel Electrophoresis and then transferred to NC membranes (CNI, Canada). Membranes were then blocked in Tris-buffered saline containing 0.1% (v/v) Tween 20% and 5% (w/v) nonfat dried milk, after which they were incubated for 2 h with the primary antibody at 1:500 in blocking buffer, and then for 1 h with horseradish peroxidase-conjugated secondary antibody at 1:2000. The blots were developed using enhanced chemiluminescence and then

recorded by Kodak films. The intensities of the target protein bands were normalized by the intensity of α-tublin or phospho-kinase/total kinase.

Flow cytometry

The cells were collected in 10 mL tubes (1×10^6 cells/mL to 5×10^6 cells/mL). After 500 rpm to 1000 rpm centrifugation for 5 min, the culture medium was discarded. The cells were washed with incubation buffer, followed by 1000 rpm centrifugation for 5 min. The cells were then re-suspended by a 100 μL labeling solution, followed by incubation at room temperature (away from light) for 20 min. Subsequently, the cells were precipitated by 1000 rpm centrifugation and then incubated in washing buffer for 5 min. Finally, the cells were analyzed by flow cytometry using an excitation wavelength of 488 nm. AnexinV-FITC fluorescence was detected at 515 nm wavelength filter, and PI fluorescence was detected at >560 nm wavelength filter.

Statistics

In this study, all data were presented as mean ± standard deviation (X ± SD) and were analyzed by using SPSS11.0 software. Differences among the three groups were compared using a single-factor analysis of variance test. Differences between two groups were compared using a t-test. A probability value of $p < 0.05$ was considered statistically significant.

Results

1. EP decreases the AST and ALT levels in the cell culture media of hepatic L02 cells under hypoxia/reoxygenation

To assess the degree of cell damage in L02 cells upon hypoxia/reoxygenation, AST and ALT concentrations in the cell culture media were examined. The AST and ALT levels in the EP group decreased significantly compared with the Con and LR groups ($p < 0.05$) (Figures 1A and B).

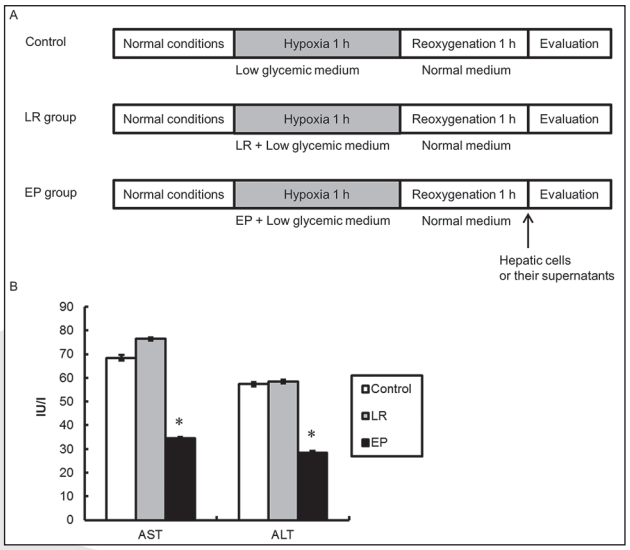

*Figure 1. Reduction of the concentration of AST and ALT in the supernatant of hepatic cells under hypoxia/reoxygenation by EP treatment. (A) Experimental process diagram. (B) Colum table of each group's liver function tests. AST and ALT concentrations in the EP group are significantly lower than those in the Con and LR groups*p < 0.05 (EP vs Con or LR)*

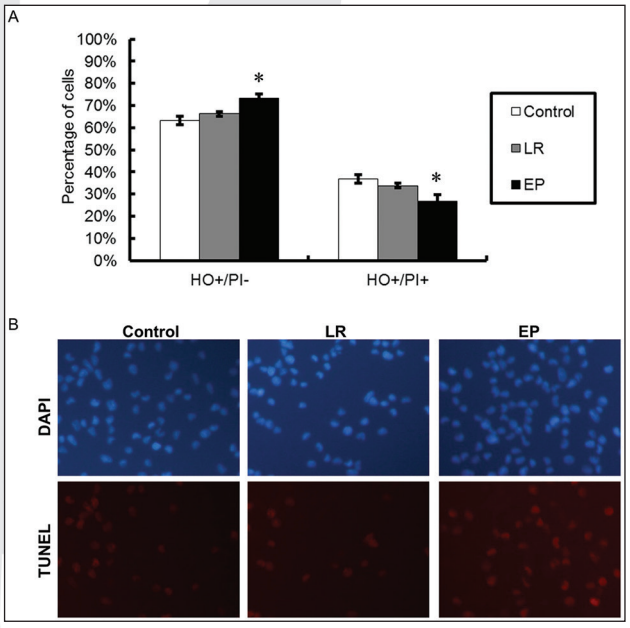

Figure 2. Alteration of the ratio of necrosis and apoptosis of hepatic cells upon hypoxia/reoxygenation by EP treatment. (A) HO/PI double staining showing that EP treatment reduced the PI-positive cells (necrosis and late apoptosis in cells) ratio. (B) TUNEL assay showing that EP treatment increased the percentage of apoptotic cells

2. EP alters the balance of necrosis and apoptosis in hepatic cells under hypoxia/reoxygenation

HO/PI double staining revealed that the percentage of HO^+/PI^- living cells in the EP group was significantly higher than those in the Con and LR groups (73.2% ± 0.2% vs. 63.2% ± 0.2% and 66.2% ± 0.1%, $p > 0.05$), whereas the percentage of HO^+/PI^+ cells (necrotic cells) in the EP group was markedly lower than those in the Con and LR groups (26.8% ± 0.3% vs. 36.8% ± 0.2% and 33.8% ± 0.1%, $p < 0.05$) (Figure 2A). Moreover, the TUNEL assay showed that EP treatment increased the percentage of TUNEL-positive apoptotic cells (Figure 2B).

Flow cytometry was also used to distinguish the apoptosis and necrosis of liver cells under hypoxia/reoxygenation. Figure 3 shows that the proportion of living cells ($AnexinV^-/PI^-$) in the EP group was significantly higher than those in the Con and LR groups (77.2% ± 0.3% vs. 61.3 ± 0.3% and 71.3% ± 0.2%, $p > 0.05$). The proportion of necrotic cells in the EP group was 10.3% ± 0.3%, which is significantly lower than those of the Con and LR groups (37.2% ± 0.1% and 26.2% ± 0.3%, $p < 0.05$). The proportion of apoptotic cells in the EP group was 14.1% ± 0.4%, which is significantly higher than those of the Con and LR groups (7.3% ± 0.2% and 6.8% ± 0.4%, $p < 0.05$).

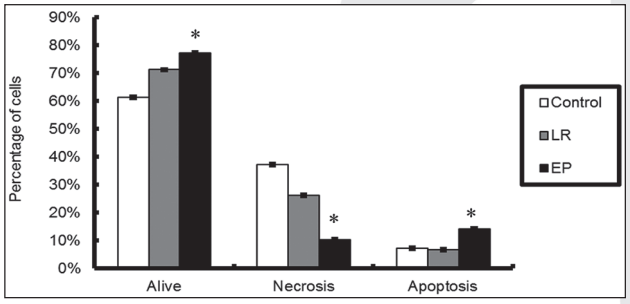

Figure 3. Flow cytometry analysis of apoptosis and necrosis in different group cells upon hypoxia/reoxygenation

3. EP activates the apoptosis pathway in hepatic L02 cells under hypoxia/reoxygenation

The expression of the activated forms of cleaved Caspase9, Caspase3, and PARP was detected by Western blot analysis. The optical density values of the cleaved Caspase9, Caspase3, and PARP bands in the EP group were significantly higher than those of the other two groups (0.92 ± 0.01 vs. 0.25 ± 0.02 and 0.87 ± 0.01, $p < 0.05$) (Figures 4A and B).

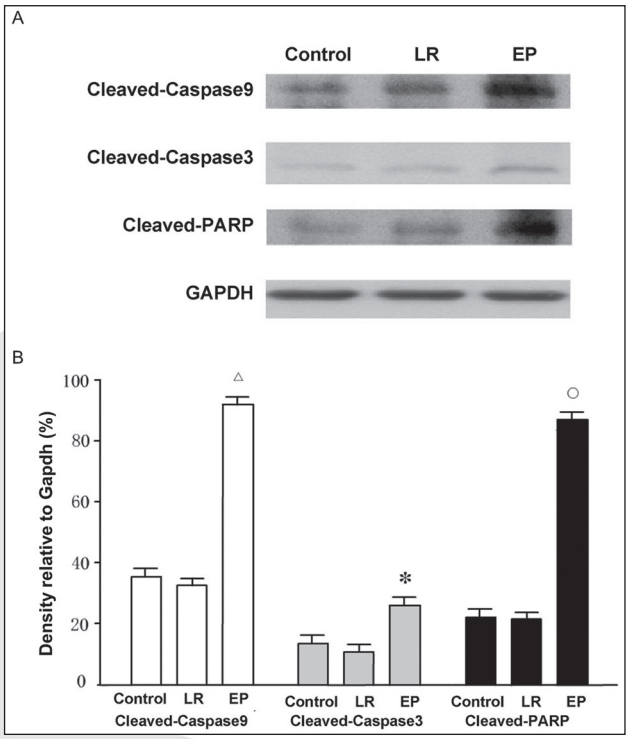

Figure 4. Western blot analysis of the expression of apoptotic proteins in different group cells. (A) Representative result of Western blot analysis. GAPDH was used as internal control. (B) Relative optical density of the bands of active cleaved Caspase3, Caspase9, and PARP from three independent experiments. "△," "," and "○," $p < 0.05$ (EP group vs. Con or LR groups).*

4. EP increases the intracellular ATP concentration in hepatic L02 cells under hypoxia/reoxygenation

Figure 5 shows that the intracellular ATP concentration was 1.78 μmol/g ± 0.3 μmol/g in the Con group and 1.73 μmol/g ± 0.4 μmol/g in the LR group, whereas it was 3.476 μmol/g ± 0.5 μmol/g in the EP group. The intracellular ATP concentration in the EP group was significantly higher than those in the Con and LR groups ($p < 0.05$).

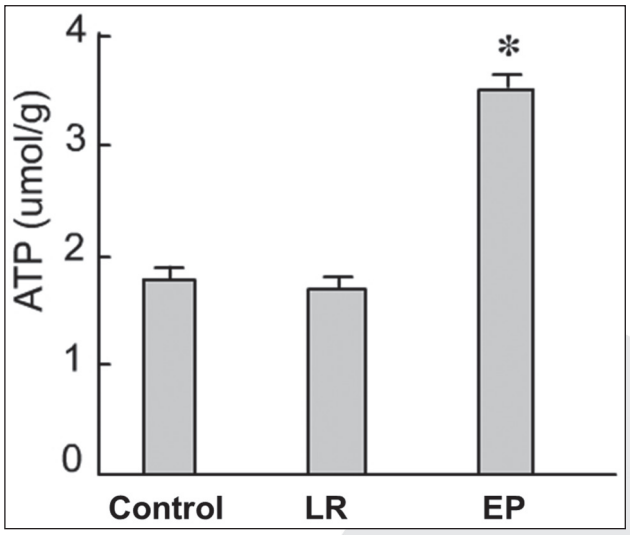

*Figure 5. Intracellular ATP concentration in different cell groups. *p < 0.05 (EP group vs. Con or LR groups).*

Discussion

In this study, EP was found to reduce significantly the concentration of aminotransferases in the supernatant culture of L02 cells under hypoxia/reoxygenation. EP treatment activates the apoptosis pathway while reducing cell necrosis. Finally, the intracellular ATP concentration increased when L02 cells were treated with EP. Together, the findings suggest that EP protects hepatocytes against hypoxia/reoxygenation injury by decreasing cell necrosis while enhancing apoptosis.

Salahudeen et al. showed that sodium pyruvate solution can alleviate renal injury in ROS-induced acute renal failure models (29). Other studies also suggest that pyruvate can reduce the damage caused by ischemia-reperfusion in multiple organs, including the heart, small intestine, and liver (30-32). However, sodium pyruvate solution is unstable and easily generates pyruvate (parapyruvate), which hinders its clinical application (15). To mitigate the instability problem, Sims et al. (24) first used EP, the esterification of pyruvate, in animal models of mesenteric IRI and found that EP can decrease the damage in the small intestine and that its effect is better than that of an equivalent sodium pyruvate solution. Varma et al. (33) also obtained similar results in oxidative stress experiments in vitro.

The protective mechanism of EP includes two aspects, namely, scavenging of free radicals and anti-inflammatory effects. The scavenging of free radicals by EP was found to be related to its α-keto carboxylic structure. In early studies, free radical scavenging was emphasized to play a role in hemorrhagic shock and acute renal failure or mesangial cell IRI. However, in a recent study, EP was found to display a more significant cell protection effect compared with other materials that have a α-keto carboxylic structure in animal experiments on IRI. The lipophilic characteristic of EP also plays a role in its protective property (34).

Yang et al. (17) first confirmed the anti-inflammatory effect of EP in an animal model of hemorrhagic shock and found that EP reduces the expression of pro-inflammatory transcriptional factor Nuclear Factor-KappaB (NF-KB) and of other pro-inflammatory genes. Subsequent studies also confirmed that EP can reduce the expression of a variety of inflammatory signaling molecules, including c-Jun N-terminal kinases, mitogen-activated protein kinases, and many others (35). Further research suggested that EP regulates the NF-KB signaling pathway via acting on the p65 subunit of NF-KB and decreases the expression of related inflammatory factors (36). Some free radicals are considered to be the second signal of inflammation, regulating the downstream inflammatory response, including the NF-KB and tumor necrosis factor, among others. Therefore, radical scavenging by EP is an important mechanism for its anti-inflammatory effects (37).

HO/PI staining and flow cytometry analysis in this study confirmed that EP can intervene in the hepatic injury of L02 cells under hypoxia/reoxygenation. Moreover, EP was found to facilitate an increase in living and apoptotic cells but a significant decrease in necrotic cells. Although apoptotic cell death and necrosis are difficult to distinguish, the outcomes of different kinds of cell death obviously differ. Apoptosis is an active process of cell shrinkage, organelle condensation, and loss of cell function, but does cause cell collapse. Moreover, apoptosis can be eventually cleared by immune cells. Necrosis is a passive process, during which the cell body collapses and a variety of substances is released to the interstitial cells of the surrounding tissues, resulting in pathogenic effects. Thus, the prevention of necrosis has recently become a primary protective strategy. A study on lung cancer cells in vitro suggested

that EP can reduce cell necrosis and stimulate the apoptosis pathway under glucose-free conditions. Although apoptosis was increased, the surrounding cell tissue was less stimulated (38), a finding that is consistent with our results.

By using electronic microscopy imaging techniques, the liver cells in each group were found to have different degrees of mitochondrial swelling and deformation, suggesting that mitochondrial permeability transition (MPT) occurred. According to the "necrosis/apoptosis" theory, MPT determines the occurrence of cell death after injury, whereas the intracellular ATP levels decide which kind of death happens (39). When the normal level of intracellular ATP reaches 10% to 15%, it can activate the apoptotic pathway via Caspase9 and Caspase3 (9). A number of studies suggested that the use of fructose to increase intracellular ATP levels during hepatic IRI can reduce cell death but can increase apoptosis (40, 41). ATP provides the necessary energy for cell apoptosis (42, 43). If ATP was absent, the apoptosis pathway will be inhibited, thus making the cell necrotic (44, 45). The phenomenon of ATP-dependent cell death/apoptosis was also confirmed in an experiment involving overloading calcium ionophore and acetaminophen poisoning (41, 46). An animal experiment on hemorrhagic shock suggested that early recovery can provide sufficient ATP, thus promoting the damaged cells to apoptosis and reducing the number of necrotic cells (47).

In summary, EP was found to protect L02 cells against hypoxia/reoxygenation injury in vitro by decreasing the number of necrotic cells while increasing the number of apoptotic cells. On one hand, EP increased the intracellular ATP levels possibly via "apoptosis/necrosis common pathway"-activated apoptosis. On the other hand, EP reduced the necrotic cells, thereby reducing the expression of endogenous material injury to play a protective role.

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Reference

1. *Tsung A, Kaizu T, Nakao A, et al: Ethyl pyruvate ameliorates liver ischemia-reperfusion injury by decreasing hepatic necrosis and apoptosis. Transplantation 79: 196-204, 2005.*
2. *Fondevila C, Busuttil RW and Kupiec-Weglinski JW: Hepatic ischemia/reperfusion injury--a fresh look. Exp Mol Pathol 74: 86-93, 2003.*
3. *Olthoff KM: Molecular pathways of regeneration and repair after liver transplantation. World J Surg 26: 831-837, 2002.*
4. *Selzner N, Rudiger H, Graf R and Clavien PA: Protective strategies against ischemic injury of the liver. Gastroenterology 125: 917-936, 2003.*
5. *Clavien PA, Rudiger HA and Selzner M: Mechanism of hepatocyte death after ischemia: apoptosis versus necrosis. Hepatology 33: 1555-1557, 2001.*
6. *Fan C, Zwacka RM and Engelhardt JF: Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med (Berl) 77: 577-592, 1999.*
7. *Gujral JS, Bucci TJ, Farhood A and Jaeschke H: Mechanism of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? Hepatology 33: 397-405, 2001.*
8. *Jaeschke H: Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol 284: G15-26, 2003.*
9. *Jaeschke H and Lemasters JJ: Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology 125: 1246-1257, 2003.*
10. *Zhang JX, Jones DV and Clemens MG: Effect of activation on neutrophil-induced hepatic microvascular injury in isolated rat liver. Shock 1: 273-278, 1994.*
11. *Zhou W, Zhang Y, Hosch MS, Lang A, Zwacka RM and Engelhardt JF: Subcellular site of superoxide dismutase expression differentially controls AP-1 activity and injury in mouse liver following ischemia/reperfusion. Hepatology 33: 902-914, 2001.*
12. *Kohli V, Selzner M, Madden JF, Bentley RC and Clavien PA: Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation 67: 1099-1105, 1999.*
13. *O'Donnell-Tormey J, Nathan CF, Lanks K, DeBoer CJ and de la Harpe J: Secretion of pyruvate. An antioxidant defense of mammalian cells. J Exp Med 165: 500-514, 1987.*

14. *Brand KA and Hermfisse U: Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB J 11: 388-395, 1997.*

15. *Fink MP: Ethyl pyruvate. Curr Opin Anaesthesiol 21: 160-167, 2008.*

16. *Tawadrous ZS, Delude RL and Fink MP: Resuscitation from hemorrhagic shock with Ringer's ethyl pyruvate solution improves survival and ameliorates intestinal mucosal hyperpermeability in rats. Shock 17: 473-477, 2002.*

17. *Yang R, Gallo DJ, Baust JJ, et al: Ethyl pyruvate modulates inflammatory gene expression in mice subjected to hemorrhagic shock. Am J Physiol Gastrointest Liver Physiol 283: G212-221, 2002.*

18. *Venkataraman R, Kellum JA, Song M and Fink MP: Resuscitation with Ringer's ethyl pyruvate solution prolongs survival and modulates plasma cytokine and nitrite/nitrate concentrations in a rat model of lipopolysaccharide-induced shock. Shock 18: 507-512, 2002.*

19. *Ulloa L, Ochani M, Yang H, et al: Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc Natl Acad Sci U S A 99: 12351-12356, 2002.*

20. *Dong YQ, Yao YM, Wei P, et al: [Effects of ethyl pyruvate on cell-mediated immune function in rats with delayed resuscitation after burn injury]. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 17: 12-15, 2005.*

21. *Yang R, Uchiyama T, Alber SM, et al: Ethyl pyruvate ameliorates distant organ injury in a murine model of acute necrotizing pancreatitis. Crit Care Med 32: 1453-1459, 2004.*

22. *Cheng BQ, Liu CT, Li WJ, et al: Ethyl pyruvate improves survival and ameliorates distant organ injury in rats with severe acute pancreatitis. Pancreas 35: 256-261, 2007.*

23. *Uchiyama T, Delude RL and Fink MP: Dose-dependent effects of ethyl pyruvate in mice subjected to mesenteric ischemia and reperfusion. Intensive Care Med 29: 2050-2058, 2003.*

24. *Sims CA, Wattanasirichaigoon S, Menconi MJ, Ajami AM and Fink MP: Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats. Crit Care Med 29: 1513-1518, 2001.*

25. *Epperly M, Jin S, Nie S, et al: Ethyl pyruvate, a potentially effective mitigator of damage after total-body irradiation. Radiat Res 168: 552-559, 2007.*

26. *Shen H, Hu X, Liu C, et al: Ethyl pyruvate protects against hypoxic-ischemic brain injury via anti-cell death and anti-inflammatory mechanisms. Neurobiol Dis 37: 711-722, 2010.*

27. *Bennett-Guerrero E, Swaminathan M, Grigore AM, et al: A phase II multicenter double-blind placebo-controlled study of ethyl pyruvate in high-risk patients undergoing cardiac surgery with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 23: 324-329, 2009.*

28. *Kao KK and Fink MP: The biochemical basis for the anti-inflammatory and cytoprotective actions of ethyl pyruvate and related compounds. Biochem Pharmacol 80: 151-159, 2010.*

29. *Salahudeen AK, Clark EC and Nath KA: Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo. J Clin Invest 88: 1886-1893, 1991.*

30. *Bunger R, Mallet RT and Hartman DA: Pyruvate-enhanced phosphorylation potential and inotropism in normoxic and postischemic isolated working heart. Near-complete prevention of reperfusion contractile failure. Eur J Biochem 180: 221-233, 1989.*

31. *Matthews BD and Williams GB: Initial experience with the advanced breast biopsy instrumentation system. Am J Surg 177: 97-101, 1999.*

32. *Sileri P, Schena S, Morini S, et al: Pyruvate inhibits hepatic ischemia-reperfusion injury in rats. Transplantation 72: 27-30, 2001.*

33. *Varma SD, Devamanoharan PS and Ali AH: Prevention of intracellular oxidative stress to lens by pyruvate and its ester. Free Radic Res 28: 131-135, 1998.*

34. *Cruz RJ, Jr., Harada T, Sasatomi E and Fink MP: Effects of ethyl pyruvate and other alpha-keto carboxylic acid derivatives in a rat model of multivisceral ischemia and reperfusion. J Surg Res 165: 151-157, 2011.*

35. *Mollen KP, McCloskey CA, Tanaka H, et al: Hypoxia activates c-Jun N-terminal kinase via Rac1-dependent reactive oxygen species production in hepatocytes. Shock 28: 270-277, 2007.*

36. *Johansson AS and Palmblad J: Ethyl pyruvate modulates adhesive and secretory reactions in human lung epithelial cells. Life Sci 84: 805-809, 2009.*

37. *Sappington PL, Fink ME, Yang R, Delude RL and Fink MP: Ethyl pyruvate provides durable protection against inflammation-induced intestinal epithelial barrier dysfunction. Shock 20: 521-528, 2003.*

38. *Lim SC, Choi JE, Kim CH, et al: Ethyl pyruvate induces necrosis-to-apoptosis switch and inhibits high mobility group box protein 1 release in A549 lung adenocarcinoma cells. Int J Mol Med 20: 187-192, 2007.*

39. *Kim JS, He L and Lemasters JJ: Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Commun 304: 463-470, 2003.*

40. *Kim JS, Qian T and Lemasters JJ: Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. Gastroenterology 124: 494-503, 2003.*

41. *Kon K, Kim JS, Jaeschke H and Lemasters JJ: Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology 40: 1170-1179, 2004.*

42. *Eguchi Y, Shimizu S and Tsujimoto Y: Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 57: 1835-1840, 1997.*

43. *Genini D, Budihardjo I, Plunkett W, et al: Nucleotide requirements for the in vitro activation of the apoptosis protein-activating factor-1-mediated caspase pathway. J Biol Chem 275: 29-34, 2000.*

44. *Richter C, Schweizer M, Cossarizza A and Franceschi C: Control of apoptosis by the cellular ATP level. FEBS Lett 378: 107-110, 1996.*

45. *Leist M, Single B, Castoldi AF, Kuhnle S and Nicotera P: Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J Exp Med 185: 1481-1486, 1997.*

46. *Mertz RJ, Worley JF, Spencer B, Johnson JH and Dukes ID: Activation of stimulus-secretion coupling in pancreatic beta-cells by specific products of glucose metabolism. Evidence for privileged signaling by glycolysis. J Biol Chem 271: 4838-4845, 1996.*

47. *Paxian M, Bauer I, Rensing H, et al: Recovery of hepatocellular ATP and "pericentral apoptosis" after hemorrhage and resuscitation. FASEB J 17: 993-1002, 2003.*

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Parameters influencing renal function in the subject with metabolic syndrome

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Abstract

Introduction: The criteria for the definition of the metabolic syndrome are different. The metabolic syndrome is described as the risk factor which can accelerate the development and progression of the damaged kidney.

The objective of the study is to assessment of parameters affecting renal function in subject with metabolic syndrome.

Material and Methods: From 46 analyzed patients in kidney diseases and the metabolic syndrome defined by the criteria of AHA/NHLBI, NCEP/ATP and the criteria of IDF. The renal function has been defined by the help of the MDRD formula and the patients have been divided into two groups with GFR < 59.9ml/min/1.73m² and GFR > 60ml/min/1.73m².

Results: Out of 46 examined patients with the presence of the metabolic syndrome by the criteria of AHA/NHLBI is at 37%, NCEP/ATPIII at 32.6% and IDF is at 39.1% of the patients. In the group of the patients defined by the criteria of IDF is 27.8 of the patient has GFR < 59.9ml/min/1.73m², the values of uric acid (males> 430μmol/L and females>360μmol/L) was found at 23.5%, creatinine (males>106.1μmol/L and females>97.2μmol/L) was found at 27.8 at female and 28.6% male patients. The logistic regression analysis showed that the older age, female gender and larger waist circumference has a statistically significant influence (CI 95%, p<0.001) on the decrease of the renal function.

Conclusion: The research has shown that the older age, the female gender and the larger waist size have a significant influence at the patients with the metabolic syndrome.

Key words: The metabolic syndrome, renal function.

Introduction

The mechanism of appearing of the metabolic syndrome is complex and still it is not known and the components of the metabolic syndrome are grouped in the basic risk factors for the cardiovascular disease. The manifestations of the kidneys in the metabolic syndrome are the micro-albuminuria, the reduction in the glomerular filtration rate (GFR) (1). Different criteria for the diagnosis of the metabolic syndrome have been suggested so far. So, in 2001 year, The National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPI-II) gave the suggestion for the diagnosis of the metabolic syndrome that has been widely used. According to them, the diagnosis based on the existence of three or more of the suggested criteria is given: abdominal obesity defined by the waist circumference, at men >102cm and at women >88cm, triglycerides>150mg/dl (1.7mmol/L), the concentration of HDL<40mg/dl (1.03mmol/L at men) and at women <50mg/dl (1.29mmol/L), blood pressure > 130/85mmHg and fasting plasma glucose > 100mg/dl (>5.6mmol/L) (2). Unlike them, The National Heart, Lung and Blood Institute and The American Heart Association (NHLBI/AHA) suggest the lower limit values for the abdominal obesity and fasting plasma glucose at men and they suggest that the diagnosis can have minimally two criteria of the ones suggested (3). Then, The International Diabetes Federation (IDF) a new definition of the metabolic syndrome which includes a central obesity plus two or more factors: triglycerides > 150mg/dl (1.7mmol/L), the lower values of HDL cholesterol < 40mg/dl (1.03mmol/L), at men < 50mg/dl (1.29mmol/L), and at women systolic blood pressure>130mmHg or diastolic blood pressure > 85mmHg and higher value of the fasting plasma glucose > 100mg/dl (5.6mmol/L) or the previously diagnosed diabetes type 2 with the waist size which characterizes a central obesity is at European men > 94cm an European women > 80cm, and at Asian

men > 90cm and Asian women > 80cm (4). The basic mechanism of the pathological and pathophysiological changes in a kidney and its relation to a the metabolic syndrome hasn't been still clarified but it involves the insulin resistance, inflammation, the endothelial dysfunction, the oxidative stress, the altered hemodynamics in the kidney, the activation of the rennin angiotensin aldosterone system and the sympathetic nervous system (5,6).

The aim of the study is to assessment of parameters affecting renal function in subject with metabolic syndrome.

The patients and the methods

From 46 analyzed patients in kidney diseases and the metabolic syndrome defined by the criteria of AHA/NHLBI, NCEP/ATP and the criteria of IDF. {The working diagnosis of the World Health Organization (WHO) hasn't been taken into account nor the diagnosis of the American Association of the Clinical Endocrinologists (AACE) due to micro-albuminuria which is not available in everyday work.} The renal function has been defined by the help of the MDRD formula and the patients have been divided into two groups with GFR < 59.9ml/min/1.73m² and GFR > 60ml/min/1.73m², the limit value for serum creatinine have been taken for males Cr > 106.1μmol/L and for females Cr > 97.2μmol/L and uric acid for males > 430μmol/L and for females > 360μmol/L.

The Results

In the Table 1, the general demographic characteristics of both groups of patients with the metabolic syndrome have been shown. It has been noted that the patients with GFR>59ml/min/1.73m² are older and have larger body mass index (BMI), but without any statistical significance and there is statistical difference between the values of uric acid and the systolic blood pressure parameters between the gropus. The statistical significance in the prevalence of the metabolic syndrome relating different criteria hasn't been recorded although there is the greatest difference between the criterion of NCEP/ATPIII 32.6% and IDF 39.1% (Figure 1).

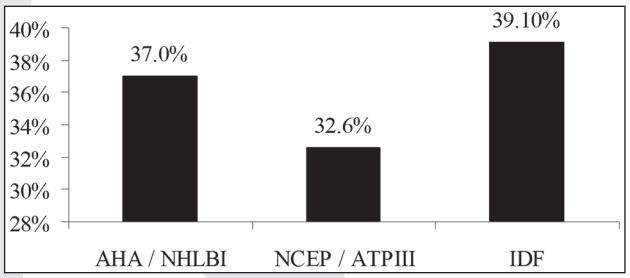

Figure 1. Presence of MS according different criteria

The metabolic syndrome has been defined at 27.8% persons with the glomerular filtration (GFR< 59.9ml/min/1.73m²), the uric acid with the defined criteria larger than the limit value appeared at 23.5%, creatinine at 27.8% of women and at 28.6% of men (Figure 2).

Table 1. General characteristics of the patients according to value of GFR

	GFR>60ml/min/1.73m²	GFR<59.9ml/min/1.73m²	p
Age (years)	41.89 ± 14.30	61.10 ± 10.70	0.0000
Weist (cm)	98.03 ± 10.33	99.55 ± 10.31	0.6820
Plasma glucose levels (mmol/l)	5.10 ± 0.81	5.13 ± 0.59	0.9000
BMI (kg/m²)	25.13 ± 4.25	26.33 ± 4.37	0.4330
Smoking (years)	7.64 ± 11.26	7.40 ± 10.60	0.9520
HOL (mmol/l)	5.61 ± 1.55	5.45 ± 1.06	0.7750
TG (mmol/l)	2.04 ± 1.16	2.28 ± 1.46	0.5930
HDL (mmol/l)	1.30 ± 0.36	1.12 ± 0.25	0.2280
LDL (mmol/l)	3.35 ± 1.44	3.29 ± 0.74	0.9110
Urates (mmol/l)	300.52 ± 85.07	402.71 ± 131.38	0.0140
SBP (mm Hg)	129.03 ± 15.98	144.50 ± 16.41	0.0100
DBP (mm Hg)	75.56 ± 8.18	81.00 ± 8.10	0.0690
PP (mm Hg)	53.47 ± 13.62	63.50 ± 13.13	0.0440

BMI - Body Mass Index (kg/m²), HOL − Holesterol (mmol/l), TG − Trigliceride (mmol/l), HDL − High-Density Lipoprotein (mmol/l), LDL − Low-Density Lipoprotein (mmol/l), SBP − Systolic Blood Pressure (mm Hg), DBP − Diastolic Blood Pressure (mm Hg), PP − Pulse Pressure (mm Hg)

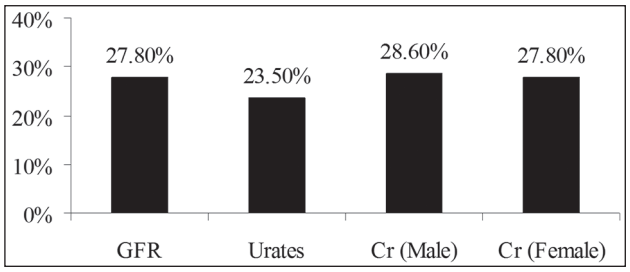

Figure 2. Parameters influencing renal function in the subject with MS (according IDF criteria of MS)

The logistic regression analysis has shown that the patients of older age, larger wais size and of female gender are under a significant risk of having a kidney damage in the metabolic syndrome ($p<0.001$) (Table 2).

Table 2. Results of the multiple regression analysis

	B	95% CI for B		p
		Lower	Upper	
(Constant)	101.83	48.74	154.93	<0.001
Age (years)	-1.07	-1.44	-0.71	<0.001
Waist (cm)	0.98	0.49	1.48	<0.001
Sex	-23.20	-34.51	-11.89	<0.001

Discussion

The relation between the metabolic syndrome and the renal insufficiency have been examined by the numerous studies (7–11). In the HANES III study, 4.6% patients older than 20 years have a chronic renal insufficiency while 23.7% has the metabolic syndrome. In our study, the chronic renal insufficiency risk at the patient with the metabolic syndrome has been examined. The patients with the decreased renal function were, in average, 61.1±10.7 years, the body mass index 26.33±4.37kg/m². According to HANES III study by the diagnostic criteria of NCEP-ATPIII definition, 34.5% patients 33.7% at men and 35.4% at women had the prevalence of the metabolic syndrome, and according to IDF criteria, 39% of the patients (39.9% at men and 38.1% at women). According to our study 39.1% of the patients have been diagnosed by the criteria of IDF for the metabolic syndrome and 32.6% by the criteria of NCEP/ATPIII. According to HANES III study, the greatest difference has been found in the prevalence at the males of Mexican origin whereby the prevalence in the older age group is 40.3% by the criteria of NCEP-ATPIII and if the definition IDF is used, then it is 50.6%. Our research has shown that the prevalence at male persons with the metabolic syndrome and the decreased renal function by the IDF criteria, for the metabolic syndrome is 28.6% at men and 27.8% at women. For example, Rashidi A at al. have shown that there is a 88 % high risk for the development of the renal insufficiency at the patients with the metabolic syndrome. Our results show that, at the patients with the metabolic syndrome diagnosed by the criteria of IDF, 27.8% have the low estimated glomerular filtration rate and 23.5% have elevated uric acid by the standard criteria (12). Then, Kurella M at al. claim that when the patients with hypertension are left out, the patients with the metabolic syndrome have a significantly higher blood pressure. Our results show that all the patients with the metabolic syndrome have a high blood pressure but the high blood pressure values appear at the patients with a lowered renal function (systolic blood pressure (SBP) 144.5±16.41mmHg, diastolic blood pressure (DBP) 81.0±8.1mmHg, pulse pressure (PP) 63.5±13.13mmHg) but without any statistical significance in the relation to the group with normal renal function (13). Kitiyakara C at al. suggest that the renal insufficiency risk is in the relation to the criteria of the metabolic syndrome diagnosis (14), which is indirectly confirmed by our research as well.

Conclusion

Our research has shown that, at the patients with the defined metabolic syndrome, the older age, the waist size and the female gender a significant influence on the decrease of the renal function.

References

1. *Ritz E. Metabolic syndrome: an emerging threat to renal function. Clin J Am Soc Nephrol 2007; 5(2): 869-71.*
2. *Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adult. 3rd Report of the NCEP ATP III. JAMA 2001; 285(19): 2486-97.*
3. *Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA et al. Diagnosis and management of the metabolic syndrome. An NHLBI/AHA Report. Circulation 2005; 112(17): 285-90.*
4. *IDF Epidemiology Task Force Consensus Group. Lancet 2005; 366: 1059-62.*
5. *Ritz E. Metabolic syndrome and kidney disease. Blood Purif 2008; 26: 59-62.*
6. *Locatelli F, Pozzoni P, del Vecchio L. Renal manifestations inthe metabolic syndrome. J Am Soc Nephrol 2006; 17: S81-S85.*
7. *Thomas G, Sehgal AR, Kashyap SR, Srinivas TR, Kirwan JP, Navaneethan SD. Metabolic syndrome and kidney disease: a systematic review and meta-analysis. Clin J Am Soc Nephrol 2011; 6(10): 2364-73.*
8. *Tuttle KR. Renal manifestation of the metabolic syndrome. Nephrol Dial Transplant 2005; 20(5): 861-64.*
9. *Alexander MP, Patel TV, Farag YMK, Florez A, Rennke HG, Singh AK. Kidney pathological changes in metabolic syndrome: a cross-sectional study. Am J Kidney Dis 2009; 5(53): 751-59.*
10. *Cirillo P, Sato W, Reungjui S, Heinig M, Gersch M, Sautin Y et al. Uric acid, the metabolic syndrome, and renal disease. J Am Soc Nephrol 2006; 17(12 Suppl 3): S 165-8.*
11. *Tasic D, Najman S. Certain experimental models in biomedical research of hypertension. Facta Universitatis, Medicine and Biology 2008; 15(3): 81-84.*
12. *Rashidi A, Ghanbarian A, Azizi F. Are patients who have metabolic syndrome without diabetes at risk for developing chronic kidney disease? evidence based on data from a large cohort screening population. Clin J Am Soc Nephrol 2007: 2(5): 976-83.*
13. *Kurella M, Lo JC, Chertow GM. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. J Am Soc Nephrol 2005; 16(7): 2134-40.*
14. *Kitiyakara C, Yamwong S, Cheepudomwit S, Domrongkitchaiporn S, Unkurapinun N, Pakpeankitvatana V at al. The metabolic syndrome and chronic kidney deisease in a Southeast Asian cohort. Kidney Int 2007; 71(7): 693-700.*

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An unusual spinal mixed glioneuronal tumor with BRAF^{V600E} mutation analysis: Case report and literature review

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Abstract

Mixed glioneuronal tumor (GNT) is a heterogeneous group of primary central nervous system (CNS) neoplasm originating from neoplastic cells that can differentiate along both glial and neuronal cell lines. There were only 12 unusual spinal mixed GNTs except well-defined ganglioglioma reported in the literature. Herein, we report one more unusual spinal mixed GNT in a 22-year-old female with signs of sensor-motor deficits. The lesion resembled pilocytic astrocytoma (PA) and presented no sign of morphologic neuronal rosette. Deoxyribonucleic acid (DNA) sequencing for BRAF^{V600E} mutational hot-spot was firstly performed in these unusual spinal mixed GNTs. However, this mutation was not identified in our case, which might be useful to differentiate extra-cerebellar PA.

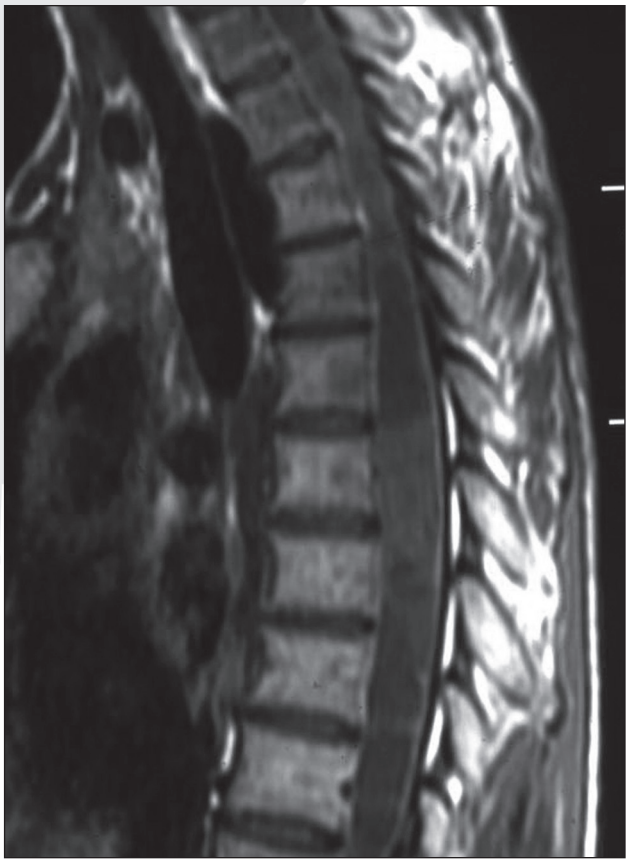
Key words: BRAF^{V600E} mutation, Leptomeningeal dissemination, mixed glioneuronal tumor, Spinal cord.

Introduction

Mixed GNTs are rare primary CNS neoplasms containing a variable extent of neuronal and glial elements. So far, only 12 spinal mixed GNTs except gangliogliomas have been reported in the literature.[1-4] BRAF^{V600E} mutation, characterized by a substitution of valine by glutamic acid at position 600, accounts for approximately 90% of BRAF mutations in human cancers. Analyze the molecular genetics of these tumors may provide novel insight in differential diagnosis, treatment and prognosis. Herein, we report a spinal mixed GNT with BRAF^{V600E} mutation analysis and also review the related literature.

Case report

A previously healthy 22-year-old female was admitted with a two-year history of back pain. Neurological examination revealed motor weakness of 4/5 on the right side and 2/5 on the left side and sensory loss below T6 segment. Bilateral Hoffmann and Babinski signs were also positive. Spinal magnetic resonance imaging (MRI) revealed a slightly enhanced intramedullary lesion in T6-7 region with obvious syringomyelia (Figure 1). A thoracic laminectomy and myelotomy were performed and the lesion was nearly totally removed. No evidence of tumor recurrence and leptomeningeal metastases was noted at 10 months postoperatively.



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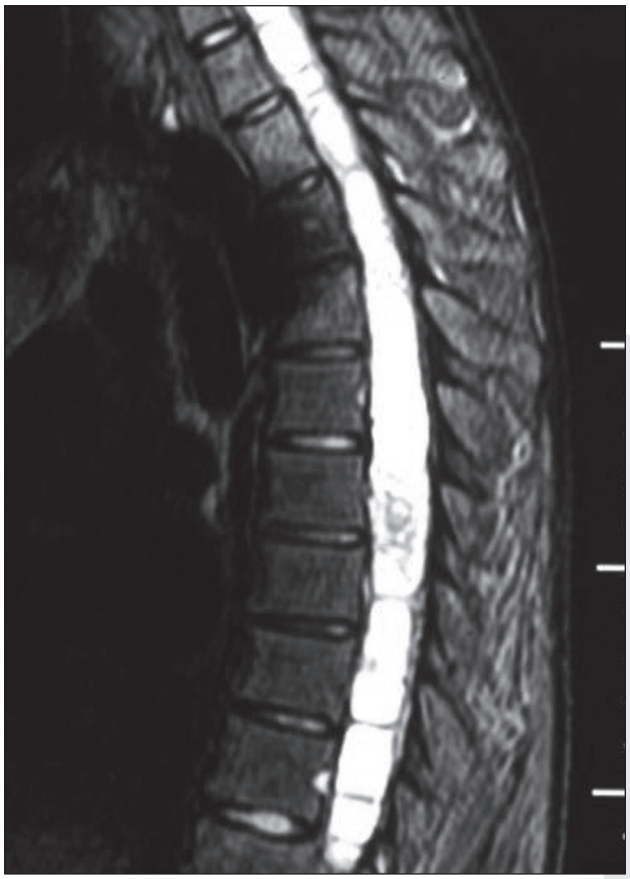

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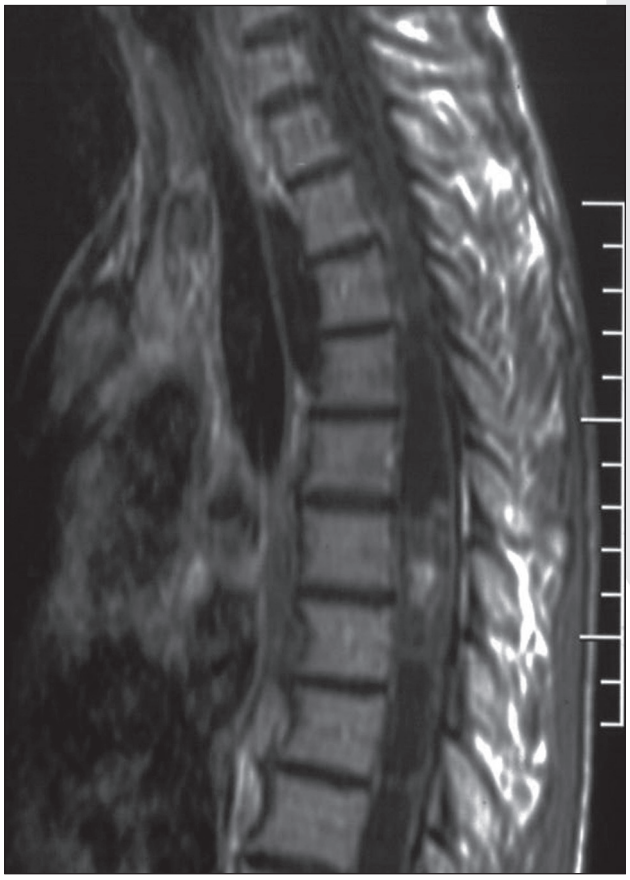
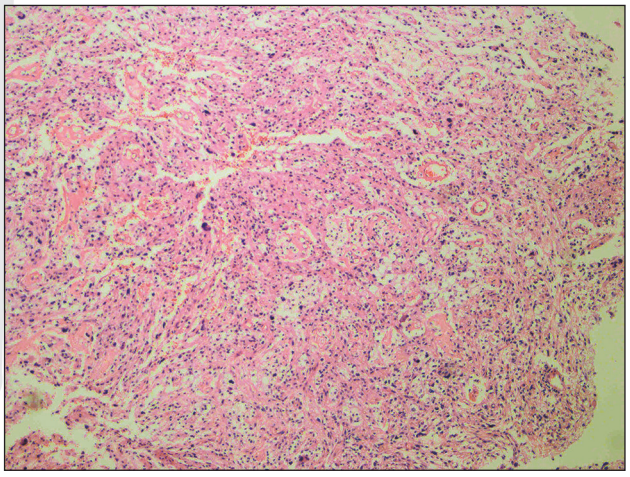


c)

Figure 1. Preoperative spinal MRI scan: (a) The T1-weighted image showing an isointense mass in T6-7 region with slightly enhanced after Gadolinium administration (c); (b) Sagittal T2-weighted image demonstrating a heterogeneous hyperintense neoplasm in the thoracic spinal cord with obvious syrinx and thinner of the spinal cord

The lesion was light-red, fish-like and well-circumscribed on macroscopic observation. Histological examination revealed the lesion containing both glial and neuronal elements. The glial element was mainly composed of bipolar cells with bland, oval to elongated nuclei and piloid (hair-like) processes, reminiscent of PA (Figure 2a). Meanwhile, a number of oligodendrocyte-like cells with small, prominent, dark round nuclei and perinuclear halos were likewise noted. The neuronal element composed of scattered neuron-like cells with abundant eosinophilic cytoplasm, large nuclei and prominent nucleoli (Figure 2b). Rosenthal fibers (RFs) and eosinophilic granular bodies (EGBs) were absent. Glomeruliod vessels, mitoses and necroses were not observed. Immunohistochemically, the astrocytic element showed positivity for glial fibrillary acidic protein (GFAP), S-100 protein, and Olig2 (Figure 2c). Neuron-like cells were synaptophysin and neurofilament positive, and the Ki67 labeling index was less than 1% (Figure 2d, 2e).



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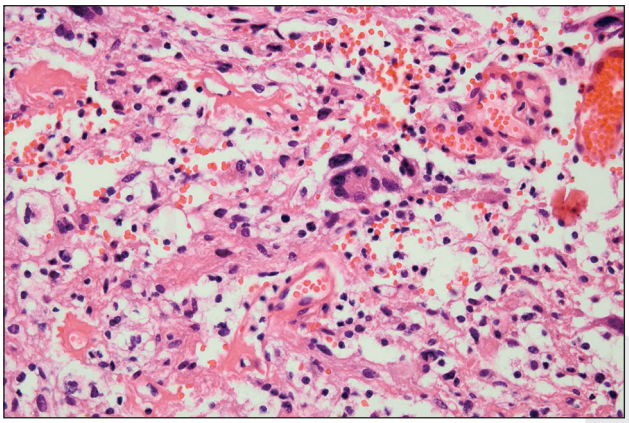

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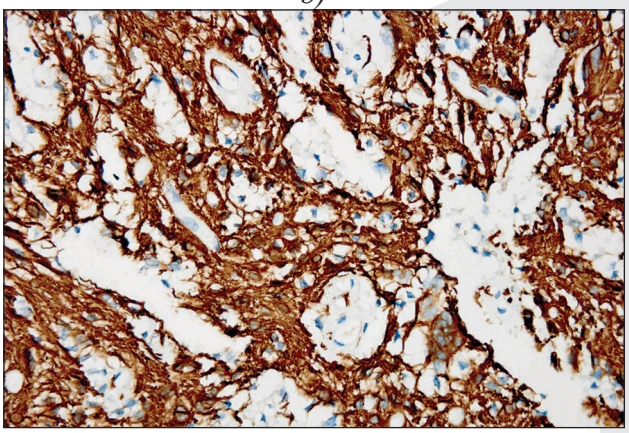

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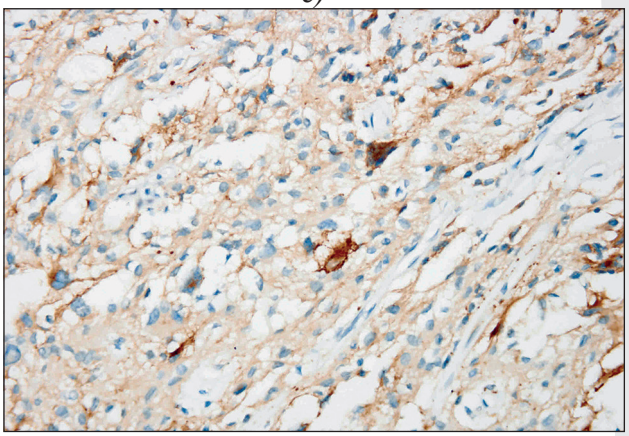

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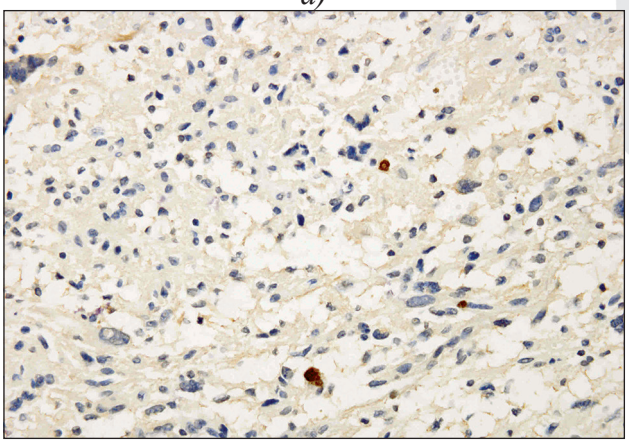

e)

Figure 2. Histopathological features of the mixed GNT: (a) The lesion is revealing bipolar cells with bland, oval to elongated nuclei and piloid (hair-like) processes, reminiscent of PA (hematoxylin and eosin, ×100); (b) Tumor cells are exhibiting multinucleate or "wreath-like" nuclei. Mitoses, significant nuclear pleomorphism, vascular proliferation and areas of necrosis are absent (hematoxylin and eosin, ×400); (c) The astrocytic element is displaying immunoreactivity for GFAP, (d) and rare neuron-like cells are showing synaptophysin positive (immunohistochemistry, ×400); (e) the Ki67 labeling index is less than 1% (immunohistochemistry, ×400)

Informed consent for analysis of the $BRAF^{V600E}$ mutation was obtained from the patient and his family, and this study was also approved by our institutional ethical committee. The DNA sample was extracted from formalin fixed and paraffin embedded tissues. DNA sequencing of hotspot codon 600 (exon 15) of the BRAF gene for mutation was performed as previously described.[5] Our case was negative for a mutation at this codon.

Discussion

Spinal mixed GNTs are uncommon entity that displays GFAP-positive glial and synaptophysin-positive neuronal elements. They are tentatively classified into three groups: Spinal gangliogliomas, spinal glioneuronal tumors and other unclassified.[6] Spinal gangliogliomas, the same as their brain counterpart, are well documented and will not be discussed in this article. Most of spinal mixed GNTs are glioneuronal tumors (75%), whereas unclassified ones only account for about 25% of these tumors. The former usually exhibits well-formed or less structured neuronal rosettes in glial background while the latter presents no sign of morphologic neuronal rosette. They are frequently located in the thoracic region, followed by cervicothoracic and cervical region of the spinal cord. The radiological feature of spinal mixed GNTs is usually iso/hypointense on T1-, iso/hyperintense on T2-weighted images with heterogeneous enhancement. Calcification and edema are generally absent while extensive syringomyelia is commonly noticed.[1-2,4,7,8]

Pathologically, spinal GNTs exhibit benign histological features like rosette-forming glioneuronal tumor of the fourth ventricle suggesting that it may represent a distinctive clinical-pathological entity different from their cerebral counterparts.[2,6] However, Scholz et al. reported a spinal GNT with diffuse infiltrating astrocytic component.[8] Therefore, further more cases with genetic analysis and clinical follow-up are needed to consider these lesions as a distinct new entity. In our case, the lesion was initially considered as a PA, but the presence of scattered synaptophysin-positive tumor cells is distinguished from PA. There are four spinal mixed GNTs resembled PA, and all of them have neuronal rosettes. No sign of neuronal rosette and lack of anaplastic features make our case an extremely rare low-grade mixed glioneuronal tumor of the spinal cord.

Spinal mixed GNTs are thought to originate from a primitive neuroectodermal stem cell that able to differentiate along both glial and neuronal cell lines.[8] Cytogenetic analyses are only performed in three spinal mixed GNTs, which revealed a variable extent of the chromosomal deletion of 1p.[3,8] Loss of 1p may serve as common genetic alterations affected multipotent precursors and have a role in tumorigenesis of these tumors.[3,8] The molecular genetics of these tumors, however, has never been investigated to date. BRAF is a partner of the RAF family of serine/threonine kinases, and is the principal downstream target of Ras in the pro-oncogenic MAPK pathway. In animal study, the activated BRAF kinase domain can induce genesis of PA, and concomitant $BRAF^{V600E}$ mutation with homozygous deletions of cyclin-dependent kinase inhibitor 2A can result in malignant gliomas.[9] Most spinal mixed GNTs show PA-like histology, raising the possibility that these tumors may not be a specific tumor entity, but are a variant of PA. Our result consistent with other recent large-scale mutation analysis of CNS tumors, suggesting that $BRAF^{V600E}$ mutation is not a frequent event in GNTs and these tumors may represent a distinct entity with molecular features differs from PA.[5] Higher mutation frequencies in extra-cerebellar PA (20%) than cerebellar PA (2%) implicate that $BRAF^{V600E}$ mutations may serve as a valuable diagnostic marker for differentiating PA from mixed GNTs in the spinal cord.[5] However, further large-scale analysis should address whether $BRAF^{V600E}$ mutation may happen in GNTs.

Though these tumors reveal benign histopathological features, the long-term outcome is relatively poor. Surgical resection is the best treatment, and gross total resection usually result in a favorable outcome.[2,7] However, aggressive total resection may result in a serious neurological deficits, therefore, adjuvant therapy including radiation and chemotherapy should be considered for those residual or recurrent ones, which might lead to some degree of clinical remission.[3] Duration of follow-up range from 9 to 57 months (average: 18.7 months), and leptomeningeal dissemination is usually presented (68%) and affect patients' long-term survival.[1,4,7,8] Relatively adverse outcomes suggest that long-term follow-up is advisab.

Reference

1. *Harris BT, Horoupian DS. Spinal cord glioneuronal tumor with "rosetted" neuropil islands and meningeal dissemination: a case report. Acta Neuropathologica 2000; 100: 575-9.*

2. *Anan M, Inoue R, Ishii K, Abe T, Fujiki M, Kobayashi H, Goya T, Nakazato Y. A rosette-forming glioneuronal tumor of the spinal cord: the first case of a rosette-forming glioneuronal tumor originating from the spinal cord. Human pathology 2009; 40: 898-901.*

3. *Fraum TJ, Barak S, Pack S, Lonser RR, Fine HA, Quezado M, Iwamoto FM. Spinal cord glioneuronal tumor with neuropil-like islands with 1p/19q deletion in an adult with low-grade cerebral oligodendroglioma. Journal of neuro-oncology 2011; 107: 421-6.*

4. *Ruppert B, Welsh CT, Hannah J, Giglio P, Rumboldt Z, Johnson I, Fortney J, Jenrette JM, Patel S, Scheithauer BW. Glioneuronal tumor with neuropil-like islands of the spinal cord with diffuse leptomeningeal neuraxis dissemination. Journal of neuro-oncology 2011; 104: 529-33.*

5. *Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, Schmieder K, Wesseling P, Mawrin C, Hasselblatt M. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathologica 2011; 121: 397-405.*

6. *Raghavan R. Pediatric Mixed Glioneuronal Tumors in the Spinal Cord. In Hayat MA, editor. Tumors of the Central Nervous System. Netherlands : Springer Netherlands; 2012. P. 7-17.*

7. *Syed S, Rajaram V, Leonard JR, Perry A, Raghavan R. Mixed glioneuronal tumors of the spinal cord in two children. Acta Neuropathologica 2006; 111: 53-5.*

8. *Scholz M, Hoischen A, Radlwimmer B, Weber RG, Harders A, Reifenberger G, Riemenschneider MJ. Rosetted glioneuronal tumor of the spine with overtly anaplastic histological features. Acta Neuropathologica 2009; 117: 591-3.*

9. *Huillard E, Hashizume R, Phillips JJ, Griveau A, Ihrie RA, Aoki Y, Nicolaides T, Perry A, Waldman T, McMahon M. Cooperative interactions of BRAFV600E kinase and CDKN2A locus deficiency in pediatric malignant astrocytoma as a basis for rational therapy. Proceedings of the National Academy of Sciences 2012; 109: 8710-5.*

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Evaluation of ST segment and T-wave changes in the anterior, inferior and posterior derivations of patients with isolated acute occluded left circumflex artery

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Abstract

Background: The aim of this study was to investigate the possible ECG changes in the isolated acute LCx occlusion and the relation between these changes and the site of the occlusion.

Methods: This retrospective study included 175 patients, who were appropriate for the inclusion and exclusion criteria, among 21855 patients who underwent coronary angiography. LCx was divided into two parts as central and peripheral.

Results: 75 patients (42.9%) had ST-elevation myocardial infarction (STEMI); 77 patients (44%) had non-ST-elevation myocardial infarction; and 23 patients (13.1%) had unstable angina pectoris. It was observed that inferior acute myocardial infarction (AMI) was often seen in the peripheral occlusion while inferoposterolateral AMI and anterolateral AMI were often seen in the central occlusion (46.5%, 40.6%, 12,5% respectively and $p<0.05$). The extent and frequency of the ST-segment depression in the precordial V1 and V2 derivations were significantly higher in the patients with the central occlusion ($p<0.05$). In the STEMI subgroup, the extent and frequency of the ST-segment depression in the V1-V3 derivations and the ST-segment elevation in the V6, V8 and V9 derivations were significantly higher in the patients with the central occlusion than the patients with the peripheral LCx occlusion ($p<0.05$).

Conclusion: The determination of the type and the extent of ST segment changes in the records of ECG of the patients with ACS may help to estimate the presence and site of the acute isolated LCx occlusion.

Key words: Isolated acute circumflex occlusion, ST segment change, T wave change.

Introduction

The classical myocardial infarction findings may not be seen in the electrocardiography (ECG) records of most of the patients with the acute coronary syndrome (ACS) caused by the left circumflex artery (LCx) occlusion, which results with delay of the necessary treatment and greater affected myocardial area.[1] The ECG has been used for decades as a reliable and inexpensive tool to diagnose ACS. However, LCx occlusions are not often recognized. A 12-lead ECG detects acute occlusions of the left anterior descending artery (LAD) and right coronary artery (RCA) in 70% to 92% of cases; however, the sensitivity for acute LCx occlusion ranges from 32% to 48%, likely because of its posterolateral location.[2, 3] The standard ECG may miss more than 50% of the patients with LCx occlusion if evaluated only for ST-segment elevation.[4] Therefore, more detailed evaluation for other potential changes due to LCx occlusion is necessary if suspected. In the literature, there is limited data showing the frequency and extent of the ST segment deviation and T-wave changes on ECG and their relation to occlusion site in patients with isolated LCx occlusion. [5-10] We aimed to investigate possible ECG changes in the isolated LCx occlusion and the relation between these changes and the site of the occlusion.

Methods

Study population: This study was carried out retrospectively in our catheterization laboratory by evaluating the files of 21855 patients who underwent selective coronary angiography between February 2002 and September 2010. The flow

chart about the study design is showed in figure 1. As a result, the study population was composed of 175 patients with ACS (with TIMI-0 and TIMI-I coronary blood flow) who admitted in the first 24 hours after beginning of the symptoms, based on the ECG records and the clinical and laboratory data provided by the patient file. Our study was approved by the local ethics committee.

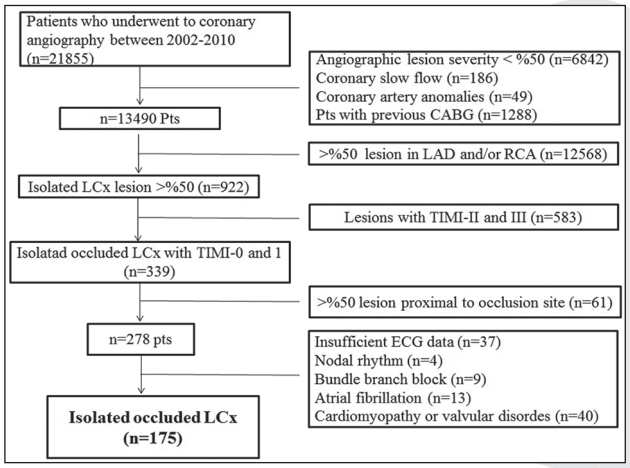

Figure 1. The flow chart about the study design. CABG: coronary artery bypass graft, ECG: electrocardiography, LAD: left anterior descending artery, LCx: left circumflex artery, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction

Angiographic analysis: Coronary angiograms were assessed by 2 experienced cardiologists who were blinded to all clinical and electrocardiographic data. Total occlusion was defined as the observation of TIMI-0 or TIMI-1 flow on selective coronary angiography. TIMI-0 flow was accepted as a complete occlusion and TIMI-1 flow was accepted as the functional occlusion.[11] LCx was divided into 5 segments as the proximal circumflex, the obtuse marginal branch, the distal circumflex, the posterolateral branch and the posterior descending branch. When the left coronary artery gives off three branches, the intermediary branch was accepted as the 6th segment of LCx. Proximal LCx was defined as the part from the ostium of the LCx to the obtuse marginal branch takeoff and distal LCx was defined as the part from the obtuse marginal branch to the origin of the posterior descending branch (Figure 2). The occlusion in LCx was defined with regard to its location as central (proximal segment, obtuse marginal branch and intermediary branch) or peripheral (distal segment, posterolateral branch and the posterior descending branch).[5]

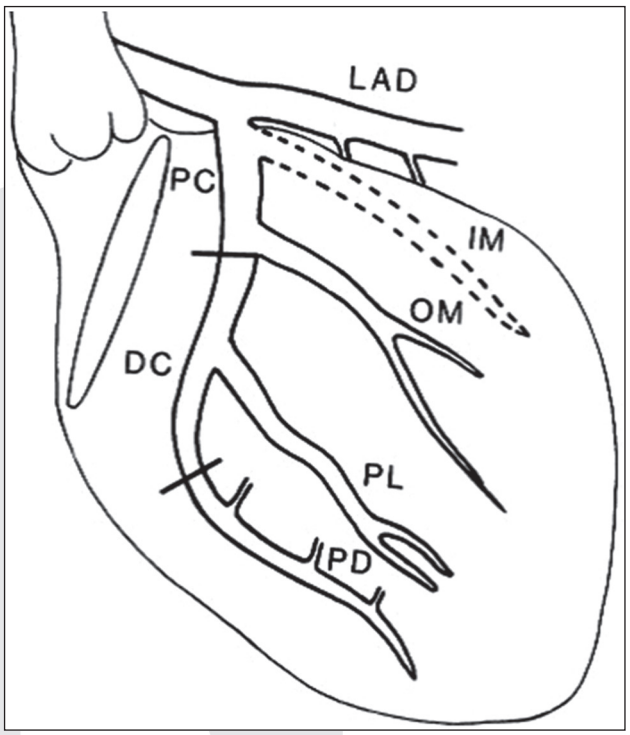

Figure 2. Cx map used in our study. PC: Proximal circumflex, DC: Distal circumflex, OM: Obtuse marginal branch, PL: Posterolateral branch, PD: Posterior descending branch, IM: Intermediary arter and LAD: Left anterior descending artery

Electrocardiographic analysis: The standart 12-lead ECG and the posterior ECG were assessed by 2 experienced cardiologists who were blinded to all clinical and angiographic data. The line between T and P wave was accepted as isoelectric line; and the interval between J point and the beginning of T wave was defined as ST segment on ECG.[12] J point was used for determining the extent of ST changes; the pathological Q wave, "ST elevation" and "ST depression" was defined with regard to the criteria stated in ESC/ACC. The localization of acute myocardial infarction (AMI) was determined according to ST-segment deviation was observed in at least two consecutive corresponding leads.[13] While D2-D3-aVF derivations were a measure for inferior AMI, ST-segment depression associated with high and sharp T waves in V1-2 and R/S>1 in V1 and V2 was used as a criteria for the diagnosis of true posterior AMI. Inferior AMI with posterior AMI was accepted as

Inferoposterior AMI.[12, 14] ST elevation in D1, aVL, V5 and V6 derivations for Anterolateral AMI [5], ST elevation in D2-D3-aVF and V5-V6 derivations for inferolateral AMI, ST elevation in D2-D3-aVF and V5-V6 derivations and ST depression in V1-V4 derivations (V1>V4) for inferoposterolateral AMI were used as diagnostic ECG criterias.[15]

Statistical Analysis

The statistical evaluation was made by SPSS 11.5 computer program. Quantitative variables were showed as arithmetic mean± standard deviation while qualitative variables were frequency and percentage. The comparison of two different parametric groups was carried out by "Student's t test". "Chi-square or Fisher's exact test" (two-way) was used for comparing the qualitative variables. $p<0.05$ was considered significant.

Results

Clinical presentation: The mean age of the study population was 58.9 (range 32 to 83) years. 138 patients (78.9%) were male and 37 patients (21.1%) were female. DM was present in 30 patients (17.1%), hypertension in 56 (32.0%), dyslipidemia in 32 (18.3%), positive family history in 11 (6.3%), and smoking in 64 (36.6%). 75 (42.9%) patients had ST-elevation myocardial infarction (STEMI), 77 (44%) had non-ST-elevation myocardial infarction (NSTEMI), while 23 (13.1%) patients had the diagnosis of unstable angina pectoris (UAP). 58 of the patients (33.1%) were in the central occlusion group, while 117 patients (66.9%) were assessed in the peripheral occlusion group. No significant difference was observed between the central and the peripheral occlusion groups in terms of the clinical diagnosis and the demographic data of the cases (Table 1). Of the patients with STEMI, 32.0% had inferior AMI, 13.3% had inferolateral AMI, 26.7% had inferoposterolateral AMI, 20.0% had inferoposterior AMI and 8.0% had anterolateral AMI. 32 (42.7%) patients from the patient subgroup with STEMI were in the central group while the rest 43 (57.3%) patients were in the peripheral occlusion group. It was observed that inferior AMI was more often in peripheral LCx occlusion while inferoposterolateral AMI and anterolateral AMI were more often in central LCx occlusion (46.5%, 40.6%, 12,5% respectively and $p<0.05$).

Table 1. The demographic characteristics with regard to site of left circumflex artery (LCx) occlusion

	Central LCx (n=58)	Peripheral LCx (n=117)	p value
Age	60,4±10,7	58,1±10,8	0,18
Male	46(79.3)	92(78.6)	0.91
Diabetes(%)	6(10.3)	24(20.5)	0.09
Hypertension(%)	14(24.1)	42(35.9)	0.11
Smoking(%)	27(46.6)	37(31.6)	0.054
Family history(%)	5(8.6)	6(5.1)	0.37
Dyslipidemia(%)	11(19.0)	21(17.9)	0.87

Electrocardiography: It was detected that there was a significant difference in the only V1 and V2 derivations of the cases in terms of ST segment changes (Table 2). The frequency and the extent of ST segment depression in V1-V2 derivations were significantly higher in the patients with central LCx occlusion than in the patients with peripheral LCx occlusion. In the STEMI subgroup, significant difference was found only in V1-V3, V6 and V8, V9 derivations in terms of ST segment changes (Table 3). The frequency and extent of the ST segment depression in precordial V1-V3 derivations were considerably higher in the patients with the central LCx occlusion than the patients with the peripheral LCx occlusion. The frequency and extent of the ST elevation in V6 and the posterior V8, V9 derivations were again considerably higher in the patients with the central LCx occlusion than in the patients with the peripheral LCx occlusion. The highest frequency and the amount of precordial ST segment depression were in V2 derivation, and the least amount was in V6. It was observed that the frequency and extent of the precordial ST segment depression was decreasing from V2 to V6 excluding V1. Table 4 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with regard to the estimation of proximal LCx occlusion of ≥1mm ST depression in V1-V3 derivation and ≥1mm ST elevation in V9 derivation in the patient subgroup with STEMI. No significant difference regarding electrocardiographic changes in the patients with NSTEMI and UAP was detected between the groups. Also, T wave changes in an-

terior, inferior, posterior and the right derivations was evaluated and no significant difference was found between the groups.

Discussion

This is a study investigate the relation between the site of occlusion in the isolated LCx acute occlusion and the ST segment and T wave changes in anterior, inferior, and posterior derivations without being influenced by other possible effects on ECG. In this study, we also identified the clinical, demographic and the angiographic characteristics of the isolated LCx occlusion on a larger population than the previous studies.

The Clinical, Demographic and the Angiographic Characteristics: The obtuse marginal branch of LCx supplies the posterolateral myocardium, the proximal LCx supplies both posteroinferior and the posterolateral myocardium and the distal LCx supplies the posteroinferior myocardium. It was reported in the previous studies that the more frequent inferior wall hypokinesis and the more frequent abnormalities in the inferior derivations on ECG were

Table 2. The relation between ST-segment deviation and site of left circumflex artery occlusion

	Central LCx (n=58)	Peripheral LCx (n=117)	p value
≥1mm ST depression			
V1(%)	23(39.7)	28(23.9)	0.031
V2(%)	24(41.4)	29(24.8)	0.025
V1 ST depression(mm)	-0.58±0.91	-0.35±0.59	0.044
V2 ST depression(mm)	-0.93±1.46	-0.55±0.97	0.045

Table 3. The relation between ST-segment deviation and site of left circumflex artery occlusion in the STEMI subgroup

	Central LCx (n=32)	Peripheral LCx (n=43)	p value
≥1mm ST depression			
V1(%)	28(87.5)	22(51.2)	0.001
V2(%)	28(87.5)	23(53.5)	0.002
V3(%)	26(81.3)	21(48.8)	0.004
≥1mm ST elevation			
V6(%)	21(65.6)	15(34.9)	0.021
V8(%)	17(53.1)	13(30.2)	0.045
V9(%)	16(50.0)	9(20.9)	0.008
V1 ST depression(mm)	1,33±0,82	-0,73±0,70	0,001
V2 ST depression(mm)	-2,07±1,42	-1,17±1,14	0,003
V3 ST depression(mm)	-1,58±1,37	-0,90±1,01	0,017
V6 ST elevation(mm)	1,00±1,21	0,44±1,12	0,043
V8 ST elevation(mm)	0,62±0,66	0,35±0,52	0,046
V9 ST elevation(mm)	0,53±0,57	0,23±0,48	0,016

Table 4. The sensitivity, specificity, positive and negative predictive value of different derivations for the prediction of the central circumflex artery occlusion in the STEMI subgroup

	Sensitivity	Specificity	PPV	NPV
≥1mm ST depression				
V1(%)	87,50	48,84	56,00	84,00
V2(%)	87,50	46,51	54,90	83,33
V3(%)	81,25	51,16	55,32	78,57
V9 ≥1mm ST elevation	50,00	79,07	64,00	68,00

Abbreviations: PPV = positive predictive value, NPV = negative predictive value.

seen in the patients with the distal LCx occlusion than the patients who had AMI due to the proximal LCx occlusion.[5, 16] In our study, it was detected that the inferior AMI was more often seen in the peripheral LCx occlusion while the inferoposterolateral AMI and anterolateral AMI were more often in the central LCx occlusion.

From et al [1] have found that the incidence of isolated LCx occlusion in patients with AMI was 19.5%, and from the patients with the isolated LCx occlusion 127 had STEMI and 166 had NSTEMI. Berry et al[2] performed surface and intracoronary ECG during percutaneous transluminal coronary angioplasty. In 19 patients with balloon inflation of the LCx, all of whom had intracoronary lead ST-segment elevation, only 32% manifested ST-segment elevation on the surface ECG, 42% had ST-segment depression, and 47% had no ST-segment deviation. Gibson et al [17] described the angiographic characteristics of the patients with the isolated precordial ST segment depression from whom had non-ST segment acute coronary syndrome; and detected that LCx was the responsible in almost half of the 314 patients (%48.2) who had AMI due to an occluded vessel. Our study has shown smilar findings with these previous studies. The protective effect of collateral circulation doesn't explain the more frequent NSTEMI due to the LCx occlusion as it doesn't have good collateral flow. These findings can be especially explained by the fact that the spatial resolution of 12-lead ECG is limited in terms of determining the posterior and lateral wall infarction of the left ventricule[1] The use of the posterior ECG derivations increases the sensitivity of ECG in diagnosis.[18]

The isolated LCx disease is not often in angiography. Dunn et al [5] defined the isolated LCx disease as the ≥%70 reduction in vessel lumen diameter and determined isolated LCx disease in 94 from 3900 patients (2.4%) who underwent angiography. From et al [1] detected that the responsible lesion in the coronary angiography of 1500 patients with the diagnosis of AMI was the most common in RCA (44.7%); the second most common location was LAD (35.8%) while culprit LCx lesions were less frequently seen (19.5%). We detected in our study that from 21855 patients who underwent coronary angiography, the isolated stenosis more than 50% was present in 922 patients (4.2%); and 339 ones (1.5%) had total occlusion. The proportion of 175 patients included in the study to whole population was 0.80%, to the ones diagnosed with coronary artery disease was 1.29%, to the isolated LCx disease was 18.9%, and to the patients with the isolated LCx occlusion was 51.6%. Thereby, we have demonstrated the prevalence of the isolated LCx disease and total occlusion among a larger number of patients than the previously reported data. It must be especially pointed out that the low frequency of LCx occlusion in the patients with AMI may be due to the fact that LCx and its branches are less prone to plaque rupture because of their geometry in comparison with the other vessels, and that less angiography is referred as a result of ignoring the ischemic cases occurred due to LCx occlusion.

Dunn et al [5] detected that the isolated coronary artery disease was encountered more often in distal LCx. Stadius et al [19] determined that 80% of the LCx lesions were in the main portion and 20% was in the obtuse marginal branch. As to our study, from 175 patients included 58 ones (33.1%) were in the central group and the rest 117 (66.9%) were in the peripheral group. From the patient subgroup with STEMI, 32 patients (42.7%) were in the central group and 43 ones (57.3%) were in the group with the peripheral occlusion. We found in our study that the occlusion was more often seen in the localization of peripheral zone. The occlusion zones refer to the locations more prone to atherosclerotic disease in the patients with the isolated LCx.

Electrocardiographic Changes: Although there are many studies defining different electrocardiographic criteria to determine the artery related to the infarction in the patients with the acute inferior AMI, and to determine proximal and distal RCA occlusion in the literature [9,10,20,21], the studies researching the electrocardiographic differences between the individual LCx occlusion zones are limited. Mikuriya et al [6] detected that the extent of ST segment depression in V2 derivation in the patients with the acute inferoposterior AMI, and the total of ST deviation in DII-V2 and aVF-V2 derivations are significantly higher in the proximal LCx occlusions in proportion to the distal LCx occlusions. Jim et al [7] detected that in the patients with the acute inferoposterior AMI the frequency of ≥1mm ST segment depression in V1-V4 deri-

vations, ≥1mm ST elevation in DI an V6 derivations, and ≥1mm ST segment depression in V4R derivation are significantly higher in the proximal LCx occlusions in proportion to the distal LCx occlusion. In our study, while significant difference in terms of ST segment changes was seen in only V1 and V2 derivations of the cases according to the location of total occlusion in the LCx, significant differences between the groups in terms of ST segment changes were detected in V1-V3, V6 and V8, V9 in the patient subgroup with STEMI. ST segment depression detected in precordial derivations shows the ischemia on the left ventricular anterior wall. At the same time, it can be seen as the reciprocal change due to the ischemia on the posterior wall.[22, 23] The fact that ST segment depression in precordial derivations and ST elevation in the posterior V8, V9 was more apparent in the central LCx occlusions is due to the more intense exposure of the posterior wall to ischemia in this group in proportion to the peripheral LCx occlusions.

There are several important limitations in our study. The type of the study was retrospective and there are wide range of anatomical variations of LCx. The factors such as LCx dominance and the obtuse marginal branch size which may differ among patients may lead to the mistakes in the evaluation of ECG changes.

Conclusion

The determination of the type and the extent of ST segment changes in the records of ECG of the patients with ACS may help to estimate the presence and site of the acute isolated LCx occlusion.

References

1. *From AM, Best PJ, Lennon RJ, et al. Acute myocardial infarction due to left circumflex artery occlusion and significance of ST-segment elevation. Am J Cardiol 2010; 106: 1081-1085.*

2. *Berry C, Zalewski A, Kovach R, et al. Surface electrocardiogram in the detection of transmural myocardial ischemia during coronary artery occlusion. Am J Cardiol 1989; 63: 21–26.*

3. *Huey BL, Beller GA, Kaiser DL, et al. A comprehensive analysis of myocardial infarction due to left circumflex artery occlusion: comparison with infarction due to right coronary artery and left ante-rior descending artery occlusion. J Am Coll Cardiol 1988; 12: 1156 –1166.*

4. *Krishnaswamy A, Lincoff AM, Menon V. Magnitude and consequences of missing the acute infarct-related circumflex artery. Am Heart J 2009; 158: 706 –712.*

5. *Dunn RF, Newman HN, Bernstein L et al. The clinical features of isolated left circumflex coronary artery disease. Circulation 1984; 69: 477-484.*

6. *Mikuriya Y, Tatsukawa Y, Tamura A, et al. Electrocardiographic diagnosis of left circumflex artery occlusion and the occlusion site. Intern Med 1996; 35: 261-265.*

7. *Jim MH, Ho HH, Siu CW, et al. Value of ST-segment depression in lead V4R in predicting proximal against distal left circumflex artery occlusion in acute infero-posterior myocardial infarction. Clin Cardiol 2007; 30: 36-41.*

8. *Zhan ZQ, Wang W, Dang SY, et al. Electrocardiographic characteristics in angiographically documented occlusion of the dominant left circumflex artery with acute inferior myocardial infarction: limitations of ST elevation III/II ratio and ST deviation in lateral limb leads. J Electrocardiol 2009; 42: 432-439.*

9. *Chia BL, Yip JW, Tan HC, et al. Usefulness of ST elevation II/III ratio and ST deviation in lead I for identifying the culprit artery in inferior wall myocardial acute myocardial infarction. Am J Cardiol 2000; 86: 341–343.*

10. *Herz I, Assali A, Alder Y, et al. New electrocardiographic criteria for predicting either the right or left circumflex artery as a culprit coronary artery in inferior wall acute myocardial infarction. Am J Cardiol 1997; 80: 1343–1345.*

11. *Pompa JJ. Coronary Arteriography and Intravasculer Imaging. In Braunwald E (eds): Heart Disease: A Textbook of Cardiovascular Medicine. WB Saunders Company, Philadelphia 2008, pp . 489-492.*

12. *David M. Mirvis and Ary L. Goldberger. Elektrocardiography. In Braunwald E (eds): Heart Disease: A Textbook of Cardiovascular Medicine. WB Saunders Company, Philadelphia 2008, pp.148-195.*

13. *Thygesen K, Alpert JS, White HD. Universal Definition of Myocardial Infarction. J Am Coll Cardiol 2007; 50: 2173-2195.*

14. *Peterson ED, Hathaway WR, Zabel KM, et al. Prognostic significance of precordial ST segment depression during inferior myocardial infarction in the thrombolytic era: results in 16,521 patients. J Am Coll Cardiol 1996; 28: 305-312.*

15. *Correale E, Battista R, Martone A, et al. Electrocardiographic patterns in acute inferior myocardial infarction with and without right ventricle involvement: classification, diagnostic and prognostic value, masking effect. Clin Cardiol 1999; 22: 37-44.*

16. *Sheehan FH. Left ventricular dysfunction in acute myocardial infarction due to isolated left circumflex coronary artery stenosis. Am J Cardiol 1989; 64: 440-447.*

17. *Gibson C, Pride Y, Mohanayelu S, et al. Angiographic and clinical outcomes among patients with acute coronary syndrome presenting with isolated anterior ST-segment depressions. Circulation 2008; 118: 654.*

18. *Schmitt C, Lehmann G, Schmieder S, et al. Diagnosis of acute myocardial infraction in angiographically documented occluded infarct vessel: limitations of ST segment elevation in standard and extended ECG leads. Chest 2001; 120: 1540-1546.*

19. *Stadius ML, Maynard C, Fritz JK, et al. Coronary anatomy and left ventircular function in the first 12 hours of acute myocardial infarction: The western Washington randomized intracoronary streptokinase trial. Circulation 1985; 72: 292-301.*

20. *Misumi I, Shono H, Nakao K, et al. Electrocardiographic discrimination of infarct-related artery between left circumflex and right coronary artery: Comparison of ST elevation between leads II and III. J Cardiol 2003; 41: 271 – 276.*

21. *Fiol M, Carrillo A, Cygankiewicz I, et al. New criteria based on ST changes in 12-lead surface ECG to detect proximal vs. distal right coronary artery occlusion in case of acute inferoposterior myocardial infarction. Ann Noninvasi ve Electrocardiol 2004; 9: 383 – 388.*

22. *Nishian K, Nomoto Y, Naruse H, et al. Precordial ST-segment depression in acute inferior myocardial infarction: The importance of posterolateral wall infarction. J Cardiol 1989; 19: 413–424.*

23. *Sigiura T, Nagahama Y, Takehana K, et al. Prognostic significance of precoridal ST-segment changes in acute inferior wall myocardial infarction. Chest 1997; 111: 1039-1044.*

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Endoscopic treatment of bile duct lithiasis after Billroth-II gastroenterostomy

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Abstract

Objective: To evaluate endoscopic retrograde cholangiography (ERCP) and ensuing treatment for bile duct lithiasis after Billroth-II gastroenterostomy.

Methods: ERCP was performed in 137 patients with bile duct lithiasis after Billroth-II gastroenterostomy. Of the 137 patients, 34 also received endoscopic sphincterotomy (EST) and 4 received endoscopic papillary balloon dilation (EPBD).

Results: ERCP was successfully performed in 109 (79.6%) out of 137. Bile duct stones were successfully removed based on EST and EPBD in 35 patients. Digestive duct bleeding was observed in 4 patients. There were no serious complications in patients receiving endoscopic treatments.

Conclusion: The successful rate of EST is high in patients with bile duct lithiasis after Billroth-II gastroenterostomy. Endoscopic treatment or cholangioduodenostomy has good therapeutic effects.

Key words: Bile duct lithiasis, Billroth-II gastroenterostomy, endoscopic sphincterotomy, treatment.

Introduction

It is common in clinical practice that the patients after subtotal gastrectomy with Billroth-II gastrojejunostomy suffer from calculus of bile duct later [1]. Because of the changes in anatomy and special diagnosis and treatment, it is insufficient to treat and operate relying solely on the findings of ultrasound, computerized tomography (CT), magnetic resonance imaging (MRI). However, it may have difficulties in endoscopic retrograde cholangiopancreatography (ERCP). We have performed ERCP on 4151 patients between May 1984 and Jun 2012. Among those, there are 137 (3.30%) patients with Billroth-II gastroenterostomy. Now, the following is the experience on diagnosis and treatment.

Patients and methods

General data

A total of 76 males and 61 females were enrolled with ages ranging from 34 to 74 years (mean age 47.5 years). Clinical manifestations: there were 83 patients who suffered from right upper quadrant pain with chills, fever, jaundice and other cholangitis symptoms, 31 patients with recurrent right upper quadrant pain, 23 patients with obstructive jaundice. The finding of ultrasound and CT showed calculus of bile duct in all cases. All the patients had the history of Billroth-II subtotal gastrectomy, including 23 cases with gastrojejunostomy in front of the colon with afferent jejunal loop of 25~40cm (average 32.4 cm), 114 patients with gastrojejunostomy behind of the colon with efferent jejunal loop of 5~8cm (average 6.4cm), 97 cases with the proximal end of jejunum suturing to greater gastric curvature and 40 cases with the proximal end of jejunum lesser gastric curvature suturing to lesser gastric curvature. There were 29 patients with Billroth-II surgery morethan 30 years, 76 patients more than 20 years, 27 patients more than 10 years and 5 patients less than 10 years.

Diagnosis and treatment by ERCP

The instrument used in the ERCP examinations was side-viewing duodenofiberscope (model JF, Olympus). The patients were given the same premedication as that of normal ERCP and were placed in the left lateral decubitus position. The duodenofiberscope was advanced to stomach through oral cavity and esophagus, observing whether there were lesions in remnant stomach and stoma, then entered into duodenum retrograde and gradually along cavity through stoma and afferent jejunal loop, finding the papilla near the closed duodenal stump. Firstly, we performed ERCP examination with catheter through papilla. The instant we determined bile duct stones, we performed endoscopic sphincterotomy (EST) or Endoscopic papillary bal-

loon dilatation (EPBD) examinations and then performed endoscopic stone extraction technique and Endoscopic nasobiliary catheter drainage (ENBD) based on practical conditions.

Results

Examination results

Among 137 patients who received ERCP, 109 cases were successful (79.6%) and 28 patients failed (20.4%). The reasons for failure: endoscopic length was not enough in 20 patients because of the gastrojejunostomy in front of the colon, endoscope didn't transit Treitz ligament in 5 patients and examinations were suspended in 3 patients due to violent reaction. In the 109 patients with successful ERCP, there were 36 patients with simple common bile duct stones, 23 patients with calculi of intrahepatic duct, 17 patients with calculi both in intrahepatic duct and extrahepatic duct. 85 patients showed gallbladder visualization and calculi were identified in 23 patients; among those, pyema and delay emptying appeared in 61 patients. Only 16 cases suffered from moderate fever after ERCP examination.

Treatment results

38 cases were treated with endoscope including 34 patients who were performed on EST and stone extraction and the numbers of successful cases were 31 (91.1%). Among those, 6 cases were performed on stone extraction by emergency ERCP and 4 cases were successful both in EPBD and stone extraction. 4 cases suffered from massive gastrointestinal tract hemorrhage after therapeutic endoscopy and were cured by non-surgical treatment. Cholangiography showed all calculi were out of papillae at 1 week after stone exaction followed by ENBD in 11 patients. No gastrointestinal perforation and dead cases in our group. There were no complications during follow-up of 1 to 19 years in 35 of 38 patients except that bile duct stones reoccurred in 12 cases.

Discussion

The normal anatomy of upper gastrointestinal tract has been changed after subtotal gastrectomy with Billroth-II gastrojejunostomy. Although endoscopic technicians have done considerable exploration with the success rate of ERCP around 80% in the past 30 years [2], post-Billroth II gastrectomy ERCP and EST are still regarded as the most complex and difficult endoscopic techniques. However, conducting ERCP when performing therapeutic endoscopy after Billroth II gastrectomy is safety and feasible [3]; the success rate of post-Billroth II gastrectomy ERCP cannulation is 55% ~88% while the success rate of the therapeutic endoscopy is 66%~100% [4]. Park [5] reported all EST performed on 10 patients after the success of ERCP were successful (100%). Yin et al. [6] said that, 21 cases in 25 patients who were performed on ERCP were successful (84.0%) and 21 patients who were treated by EST＋ENBD were successful (100%). Jia et al. [7] successfully cut off and exacted stones with needle-knife guided by the plastic stent which was inserted in after successful intubation in 2 patients. Huang et al. [8] performed stone extraction on 2 patients and stent insertion on 1 patient after EPBD with front-viewing endoscope. Zhang et al. [9] reported 22 patients acquired good effects. The author has further investigated the operation of EST technology based on the success of post-Billroth II gastrectomy ERCP for many years [10].The experiences in the encountering problems and how to conquer difficulties are following.

Determine the afferent and efferent ends of gastrointestinal stoma correctly: 1)the operation methods of Billroth-II gastrojejunostomy are divided into the proximal end of jejunum suturing to greater gastric curvature and the proximal end of jejunum suturing to lesser gastric curvature. Generally, patients are placed in left lateral decubitus position when endoscope is operated. If the proximal end of jejunum is sutured to lesser gastric curvature, the opening above the stoma is the afferent jejunal loop and the opening below is efferent jejunal loop in the remnant stomach under the visual field of endoscope, which are opposite when the proximal end of jejunum is sutured to greater gastric curvature, i.e. efferent jejunal loop lies above the opening while afferent jejunal loop lies below the opening. We can immediately judge according to pervious surgical records of patients before examination, however, it is difficult to obtain such information in practical clinical work. Because the proximal end of jejunum suturing to greater gastric curvature is regarded as antegrade peristalsis

in Gastrointestinal Operative Surgery, most of surgeon trend to choose this method [11]. That is worth referring by endoscopic technician. 2) the author usually uses two methods to advance endoscope. First, observe two openings of gastroenterostomy for several minutes, the place where the bile and bubble flow is the opening of afferent jejunal loop. But this condition is not too much, only 21cases in our group. Second, the endoscope is advanced along two openings respectively, but when the duodenal stump is not seen until all the endoscope run out, return to stoma and enter in another opening where is the afferent jejunal loop. The character of efferent jejunal loop under endoscope: the enteric cavity is clean so the endoscope would not be blocked when advancing. The character of afferent jejunal loop: bile and gas bubbles are common in enteric cavity and the color of the intestinal mucosa is slightly yellow; if the endoscope is easily to be blocked when being advanced, especially when suddenly be blocked with difficulty in seeing the front enteric cavity, the endoscope is likely in afferent jejunal loop at the moment.

Side-viewing duodenoscope through the opening of afferent jejunal loop: It is difficult to enter in the efferent jejunal loop for side-viewing duodenoscope, which is the same to passing through the pylorus; the essential for endoscope passing through the opening of afferent jejunal loop is "the meaning of sun goes down". At this time, it is comparatively smooth for the proximal end of jejunum suturing to greater gastric curvature while it is more difficult for the proximal end of jejunum suturing to lesser gastric curvature, which is because the opening of afferent jejunal loop is higher and often shows acute angle. If change patients' positions or repeatedly adopt hooking-pulling method, it may help endoscope enter in the afferent jejunal loop. Bergman et al. [12] reported that it was easy to pass through the opening of efferent jejunal loop for front-viewing duodenoscope which has clear visual field and is easier to reach duodenal papilla than side-viewing duodenoscope, increasing the success rate of ERCP; the drawbacks of front-viewing duodenoscope are there is no lifting device and the size of biopsy hole is small, so therapeutic operations such as EST are more difficult. Recently, an increasing number of foreign scholars recommend adopting side-viewing duodenoscope for therapeutic ERCP following Billroth II gastrectomy [13].

Endoscopy pass through the Treitz ligament: Treitz ligament is the extension of peritoneum of duodenojejunal flexure which is located in front of spine and comparatively fixed, toward the beginning part of jejunum and spine. Billroth-II gastrojejunostomy changes the physiological direction of Treitz ligament, and also changes the degree of curvature of duodenojejunal flexure, which directly influences the complexity for endoscope passing through Treitz ligament; that is the jejunum curvature generated by afferent jejunal loop suturing to greater gastric curvature is different from that generated by afferent jejunal loop suturing to lesser gastric curvature [14]. Jejunum curvature of Treitz ligament significantly blocks the endoscope, which is similar to colonoscope passing through splenic flexure or hepatic flexure. At this time, the operation methods are different from that of colonoscope when passing through splenic flexure or hepatic flexure, i.e. do not blindly advance endoscope without seeing the enteric cavity, lift the front-end of endoscope slowly to search enteric cavity in the anterior wall and advance endoscope when the side view field of enteric cavity sliding toward the intestinal mucosa in anterior wall. If the enteric cavity can't be seen, adopt hooking-pulling method, i.e. lift upward large knob to hook intestinal canal and draw back endoscope aided with ventral massage shot or position adjusting for several times, the intestinal canal can be seen and the endoscope can pass through Treitz ligament.

Search for duodenal papilla: When the endoscope is smoothly advanced through the stoma in jejunum and it completely enter in enteric cavity, but the papilla can't be found, the endoscope may lies in efferent jejunal loop. At this time, we should withdraw the endoscope to stoma and advance it from another opening. If the endoscope is completely enter in enteric cavity without seeing papilla, the reasons may be: first, afferent jejunal loop in front of the colon is too long; second, there is side-to-side anastomosis between efferent and afferent jejunal loops, which can make endoscope lose direction. We found the previous surgical record of the patient who is failing in our group after endoscopy showing side-to-side anatomosis between and afferent jejunal loops. Post-Billroth

II gastrectomy duodenal stump presents blind-end whose mucosa is smooth and flat without mucosal folds. Papilla usually lies in 3~5cm of the closed stump and the characteristics are that papilla is located on the wall opposite to visual field of endoscope, its opening direction is on the top of visual field and the ring-shaped intestinal mucosa on the site of papilla uplift sits on the bottom of visual field. Because gastric fluid and food don't pass through duodenum which has been closed after Billroth II gastrectomy, duodenal lumen becomes narrow and papilla becomes smaller with covert opening, the papillae in some patients seem stiff.

Overcome the bottleneck of insufficient length of endoscope: That insufficient length of endoscope in patients with Billroth II gastrojejunostomy often confuses operators. The length of afferent jejunal loop in patients with gastrojejunostomy in front of the colon ranges from 25 to 40cm while the length of endoscope is sufficient in patients with gastrojejunostomy behind of the colon. Among 28 patients who are failing in operations in our study, 20 cases have surgical histories of gastrojejunostomy in front of colon. So, the success rate of ERCP and EST in patients with Billroth II gastrojejunostomy in front of the colon is very low, only 3 cases were successful in our group (13.0%). Be cautious in choosing endoscopy for treating and diagnosing patients with biliopancreatic disease if they have such surgical approaches. Accordingly, we suggest surgeons choose anastomosis behind the colon when performing Billroth II gastrojejunostomy to increase success rate with endoscope in future. Sometimes, the length of endoscope is insufficient even if in patients with gastrojejunostomy behind the colon, which can be partly conquered with the methods of hooking-pulling and encasing endoscope into jejunum; if duodenal stump still can't be seen when all the endoscope is advanced into enteric cavity, we should cease continuing to operate to avoid complications due to long time operation.

(6) Intubate through papilla by opposite phase of side-viewing endoscope: The endoscope is retrograde inserted through reconstructed digestive tract, so the papilla and its opening direction faced in the visual field of endoscope are opposite to those of common endoscope; however, the side-viewing duodenoscope is designed to be inserted upward through the opening of inferior papilla. But, post-Billroth II gastrojejunostomy papilla and the opening direction are opposite to the visual field of endoscope and intubation direction, which lead to difficult intubations. The author adopt four methods to make endoscope close to papilla: 1 change patients' position, such as Left lateral position, semiprone position and prone position; 2 turn operators' body greatly to coordinate with the adjustment of knobs; 3 assistants massage the lens through abdomen; 4 put the top end of catheter on the opening of papilla which is extended, adjust knobs and change the directions of patients and operators. The catheter is easily inserted into pancreatic duct rather than bible duct. To visualize bile duct, the endoscope should be inserted upward in line with opening direction of bile duct in principle. We can make the campiscope surface close to intubation tube or visualize bile duct with the kissing-style method.

Cut-off papilla and remove stones: The position of papilla and the opening in visual field of endoscope are opposite to normal position due to the endoscope is conversely inserted the closed duodenal stump, plus the closed stump limits upward movement, the difficulty of operation will be increased. We take common bow-shaped knife for example which has few opportunities in endoscopic sphincterotomy, only 2 cases in our study. Other operations mainly depend on needle-knife, because needle-knife is more convenient to cut off the sphincter with endoscope at 5-6 o'clock position. Bow-shaped knife is difficult to adjust the endoscope to 5-6 o'clock position in patients with Billroth II gastrojejunostomy. Sometimes, we combine needle-knife with bow-shaped knife in some patients. The author choose three methods on the basis of practical situations of the duodenal stump and papilla: (1) Antegrade incision. It is suitable for the patients with obvious papilla openings. Aim the anterior wall of papilla opening with needleeknife which then is slid upward (the direction of bile duct) stably and accurately to cut off the papilla for about 1cm once. Dolay et al. [15] suggested that the operation is safer when the incision length of EST in post- Billroth II gastrojejunostomy patients is 0.5~1.0cm, and don't make it more than 1.5cm. (2) Retrograde incision. It is suitable for patients with concealed papilla.

Stay the needle-knife directly on the papilla surface of junction between papilla and duodenal mucosa and slide it downward (the direction of papilla opening) stably and accurately to cut all the papilla once. (3) Fenestration. It is suitable for patients with deformation of papilla eminence and other structures. The needle-knife faces the most obvious of eminence and cuts the anterior wall of papilla for 2~3cm to open a small window. After that we verify the directions of papilla and opening through the small window with catheter. At this time, the papilla below the window (opening direction of papilla) is easily cut with bow-shaped knife while it is more safe to cut the papilla above the window (direction of bile duct) with needle-knife. We eliminate stones with stone blanket after EST and EPBD. The operation of endoscopic nasobiliary drainage (ENBD) is similar to common endoscopic therapy, while the only difference is that we draw back endoscope when withdrawing the calculi rather than advance endoscope.

The calculi after Billroth II gastrectomy mainly are bile pigment calculi with few cholesterol gallstones. With the development of endoscope and minimally invasive techniques, the therapeutic effects of stone extraction by stone basket on simple bile duct stones following EST or EPBD are given fully affirmation. However, there is a certain difficulty in post-Billroth II gastrectomy therapeutic endoscopy, the success rate of treatment based on ERCP depends on the anatomical relationship between the papilla and the sphincter of Oddi and also the experience and proficient technology of operators.

References

1. *Peng GF, Song Y, Liang YC. Biliary tract stones occnrred after subtotal gastrectomy: a report of 36 cases. Chin J Gen Surg 2003; 12(2): 158-159.*
2. *Zhang ST, Ji M, Yu ZL. Standadization of endoscopic retrograde cholangiopancreatography. Chin J Dig Endosc 2009; 26(7): 337-338.*
3. *Swarnkar K, Stamatkis JD, Young WT. Diagnostic and therapeutic endospic retrograde cholangiopancreatcography after Billroth II gastrectomy-safe provision in a district general hospital. Ann R Coll Surg Engl 2005; 87(5): 274-276.*
4. *Reviakinvi Cotton PB. Endoscopic retrograde pancreaticochangiography and papillosphincterotomy after resection of the stomach by the Billroth II method. Sov Med 1986; 31(1): 111-115.*
5. *Park CH, Lee WS, Joo YE, et al. Cap-assisted ERCP in patients with a Billroth II gastrectomy. Gastrointest Endosc 2007; 66(3): 612-615.*
6. *Yin P, Ma EW, Bao WM, et al. Duodenoscopic treatment of biliary tract diseases in patients after gastrectomy and Billroth II gastrojejunostomy. Chin J Hepatob Surg 2005; 11(9): 609-611.*
7. *Jia GF, Song LS, Wang ML. Application of precut sphincterotomy in difficult common bile duct cannulation of ERCP. Chin J Diges Endosc 2007; 24(3): 209-211.*
8. *Huang YH, Che ZP, Sun CY. Double-channel gastroscopy for biliary tract diseases after Billroth-II gastroenterostomy. Chin J Dig Endosc 2003; 20(3): 187-189.*
9. *Zhang BY, Tian FZ, Tang LJ. Endoscopic sphincteropapillotomy after Billroth-II gastroenterostomy. Chin J Dig Endosc 2003; 20(1): 46-47.*
10. *Zhang GQ, Yang HX, Cheng TX. Billroth II subtotal gastrectomy after endoscopic retrograde cholangiography. Chin J Gen Surg 1994; 3(4): 223-226.*
11. *Zhang QY, Qian L. Abdominal Surgery[M]. Beijing: People's Medical Publishing House, 2006: 175.*
12. *Bergman JJ,van Berkel AM, Bruno MJ,et al. A randomized trial of endoscopic balloon dilation and endoscopic sphincterotomy for removal of bile duet stones in patients with a prior Billroth II gastrectomy. Gastrointest Endosc 2001; 53(1): 19-26*
13. *Kasapidis P. Cannulating the papilla from the reverse position. Therapeutic ERCP in patients with Billroth II gastrectomy. Ann Gastroenterol 2006; 19(2): 121-124.*
14. *Zhu SP, Zhong XY, Li MQ. Significance of Treitz ligament for Billroth II subtotal gastrectomy. J Abdom Surg 2002; 15(4): 226-227.*
15. *Dolay K, Soylu A. Easy sphincterotomy in patients with Billroth II gastrectomy: A new technique. Turk J Gastroenterol 2008; 19(2): 109-113.*

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Effects of autohemotherapy on hematological responses in Wistar female rats
Autohemotherapy in rats

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Abstract

Introduction: Autohemotherapy is a type of treatment that have acquired an opposite role and have presented its efficiency strived by the medical community for many reasons. In this study we aimed to evaluate the effects of authohaemotherapy on hematological response.

Method: We used Wistar female rats (300g). The study consisted in a control group and a treatment group, blood samples were collected at the first day and at the eighth day after the application. In the both groups we collected 300 µl of blood from each rat through a syringe with a previously prepared solution of 30 µl of sodium citrate 3.2%. In the autohemotherapy group the blood sample was immediately injected in the quadriceps muscle on the back of the thigh hind limb. Rats from the control group did not receive intramuscular blood application. The cellular count was done through flow cytometry and the samples were dosed for immunoglobulin.

Results: In the both groups we observed increased production of erytrocites, hemoglobin and platelet ($p<0.05$). However, there was reduction of basophil in the control group and reduction of lymphocyte, monocyte and neutrophil in the both groups. No effects were observed in IgA, IgG and IgM levels.

Conclusion: Autohemotherapy did not influence hematological responses in Wistar female rats.

Key words: Therapy; Blood; Cytokines, Chemotactic; Hemoglobins; Blood platelets.

Introduction

The literature investigated some components of the blood as adjunctive therapy in some treatments [1-4]. The procedure in which the blood is removed and the same blood is administrated through intra muscular or subcutaneous injection is known as autohemotherapy. This procedure may still be accomplished with the application of whole blood [5].

The degradation products of erythrocytes are erythropoiesis [6] and immune [7] stimulators. It was observed an increase in 10% or more in the number of platelet-forming cells in the blood and organs from 15 to 30 minutes after application of this procedure [8]. It was also observed an increase of antibodies responsible for neutralizing the cytotoxic products of cell degradation [5].

The immune stimulation caused by the blood was also observed in allergic diseases [7]. A positive clinical effect was obtained in patients with bronchial asthma induced by infection. The production of antibodies against antigens in the lung tissue was suppressed [9].

Autohemotherapy was applied in patients with systemic lupus erythematosus resistant to costicoesteroids. The patients presented clinical remission with decreased activity of the DNase enzyme, the same effect of immunosuppressors treatment [10]. Triquet et al performed autohemotherapy in HIV-negative patients presenting chronic ulcers in the lower limbs. The blood collected from the patient was heparinized and applied to the wound. The result was the ease of removal of the fibronecrotic tissue and faster formation of granulation tissue on the wound [11].

One simple report of clinical outcomes is not enough to establish the actual effects of this technique and there are no clinical studies that have sufficient data to discard the side effects or complications of this procedure.

In 2007 the National Agency of Sanitary Surveillance (ANVISA) through the Management of Blood and Blood components (GGSTO) banned this procedure in Brazil. Autohemotherapy is included in the item V, Article 2 of Decree 77.052/76, and it constitutes a sanitary infraction, subject to the penalties provided in Section XXIX of Article 10 of Law no. 6437, 20 August 1977 [12]. One of the arguments for the prohibition contained in the note was the absence of scientific evidence and indexed reports to prove the efficacy and safety of this procedure. In fact, the literature has not been able to conclude whether autohemotherapy may cause immediate or delayed adverse reactions. In addition, it is not confirmed yet the physiological responses of female subjects treated with autohemotherapy. Therefore, we aimed to evaluate the effects of autohemotherapy on hematological responses in female rats.

Method

Animals

The experiments were performed in Wistar female rats (n=15, 300 g). Rats were housed individually in plastic cages under standard laboratory conditions. We divided the animals into two groups: Control (n=5) and autohemotherapy (n=10) groups. They were kept under a 12-h light/dark cycle and had free access to food and water. Housing conditions and experimental procedures were approved by our institution's Animal Ethics Committee (protocol number 009/2008). Efforts were made to minimize the number of animals used.

Protocol Procedures

Blood samples was collected through the tail vein with a needle injection of 1 ml with a needle (35x0.7 mm) with a previously prepared solution of sodium citrate 3, 2%, looking for a 1: 10 ratio of the volume of anticoagulant in the volume of blood collected. The animals were anesthetized in order to avoid stress during the procedure using a combined solution of 1ml of ketamine (100mg/ml), 0.5 ml of xylazine (20mg/ml) and 8.5 ml of saline 0.9%. The volume of blood collected from each animal did not exceed 0.05 ml/kg or 7.5% of blood volume from each animal.

In the autohemotherapy group we collected 300 µl of blood from each rat through a syringe with a previously prepared solution of 30 µl of sodium citrate 3.2%. The volume was immediately injected in the quadriceps muscle on the back of the thigh hind limb. The application of blood was considered the autohemotherapy treatment, this procedure was performed once in each animal.

In the control group we collected 300 µl of blood from each rat, however, we did not apply the intramuscular injection.

We collected blood samples for laboratory analysis equivalent to 7.5% of volume of each animal before the treatment and on the 8th day after the treatment, respecting the period of one week for hematopoietic recovery.

Blood samples were analyzed through complete blood count and it was measured immunoglobulin (Ig) G, Ig A and Ig M.

Cells were counted with the flow cytometer (ABX Pentra 60) and blades were also performed using the technique of blood smear and subsequent staining with Leishman (eosinophilic stain basophilic) for the qualitative and quantitative morphological evaluation of peripheral blood cells.

After the complete blood count, the samples were subjected to centrifugation in order to determine the concentrations of immunoglobulins IgG, IgA and IgM through radial immunodiffusion. After inoculation of the plasma above the plates, halos were measured for 72 h incubation at 25 ° C.

Statistical Analysis

To verify the normality of the distributions we applied the Kolmorogov-Smirnov normality test. We applied the parametric paired Student T test to compare the variables between before and after the treatment, because all distributions were parametric. Differences were considered significant when the probability of a Type I error was less than 5% ($p < 0.05$). We used the statistical package GraphPad Prisma®.

Results

Hematocrit, Hemoglobin and Platelet

Figure 1 presents data regarding hematocrit, hemoglobin and platelet levels in the control group before and after blood collection. We observe that all parameters were increased ($p<0.05$) in the second blood sample.

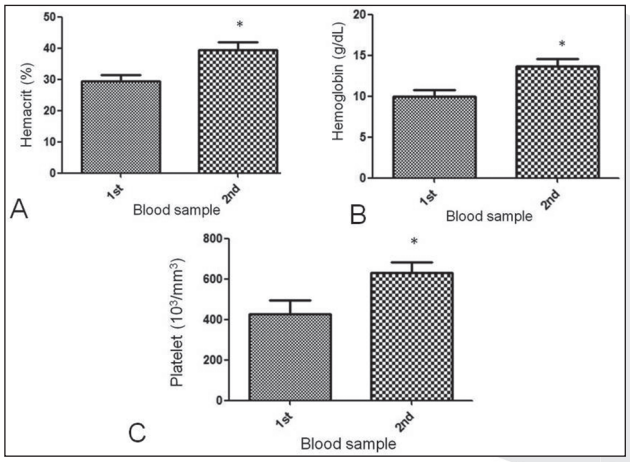

*Figure 1. Hematocrit (A), hemoglobin (B) and platelet (C) before (1st) and after (2nd) blood collection in the control group. *p<0.05: 1st vs. 2nd*

Similar to the control group, hematocrit, hemoglobin and platelet parameters were also increased after autohemotherapy treatment (Figure 2).

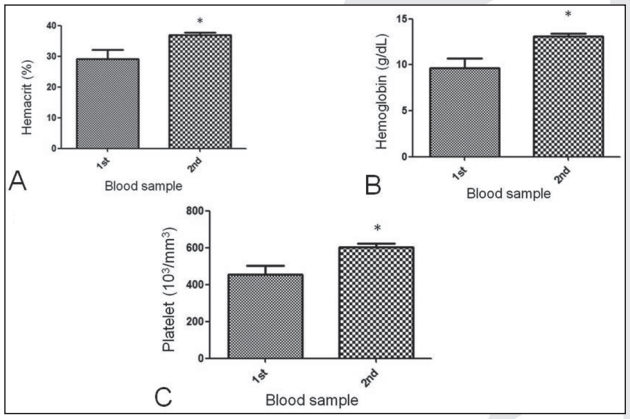

*Figure 2. Hematocrit (A), hemoglobin (B) and platelet (C) before (1st) and after (2nd) autohemotherapy treatment. *p<0.05: 1st vs. 2nd*

Basophil, Eosinophil, Lymphocyte, Monocyte and Neutrophil

According to Figure 3, the control group presented lower values after the 1st blood collection in relation to plasma basophil, lymphocit, monocyte and neutrophil levels.

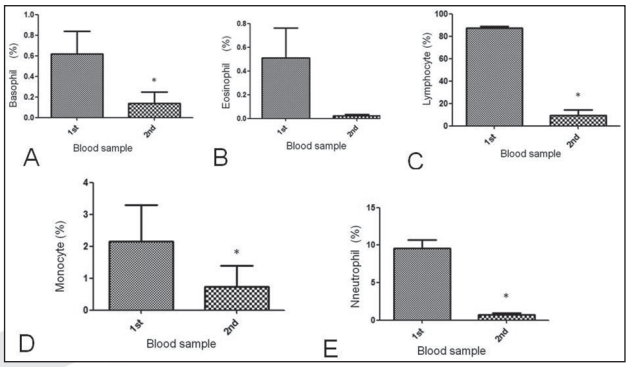

*Figure 3. Basophil (A), eosinophil (B), lymphocyte (C), monocyte (D) and neutrophil (E) before (1st) and after (2nd) blood collection in the control group in the control group. *p<0.05: 1st vs. 2nd*

In relation to the treated group, basophil levels tended to be decreased after autohemotherapy treatment, however, it did not reach statistical significance. Similar to the control group, eosinophil was not significantly reduced after autohemotherapy. Lymphocyte, monocyte and neutrophil were significantly decreased after autohemotherapy treatment (Figure 4).

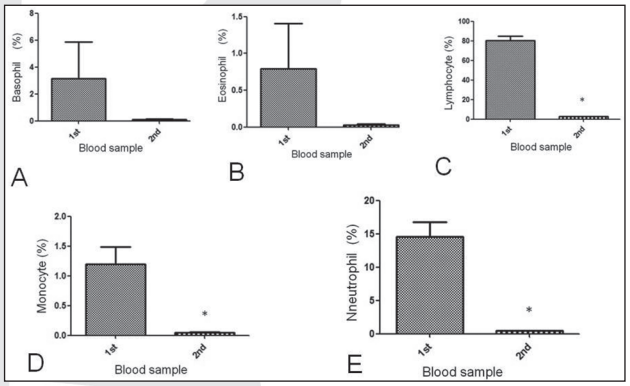

*Figure 4. Hematocrit (A), hemoglobin (B) and platelet (C) (1st) and after (2nd) autohemotherapy treatment. *p<0.05: 1st vs. 2nd*

IgA, IgG and IgM

IgA, IgG and IgM were not changed after blood collection in the treated group (Figure 5). In the treated group, the same cells were not changed after authohemotherapy treatment (Figure 6).

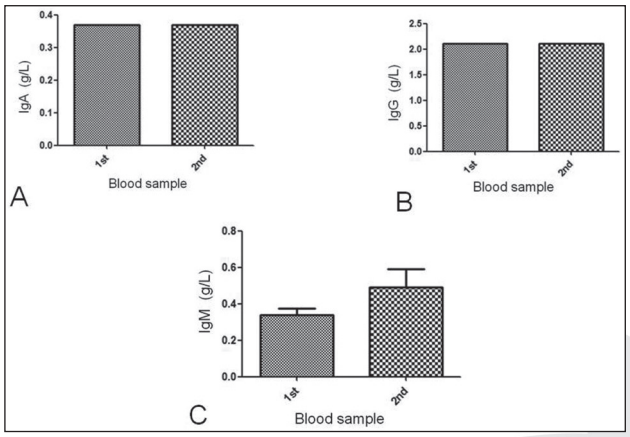

Figure 5. IgA (A), IgG (B) and IgM (C) before (1st) and after (2nd) blood collection in the control group in the control group

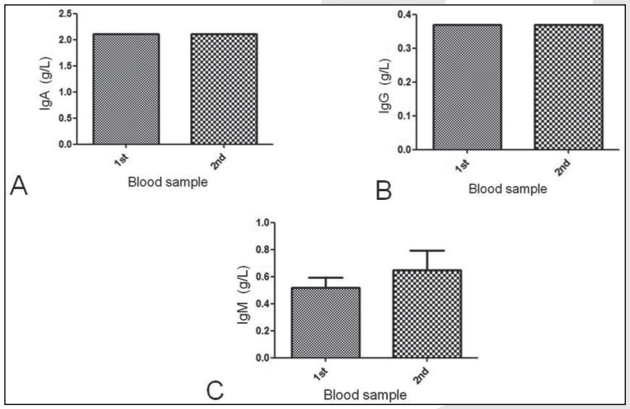

Figure 6. IgA (A), IgG (B) and IgM (C) (1st) and after (2nd) autohemotherapy treatment

Discussion

This investigation was undertaken to evaluate the effects of autohemotherapy on hematological responses in female rats. As a main finding, there was no difference between the control and treated groups regarding hematocrit, hemoglobin, platelet, basophil, eosinophil, lymphocyte, monocyte, neutrophil, IgA, IgG and IgM. We suggest that the treatment presented the same effects of the control group. Blood removal is responsible for inducing blood cell production, since blood loss lead to decreased tissue oxygenation and it is a stimulus for erythropoiesis [13]. This mechanism also occurred with a small volume of blood applied, such as the procedure used in our study.

In relation to the leukocyte count, we observed no difference between the autohemotherapy and control groups. Our finding indicates that blood withdrawal reduced the production of leucocyte. Moreover, a previous investigation concluded that ozonated autohemotherapy in a dose of 50 mg/mL does not present significant influence on natural killer cell function in hemodialyzed patients [14]. No differentiation was made between the types of lymphocytes, we suggest future studies to perform this procedure. To the best of our knowledge, no previous study specifically examined the induction of leukocyte cell by cell. Thus, there is no guesswork in the literature regarding the pathophysiology of this mechanism.

A possible explanation for the phagocyte behavior found in our study is that intramuscular application of blood as well as blood withdrawal cause a local inflammatory process by stimulating phagocytosis through tissue macrophages [15]. Tissue macrophages present antigens to T lymphocytes that are activated and are involved in the process of B cells activation that initiate the process of clonal division with the production of antibodies and globulins [16]. Neutrophils are recruited from peripheral blood after inflammatory mediators secreted by damaged tissues [15]. The result of the process is a decreased number of lymphocytes induced by local stimulation, which is responsible for the response modulation and is detected in peripheral blood. Since neutrophils and macrophages recruited into the tissues phagocytize the injured tissues and blood cells inserted in place with cleaning function until the damaged cells and tissues are properly removed [15], it is observed a lower removal to the lymphatic system are poorly detected in peripheral blood.

Bocci et al [17] published the first study to show that autohaemotherapy may activate an immunological marker in normal subjects without procuring any toxic effects. However, according to our data, in the comparison between control and autohemotherapy groups regarding immunoglobulins, there was no difference. We noted that in the both groups immunoglobulins production was not changed. It indicates that autohemotherapy does not induce the production of acute-phase immunoglobulin. Furthermore, induced desensitization plays an important part in the mechanism of action of autohaemotherapy. The administration of large doses of erythrocytes or erythrolysate results in immunosuppression [5]. Nevertheless, according to Klemparskaya et al [5], autohaemotherapy does

not cause side effects and is feasible both on an in- and out-patient basis.

In this study, the maximum amount of blood that could be injected into the muscles of the animals was collected. This volume of blood did not affect blood oxygenation because the animals did not present symptoms related to this mechanism such as cyanosis and reduced blood hemoglobin levels. However, although this volume is not dangerous to blood oxygenation, it may have been responsible for the induction of hematopoietic responses observed in the autohemotherapy and in the control groups.

Based on our findings, Wistar female rats treated with autohemotherapy presented the same responses described in previous studies such as induction of erytropoiesis [5]. Also, similar responses were observed related to Ig M production. Nonetheless, we found no reference regarding this mechanism in female rats. Observing the principles of erythropoiesis induction, this mechanism occurs when there is any condition that decreases tissue oxygenation such as low blood volume, anemia, low hemoglobin levels, reduced blood flow, low atmospheric oxygen or lung disease [18]. In these cases, renal hypoxia stimulates the production of erythropoietin inducing factor from bone marrow to produce erytrocites [19]. Removal of 5 ml of blood during autohemotherapy performed in humans does not induce renal hypoxia [20].

We observed that autohemotherapy and blood removal increased blood platelet levels. However, a previous study reported opposite findings in humans [21]. Tylick et al evaluated the effects of ozonated autohaemotherapy on the platelet function in chronically haemodialysed patients with peripheral arterial disease. They found that autohemotherapy with ozone concentration of 50 microg/ml and citrate as an anticoagulant does not induce platelet aggregation.

Our data demonstrated that autohemotherapy presented the same effects of no treatment regarding hematological responses. This data does not provide evidence enough to implement changes to human treatment. We suggest further studies to justify the application of this technique in humans for treatment or cure of any disease.

These data provide useful information given that currently many types of animals are widely studied in order to develop new therapies for the prevention of several cardiovascular disorders in humans [22-25].

Conclusion

Autohemotherapy did not influence hematological responses in Wistar female rats.

Acknowledgement

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References

1. *Bucioli SA, Abreu LC, Valenti VE, Vannucchi H. Vitamin e and carnitine suplementation effects on blood glucose levels in young rats submitted to exhaustive exercise stress. HealthMED J 2012; 6: 136-139.*

2. *Centofanti G, Breda JR, Valenti VE, Abreu LC, Fujiki EN, Audi SG, Pereira VX, Correa JA. Vascular access for hemodialysis: an experience report. HealthMED J 2011; 5: 1959-1962.*

3. *Moafi A, Rahgozar S, Hajian M, Ghias M, Ghorbani N, Hassanzadeh A. The Effects of Supplemental Iron on Educational Achievements of Students with "Iron Deficiency without Anemia": A Randomized, Double-Blind, Placebo-Controlled Trial. HealthMED J 2012; 6: 2047-51.*

4. *Zheng Z, Zhan J, Zhang Y, Wei Y, Wang M, Zheng Z, Peng J. Serum fgl2 levels elevated in patients with acute coronary syndrome (ACS). HealthMED J 2012; 6: 2062-65.*

5. *Klemparskaya NN, Shalnova GA, Ulanova AM, Kuzmina TD, Chuhrov AD. Immunomodulating effect of autohemotherapy (a literature review). J Hyg Epidemiol Microbiol Immunol 1986; 30: 331-6.*

6. *Mikhailov SN, Novikov NM. Patologicheskaya foziolgiya i eksperimentalnaya terapiya. Eksperimental'naya Terapiya 1981; 5: 62.*

7. *Klemparskaya NN, Shalnova GA. Normal autoantibodies as radioprotective factors. Atomizdat 1978; 3: 134-9.*

8. *Klemparskaya NN. Zhurnal mikrobiologii, epidemiologii i immunologii. Eksperimental'naya Terapiya 1973; 9: 47.*

9. *Gurgenidze GV, Kilasoniya LO. Terapevticheskii arkhiv. Eksperimental'naya Terapiya 1971; 12: 83.*

10. *Domz CA, Fay KJ, Hoag CL. Hemotherapy in Suspected Dermatomyositis. Calif Med 1957; 87: 108–111.*

11. *Ruffiex TB, Mainetti P, Solomon C, Saudat D. Topical Haemotherapy for Leg Ulcers. Dermatology 1994; 189: 418-420*

12. *Sangue, Tecidos e Órgãos; Nota Técnica nº 1 de 13 de abril de 2007 - 18h50 Auto-Hemoterapia, disponível em: http: //www.anvisa.gov.br/sangue/informes/01_130407.htm - acessado em 2 setembro de 2008*

13. *Jagannath VA, Fedorowicz Z, Al Hajeri A, Hu N, Sharma A. Hematopoietic stem cell transplantation for people with ß-thalassaemia major. Cochrane Database Syst Rev. 2011; 10: CD008708.*

14. *Biedunkiewicz B, Tylicki L, Rachon D, Hak L, Nieweglowski T, Chamienia A, Debska-Slizien A, Mysliwska J, Rutkowski B. Natural killer cell activity unaffected by ozonated autohemotherapy in patients with end-stage renal disease on maintenance renal replacement therapy. Int J Artif Organs 2004; 27: 766-71.*

15. *Skyler JS. Immune intervention for type 1 diabetes mellitus. Int J Clin Pract Suppl 2011; 2: 61-70.*

16. *Pammi M, Haque KN. Oral immunoglobulin for the treatment of rotavirus diarrhea in low birth weight infants. Cochrane Database Syst Rev 2011; 10: CD003742.*

17. *Bocci V, Luzzi E, Corradeschi F, Paulesu L. Studies on the biological effects of ozone: 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy. Biotherapy 1993; 7: 83-90.*

18. *Okebe JU, Yahav D, Shbita R, Paul M. Oral iron supplements for children in malaria-endemic areas. Cochrane Database Syst Rev 2011; 10: CD006589.*

19. *Lee GR, Bithell TC, Foerster J, Lukens JN. Erithropoiesis In: Wintrobe's Clinical Hematology. ed. 9 Lea & Febiger, Pennsylvania – E.U.A., 1993, vol 1. Part 3, section 2: 169-267.*

20. *Deen PM, Robben JH. Succinate receptors in the kidney. J Am Soc Nephrol 2011; 22: 1416-22.*

21. *Tylicki L, Lizakowski S, Biedunkiewicz B, Skibowska A, Nieweglowski T, Chamienia A, Debska-Slizien A, Rutkowski B: Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients. Blood Coagul Fibrinolysis 2004, 15: 619-22.*

22. *Valenti VE, Ferreira C, Meneghini A, Ferreira M, Murad N, Ferreira Filho C, Correa JA, Abreu LC, Colombari E. Evaluation of baroreflex function in young spontaneously hypertensive rats. Arq Bras Cardiol 2009; 92: 205-15.*

23. *Cisternas JR, Valenti VE, Alves TB, Ferreira C, Petenusso M, Breda JR, Pires AC, Tassi N, de Abreu LC. Cardiac baroreflex is already blunted in eight weeks old spontaneously hypertensive rats. Int Arch Med 2010; 3: 2.*

24. *Valenti VE, Imaizumi C, de Abreu LC, Colombari E, Sato MA, Ferreira C. Intra-strain variations of baroreflex sensitivity in young Wistar-Kyoto rats. Clin Invest Med 2009; 32: E251.*

25. *Valenti VE, De Abreu LC, Colombari E, Sato MA, Ferreira C. The variability of baroreflex sensitivity in juvenile, spontanebously hypertensive rats. Cardiovasc J Afr 2011; 22: 14-7.*

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Subglottic high frequency jet ventilation in management of bilateral vocal fold paralysis: A case report

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Abstract

We present the use of subglottic high frequency jet ventilation (HFJV) in treatment of bilateral vocal fold palsy in 36-year-old female who underwent thyroidectomy on the previous day. After intravenous anesthesia induction, endotracheal tube was exchanged with jet catheter inserted through the vocal cords into the trachea. The surgical procedure was performed endoscopically using HFJV and the special endo-extralaryngeal suture technique by Lichtenberger. At the end of procedure, jet catheter was exchanged with LMA until spontaneous breathing was established. There was no hypoxia or barotrauma to the lungs during the surgery. The postoperative course was uneventful.

Key words: High frequency jet ventilation, Thyroid gland, Postoperative Complications, Airway Obstruction, Vocal Fold Paralysis.

Introduction

Lesion of recurrent laryngeal nerves as consequence of thyroid surgery, results in bilateral vocal fold paralysis (BVFP) and respiratory obstruction [1,2].

The initial treatment involves obtaining an adequate airway with endo-extralaryngeal laterofixating operations undergoing general anesthesia [3]. This revision surgery of bilateral vocal fold palsy has been conducted in suspension laryngoscope with subglottic high frequency jet ventilation (HFJV). Applying these operative techniques satisfactory breathing can be achieved without tracheotomy [4-6].

Endolaryngeal microsurgery is challenging for the anesthesiologist and the laryngologist since they share access to the larynx [7]. The good exposure of the larynx, immobility of the vocal cords, satisfactory ventilation and oxygenation are crucial. High frequency jet ventilation as ventilator approach in laryngeal microsurgery provides application of small tidal volume at high rates, followed by passive expiration [8,9]. Subglottic HFJV with narrow tracheal catheters is described for surgery of the endolarynx as a safe and less obstructive ventilation technique [10,11]. However, the technical skills and equipment are the reasons for HFJV limited use.

We report a patient with BVFP undergoing endo-extralaryngeal laterofixating operation without tracheotomy with subglottic HFJV on first day after total thyroidectomy.

Case report

A 36-year old female (BMI 24 kg/m^2) was admitted from outside hospital after total thyroidectomy, performed on the previous day. Postoperatively, patient developed severe inspiratory stridor, worsening progressively with decreased arterial oxygen saturation (from 100% to 82 %) after tracheal tube removal. After mask ventilation with 100 % oxygen, flexible laryngoscopy revealed paradoxical behavior of the vocal folds that caused near total obstruction of the larynx on forced inspiration. Trachea was reintubated over flexible laryngoscope. Chest radiograph, electrocardiography (ECG) and laboratory findings including potassium, calcium and glucose level were normal. Due to the logistic problem, laryngologist and equipment were unavailable; patient was transferred on the following day to our institution.

Premedication consisted of intravenous (IV) atropine 1 mg and dexamethason 10 mg. Monitoring included noninvasive blood pressure, ECG, capnography, pulse oximetry and entropy. Anesthesia was induced with propofol 2.5 mg/kg IV

followed by remifentanil infusion (0.2-0.5 μg/kg/min) (ARGUS Medical, Switzerland) and rocuronium (0.5 mg/kg) (ARGUS Medical, Switzerland). Maintenance anesthesia was achieved with an infusion of propofol and remifentanil. Patient was ventilated with 100% oxygen during induction. After two minutes, when satisfactory level of anesthesia was achieved and justified by entropy (RE/SE 35/36) and the loss of eyelash reflex, tracheal tube was exchanged with jet catheter (Dual Lumen Jet Ventilation Catheter acc. Biro Acutronic Medical System, Switzerland) under laryngoscopy with Miller 3 blade. The jet catheter was advanced by direct laryngoscope and positioned in the posterior commissure. Subglottic HFJV was applied with automatic commercial Mistral jet ventilator (Acutronic Medical System, Switzerland) attached to the jet catheter.

The initial ventilator settings were: FiO_2 0.6 respiratory frequencies 120 cycles/min, 40% inspiration duration, and driving pressure 1.2 bar. After initiation HFJV was monitored by bilateral chest movements and auscultation of the lungs. Muscle relaxation was maintained based on train-of-four (TOF) stimulation of the ulnar nerve.

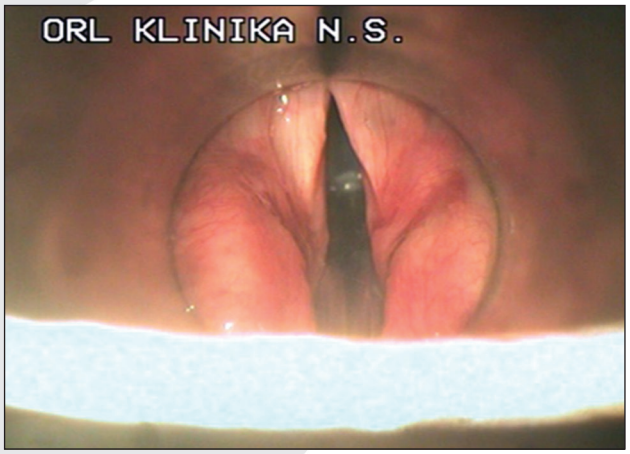
The surgical procedure consisted of right endo-extralaryngeal laterofixation. Duration of surgery was 30 minutes. At the end of the surgical procedure, propofol and remifentanil infusions were stopped. When TOF of 0.9 was achieved block was reversed with IV neostigmine (2.5 mg) and atropine (1mg). Ketorolac 30 mg IV was given for postoperative pain treatment. No laryngeal edema was noticed on repeated postoperative endoscopic laryngeal examination. Jet catheter was removed and LMA (LMA-Classic™, The Laryngeal Mask Company Limited, Nicosia, Cyprus) was used to secure a supraglottic airway until the patients regained protective reflexes and spontaneous breathing (patients lungs were ventilated by mask until awakening).

Postoperatively, the patient denied procedural recall, and expressed a high degree of satisfaction. Clinical signs, spirometry and flow/volume curve verified absence of airway obstruction. Complications resulting from ventilation with subglottic HFJV such as barotrauma or subcutaneous emphysema, seeding of blood into tracheobronchial tree were not observed in patient. Within 8 days patient was discharged home.

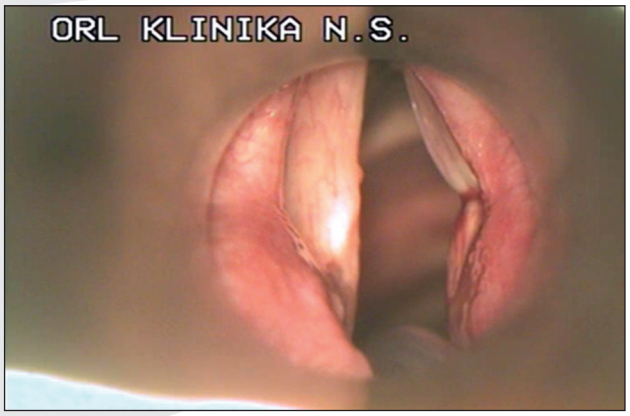
Discussion

We presented the use of subglottic HFJV during the revision surgery of BVFP in order to establish the best surgical field and safe approach to the airway on previously intubated trachea.

Laryngeal obstruction due to BVFP may cause dyspnea, hoarseness and dysphagia. If left untreated, patients may become tracheotomy depended [3,5,6]. In this patient immediate airway obstruction due to the bilateral recurrent laryngeal nerve damage and inability to phonate required tracheal reintubation.



a)



b)

Figure 1. Views of a jet catheter in the glottis providing unobstructed access to the BVFP before (a) and after (b) endo-extralaryngeal fixation procedure

Our decision to extubate trachea and apply HFJV was based on previous date about easy intubation and HFJV suitability for this procedure. Suspension microlaryngeal surgery requires good exposure of the larynx, continuous control of the

airway patency and immobility of the vocal cords. Even a small endotracheal tube impedes access and visibility of the operative field, especially posterior part of the glottis [10,12,13]. A good access to the surgical field due to small diameter of the jet catheter is main advantage of subglottic jet ventilation [11]. Narrow jet catheters are preferable to provide space for observation and surgical manipulation, and permit a sufficient outflow of respiratory gases.

This is particularly important in patients with difficult or partially occluded airways with point out on its use in the setting of bilateral vocal cord immobility. Although certain vibration of the vocal folds may occur, the rigid laryngoscope remains unmoved and the operating field is quiet [10,14-16]. The jet catheter tip position above carina enables a limited entrainment of ambient air only, HFJV creates a constant PEEP, which prevents bronchial and alveolar collapse and increase functional residual capacity [17]. The most serious complication of HFJV is lung barotraumas that may occur to an obstructed airway. This can happen in case of accidental displacement of the jet catheter into a bronchus, obstruction of the larynx by instruments or inadvertent closure of the glottis when muscular relaxation is diminished. Therefore, it is essential to monitor airway pressure to avoid barotraumas [18,19].

Dexamethason was given for prevention of laryngeal edema as the possible complication of surgery [1]. Use of remifentanil with propofol for TIVA is safe and the patient is protected from delayed respiratory depression [20].

Although subglottic HFJV is suitable to maintain adequate ventilation throughout the procedure, we decided to insert LMA at the end of the procedure. The plan was based on endoscopic examination and decision that trachea should be extubated at the end of the procedure if adequate postoperative airway is achieved. This enables good airway control, smooth and safe tracheal extubation in patient who experienced previous emergency reintubation. Moreover LMA provides adequate views of vocal cords and facilitates repeated endoscopic exam [15,16,21,22].

In conclusion, this case demonstrates that subglottic HFJV is safe and effective in maintaining gas exchange, providing optimal visibility of laryngeal structures, offering adequate space for surgical manipulation without tracheotomy. Overall, combination of HFJV and conventional ventilation with LMA seems uniquely suited for this specific use, jet prospective comparison of anesthetic techniques is needed to establish superiorly.

References

1. *Gardner GM, Benniger MS. Vocal fold paralysis. In: Rubin JS, Sataloff RT, Korovin GS (Eds). Diagnosis and treatment of voice disorders. San Diego: Plural Publishing, Inc. 2006; 471-93.*
2. *Goncalves Filho J, Kowalski LP. Surgical complications after thyroid surgery performed in a cancer hospital. Otolaryngol Head Neck Surg 2005; 132: 490-4.*
3. *Jori J, Rovo L, Czigner J. Vocal cord laterofixation as early treatment for acute bilateral abductor paralysis after thyroid surgery. Eur Arch Otorhinolaryngol 1998; 255: 375–8.*
4. *Shvero J, Koren R, Stern Y, Segal K, Feinmesser R, Hadar T. Laser posterior ventriculocordectomy with partial arytenoidectomy for the treatment of bilateral vocal fold immobility. J Laryngol Otol 2003; 117: 540-3.*
5. *Lichtenberger G. Reversible lateralization of the paralyzed vocal cord without tracheostomy. Ann Otol Rhinol Laryngol 2002; 111: 21-6.*
6. *Leitersdorfer S, Lichtenberger G, Bihari A, Kovacs I. Evaluation of the lung function test in reversible glottis-dilating operations. Eur Arch Otorhinolaryngol 2005; 262: 289-93.*
7. *Rezaie-Majd A, Bigenzahn W, Denk DM, Burian M, Kornfehl J, Grasl MCh, Ihra G, Aloy A. Superimposed high-frequency jet ventilation (SHFJV) for endoscopic laryngotracheal surgery in more than 1500 patients. Br J Anaesth 2006; 96: 650-9.*
8. *Ihra G, Gockner G, Kashanipour A, Aloy A. High-frequency jet ventilation in European and North American institutions: developments and clinical practice. Eur J Anaesthesiol. 2000; 17(7): 418-30.*
9. *Bacher A, Pichler K, Aloy A. Supraglottic combined frequency jet ventilation versus subglottic monofrequent jet ventilation in patients undergoing microlaryngeal surgery. Anesth Analg 2000; 90: 460-5.*
10. *Davies JM, Hillel AD, Maronian NC, Posner Lk. The Hunsaker Mon-Jet tube with jet ventilation is effective for microlaryngeal surgery. Can J Anaesth 2009; 56: 284-90.*

11. *Bourgain JL, Chollet M, Fischler M, Gueret G, Mayne A. Guideline for the use of jet ventilation during ENT and oral surgery. Ann Fr anaesth Reanim 2010; 29(10): 720-7.*

12. *Bacher A, Lang T, Weber J, Aloy A. Respiratory efficiancy of subglottic low-frequency, subglottic combined-frequency, and supraglottic combined-frequency jet ventilation during microlaryngeal surgery. Anesth Analg 2000; 91: 1506-12.*

13. *Bourgain JL, Desruennes E, Fischler M, Ravussin P. Transtracheal high frequency jet ventilation for endoscopic airway surgery: a multicentre study. Br J Anaesth 2001; 87(6): 870-5 .*

14. *Barakate M, Maver E, Wotherspoon G, Havas T. Anaesthesia for microlaryngeal and laser laryngeal surgery: impact of subglottic jet ventilation. J Laryngol Otol. 2010; 124(6): 641-5.*

15. *Rubin JS, Patel A, Lennox P. Subglottic jet ventilation for suspension microlaryngoscopy. J Voice 2005; 19: 146-50.*

16. *Patel A, Rubin JS. The difficult airway: the use of subglottic jet ventilation for laryngeal surgery. Logoped Phoniatr Vocol 2008; 33: 22-4.*

17. *Mausser G, Schellauf A, Scherubl M, Arrer A. Schwarz G. Experimental model of laryngotracheal stenosis in infants: effect of different high-frequency jet ventilation patterns on pulmonary parameters. Paediatr Anaesth 2011; 21: 894-9.*

18. *Jaquet Y, Monnier P, Van Melle G, Ravussin P, Spahn DR, Chollet-Rivier M. Complications of different ventilation strategies in endoscopic laryngeal surgery: a 10 – year review. Anesthesiology 2006; 104: 52-9.*

19. *Ihra GC, Heid A, Pernerstorfer Th. Airway stenosis-related increase of pulmonary pressure during high-frequency jet ventilation depends on injector's position. Anesth Analg 2009; 109: 461-5.*

20. *Mertens MJ, Engbers FH, Burm AG, Vuyk J. Predictive performance of computer-controlled infusion of remifentanil during propofol/remifentanil anaesthesia. Br J Anaesth 2003; 90: 132-41.*

21. *Brimacombe JR, Berry AM, White PF. The laryngeal mask airway: limitations and controversies. Int Anesthesiol Clin 1998; 36: 155-82.*

22. *Brimacombe J, Sher M, Laing D, Berry A. The laryngeal mask airway: a new technique for fiberoptic guided vocal cord biopsy. J Clin Anaesth 1996; 8: 273-5.*

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Effect of yoga exercises on quality of life of postmenopausal women

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Abstract

Background: As a physiological event, menopause has complications which influence women's quality of life. Several measures such as hormone therapy and non-pharmacological therapies are recommended to reduce menopausal symptoms and improve quality of life (QoL) in this period. One of the non pharmacological methods is complementary medicine i.e. yoga. The present study aimed to determine the impact of yoga exercises on the quality of life of postmenopausal women.

Methods: This was a randomized clinical trial in which 136 postmenopausal women aged 45-60 were divided into experimental and control groups through random allocation method based on the study environment. The experimental group participated in basic Hatha Yoga training classes (twice a week and 90 minutes for each session); a combination of *Asana, Pranayama* and *Shavasana*. Data collection tools included a Menopause-specific Quality of Life (MENOQOL) developed by Hilditch et al. The quality of life of the subjects was evaluated before and two months after the intervention (in vasomotor, psychosocial, physical and sexual dimensions). Data were analyzed through SPSS Software version 16 through the statistical methods of Mann-Whitney and independent t-test ($P < 0.05$).

Results: The subjects were similar in terms of individual variables before the intervention. Mean QoL score before the intervention in the experimental and control groups was 103.69 (33.27) and 100.28 (36.27) respectively which did not have a significant difference. Two months after the intervention, this reached 84.75 (32.10) and 105.73 (37.22) respectively for the experimental and control groups. Independent t-test showed improvement of the QoL score in the experimental group ($P = 0.002$). The results of statistical tests showed that the yoga exercises were significant in three vasomotor ($P = 0.039$), psychosocial ($P = 0.009$) and physical ($P = 0.006$) dimensions.

Conclusion: Considering that a large number of women are reaching menopausal age, understanding the effect of this stage of life on mental health and QoL in this population and using therapeutic strategies such as physical activities for their sense of well-being should be taken into account as an important health goal.

Key words: Yoga, quality of life, menopause.

Introduction

Menopause is the cessation of menstruation resulting from loss of ovarian follicular function which is a recognized physiological event in women's lives. In addition to hormonal changes particularly reduction of estrogen levels with biological, psychological and social changes (1), this physiological process is associated with complications such as sleep disorder, night sweats, headaches, hot flashes, vaginal dryness, anger and depression, increase in cholesterol levels and cardiovascular diseases (2) which influence women's quality of life (QoL) (3-5). Due to an increase in life expectancy in the world and Iran, it is expected that women spend 20-30 years of their life in postmenopausal period during which they are faced with many problems, symptoms and complications (6) and has significant consequences for families and society arising from physical, mental, social and economic issues (7). 75 to 80% of women experience menopause-

related problems and complications(8) approximately 50% of them have these symptoms averagely four years after complete cessation of menstruation (9) and 10 to 30% of them have these symptoms their entire life so that 60% of women are seeking a solution and treatment for such problems (10). Different actions such as hormone therapy, physical activity and exercises can be applied to decrease menopausal symptoms (11). Although hormone therapy is a selective treatment for reduction of menopausal symptoms (12), its harmful side effects in long-term use, including increased risk of cataract (13), endometrial and breast cancer, coronary artery disease, MI and thromboembolism have been proposed in different studies (14-17). However, Blake has announced that there is no evidence to confirm the side-effects of hormone therapy (18). Nonetheless, the concerns about safety of hormone therapy, incentive in the health lifestyle and behavior changes and alternative and complementary approaches during middle ages have been increased (14).

One of the types of complementary medicine is yoga. It is considered to be an exercise for the mind, body and spirit that can significantly reduce stress and anxiety (19), depression (20) and problems related to musculoskeletal disorders such as osteoarthritis, carpal tunnel syndrome, hyperkyphosis, backache and of motor skills and physiological variables such as hypertension, heart rate and body weight (21). QoL improvement and sense of mental well-being have been reported in yoga (22). Furthermore, in many studies yoga has been reported to be effective in reduction of menopausal symptoms (23-26). In addition to the mentioned advantages of yoga, it has some other benefits in terms of cost, efficacy, non-invasiveness, minimum risk compared with side-effects of drugs, and physical fitness that can improve QoL (20). On the other hand, the effect of exercises and physical activity has been proposed on mental and physical health; so that a systematic review of 14 studies has shown a positive relationship between physical activity and health related quality of life (27). Moreover, it has been suggested that women who exercise regularly experience less vasomotor symptoms, negative mood, decreased sexual desire and a higher feeling of well-being (28). Other researchers have also proposed that sports activities are related to quality of life in postmenopausal women (29) and have paid attention to exercise and lifestyle change as important factors reducing menopause symptoms (30).

Despite findings proposing that exercise can be an effective treatment for menopausal problems, Borrelli reported that there are very limited evidences to prove positive impacts of exercise on symptoms and problems experienced during postmenopausal period (12). Therefore, the present study aimed to determine the impact of yoga exercises on quality of life of postmenopausal women in order to improve their physical and mental health and promote their QoL in this period. Considering the mentioned cases, the question of the present study was "what is the impact of implementing two months of yoga exercises on quality of life of postmenopausal women?"

Materials and methods

Experimental Design

This was a randomized experimental trial with a control group. The researchers evaluated the impact of yoga exercises on four dimensions of QoL in postmenopausal women by inserting the independent variable (yoga exercises) and also considering a control group. Thus, 136 postmenopausal women aged 45-60 participated in 8 weeks of basic Hatha yoga; a combination of Asana (physical exercise), Pranayama (breathing exercises) and Shavasana (relaxation training). Randomization was based on the study environment and conducted through selecting 5 districts from the 22 geographical districts of Tehran municipality by the use of a random number table and all the health centers in the mentioned districts were identified (totally; n = 29). Thereafter, each of the health centers was given a special number and four health centers were randomly selected through the random number table. After random selection of the four health centers, in order to prevent pollution of information among the experimental and control groups, the subjects of the experimental and control groups were selected from separate health centers; thus, two health centers were randomly allocated to the experimental and control groups. In addition, in order to prevent from pollution of the information, the two health centers which were almost in a geographic district were considered as the experimental group.

Subjects

The eligible subjects with inclusion criteria were selected through continuous method from the selected health centers. The inclusion criteria of this study were: Married menopausal Iranian women aged 45-60, at least a year and a maximum of four years had passed from their menopause, with common symptom of menopause and lowest literacy, BMI between 20-30, their menopause happened naturally not due to surgery, no regular physical activity currently, no history of HRT in past 6 months and no known physical (hypertension, diabetes, hypothyroidism and hyperthyroidism) and mental illness. The exclusion criteria of this study were: Fail to attend yoga classes as four sessions, the occurrence of any crisis during the two months of the study, use of hormonal medications and entire in exercise program during the research period and lack of willingness to continue in the study.

Instrumentation

This study was approved by the Research Deputy of Tehran University of Medical Sciences. Informed written consent was obtained from all the study subjects before starting the study. All participants are first accepted the questionnaire. The questionnaire consisted of two parts; the first part included the basic information such as age, education, occupation status, the number of children, menopausal age and the time elapsed from their menopause. The second part also included 31 questions related to the QoL assessment based on Menopause-specific Quality of Life (MENOQOL).This questionnaire has been developed by the Department of Family and Community Medicine, University of Toronto, Canada in 1992(31) . It was used in Iran after cultural adaptation with Iranian the community; so that after determining the validity of this questionnaire by Golyan et al. in Iran, its reliability was calculated with the correlation of 0.95(32) . Its reliability in the present study was also obtained (r = 0.91). The questions with 8-degree Likert scale were scored based on how much the mentioned symptoms have been problematic during the past month. Scoring was implemented through the following method: answer "*No"* scored 1 and answer "*Yes"* scored 0. In choices identifying the intensity of the symptoms the scores were: zero equals 2 scores, one equals 3 scores, two equal 4 scores, three equal 5 scores, four equal 6 scores, five equal 7 scores and six equal 8 scores. As a result, the total score of QoL in this tool was maximum 248 and minimum 31 scores. In order to evaluate QoL of postmenopausal women in vasomotor, psychosocial, physical and sexual dimensions, the mean score of each dimension was calculated and compared; thus, mean score in each dimension was calculated to be 1-8. It was a self-report questionnaire.

Training Program

The study subjects in the experimental group participated in yoga exercises for eight weeks (2 months), twice a week and each session for 90 minutes. The exercises were presented by the experienced instructor under the supervision of Yoga Association and also had the certificate of the provided trainings. The conditions and positions effective on relief of menopausal symptoms were extracted from Hatha yoga focusing on deep relaxation techniques. The exercises consisted of stretching and endurance in standing, sitting and sleeping, and breathing position and breathing exercises with each position. These types of exercises have been selected based on the recommendation of current articles on women with menopausal symptoms.The sessions consisted of checking the presence of women, controlling the exercises in the previous sessions, warm-up techniques and breathing, yoga Asana gestures and exercises, and eventually relaxation techniques. Furthermore, the experimental group was recommended to exercise for 15 minutes at home based on the provided pamphlet including joint rotation exercises of wrist, elbow, shoulders and abdominal breathing due to the importance and ease of implementing these exercises. Moreover, at the beginning of each session, their feedback has been analyzed concerning the home exercises recommended to them.

The samples of the exercises during the sessions were: corpse pose (5-7 min), Pawan Mukta Asana (wind relieving pose) for hip and feet such as ankle rotation, knee crank, cycling, leg rotation, raised legs pose (20-25 min), backward bending movements and poses such as half locust pose, snake pose, Sphinx asana, Cobra pose (10-15 min totally), rotating poses such as sleeping abdominal stretching, leg log pose (2-3 min), Pawan Mukta Asana for hands such as wrist bending, elbow

bending, shoulder socket pose, and stretching poses in this area such as hand raising pose, bow and arrow pose (30-35 min), rotational viewing (2-3 min) and asana corpse poses (5-10 min).

Menopause-specific Quality of Life (MENOQOL) was repeated two times for both groups; one before and another at the end of the intervention (end of week 8). It should also be noted that in order to complete the questionnaires at the end of week 8 for the control group, all the coordination were conducted through the phone. No intervention was conducted for the control group; therefore, the control group was given a yoga training book as a gift to show appreciation and to enhance their knowledge on yoga so that they can participate in yoga classes if willing to do so.

Statistical analysis

Data were analyzed through Software SPSS version 16. In order to describe the data from the central and dispersion parameters for inferential analysis of research findings, first we used Kolmogorov-Smirnov test to determine the normal data.The homogeneity of demographic and baseline data between the experimental and control groups was examined through chi-square, t and fisher's exact tests. Then, in order to determine the effect of independent variable on the experimental and control groups, we used statistical normal distribution of non-parametric Mann-Whitney and independent t-test at significance level of $P < 0.05$.

Results

Out of 136 participants, 13 subjects were excluded from the study due to reasons including unforeseen events for families (7 subjects), caring for diseased husband (2 subjects), unwillingness to participate in a yoga class (1 subject), and failure to attend more than 4 sessions of the classes (3 subjects). In total 123 subjects (62 subjects in the experimental and 61 subjects in the control group) continued the study (Figure 1). Table 1 illustrates the statistical indicators related to mean age and menopausal age and the highest frequency percentage of individual characteristics.There was no significant difference between the experimental and control groups in mean score of QoL and its dimensions (vasomotor, psychosocial, physical and sexual dimensions) before the intervention (Table 2).Mean changes of the QoL score in three vasomotor, psychosocial and physical dimensions showed a significant reduction two months after the intervention, compared with the pre-intervention phase, between the two groups. However, this difference was not seen in the sexual dimension (Table 3).

Discussion

The findings of the study showed no statistically significant difference between the experimental and control groups in terms of age, education, number of children, menopausal age and years passed from

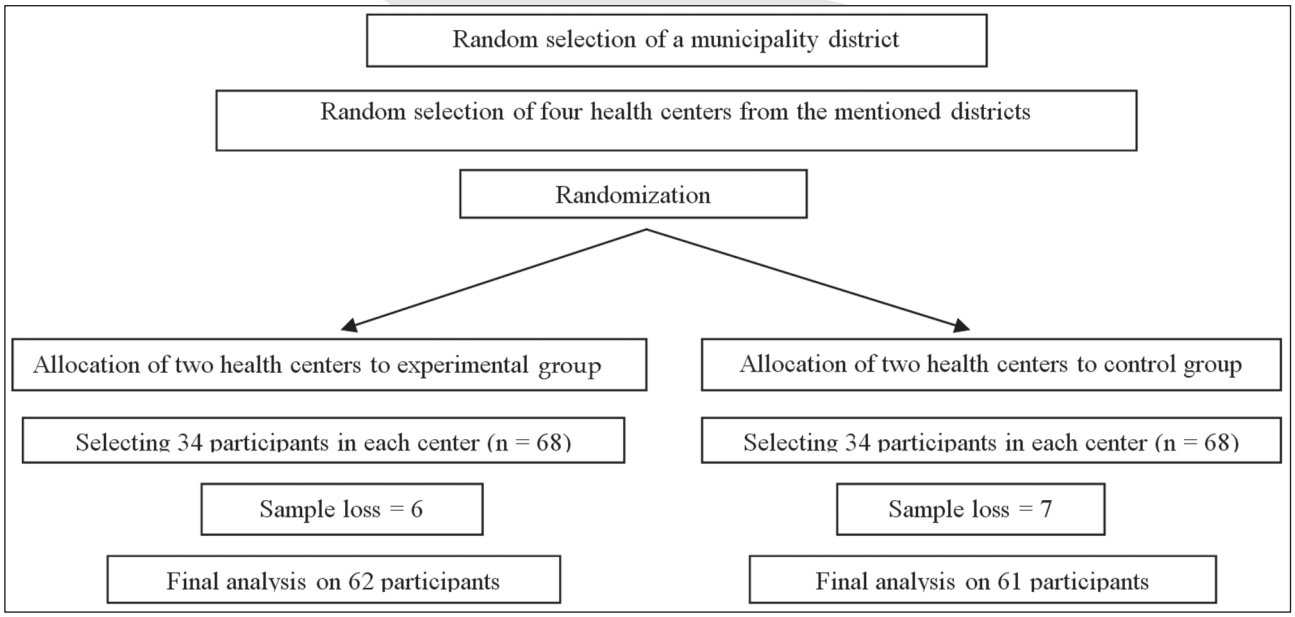

Figure 1. Random selection of a municipality district

Table 1. Statistical indicators related to individual characteristics of the study subjects

Characteristics	Experimental group	Control group	Total	P value
Age, year (mean, SD) (frequency percentage)	53.04 ± 2.98 48.5%	53.64 ± 3.49 47.1%	47.8%	P = 0.281
Menopausal age (mean, SD) (frequency percentage)	50.35 ± 2.61 73.5%	5.73 ± 3.04 66.2%	69.9%	P = 0.786
Education at primary or high school (frequency percentage)	64.7%	66.2%	65.4%	P = 0.188
Occupational status housekeeper, (frequency percentage)	80.9%	86.8%	83.8%	P = 0.352
Number of children, 3 or less (frequency percentage)	58.8%	58.8%	58.8%	P = 0.295
Years passed from menopause, 3-4 years (frequency percentage)	63.2%	67.6%	65.4%	P = 0.589

Table 2. Total mean score of vasomotor, psychosocial, physical and sexual dimensions in QoL of women in both experimental and control groups before the intervention

Groups Descriptive indices Dimension (before)	Experimental n = 68		Control n = 68		Independent t or *Mann-Whitney test
	Mean	SD	Mean	SD	
Vasomotor	9.66	3.90	9.14	5.60	P = 0.536 t = 0.621
Psychosocial	30.58	13.33	28.54	13.18	P = 0.370 t = 0.699
Physical	48.76	16.72	47.39	18.10	P = 0.648 t = 0.5457
Sexual	14.47	7.31	15.19	7.65	P = 0.689 t = 0.512
Total score of QoL	103.69	33.27	100.28	36.27	P = 0.569 Z* = -0.738

Table 3. Total mean score of vasomotor, psychosocial, physical and sexual dimensions in QoL of women in both experimental and control groups 8 weeks after the intervention

Groups Descriptive indices Dimension (After)	Experimental n = 62		Control n = 61		Independent t or Mann-Whitney* test
	Mean	SD	Mean	SD	
`Vasomotor	7.08	3.80	9.59	5.97	P = 0.039 Z* = -2.065
Psychosocial	24.25	11.49	30.34	13.80	P = 0.009 t = -2.659
Physical	39.51	18.35	48.91	18.90	P = 0.006 t = -2.798
Sexual	13.90	6.58	16.18	7.37	P = 0.073 t = -1.807
Total score of QoL	84.75	32.10	105.73	37.22	P = 0.002 t = -3.237

menopause. In other words, there was no difference between the two groups in terms of the above factors which can affect QoL of in this period.

The results showed that yoga exercises had a significant impact on reduction of vasomotor symptoms (P = 0.039). The results of this part of the study were in accordance with the results of Chattha in India(24), Elavsky (33) and Cohen in the U.S. (34). Cohen et al. believed menopause-related hot flashes were due to decreased estrogen level, which causes changes in sympathetic nervous system neurotransmitter level and they also believed that reduction of

hot flashes has been due to decreased sympathetic tone following implementation of yoga. The above mentioned results were not in accordance with the results of Arian (11) and Shaanabi in Iran (35). The reason for inconsistency of the results might be due to use of educational method, type of presented exercises and their intensity. Thus, physical activity with appropriate intensity can be effective on neural transmitters influencing the central body temperature regulators (36).

Comparing the mean difference of scores in psychosocial dimension in the two groups two months after the intervention showed a statistically significant difference (P = 0.009). Therefore, yoga exercises improved psychosocial dimension in the experimental group. The above finding was in accordance with the results of Chattha et al. in India (24) based on improvement of most mental symptoms of postmenopausal women following yoga exercises. Sunsern also suggested that exercise and regular physical activity postpone age-related changes and improve mental status of postmenopausal women (37). Mechanism of increasing the level of anxiety, depression and menopausal syndrome is through sympathetic stimulation leading to increased levels of cortisol and catecholamine via the adrenal-pituitary-hypothalamus pathway. Thus, yoga exercises through electrophysiological and neurohormonal changes lead to decreased sympathetic stimulation and consequently improvement of depression, anxiety and psychosocial symptoms of menopause (24). Considering the above findings concerning physical activity and relaxation exercises such as yoga which can have positive effects on psychosocial aspects of menopause including depression and anxiety, the role of factors such as lifestyle, body self-image, interpersonal relations, role of women and other socio-cultural factors on psychosocial aspects of menopause should not be ignored. Perhaps, the inconsistency of the results of the present study with the study of Cohen et al. regarding lack of effect of yoga on psychosocial aspects of QoL could be due to the above mentioned factors.

It was also seen that yoga exercises had a significant impact on improvement of physical symptoms in postmenopausal women (P = 0.006). The present study was in accordance with the studies of Booth La Force in the U.S. (38), Forouhari in Iran (39), Arian in Iran(11) and Mc Andrew et al. in the U.S. (40). The results of this part of study were not in accordance with the results of Chattha et al. in India (24) regarding the intra-group comparison of impact of yoga on physical symptoms of menopause. A reason for this inconsistency with the present study might be due to selection of the control group. Similar to the results of Li et al. who announced that moderate physical activity can have a protective effect against physical symptoms of menopause (41), so that the control group in the mentioned study did a series of simple and regular physical exercises; therefore insignificant statistical difference between the two groups has been due to implementation of exercise activities in both groups.

In terms of sexual dimension, mean score in the experimental group after 8 weeks of intervention decreased; while in comparison with mean difference of sexual score two months after the intervention, there was no significant difference between the two groups (P = 0.073). Improvement of sexual symptoms in postmenopausal women who participated in physical activity might be due to increased muscle tone and also increased perceived physical attractiveness following participation in the exercises. Probably, insignificance of difference between the two groups in sexual dimension could be due to socio-cultural issues affecting the attitude of women toward sexual symptoms, which was in accordance with the results of Shaabani (35). Arian also confirmed this subject in Iranian postmenopausal women. Thus, in his conclusion he stated that decreased sexual desires and motivations in menopausal women is a multidimensional matter influenced by physiologic, cultural and social factors (11). Mc Andrew also did not mention any improvement in sexual dimension of menopausal women following physical activity with appropriate intensity (40). In general, reviewing the results showed that exercise programs in the present study could improve the QoL of postmenopausal women (P = 0.002). The results of the present study were in accordance with the results of Mastrangelo et al. in the U.S. (28), Elsvasky and Mc Auley in the U.S. (33) and Vaez Mosavi in Iran (42).

Conclusion

In this study, QoL in three dimensions (vasomotor, psychosocial and physical dimensions) significantly changed after implementation of yoga exer-

cises. Therefore, yoga exercises can be considered as an effective and acceptable intervention in order to reduce symptoms and problems of this period, improve physical and mental health, and ultimately promote quality of life in postmenopausal women. On the other hand, considering that a large number of women are reaching menopause age, understanding the effect of this stage of life on mental health and QoL in this population and using therapeutic strategies such as physical activities for their sense of well-being should be taken into account as an important health goal.

Acknowledgment

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References

1. *Zapantis G, Santoro N. The menopausal transition: characteristics and management. Best Practice & Research Clinical Endocrinology & Metabolism. 2003; 17(1): 33-52.*
2. *Williams RE, Levine KB, Kalilani L, Lewis J, Clark RV. Menopause-specific questionnaire assessment in US population-based study shows negative impact on health-related quality of life. Maturitas. 2009; 62(2): 153-9.*
3. *Fallahzadeh H. Quality of life after the menopause in Iran: a population study. Quality of Life Research. 2010; 19(6): 813-9.*
4. *Takamatsu K, Makita K, Nozawa S. Study of psychosocial factors in japanese patients suffering from menopausal disorders. Journal of Obstetrics and Gynaecology Research. 2004; 30(4): 309-15.*
5. *Blumel J, Castelo-Branco C, Binfa L, Gramegna G, Tacla X, Aracena B, et al. Quality of life after the menopause: a population study. Maturitas. 2000; 34(1): 17-23.*
6. *Rapkin AJ. Vasomotor symptoms in menopause: physiologic condition and central nervous system approaches to treatment. American journal of obstetrics and gynecology. 2007; 196(2): 97-106.*
7. *Kjerulff KH, Frick KD, Rhoades JA, Hollenbeak CS. The cost of being a woman: a national study of health care utilization and expenditures for female-specific conditions. Women's Health Issues. 2007; 17(1): 13-21.*
8. *MacLennan AH. Evidence-based review of therapies at the menopause. International Journal of Evidence-Based Healthcare. 2009; 7(2): 112-23.*
9. *Politi MC, Schleinitz MD, Col NF. Revisiting the duration of vasomotor symptoms of menopause: a meta-analysis. Journal of general internal medicine. 2008; 23(9): 1507-13.*
10. *Williams RE, Kalilani L, DiBenedetti DB, Zhou X, Fehnel SE, Clark RV. Healthcare seeking and treatment for menopausal symptoms in the United States. Maturitas. 2007; 58(4): 348-58.*
11. *Arian S. The effect of educational program on menopausal women. (Dessertation) Iran Tehran Tarbiat Modarress University. 2001.*
12. *Borrelli F, Ernst E. Alternative and complementary therapies for the menopause. Maturitas. 2010; 66(4): 333-43.*
13. *Lindblad BE, Håkansson N, Philipson B, Wolk A. Hormone Replacement Therapy in Relation to Risk of Cataract Extraction: A Prospective Study of Women. Ophthalmology. 2010; 117(3): 424-30.*
14. *Umland EM. Treatment strategies for reducing the burden of menopause-associated vasomotor symptoms. Journal of managed care pharmacy: JMCP. 2008; 14(3 Suppl): 14.*
15. *Hersh AL, Stefanick ML, Stafford RS. National use of postmenopausal hormone therapy. JAMA: the journal of the American Medical Association. 2004; 291(1): 47-53.*
16. *Faber A, Bouvy ML, Loskamp L, Van De Berg PB, Egberts TCG, De Jong-van den Berg LTW. Dramatic change in prescribing of hormone replacement therapy in the Netherlands after publication of the Million Women Study: a follow-up study. British journal of clinical pharmacology. 2005; 60(6): 641-7.*
17. *Lawton B, Rose S, McLeod D, Dowell A. Changes in use of hormone replacement therapy after the report from the Women's Health Initiative: cross sectional survey of users. BMJ. 2003; 327(7419): 845-6.*
18. *Blake J. Menopause: evidence-based practice. Best Practice & Research Clinical Obstetrics & Gynaecology. 2006; 20(6): 799-839.*
19. *Michalsen A, Grossman2CDE P, Acil1BDF A, Langhorst1AE J, Lüdtke3ACD R, Esch4DE T, et al. Rapid stress reduction and anxiolysis among distressed women as a consequence of a three-month intensive yoga program. Med Sci Monit. 2005; 11(12): 561.*
20. *Da Silva TL, Ravindran LN, Ravindran AV. Yoga in the treatment of mood and anxiety disorders: A review. Asian Journal of Psychiatry. 2009; 2(1): 6-16.*

21. *Cowen VS, Adams TB. Physical and perceptual benefits of yoga asana practice: results of a pilot study. Journal of Bodywork and Movement Therapies. 2005; 9(3): 211-9.*

22. *Damodaran A, Malathi A, Patil N, Shah N, Marathe S. Therapeutic potential of yoga practices in modifying cardiovascular risk profile in middle aged men and women. Journal of Association of Physicians of India. 2002; 50(MAY): 633-40.*

23. *Chiesa A, Serretti A. Mindfulness-based stress reduction for stress management in healthy people: a review and meta-analysis. The Journal of Alternative and Complementary Medicine. 2009; 15(5): 593-600.*

24. *Chattha R, Raghuram N, Venkatram P, Hongasandra NR. Treating the climacteric symptoms in Indian women with an integrated approach to yoga therapy: a randomized control study. Menopause. 2008; 15(5): 862.*

25. *Khalsa SBS. Treatment of chronic insomnia with yoga: a preliminary study with sleep–wake diaries. Applied psychophysiology and biofeedback. 2004; 29(4): 269-78.*

26. *Innes KE, Selfe TK, Vishnu A. Mind-body therapies for menopausal symptoms: A systematic review. Maturitas. 2010; 66(2): 135-49.*

27. *Bize R, Johnson JA, Plotnikoff RC. Physical activity level and health-related quality of life in the general adult population: a systematic review. Preventive medicine. 2007; 45(6): 401-15.*

28. *Mastrangelo MA, Galantino ML, House L. Effects of yoga on quality of life and flexibility in menopausal women: a case series. Explore: The Journal of Science and Healing. 2007; 3(1): 42-5.*

29. *Abedzade M, Taebi M, Saberi F. Quality of life related factors in postmenopausal women residing in Kashan. The Persian Gulf Biomedical Research Institiute. 2009; 12: 81-8.*

30. *Jahanfar S, Ramezani F, Sadathasemi M. Early symptoms of menopause in women in Tehran. Journal of Reproductive. 2002: 31-40.*

31. *Hilditch JR, Lewis J, Peter A, van Maris B, Ross A, Franssen E, et al. A menopause-specific quality of life questionnaire: Development and psychometric properties. Maturitas. 2008; 61(1): 107-21.*

32. *Golyan Tehrani S, Mir Mohammad A, Mahmoudi M, Khaledian z. Study of quality of life and its patterns in different stages of menopause for women in Tehran,. Institute for Humanities and Cultural Studies, Tehran University of Medical Sciences and Health Services. 2002; 8: 33-41.*

33. *Elavsky S, McAuley E. Physical activity and mental health outcomes during menopause: a randomized controlled trial. Annals of Behavioral Medicine. 2007; 33(2): 132-42.*

34. *Cohen BE, Kanaya AM, Macer JL, Shen H, Chang A, Grady D. Feasibility and acceptability of restorative yoga for treatment of hot flushes: a pilot trial. Maturitas. 2007; 56(2): 198-204.*

35. *Shaabani Bahar G, Nazem F, PourAghaie Z. Special training program on quality of life of postmenopausal women with non-athlete. Journal of Research in the Humanities. 2006; 12: 123-33.*

36. *Ivarsson T, Spetz AC, Hammar M. Physical exercise and vasomotor symptoms in postmenopausal women. Maturitas. 1998; 29(2): 139-46.*

37. *Sunsern R. Effects of exercise on stress in Thai postmenopausal women. Health Care for Women International. 2002; 23(8): 924-32.*

38. *Booth-LaForce C, Thurston RC, Taylor MR. A pilot study of a Hatha yoga treatment for menopausal symptoms. Maturitas. 2007; 57(3): 286-95.*

39. *Forouhari S, Safari Rad M, Moattari M, Mohit M, Ghaem H. The effect of education on quality of life in menopausal women referring to Shiraz Motahhari clinic in 2004. Journal of Birjand University of Medical Sciences. 2009; 16: 39-45.*

40. *McAndrew LM, Napolitano MA, Albrecht A, Farrell NC, Marcus BH, Whiteley JA. When, why and for whom there is a relationship between physical activity and menopause symptoms. Maturitas. 2009; 64(2): 119-25.*

41. *Li S, Holm K, Gulanick M, Lanuza D, Penckofer S. The relationship between physical activity and perimenopause. Health Care for Women International. 1999; 20(2): 163-78.*

42. *Vaez Mosavi M. Comparison of individual and collective quality of life for athletes. Journal of Sports Sciences. 2002; 3: 83-93.*

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Role of Bradykinin in the cardioprotective effects of Captopril and Angiotensin II receptor blockers (AT_1, AT_2) on Myocardial ischemia–Reperfusion injury in rats

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Abstract

Background: Kinins protect against ischemia–reperfusion (IR) injury. Local release of bradykinin (BK) is increased during ischemia. The aim of this study was to examine the role of bradykinin in the cardioprotective effects of ACE inhibitor captopril, AT1 and AT2 receptor blockers, losartan, and PD123319, on IR-induced myocardial infarct size in rats.

Material and Methods: To induce necrosis, a branch of the descending left coronary artery was occluded for 30 minutes, followed by 2 hours reperfusion. Captopril (3mg/kg), losartan (2mg/kg), and PD123319 (20µg/kg/min) were given I.V. 10 min before ischemia, BK (40 µg/kg) was given I.V. 1 min before ischemia and continued during ischemia, and HOE 140 (BK B2 receptor blocker) was given by I.V. bolus injection 15 min before coronary occlusion. Infarction was measured by triphenyl tetrazolium staining. The volumes of necrosis and total heart zone were determined by planimetry.

Results: Necrosis/Total heart zone ratios were significantly reduced in BK (44±2%, $p<0.05$), captopril (40±2%, $p<0.05$), losartan (42±1%, $p<0.05$), and PD123319+BK (43±2%, $p<0.05$) groups in comparison with the control (55±4%). Necrosis/Total heart zone ratios were non-significantly decreased in captopril+HOE140 (48±2%) in comparison with captopril (40±2), and (49±1%) in Losartan+HOE140 in comparison with losartan (42±1%).

Conclusion: These results supported the protective role of BK in myocardial IR injury. The non-significant decrease in the protective effects of captopril and losartan against IR injury by HOE140 suggests there may be routes other than BK in the protective effects of these drugs.

Key words: Bradykinin, Captopril, Losartan, PD123319, Myocardial ischemia–reperfusion.

Introduction

Ischemic heart disease is the most important cause of morbidity and mortality in humans (1). Myocardial ischemia may be caused by therapeutic and physiological interventions such as atherosclerosis, thromboembolism, percutaneous transluminal coronary angioplasty, coronary artery bypass, and transplantation (2,3). The mechanism of myocardial injury related to ischemic–reperfusion (IR) is not yet fully understood. Over-production of free radicals and intracellular Ca+2 ion imbalance, the roles of the renin-angiotensin system (RAS), neutrophils, platelets, and complementary system were shown to have a role in reperfusion injury (1).

RAS controls cardiovascular, renal, and adrenal functions via body fluid, electrolyte balance, and arterial pressure. Angiotensin II (Ang II), as a main mediator of RAS, causes vasoconstriction, release of aldosterone and vasopressin, sodium and fluid retention, and sympathetic activation. Angiotensin converting enzyme (ACE) inhibitors impede the breakdown of bradykinin (BK), which is a vasodilatory peptide, and are important agents in the treatment of congestive heart failure and hypertension, while the vasoconstriction inhibits the formation of the peptide Ang II (4). In addition to these features, ACE inhibitors have been shown to be "heart savers" by reducing post-ischemic enzymes, norepinephrine (5), functional and metabolic injury (6,7), and reperfusion arrhythymias (8,9) in myocardial IR animal models.

The effects of Ang II are known to occur via Ang type I (AT1) and Ang type II (AT2) receptor subtypes (10). Some studies have shown that these two receptor subtypes have opposite effects to each other (11-13). Very little is known about the functions of AT2 receptors, although it has a vasoconstrictive effect (14), while AT1 receptor is responsible for almost all of the well-known

cardiovascular and renal effects of Ang II (15). It was suggested that these effects of AT2 occur through nitric oxide and BK. Although the functions of AT2 receptors in myocardium are not very well defined, because cardiac AT2 receptors in rats and humans are upregulated in some pathological conditions such as myocardial infarction, it was shown that AT2 receptors might play an important role in tissue remodeling (16,17).

Like RAS, the Kallikrein-kinin system (KKS) plays an important role in cardiovascular and renal functions and the regulation of blood pressure (18). All components of KKS are localized to heart and vascular tissues. Kinins are released during ischemia and cause beneficial cardiac effects (19,20). Kinins act through two type receptors, type 1 (B1) and type 2 (B2) receptors. The beneficial effects of kinins in the heart could be counteracted by kinin B2-receptor antagonist HOE140 (21). Kinins with wide-spectrum activity are vasodilators and support natriuresis and diuresis, and also play a protective role in IR injury by decreasing post-ischemic leukocyte adhesion, tissue injury, and disruption of the microvascular barrier (22).

The aim of this study was to investigate the role of BK in the effects of captopril, an ACE inhibitor, AT1 selective receptor blocker, losartan, and AT2 selective receptor blocker "PD123319" on both hemodynamic and infarct size in myocardial IR.

Materials and Methods

Experimental animals

The study used a group of 64 male Wistar albino rats, (weight 250–300 g) obtained from the Research Center for Experimental Animals at Inonu University. Rats were housed in special cages under standard conditions (ventilated, constant-temperature rooms with 12 hours daylight, 12 hours dark) and provided 8-mm standard rat pellet feed.

Drugs

The following drugs were used in this study: Captopril (Sigma, USA; CAS number: [62571-86-2]; 3 mg/kg), losartan (Merck, USA; L-158086-005H067; 2 mg/kg), PD123319 (Sigma, USA P-186; 20 μg/kg/minute), BK (Sigma, USA B-3259; 40 μg/kg), HOE140(Sigma, USA H-157; 100 μg/kg).

Myocardial IR

The animals were administered 1.2–1.4 g/kg urethane intraperitoneally for anesthesia. Tracheal and jugular vein cannulations were performed for artificial respiration and drug administrations, respectively. Blood pressure, heart rate, and ECG were recorded via a cannula transducer (Harvard model, 50-8952) and a recorder (Harvard Universal Pen-recorder) placed to the carotid.

After making a 1–1.5 cm incision in the left side of the chest, toracotomy was performed, passing through subcutaneous and thoracal muscles, and cutting the fourth rib 2 mm to the left side of the sternum. As the thorax was opened, positively-pressured respiration was initiated (1.5ml/100 g and 60 atm/min room-air). Thus, the ventilation device (Harvard Animal Rodent Ventilator) was used to counteract the resulting loss of negative pressure in the interior, to maintain respiration, and preserve PCO2, PO2, and Ph at normal values.

The heart was freed after smoothly opening the pericardium. The heart was then externalized by applying pressure to the right side of the chest. A 6/0 silk suture with 10 mm, round-tipped needle was quickly passed under the left main coronary artery with slightly incorporated myocardial tissue. The heart was replaced and the animal was monitored for approximately 20 minutes, until stabilization. In cases of arrhythmia or a decrease in the mean blood pressure below pre-occlusion (70 mm Hg, according to Lambeth Convention evaluation criteria) (23), subjects were excluded from the study. The ends of the placed 6/0 silk sutures were passed through a small plastic tube (diameter 1 mm, length 1 cm). After the 20-minute period of stabilization, the string passed under the vessel was constricted via the plastic tube and a clamp, causing ischemia (occlusion), and reperfusion was achieved with release of the string. Thirty-minute ischemia and 120-min reperfusion were performed, according to previous protocols measuring necrosis area (24). The experiment was terminated via carotid arterial bleeding of the animal.

Groups

Animals were randomly assigned to eight groups, each comprising eight individuals.

1. Control group,
2. BK group,

3. Losartan group,
4. PD123319 group,
5. Captopril group,
6. Losartan+HOE140 group,
7. Captopril+HOE140 group,
8. PD123319+BK group.

Captopril, losartan, PD123319 were given 10 minutes before occlusion, BK was given 1 minute before occlusion and infused into the jugular vein at all ischemic periods with an infusion pump (Infusion Pump May INF 9601, COMMAT LTD., Ankara, Turkey); HOE140 was given as bolus injection15 minutes before occlusion.

Evaluation of Hemodynamic Parameters

ECG, blood pressure, and heart rate were recorded during preparation and occlusion–reperfusion periods. In addition, the mean arterial pressure and heart rate were evaluated. The mean blood pressure calculation was performed with 40% systolic blood pressure plus 60% diastolic blood pressure.

Measurement of Necrosis Area

Hearts were quickly removed and frozen at the end of each procedure. Hearts were sliced in 2 mm-thickness and incubated in the tampon containing 1% triphenyl tetrazolium chloride (TTC) with 7.4 Ph at 37C^{O} for 15 minutes. TTC reduces formazan pigments when nicotinamide adenine dinucleotide, dehydrogenases, and diaphorase are present in tissue. Areas of viable tissue contain these enzymes and co-factors and therefore stain dark red, whereas necrotic areas do not stain (25). After staining, the heart slices were placed between two glass plates spaced 2 mm apart. Necrotic region borders (TCC-negative tissue) and healthy tissue (TTC-positive tissue; dark red) were copied onto transparent acetate. Infarct areas and total heart areas were measured using a computer-supported planimetric method.

Statistical Analysis

Statistical evaluation was performed with SPSS (version 10.0). All results were expressed as the mean ± standard error, and $p<0.05$ was considered

Table 1. The effects of Bradykinin, Captopril, Losartane, PD123319, Losartane+HOE140, Captopril+HOE140, PD123319+Bradykinin on the mean blood pressure (BD: Before drug)

			Ischemia			Reperfusion		
Groups	**BD**	**0. min**	**10. min**	**20. min**	**30. min**	**30. min**	**60. min**	**120. min**
Control	83 ± 2,6	81± 3,4	65 ± 5,8	65 ± 5,8	65 ± 6,9	72 ± 5,6	70 ± 5,1	67 ± 3,7
Bradykinin	85 ± 2,1	85 ± 2,1	68 ± 4,4	69 ± 4,2	71 ± 4,9	75 ± 6,3	78 ± 5,9	72 ± 4,8
Captopril	82 ± 2,8	72 ± 1,6	54 ± 3,0	57 ± 3,1	55 ± 2,2	64 ± 6,5	65 ± 5,8	60 ± 5,7
Losartane	75 ± 3,0	70 ±0,2	48 ± 6,0	56 ± 5,3	51 ± 5,4	56 ± 1,0	54 ± 9,6	52 ± 6,6
PD123319	79 ± 2,6	85 ± 2,2	74 ± 2,4	74 ± 1,6	75 ± 1,6	78 ± 2,3	77 ± 2,1	70 ± 1,4
Losartane + HOE140	90 ± 5,0	77 ± 4,0	58 ± 6,5	61 ± 7	61 ± 8,3	63 ± 6,6	60 ± 4,4	56 ± 3,5
Captopril + HOE140	80 ± 3,3	71 ± 1,4	51 ± 3,4	53 ± 1	52 ± 1,9	53 ± 2,1	54 ± 2,0	54 ± 2,4
PD12331 + Bradykinin	90 ± 5,2	82 ± 4,4	71 ±5,2	74 ± 5,3	77 ± 4,7	78 ± 4,1	73 ± 2,7	62 ± 3,2

Table 2. The effects of BK, Captopril, Losartane, PD123319, Losartane+HOE140, Captopril+HOE140, PD123319+BK on the mean heart rates. (BD: Before drug)

			Ischemia			Reperfusion		
Groups	**BD**	**0. min**	**10. min**	**20. min**	**30. min**	**30. min**	**60. min**	**120. min**
Control	400± 25	430 ±18	480 ±15	480 ±15	460 ±20	410 ±28	400 ±36	410 ±42
BK	400 ±12	400 ±12	460 ±12	470 ±10	440 ±12	420 ±15	420 ±15	410 ±10
Captopril	432 ±12	408 ±12	468 ±12	468 ±12	444 ±14	444 ±14	444 ±14	432 ±12
Losartane	372 ±12	348 ±29	396 ±14	396 ±14	372 ±12	372 ±22	360 ±18	360 ±18
PD123319	408 ±12	408 ±12	468 ±12	444 ±14	444 ±14	420 ±0	432 ±12	432 ±12
Losartane + HOE140	400 ±12	400 ±12	440±12	440 ±12	440 ±12	410 ±10	420 ±0	410 ±10
Captopril + HOE140	390 ±25	390 ±25	420±34	420 ±26	410 ±28	400 ±12	410 ±24	400 ±25
PD12331 + BK	420 ±15	410 ±10	470 ±10	470 ±10	470 ±10	470 ±10	460 ±12	440 ±20

statistically significant. Heart rate, blood pressure, and the extent of necrosis were evaluated using repeated measures analysis of variance (ANOVA). The Tukey test was used for post hoc comparison.

Results

Blood Pressure and Heart Rate

In terms of the mean arterial blood pressure (MABP) and heart rate: There were no statistically significant differences between the control group and other drug groups in the period before drug administration (BD); beginning of ischemia (i.e., 0 Minutes); 10, 20, 30 minutes of ischemia; 30, 60, and 120 minutes after occlusion (reperfusion). There were also no statistical significant differences between losartan and losartan+HOE140; captopril and captopril+HOE140; PD123319 and PD123319+BK (Table 1, Table 2).

Necrosis/Total Heart Area

The effects of BK, captopril, losartane and PD123319+BK on necrosis/Total Heart area are showed in Table 3 and figure 1. BK (%44±2), captopril (%40±2), losartane (%42±1), and PD123319+BK (%43±2) reduced necrosis/Total Heart area according to the control group (%55±4) ($p<0.05$). However, HOE140 decreased the protective effects of losartane and captopril. Also, PD123319+BK (43%±2) diminished necrosis/total heart area, according to PD123319 (50%±3) ($p>0.005$).

Table 3. The effects of BK, Captopril, Losartane, PD123319, Losartane + HOE140, Captopril + HOE140, PD123319 + BK on the Necrosis/Total Heart Area

Groups	Necrosis/total heart area (%)
Control	55±4
BK	44±2*
Captopril	40±2*
Losartane	42±1*
PD123319	50±3
Losartane + HOE140	49±1
Captopril + HOE140	48±2
PD12331 + BK	43±2*

** Significantly different from the control group ($p<0.05$)*

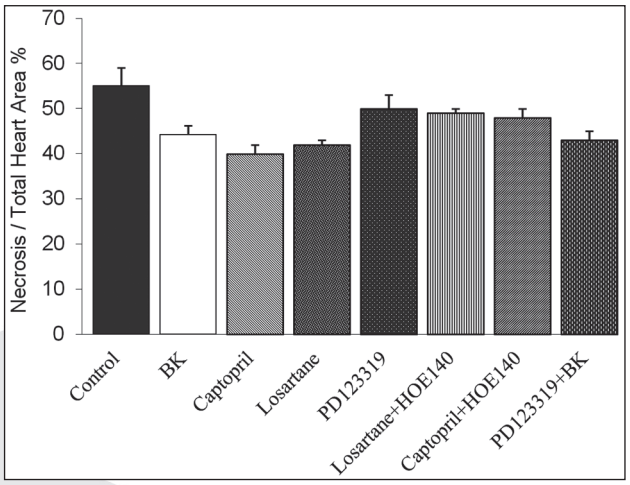

Figure 1. The effects of BK, Captopril, Losartane, PD123319, Losartane + HOE140, Captopril + HOE140, PD123319 + BK on the Necrosis/Total Heart Area

Discussion

All components of KKS are localized to heart and vascular tissues. Kinins are secreted during ischemia and produce beneficial cardiac effects (19,20). The animal models showed that kinins have both short-term and long-term cardioprotective effects. While the short-term cardiac protection effect is related to protection against IR, the long-term effect is related to mitigating the progression of heart failure and left ventricular hypertrophy (26). BK given to coronaries had decreased IR in canine (27,28), rabbit (29), and pig (30) models and its effect was inhibited by BK B2 receptor blocker, HOE140 (28,29,31). It was observed that BK improved ventricular functions after ischemia (27,28) and this effect was blocked by a nitric oxide synthase inhibitor, L-NAME, and indomethacin (32). The protective mechanisms of BK in myocardial IR are not fully elucidated. However, it has been showed that there are some mechanisms such as the protection of high-energy phosphates, increasing of glucose uptake in cardiac metabolisms, increasing of coronary perfusion, protecting ventricular contractility by reducing the release of norepinephrine (33). It was seen in our study that exogenously-administered BK reduced necrosis area compared to the control.

AT1 receptor blockage increases the amount of renin and Ang II, and increased Ang II stimulates II AT2 receptors. The stimulation of AT2 receptors leads, directly or indirectly, to growth-inhibiting,

anti-hypertrophic and proapoptotic effects through the stimulation of autocoids. When AT1 antagonists and AT2 antagonists combined in IR models, it was observed that the beneficial effects of AT1 antagonists were negated (34). In the present study, it was found that BK was effective in the role of losartan in decreasing necrosis/total heart area. This effect of losartan was reduced when combined with HOE140.

In the present study, PD123319, an AT2 blocker, was found to reduce necrosis area, relative to the control, although this was not significant, which is consistent with the literature. Ang II interacts with AT1 receptors when AT2 receptors are blocked by PD123319. However, it has been shown that AT1 receptor has BK-forming effect. But, this effect is not as far as AT2 receptors (35). When PD123319 was administered alone, Ang II maintained its effect with AT1 receptor, and stimulated BK by this receptor might have a minor beneficial effect on necrosis. In addition, some studies reported that PD123319 reduced apoptosis stimulated by Ang II (36).

Losartan and captopril may prevent harmful effects of RAS by inhibiting binding of Ang II to AT1 receptors and suppressing the formation of Ang II, respectively. ACE inhibitors also prevent the destruction of BK. This effect may play a role on limiting the IR-induced damage in myocardium by captopril. As ACE is effective in the breakdown of BK, a vasodilatory peptide, and kallidin so ACE inhibition raises the level of kinin peptides, which has a vasodilatory effect (37, 38). Birincioglu et al (39) associated the therapeutic effect of captopril in IR injury to stimulation of prostaglandin synthesis and prevention of BK breakdown. In our study, while captopril decreased necrosis area, as captopril combined with HOE 140, its protection effect was not fully removed. This suggests that pathways other than BK may be involved in the protective effect of captopril. Birincioglu et al. reported that captopril stimulates synthesis of prostaglandin and its structure containing sulfhydryl group, which provides an antioxidant properties (40).

This study has shown that both ACE inhibition and AT1 receptor blockage and, indirectly, AT2 receptor, have protective effects on necrosis area resulting from myocardial IR. However, BK plays an important role among the possible mechanisms of this cardioprotective effect, but it appears that this is not the only effective pathway in this process.

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References

1. *Moens AL, Claeys M, Timmermans JP, Vrints CJ. Myocardial ischemia / reperfusion - injury, a clinical view on a complex pathophysiological process. International Journal of Cardiology 2005; 100: 179– 90*

2. *Lai ZF. The relationship between intracellular chloride concentration and ischemia reperfusion-induced arrhythmias in myocardial cells. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2002; 24(2): 190-6.*

3. *Venardos KM, Perkins A, Headrick J, Kaye DM. Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review. Curr Med Chem 2007; 14(14): 1539-49.*

4. *Tom B, Dendorfer A, AH Jan Danser. Bradykinin, angiotensin-(1–7), and ACE inhibitors: how do they interact? Journal of Biochemistry & Cell Biology 2003; 35: 792–801*

5. *Friedrich B, Walter RK. Postischemic antiarrhytmic effects of angiotensin-converting enzyme inhibitors. Circulation 1996; 94: 1752-61.*

6. *Kawabata H, Ryomoto T, Ishikawa K. Cardioprotection with angiotensin converting enzyme inhibitor and angiotensin II type 1 receptor antagonist is not abolished by nitric oxide synthase inhibitor in ischemia-reperfused rabbit hearts. Hypertens Res. 2001 Jul; 24(4): 403-9*

7. *Toblli JE, Cao G, Derosa G, Di Gennaro F, Forcada P. Angiotensin-converting enzyme inhibition and angiogenesis in myocardium of obese Zucker rats. Am J Hypertens. 2004 Feb; 17(2): 172-80.*

8. *Jurkovicova O, Cagan S. Reperfusion arrhythmias. Bratisl Lek Listy 1998; 99(3-4): 162-71.*

9. *Zhu B, Sun Y, Sievers RE, Browne AE, Pulukurthy S, Sudhir K, Lee RJ, Chou TM, Chatterjee K, Parmley WW. Comparative effects of pretreatment with captopril and losartan on cardiovascular protection in a rat model of ischemia-reperfusion. J Am Coll Cardiol 2000; 35(3): 787-95.*

10. *De Gasparo M, Catt KJ, Inagami T, Wright W, Unger TH. International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev. 2000; 52: 415–72.*

11. *Jones A, Dhamrait SS, Payne JR, Hawe E, Li P, Toor IS, Luong L, Wootton PT, Miller GJ, Humphries SE, Montgomery HE. Genetic Variants of Angiotensin II Receptors and Cardiovascular Risk in Hypertension. Hypertension 2003; 42: 500-6.*

12. *Said AbdAlla, Heinz Lother, Ahmed M. Abdel-tawab, Ursula Quitterer. The Angiotensin II AT2 Receptor Is an AT1 Receptor Antagonist. The Journal Of Biological Chemistry 2001; 276: 39721-26.*

13. *Lan Wu, Masaru I, Hironori N, Rui C, Jun , Masahiro A, Marc De G, Masatsugu H. Effect of Angiotensin II Type 1 Receptor Blockade on Cardiac Remodeling in Angiotensin II Type 2 Receptor Null Mice. Arterioscler Thromb Vasc Biol. 2002; 22: 49-54*

14. *Johren O, Dendorfer A, Dominiak P. Cardiovascular and renal function of angiotensin II type-2 receptors Cardiovascular Research 2004; 62: 460– 7.*

15. *Horiuchi M, Akishita M, Dzau VJ. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. Brief Review. 1999; 33: 613-21.*

16. *Nio Y, Matsubara H, Murasawa S, Kanasaki M, Inada M. Regulation of gene transcription of angiotensin II receptor subtypes in myocardial infarction. J Clin Invest 1995; 95: 46 – 54.*

17. *Wharton J, Morgan K, Rutherford RA, Catravas JD, Chester A, Whitehead BF, De Leval MR, Yacoub MH, Polak JM. Differential distribution of angiotensin AT2 receptors in the normal and failing human heart. J Pharmacol Exp Ther 1998; 284: 323– 36.*

18. *Yan Tan,. Hutchison FN, Jaffa AA. Mechanisms of angiotensin II-induced expression of B2 kinin receptors Am J Physiol Heart Circ Physiol 2004; 286: 926–32.*

19. *Sharma JN. Does the Kinin System Mediate in Cardiovascular Abnormalities? An Overview. Journal of Clinical Pharmacology, 2003; 43: 1187-95.*

20. *Sharma JN. Role of tissue kallikrein-kininogen-kinin pathways in the cardiovascular system Arch Med Res. 2006 Apr; 37(3): 299-306.*

21. *Liu X, Lukasova M, Zubakova R, Lewicka S, Hilgenfeldt U. A kallidin-like peptide is a protective cardiac kinin, released by ischaemic preconditioning of rat heart. Br J Pharmacol. (2005); 146: 952–57.*

22. *Campbell DJ. The kallikrein–kinin system in humans. Clinical and Experimental Pharmacology and Physiology. 2001; 28: 1060–65.*

23. *Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, Yellon DM, Cobbe SM, Coker SJ, Harness JB, Harron DW. The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia infarction, and reperfusion. Cardiovasc Res 1988; 22 (7): 447-55.*

24. *Ozer MK, Sahna E, Birincioglu M, Acet A. Effects of captopril and losartan on myocardial ischemia-reperfusion induced arrhythmias and necrosis in rats. Pharmacol Res. 2002 ; 45(4): 257-63.*

25. *Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Merciier JC, Corday E, Ganz W. Early phase acute myocardial infarct size quantification: Validation of the triphenyltetrazorium chloride tissue enzyme staining technique. Am Heart J 1981; 101: 593-600.*

26. *Jorma O. Kokkonen, Ken A. Lindstedt, Antti Kuoppala, Petri T. Kovanen. Kinin-Degrading Pathways in the Human Heart Trends Cardiovasc Med 2000; 10: 42–5*

27. *Linz W and Scholkens BA. Role of bradykinin in the cardiac effects of angiotensin-converting enzyme inhibitors. J. Cardiovasc. Pharmacol. 1992; 20: 81–90.*

28. *Martorana PA, Kettenbach B, Breipohl G, Linz W and Scholkens BA. Reduction of infarct size by local angiotensinconverting enzyme inhibition is abolished by a bradykinin antagonist. Eur. J. Pharmacol. 1990: 182: 395–6.*

29. *Hartman JC, Wall TM, Hullinger TG and Shebuski RJ. Reduction of myocardial infarct size in rabbits by ramiprilat: reversal by the bradykinin antagonist HOE 140. J. Cardiovasc. Pharmacol. 1993; 21: 996–1003..*

30. *Tio RA, Tobe TJ M, Bel KJ, Langen CD J DE, Gilst WH VAN and Wesseling H. Beneficial effects of bradykinin on porcine ischemic myocardium. Basic Res. Cardiol. 1991; 86: 107–16.*

31. *Kanner J, Harel S And Granit R. Nitric oxide as an antioxidant. Arch. Biochem. Biophys. 1991; 289: 130–6.*

32. *Zhu P, Zaugg CE, Simper D, Hornstein P, Allegrini PR, Buser PT. Bradykinin improves postischemic recovery in the rat heart: role of high energy phosphates, nitric oxide, and prostacyclin. Cardiovasc. Res. 1995; 29: 658–63.*

33. *Schriefer JA, Broudy EP, Hassen AH. Inhibitors of bradykinin-inactivating enzyme decrease myocardial ischemia/reperfusion injury following 3 and 7 days of reperfusion. The Journal of Pharmacology and Experimental Therapeutics 2001; 298: 970-5.*

34. *Jalowy A, Schulz R, Dorge H, Behrends M, Heusch G. Infarct size reduction by AT1 receptor blockade through a signal cascade of AT2 receptor activation. 1998 Nov 15; 32(6): 1787-96.*

35. *Seyedi N, Xu XB, Nasjletti A, Hinzte TH. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. Hypertension 1995; 26: 164-70*

36. *Goldenberg I, Grossman E, Jacobson KA, Shneyvays V, Shainberg A. Angiotensin II-induced apoptosis in rat cardiomyocyte culture: a possible role of AT1 and AT2 receptors. J Hypertens. 2001; 19(9): 1681-9.*

37. *Brown NJ, Vaughan DE. Angiotensin-Converting Enzyme Inhibitors. Circulation 1998; 97(14): 1411-20.*

38. *Woo KS, Nicholls MG. High prevalence of persistent cough with angiotensin converting enzyme inhibitors in Chinese. Br J Clin Pharmacol 1995; 40: 141-4*

39. *Birincioglu M Olmez E, Aksoy T, Acet A. The role of prostaglandin synthesis stimulation in the protective effects of captopril on ischemia-reperfusion arrhytmias in rats in vivo. Pharmacol Res 1997; 36: 299-304.*

40. *Birincioglu M, Aksoy T, Olmez E, Acet A. Protective effects of ACE inhibitors on ischemia-reperfusion-induced arrhytmias in rats: is this effect related to the free radical scavenging action of these drugs? Free radic Res 1997; 27: 389-96.*

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Orthodontic treatment of class II division 1 malocclusion by Fränkel functional regulator type Ic: Case report

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Abstract

Introduction: Skeletal Class II is one of the mainly malocclusion in modern populations. Patients with this type of malocclusion could be treated in early mixed dentition by Fränkel`s functional regulator.

Case report: In this case was shown treatment by Fränkel`s functional regulator Ic (FR-Ic) in boy, age 8 years and 3 months, with malocclusion Class II division 1. Whole treatment lasted 22 months, with permanently wearing of appliance and regular orthodontic control. Age of patient has enabled to use modification of craniofacial growth and growth stimulation of the lower jaw during puberty. On lateral cephalograms changes were monitored and analysed.

Conclusion: FR-Ic has caused significant changes on skeletal and dental structures, stimulating growth of the lower jaw and causing retrusion of the upper and protrusion of the lower incisors. It caused significant improvement in facial aesthetics and corrections on the soft-tissue profile. Also, functions of swallowing and articulation of dental vocals were corrected.

Key words: Malocclusion Class II Division 1, functional appliances, growth modification, Fränkel functional regulator, cephalometric changes, early mixed dentition.

Introduction

Skeletal Class II is malocclusion of sagittal direction caused by unbalanced anteroposterior position and development of the upper and lower jaw with respect to anterior cranial base. It manifested by increasing values of angle ANB (over 4°). According to some studies the cause of this malocclusion is maxilarry prognatism in 56.3% cases, mandibular retrognatism in 27% cases and combination of them in 16.7% cases[1]. Of the total number of malocclusion Class II accounts for 27%, according to Angle. The most important characteristics of Class II division 1 malocclusion are: bimaxillary retrognatism (more visible on the lower jaw), protrusion of upper incisors, narrow upper jaw, facial growth with backward rotation, mostly[2]. Mandibular deficiency can be absolute, with smaller lower jaw in all dimensions, and relative, with posterior position of the lower jaw. Facial profile has increased convexity, overjet is enlarged, mandibular corpus and ramus are shorter[3,4]. Use of functional appliances in treatment of Class II primarily based on stimulation growth of the lower jaw. The best treatment effect is possible in early mixed dentition, during the period of intense growth, also with continuously wearing of appliances. On that way we have adequate force and response in orofacial muscles[5].

Fränkel`s functional regulator is distinctive in that is only mounted tissue functional appliance, which can effectively corrected morphological (skeletal and dental) and functional irregularities[6,7]. Operates on the principle of directional pressure applications at the contact appliance and the skeletal and dental structures, elimination of the pressure surrounding perioral musculature and causing tensile stress in the area of mucosal fornix, where exist bone apposition[8-10].

This appliance is distinctive in that it can be used very early, immediately after eruption of incisors and first permanent molars, in early mixed dentition. On that way we create conditions for early corrections of skeletal deviations and prevent the occurrence of later developmental anomalies. Anomalies are inevitable if we delayed beginning of orthodontic treatment. In this age patients are motivated for cooperate, appliance is comfortable, easy for wearing, adaptation phase is short, and there is no other unwanted side effect regarding the use of this appliance[11-13].

Case report

In this case was treated male patient in age of 8 years and 3 months with diagnosis malocclusion Class II division 1, caused by manibular retrognatism. Intraoral examination indicated occlusion in whole Class II with extremely increased overjet of 12mm, overbite of 7mm, protrusion of upper incisors with injection of the upper lip between upper and lower incisors. Functional examination indicated existing of infantile swallowing and interdental sigmatism. Extraoral examination from the front indicated shorter lower third of face, emphasissed mentolabial sulcus and potentially competent lips. Extraoral examination in profile indicated grave convexity with prominent position of the upper lip and distal position of chin. Before orthodontic treatment study cast analysis, orthopantomograph and lateral cephalogram have been done. Skeletal maturity was determinated by level of development of cervical vertebrae on lateral cephalogram[14,15]. Patient was in CvS1 stage, before puberty growth acceleration. Parameters which were analysed on lateral cephalogram suggested skeletal distal bite caused by mandibular retrognatism, horizontal facial growth, protrusion of the upper and retrusion of the lower incisors, decreased vertical interjaw (B) angle, anteinclination of both jaw and shorter manidubular corpus. After diagnostic procedures, bearing in mind patients calendar age and skeletal maturity, Fränkel's functional regulator type Ic (FR-Ic) was chosen as a method of treatment (Figure 1.). Structural bite was determined in relation Class I on the posterior teeth, without activation in vertical direction, on the level of physiological stagnancy. On that way avoided the excessive tension of neuromuscular structures. Based on structural bite and later corrections on the study casts FR-Ic was created in laboratory. Components of this appliance are: 2 posterior buccal shields, 2 pelotas for lower lip, lingual shield with pins for protrusion, labial arch, palatal arch with anchorage on first permanent molars, loops for canines in upper jaw and screw. Patient received FR-Ic and instructions on wearing appliances during the whole day, except during meals. Regular orthodontics controls were carried out at intervals of 4 to 6 weeks. At the time when overjet was decreased on 7mm, after 9 months of treatment, patient started to turn the screw every 10 days.

Table 1. Skeletal and dental changes during treatment with FR-Ic

Parameter	Before treatment	After treatment
Angle SNA	80°	79°
Angle SNB	74°	76°
Angle ANB	6°	3°
Angle I/SpP	60°	67°
Angle i/MP	83°	80°
Angle SpP/MP (B)	19°	23°
Distance Cmand	69mm	73mm

Angle SNA – sagital position of upper jaw to anterior cranial base, angle SNB – sagital position of lower jaw to anterior cranial base, angle ANB – sagital interjaw angle, angle I/SpP – inclination of upper incisors, angle i/MP – inclination of lower incisors, angle SpP/MP (B) – vertical interjaw angle and distance Cmand – length of mandibular corpus

Orthodontic procedure lasted for 22 months. After that period patient had stabile occlusion in Class I, with significant decrease of overjet and overbite (Figure 2.). After treatmen control study casts and lateral cephalogram have been done. Patient started wearing appliance only during the night, as a retention. On lateral cephalogram after treatment was observed anterior movement of lower jaw with increasing length of mandibular corpus and correction of sagittal interjaw relationship from Class II to Class I (Figure 3.). Upper incisors were remarkably retruded, vertical interjaw angle was increased, without changes in facial growth (table 1). Functional examination after treatment indicated correct articulation of dental vocals (s, z, c) and transition from infantile to mature swallowing. Extraoral examination indicated significant improvement in facial aesthetics, decrease of profile convexity and prominence of the upper lip, balanced relationship between three parts of face in vertical direction, competent lips and correctly expressed mentolabial sulcus.

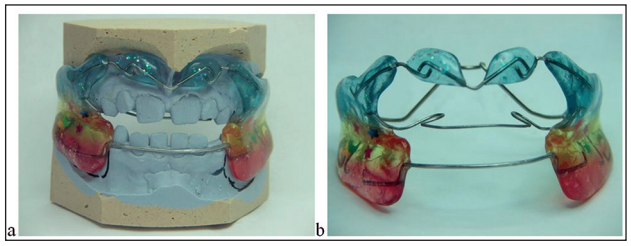
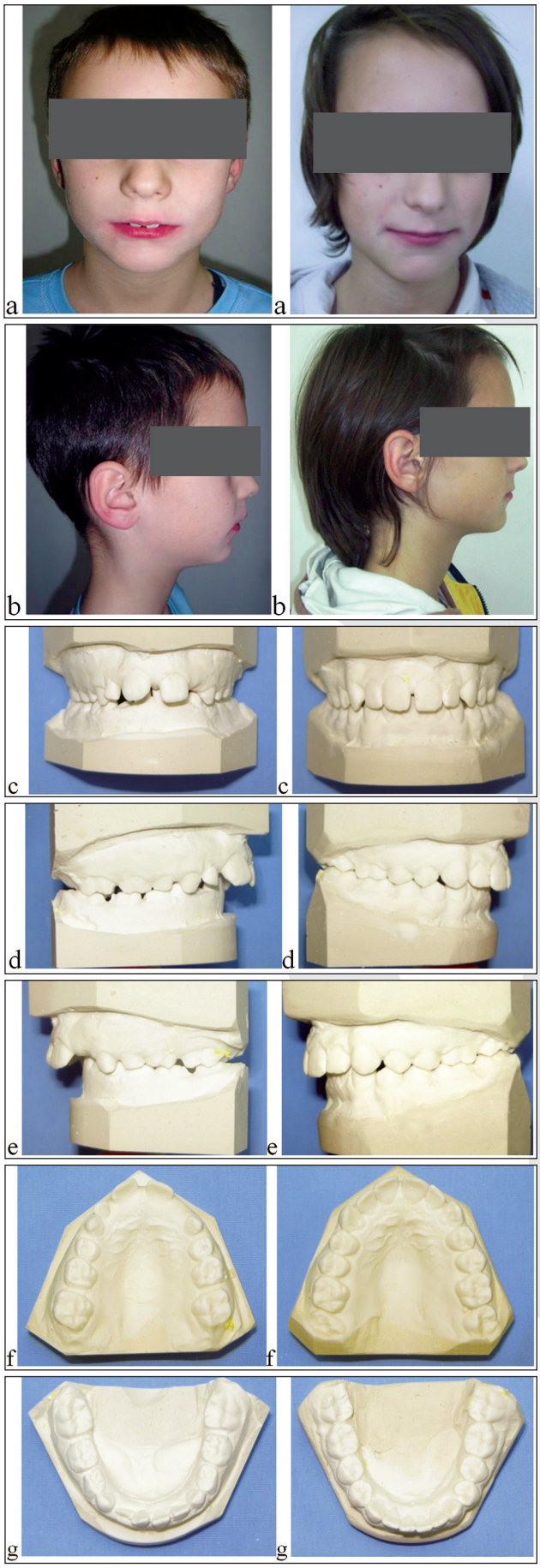


Figure 1. (a and b) Fränkel Appliance



A – before treatment *B – after treatment*

Figure 2. Patient with Class II, division 1. malocclusion before A(a-g) and after B(a-g) treatment by Fränkel I-c appliance

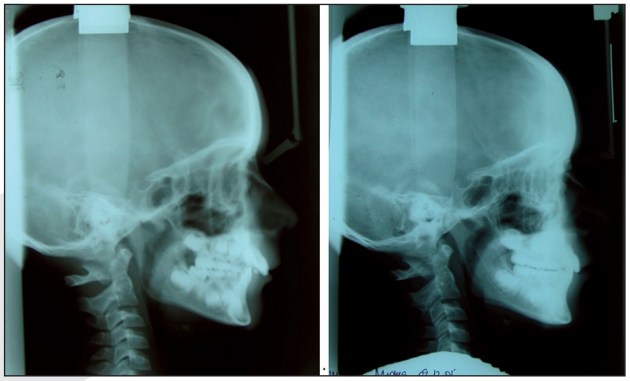


Figure 3. Lateral cephalogram a) before and b) after the treatment

Discussion

According to age when patient wanted orthodontic treatment and existing orthodontic diagnosis, using of FR-Ic was method of choise, which provides stabile therapeutic result. During orthodontic treatment sagittal interjaw relationship was significantly corrected, due to accelerating the growth of the lower jaw and increase in total length of mandibular corpus[15-19]. Stimulation of growth on the lower jaw occurs due to activity of condilar cartilage, associated with dental displacement during the treatment[20,21]. The most significant dental changes are retrusion of upper and protrusion of lower incisors, with correction of overjet[22,23]. Treatment with FR-Ic provides much better facial aesthetics, harmonic profile, correct position of lip in relation to aesthetic line and significant decrease of profile convexity[22,24,25]. Appliance causes significant changes in activity of orofacial and masticatory muscles. Muscles show increased activity, which contributes to correction of orofacial disfunctions. Ba favour of pulling force and tension in muscles generates an overall increase in growth of lower jaw[26].

Conclusion

FR-Ic is very efficient appliance for correction of mandibular retrognatism in early mixed dentition. FR-Ic induce significant changes on skeletal and dental structures, as advancement method of execution of orofacial functions and improvement of facial aesthetics, with stabile treatment result.

References

1. *Rosenblum RE. Class II malocclusion: Mandibular retrusion or maxillary protrusion? Angle Orthod 1995; 1: 49-62.*
2. *McNamara JA. Components of Class II malocclusion in children 8-10 years of age. Angle Orthod 2002; 51: 177-202.*
3. *Rakosi T, Jonas I, Graber TM. Orthodontic diagnosis. George Thieme Verlag: Stutgart – New York, 1993.*
4. *Pancherz H, Zieber K, Hoyer B. Cephalometric characteristics of Class II division 1 and Class II division 2 malocclusions: a comparative study in children. Angle Orthod 1997; 67: 111-120.*
5. *Proffit WR, Ackerman JL. A systematic approach to orthodontic diagnosis and treatment planning. In Graber TM, Vanarsdall RL (eds): Current orthodontic concepts and techniques, ed 3, St Louis, Mosby, 2000.*
6. *Frankel R. The theoretical concept underlying the treatment with the functional correctors. Trans Eur Orthod Soc 1966; 42: 233-254.*
7. *Frankel R, Frankel Ch. Orofacial Orthopaedics with the Function Regulator. Karger, Basel – Munchen – Paris – London – New York – New Delhi – Singapore – Tokyo – Sydney, 1989.*
8. *Braun S, Diers NR, Engel G, Wojtkiewicz P, Ewing SK. The effect of Fränkel II and modified Twin blok appliances on the "C"-axis: The growth vector of the dentomaxillary complex. Angle Orthod 2004; 74: 749-753.*
9. *Barton S, Cook PA. Predicting functional appliance treatment outcome in Class II malocclusions – a rewiew. Am J Orthod Dentofacial Orthop 1997; 112: 282-86.*
10. *Basciftci FA, Uysal T, Buyukrkmen A, Sari Z. The effects of activator treatment on the craniofacial structures of Class II division 1 patients. Eur J Orthod 2003; 25: 87-93.*
11. *Almeida MR, Henriques JFC, Almeida RR, Almeida-Pedrin RR, Ursi W. Treatment effects produced by the Bionator appliance. Comparison with an untreated Class II sample. Eur J Orthod 2004; 26: 65-72.*
12. *Bishara SE. Textbook of orthodontics. W. B. Saunders; Philadelphia, 2001.*
13. *Proffit WR, Fields HW, Sarver DM. Contemporary Orthodontics, 4th ed St. Louis, Mo: Mosby O'Relly T, Yanneillo G. Mandibular growth changes and maturation of cervical vertebrae – A longitudinal cephalometric study. Angle Orthod 1988; 4: 179-184.*
14. *Ball G, Woodside D, Tompson B, Hunter WS, Posluns J. Relationship between cervical vertebral maturation and mandibular growth. Am J Orthod Dent Orthop 2011; 139 (5): 455-461.*
15. *McNamara JA, Huge SA. The Frankel appliance (FR-2) model preparation and appliance construction. Am J Orthod 1981; 80: 478-495.*
16. *Almeida MR, Henriques JFC and Ursi W. Comparative study of the Frankel (FR-2) and bionator appliances in the treatment of Class II malocclusion. Am J Orthod Dentofacial Orthop 2002; 121: 458-466.*
17. *Chadwick SM, Aird C, Taylor S, Bearn DR. Functional regulator treatment of Class II division 1 malocclusions. Eur J Orthod 2001; 23: 495-505.*
18. *Creekmore TD, Radney LJ. Frankel appliance therapy: orthopedic or orthodontic? Am J Orthod 1983; 83: 89-108.*
19. *Petrovic AG, Stutzmann JJ, Lavergne JM, Shaye R. Is it possible to modulate the growth of the human mandible with a functional appliance? Bilt UOJ 1988; XXI (1): 15-20.*
20. *Thieme KM, Nägerl H, Hahn W, Ihlow D, Kubein D. Variations in cyclic mandibular movements during treatment of Class II malocclusions with removable functional appliances. Eur J Orthod 2011: 33(6): 628-635.*
21. *Stamenkovic Z. Clinical effects of Fränkel's functional regulators in treatment of distal and mesial bite. PhD thesis. Belgrade, 2011. (Serbian)*
22. *Janson GRP, Alegria Toruno JL, Rodrigez Martins D, Henriques JFC, De Freitas MR. Class II treatment effects of the Fränkel appliance. Eur J Orthod 2003; 25: 301-09.*
23. *Battagel JM. The relationship between hard and soft tissue changes following treatment og Class II division 1 malocclusions using Edgewise and Fränkel appliance techniques, Eur J Orthod 1990; 12: 154-165.*
24. *Carlos Flores-Mir and Paul WM. A systematic review of cephalometric facial soft tissue changes with the Activator and Bionator appliances in Class II division 1 subjects. Eur J Ortho. 2006; 28: 586-593.*
25. *Yamin-Lacouture C, Woodside DG, Sectakof PA, Sessle BJ. The action of three types of functional appliances on the activity of the masticatory muscles. Am J Orthod Dentofacial Orthop 1997; 112: 560-572.*

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Comparison of post-operative analgesic efficacies of Tramadol and Bupivacaine infiltration applied to multiple regions during laparoscopic cholecystectomy

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Abstract

Purpose: The purpose of this study was to evaluate the analgesic efficacy of tramadol and bupivacaine during multi-regional application.

Methods: Ninety patients who had laparoscopic cholecystectomy were included in the study. Patients in group 1 received 100 mg of tramadol was diluted with 50 mL of physiological serum and patients in group 2 received 0.125% 50 mL bupivacaine. 10 mL of each solution was infiltrated in gall bladder, right subdiaphragmatic and visceral peritoneal surface compartments and also 5 mL of each solution was infiltrated to the each trocar entry site; patients in group 3 received 1 mg/kg IV tramadol 30 minutes before the end of surgery. During the first postoperative 24 hours, pain scale, time of initial analgesic need, total analgesic dose and side effects were recorded.

Results: When pain scores from patients were compared, the mean scores from the 1st hour was 5.16 in Group 1, 5, 90 in Group 2, 5.73 in Group 3, being significantly lower for Group 1 ($p<0.05$). Pain scores from other time points were compared and their values were determined to be similar. Adverse effects were similar among the groups.

Conclusion: For the first postoperative hour, tramadol infiltration was especially more effective compared to bupivacaine, and neither drugs had any side effects. Additionally, in group 3 about 20 % of had shoulder pain.

Key words: Laparoscopic cholecystectomy, multi-regional application, Tramadol, Bupivacaine.

Introduction

Laparoscopy is currently the standard surgical procedure for cholecystectomy. The main advantage of laparoscopic cholecystectomy (LC) is less postoperative pain. However, the severity of postoperative abdominal and shoulder pain is still a significant problem for LC. The intensity of pain after LC peaks within 4–8 h after surgery (1,2). Therefore, LC is not performed as a day-care surgical procedure in a remarkable number of patients. The underlying mechanisms that contribute to postoperative pain are tissue damage resulting from surgical incisions, removal of the gallbladder from the liver bed, stretching of nerve endings and irritation of the diaphragm by carbon dioxide (3,4). Moreover, the consent of central sensitization increases postoperative perceived pain in the area of tissue damage and in the surrounding areas as well(5). Intraoperative blockade of the peripheral nerves directly before commencing LC is a promising strategy to decrease postoperative pain and prevent central sensitization. Bupivacaine as a long acting local anesthetic was investigated to decrease postoperative pain as well as central sensitization through infiltration into both surgical incisions and intraperitoneally (6).

Tramadol is a centrally-acting μ- receptor agonist cyclohexanol derivative. It is reported that tramadol provides a powerful analgesic effect and has no side effects relating to other opioid receptors. In addition to the well known central effects, recent studies have shown that tramadol also had local analgesic efficacy (2,7, 8).

The objective of this randomized prospective trial was to compare the efficacy of three methods, namely tramadol infiltration into surgical sites, bupivacaine infiltration into surgical sites and intravenous tramadol application in terms of relieving postoperative pain in patients with LC.

Methods

This study was planned as a prospective, randomized-double blind study. Before starting the study, Local Ethical Committee approval and informed consent from all patients were obtained Ninety patients aged between 30-60 who were ASA (American Society of Anesthesiologists) I-II, and had laparoscopic cholecystectomy due to gall bladder stones were included in the study. The patients were randomized into three groups each containing 30 subjects initially. We excluded the patients who had Endoscopic Retrograde Cholangiopancreatography (ERCP) and/or papillotomy in the past 3 months or those who were pregnant, lactating, having previous abdominal surgery, renal or hepatic diseases, drug or alcohol dependency and socially enable to participate in addition to ones who refused to participate. Furthermore, patients whose surgeries were not completed laparoscopically or had acute cholecistitis, history of chronic pain, allergy to bupivacaine and tramadol were not included in the study. According to CONSORT guidelines, recruitment, enrollment, retention of subjects are summarized in Figure 1. For post-operative pain evaluation, pain intensity postoperatively was evaluated with a 10 cm Visual Analog Scale (VAS; 0-10 cm) in which 0 represented no pain and 10 represented the most severe pain.

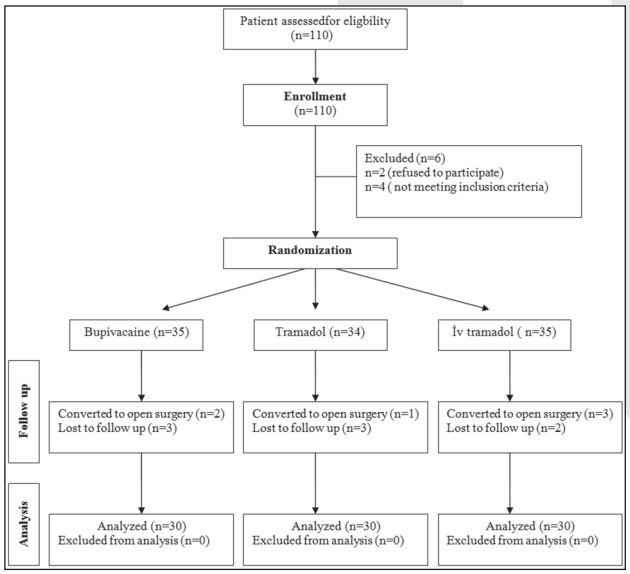


Figure 1. CONSORT flowchart summarizing enrollment and retention in the study protocol

Patients in group 1 received 100 mg of tramadol diluted with 50 mL of physiological saline and patients in group 2 received 0.125% 50 mL bupivacaine. In group 1 and group 2, the gall bladder, right subdiaphragmatic and visceral peritoneal surface compartments were infiltrated with 10 mL of solution and each trocar entry sites were infiltrated with 5 mL of solution. Patients in group 3 received 1 mg/kg IV tramadol 30 minutes before the end of surgery. Before going into the operation room the patients were pre-medicated with a dose of0,01mg/kg midazolam intravenously. General anesthesia protocol was performed for all patients. For induction, 2 mg/kg propofol, 0.1 mg/kg vecuronium bromide and 1 mcg/kg fentanyl were administered (additional analgesic dose was not required during surgery). During maintenance, vecuronium bromide, fentanyl, nitrous oxide/oxygen at 3/3 liter ratio and 1 minimum alveolar concentration (MAC) sevoflurane (concentration 2%) were used. During the surgery, electrocardiography (ECG), end-tidal carbon dioxide (ETCO2) values, oxygen saturations (SPO2), average artery pressures, pulse rates, tidal volumes, minute volumes, respiration rates, airway pressures and MAC sevoflurane values were observed.

Four port techniques were used in all operations. Pneumoperitoneum was created by inserting a Verres needle and insufflation of CO2 into the abdomen. During the surgery, internal CO2 levels were kept at 10-12 mmHg. Once the gall bladder removed, it was possible to spray the medication by entering inside the abdomen through the subcostal trocar using an endoscopic aspirator. Postoperatively, 100 mg tramadol IV was given to patients who were above VAS 5.

During the postoperative 24 hours, time of initial need for analgesic, total analgesic dose and side-effects (nausea, vomiting, headache etc.) were recorded.

Statistical Analysis

Based on the previous studies and clinical experiences, a difference of 0.5 } 0.6 was accepted to be significant. Each group should have 29 patients depending on the power ratio and type 1 error of the test as 80% and 5%, respectively. PASW (ver18) programme was used for all analyses. One-Way Analysis of Variance (ANOVA) was used for differences among the groups with regard to continuous demographic variables which are normally distributed.

In addition, Kruskal-Wallis test was used for comparison of VAS scores between groups. The P value<0.05 was considered to be statistically significant.

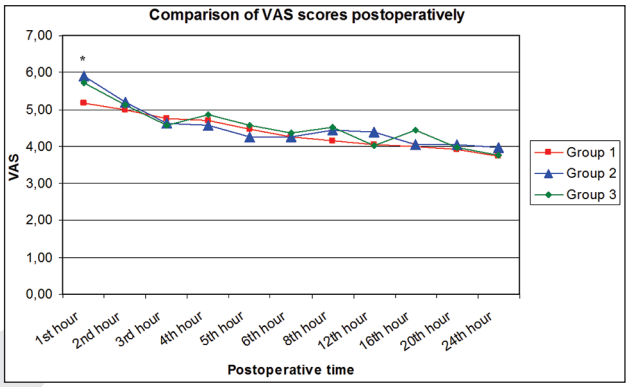
Results

When groups were compared to each other based on age, weight, ASA, length of anesthesia and length of surgery, they were found to be similar. The demographic data of patients are summarized in Table 1.

The three groups were found to be similar to each other based on SpO2, ETCO2, pulse, and average artery pressure and fentanyl consumption.

When pain scores were compared during the 1st hour, the mean pain scores were 5.16 in Group 1, 5.90 in Group 2 and 5.73 in Group 3, being significantly lower in Group 1 (p<0.05). Pain evaluations from other time points were found to be similar. Distribution of the pain scores based on different time points is summarized in Figure 2. In comparison of total analgesic consumptions of the groups were not different among all groups (p=0.107).

When side effects were evaluated, 6 patients had nausea and 3 patients had vomiting in Group 1; 7 patients had nausea and 3 had vomiting in Group 2; and 3 patients had nausea and 1 patient had vomiting in Group 3. Other side effects (headache, itching, and dizziness) were not found in any of the groups (Table 2).



** P= 0.025*

Figure 2. Comparison of VAS scores during postoperative 24 hours

Shoulder pain was observed in 6 patients in Group 3, which was statistically highly significant compared to other groups (p=0.003).

Discussion

Our study has shown that post-operative multiregionally applied tramadol to treat pain after LC was effective in reducing the need for additional analgesic and VAS scores during the first hour compared to bupivacaine and IV tramadol. We believe that insufficiency of bupivacaine was due to the application of low dose and its delay in taking effect. Since it was observed that VAS scores from the second hour and following time points were similar for groups and there was no difference between the total analgesic consumption of groups in the first postoperative 24 hours.

Table 1. Demographic data of patients (Mean Value±Standard Deviation)

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P-value
Age (year)	49,96±13.93	48,93±12.49	49.20±14.79	0.956
Weight (kg)	82.86±12.32	82.03±20.55	75.73±15.56	0.193
Height (cm)	170,23±8,20	168,73±9,19	169,43±8,64	0.799
Anesthesia time (min)	82.46±21.31	84.16±20.30	90.83±27.82	0.347
Surgery time (min)	64.00±22.33	65.83±18.89	72.00±27.24	0.376

Table 2. Comparison of complications of the patients

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P-value
Nausea	6 (20%)	7 (23.3%)	3 (10%)	0.372
Vomiting	3 (10%)	3 (10%)	1 (3.3%)	0.538
Headache	0	0	0	
Itching	0	0	0	
Dizziness	0	0	0	

Kucuk et al. applied bupivacaine100 mg (21 mL) + 1/200000 adrenaline, ropivacaine 100 mg (21 mL) + 1/200000 adrenaline and ropivacaine 150 mg (21 mL) + 1/200000 adrenaline to intraperitoneal and wound sites, and determined that the analgesic need during the initial four hours was significantly reduced in the group that was given 150 mg of ropivacaine. In our study, we found a decrease in the VAS scores in intraperitoneal tramadol group which was confined to first postoperative hour. We believe that this was because of not using adrenaline to extend the effective time for our drugs and administration of a high dose as in ropivacaine group in the study of Kucuk et al (9).

Despite these studies, there are other studies which show bupivacaine to be an ineffective analgesic. In one study, it was shown that bupivacaine administered in high doses intraperitoneally is not effective as an analgesic(10).

Papadima et al, administered 0.5% levobupivacaine before extubation and continued infusion 8 hours postoperatively by placing a subdiaphragmatic drain and determined that analgesic consumption was reduced by 50% in PCU and by 80% during the initial 24 hours compared to the control group(11). Mraovic et al, administered 15ml 0.5% bupivacaine infusion during the first postoperative 8 hours and showed that the analgesic need is much less. In this study, high VAS scores in the bupivacaine group may be due to the low dose of the drug and the type of infusion(12).

Shoulder pain is seen at 66% of patients after laparoscopic surgery. The importance of pain is lesser in the first 24 hours, but it becomes increasingly the main complaint after the second day 1. It is assumed to be the result of peritoneal stretching, diaphragmatic irritation, traumatic nerve traction, and phrenic nerve stimulation due to the release of inflammatory mediators in(13). In studies trying to reduce the shoulder pain, the duration of pneumoperitoneum, the amount of gas insufflated, peak intra-abdominal pressure, gas temperature, intraperitoneal injection of nitrous oxide, local anesthetics and the use of saline has been questioned(1,14). In our study, intraperitoneal administration of the groups (intraperitoneal bupivacaine and tramadol), shoulder pain was not observed. Also, it is concluded that application of local anesthetic drugs intraperitoneally into the subdiaphragmatic area prevented phrenic nerve excitation by creating irrigation and anesthetic effect and by this way shoulder pain was not seen.

In addition to its known central effects, recent studies showed that tramadol has also local analgesic effects(2,8,9). Golubovic et al, administered intraperitoneal saline, bupivacaine and tramadol + bupivacaine and demonstrated that the analgesic needs of the latter two groups decreased considerably compared to the saline group during the initial postoperative hours(15).

Memis et al, showed similar results in their study where they administered tramadol intraperitoneally following total hysterectomies(16). In contrast to these studies, Akinci et al, has shown that intraperitoneally administered tramadol was effective during the initial postoperative 15 minutes; however tramadol given intravenously was more effective at all postoperative time points(8). In our study, especially during the first postoperative hour, it was observed that the analgesia was more effective in tramadol group compared to bupivacaine group and shoulder pain was observed in the IV tramadol group. Mean VAS scores in tramadol group in the first postoperative hour was lower than in Group 2 and 3 and it was statistically significant ($p<005$). Therefore, it can be concluded that the analgesia was more effective in the intraperitoneal tramadol group. However, in comparison of total analgesic consumptions of the groups were not different among all groups ($p=0.107$).

In conclusion, pain increases mortality and morbidity during LC operations, thus it is an important symptom that should not be ignored. For this reason, in addition to precautions taken during operation, analgesic applied to multiple regions can reduce shoulder and visceral pain especially during the initial postoperative hours.

References

1. *Joris J, Thiry E, Paris P, Weerts J, Lamy M. Pain after laparoscopic cholecystectomy: characteristics and effect of intraperitoneal bupivacaine. Anesth Analg 1995; 8: 379-84.*

2. *Bisgaard T, Kehlet H, Rosenberg J. Pain and convalescence after laparoscopic holecystectomy. Eur J Surg. 2001; 167: 84-96.*

3. *Lindgren L, Koivusalo AM, Kellokumpu I. Conventional pneumoperitoneum compared with abdominal wall lift for laparoscopic cholecystectomy. Br J Anaesth 1995; 75: 567–572.*

4. *Lindgren L. Pain after laparoscopic cholecystectomy. Do we do our best? Acta Anaesthesiol Scand 1997; 41: 191–192.*

5. *Kehlet H, Jensen TS,Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet 2006; 367: 1618–1625.*

6. *Moiniche S, Jorgensen H, Wetterslev J, Dahl JB. Local anesthetic infiltration for postoperative pain relief after laparoscopy: a qualitative and quantitative systematic review of intraperitoneal, port-site infiltration and mesosalpinx block. Anesth Analg. 2000; 90: 899-912.*

7. *Grond S, Meuser T, Zech D, Hennig U, Lehmann KA. Analgesic efficacy and safety of tramadol enantiomers in comparison with the racemate: a randomised, double-blind study with gynaecological patients using intravenous patient-controlled analgesia. Pain. 1995; 62: 313-20.*

8. *Robaux S, Blunt C, Viel E, Cuvillon P, Nouguier P, Dautel G, Boileau S, Girard F, Bouaziz H. Tramadol added to 1.5% mepivacaine for axillary brachial plexus block improves postoperative analgesia dose-dependently. Anesth Analg. 2004; 98: 1172-7.*

9. *Kucuk C, Kadiogullari N, Canoler O, Savli S A. placebo-controlled comparison of bupivacaine and ropivacaine instillation for preventing postoperative pain after laparoscopic cholecystectomy. Surg Today. 2007; 37: 396-400.*

10. *Raetzell M, Maier C, Schroder D, Wulf H. Intraperitoneal application of bupivacaine during laparoscopic cholecystectomy--risk or benefit? Anesth Analg. 1995; 81: 967-72.*

11. *Papadima A, Lagoudianakis EE, Antonakis P, Filis K, Makri I, Markogiannakis H, Katergiannakis V, Manouras A. Repeated intraperitoneal instillation of levobupivacaine for the management of pain after laparoscopic cholecystectomy. Surgery. 2009; 146: 475-82.*

12. *Mraović B, Jurisić T, Kogler-Majeric V, Sustic A. Intraperitoneal bupivacaine for analgesia after laparoscopic cholecystectomy. Acta Anaesthesiol Scand. 1997; 41: 193- 6.*

13. *Atak I, Ozbagriacik M, Akinci OF, Bildik N, Subasi IE, Ozdemir M, Ayta NI. Active gas aspiration to reduce pain after laparoscopic cholecystectomy. Surg Laparosc Endosc Percutan Tech 2011; 21: 98–100.*

14. *Mouton WG, Bessell JR, Otten KT, Maddern GJ. Pain after laparoscopy. Surg Endosc. 1999; 13: 445–448.*

15. *Golubović S, Golubović V, Cindrić-Stancin M, Tokmadzić VS Intraperitoneal analgesia for laparoscopic cholecystectomy: bupivacaine versus bupivacaine with tramadol. Coll Antropol. 2009; 33: 299-302.*

16. *Memis D, Turan A, Karamanlioglu B. Intraperitoneal tramadol and buvacaine in totalabdominal hysterectomy. Eur J Anaesthesiol. 2005; 22: 804-5.*

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Medical University's strategic plan: Progress and related obstacles

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Abstract

Background: The survival of health organizations even Medical Universities with great mission are in close connection with application of strategic planning.

Objective: To assess the progress and related obstacles of the University strategic plan and its effective factors

Methods: This study was conducted within the Medical University's divisions during 2006-2010. The progress reporting forms and priority settings were formulated through team discussions and participation of the stakeholders.

Findings: Results showed that The most important obstacles to achieve both curative and educational goals were recognized as shortage of fiscal resources, lack of clear internal and external communication, and poor orientation of the personnel which caused the ascendant trend of achieving defined goals of University that 12% of them were shown to have been unattained at the end of the study period. In fact Systematic design of strategic committee meetings to engage those involved in planning and assessments of their related educational need were the lost circles of university strategic plan chain.

Conclusion: It is concluded that improving personnel's perception of strategic plan and their sense of responsibility will develop its process. Current study also showed, due to their severe influence on the implementation stage, clear internal and external communication, more partnership plans and continuous review of structures can be determinant.

Key words: Planning, strategic planning, health care system.

Introduction

One of the most outstanding properties of developed countries is paying attention to knowledge management as one of unavoidable necessities of improvement, development and social-economic growth. Herein, what has been discussed as the most important management function of each country in any level, is to plan and endeavor to gain anticipated goals in it. So in the recent decays, administrative manager's attention is attracted to long term planning and especially strategic ones. In fact organizations ought to have an strategic thought, convert their discoveries and understandings to effective strategies to adapt with variable environments and finally prepare the essential infrastructures to execute them since strategic planning is a process to access the organization's goals in a competitive ,dynamic and varying environment which is designed through resource allocation(1). In fact the organizations that provide strategic planning can get to their goals with a higher probability than those who don't have this planning (2). In other words it defines the future navigation by helping the organizations to select the presidencies and takes today's decision makings under their future aftereffects to provide bases of improvement of the organization's functioning.(3) A look to salient achievements of societies along different periods and preliminary study of health market's circumstances and the internal and external environment fluctuations which rules on it, also shows that strategic planning maintain health organization's durability as other organizations.

1970's strategic planning in health care was formed to design a new structure and development of healthcare services in response to population growth. By inventing payment system in 1980, strategic planning turned to a necessity for health care organizations to be able to respond the incre-

asing requirements in competing environments (4,5). Organized healthcare needed more amplification in most fields during 80 and 90 decays .healthcare cost continuously increased more than gross domestic product and competition intensified in the market. Today hospitals and other health organizations utilize strategic planning as a useful tool to assess the substitution solutions and help to prepare them for the future. Managers of health field need to understand the process of strategic planning, its goals, advantages, challenges, and key factors to assess its progression (6).

Material and methods

This descriptive-analytical study was conducted within the 14 divisions affiliated to one of the High ranked Medical Universities to assess the progress of university's strategic plan and obstacles prevented its development between2006 - 2010 for the first time in Iran. The evaluation method and the progress reporting forms were formulated through team discussions and participation of the stakeholders in the collective interviews. It took several sessions for the strategic committee to analyze the information on forms completed by the participants to set the obstacle priorities annually and recognize efficacious factors. More over the process of goal achievement was assessed and classified in 4 subgroups: unattained, completely achieved (100%), between 50 to 90% and <50% achieved. While, delayed goals have been distinguished. Finally the obstacle's influences on achieving defined goals formulated through collective interviews and the data were summarized and categorized by Spss 16.

Findings

The frequency of goal achievement in different divisions affiliated to the University is shown at tables and figures. Based on the gathered data, there was an ascendant trend on the completely achievement (100%) of goals until 2009, while it decreased (34.5 %) in 2010. On the other hand, the most unattained goals have been seen (12%) in the same year, as shown in the first figure. Moreover, subsequences of goal attainment assessed during the years surveyed and the data showed that in the first years of planning most of the goals have gained on time while in the last year (2010), there was a salient down fall and only 32% of the goals had been achieved (figure 3). Nevertheless the University have been had the best rate of 50-90% goal achievement in 2010(figure2). According to the findings, strategic planning had been faced with some obstacles and problems such as shortage of fiscal resources (44%), unclear internal and external communications (29%), manpower and their motivational problems (19%), administrative problems and personnel's Involvement to their routine activities (8%) totally. The findings suggest that besides the appearance of new problems like personnel's involvement to their routine activities, the priorities of the old obstacles has been changed completely in the last year of the study and fiscal resource's shortage has gained the 6th place (9%) (table2). So a salient down fall should be mentioned parallel with the emerged barriers and the priority setting has been done through team discussions.

Table 1. Trend of University's goal achievement

total	Total goals		percentage of Goal achievement				Year
	Achieved						
	delayed	timely	zero	0 - 50	50 - 99	100	
100	28	72	7	10	34	49	**2007**
100	31	69	4	11	32	53	**2008**
100	25	75	5	8	30	57	**2009**
100	68	32	12	16	37.5	34.5	**2010**

Table 2. Priorities of obstacles that strategic plan has been faced to during the 4 years of assessment

Degree of priority				
related obstacles	2007	2008	2009	2010
Shortage of fiscal resources and budjet	1	1	1	6
Unclear internal communications	2	5	3	3
Unclear external communications	4	3	2	5
Shortage of manpower and their motivational problems	3	2	4	4
Administrative problems	5	4	5	2
Involvement to the Routine activities	-	-	-	1

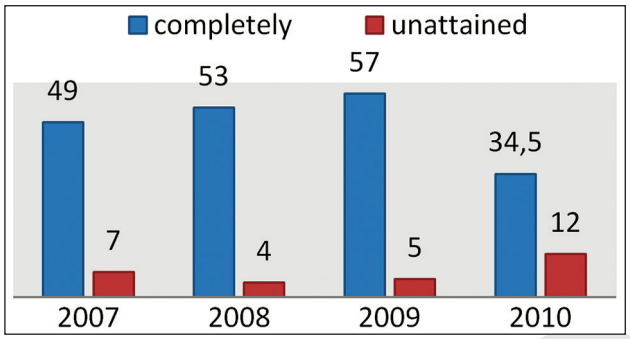

Figure 1. Percentage of goal achievement 2007 - 2010

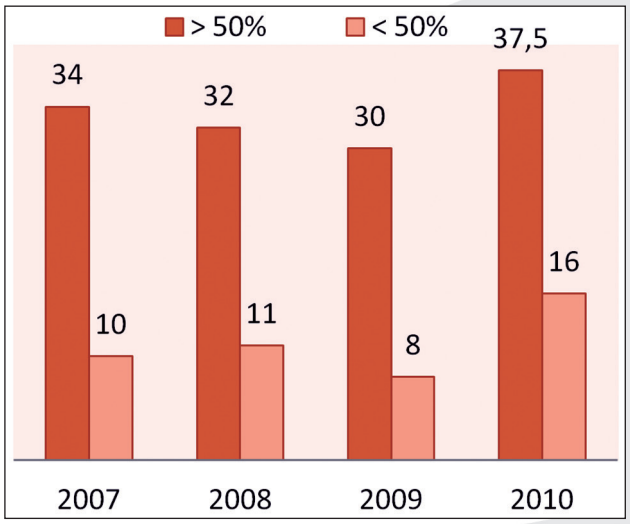

Figure 2. Goals achievement's percentage (> 50% and < 50%) 2007 - 2010

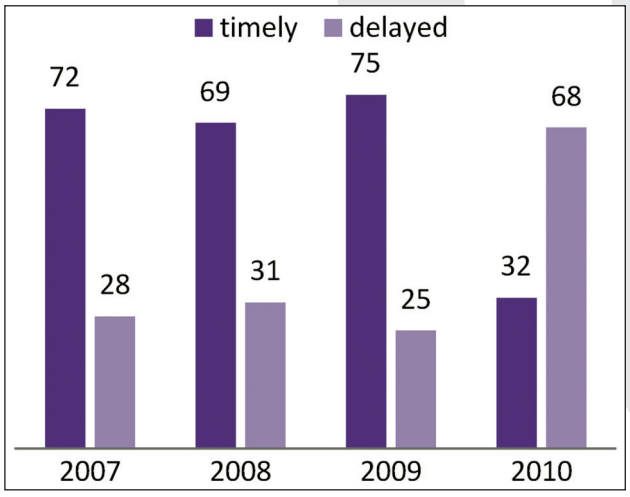

Figure 3. Percentage of goal achievement's retardation 2007 - 2010

Conclusion

Based on the medical universities scientific map's vision which is " training the best graduates, achieving health indexes as like as possible and having sufficient share in production, scientific and technological development until 2025" elementary policies are made to orient the university toward its mission and final goals. University's strategies and operational actions have been predicted also to define strategic plan. On the other hand, each strategic plan will be reformed if the related forms and reports review perpetually to all available obstacles be revealed and removed after necessary analysis. So Qazvin university's strategic plan has been assessed during 2006 – 2010 to find whether related division's activities follow mission and policies of the university or not, if not what was the reason. As findings suggest at the end of the 2006 which has been the first year of planning, about half of the defined goals completely attained (49%), while for the next years it was more than that (respectively 53% and 57%). But at the end of 2010 this trend has been changed and it descent to 37.5% because of the new obstacles which are ignored mostly. In fact the collective interviews revealed and prioritized the existing snags and problems University strategic planning has been faced to, shortage of fiscal resources categorized as the 6th priority in the last year of study while it has been the first one between 2006 to 2009 which demonstrate despite dominating fiscal obstacles, improving of strategic performance have been affected by non- financial factors which are in close relationship with the involved people perception. As the studies of Rudd JM, Greenly AT about "strategic planning and performance" (2008) showed, organization's financial, structural and technical performance are strongly affected by strategic plan's application which would have improve both financial and nonfinancial aspects of the organization (7, 8). Meanwhile, strategic committees survey showed that besides the old obstacles some new ones appeared like administrative problems and poor orientation of personnel which have influenced the strategic progression in 2010. So clear internal and external communication, partnership planning and structures review would have a se-

rious impact on personnel's motivation through Improving their perception of strategic planning and their responsibilities. On the other hand Necessity of improving knowledge level, acquaintance and proficiency of targeting revealed when some of the routine activities had been defined as the strategic goals. Tohidi (2010) also has emphasized on his research about "strategic planning of Iran's educational institutes" that participant's active role and educational standard's improvement influence the effectiveness and efficiency of strategic planning tools strongly (9). Vafaeezadeh also has researched the effective factors play an important role in strategic plan of Iran's Marine Industry (2000). According to her findings original source of each strategic plan is logical thought which force managers and workforces to respect the standards and follow them strongly (10). So Qazvin University's strategic planning committee can improve the quality of strategic targeting by providing sufficient educational courses about strategic planning and logical thought.

As findings showed, more than 50% of goals have been achieved without delay (in 11 divisions). It demonstrates that the University's strategic plan has been implemented timely based on the schedules. Gordon (2000) investigated the University of Sydney's educational situation through the SWOT analysis technique. He has also confirmed that strategic planning would improve the quality of educational services and activity's schedule (11). So, it is expected to Qazvin medical university eliminate related obstacles and improve goal achievement of 14th affiliated divisions as like as possible to have the best performance based on its scientific map. But the most important factors which have been recognized for improving the quality of strategic management in all educational, research and curative deputies of universities can be listed as below:

- Continuous review of strategic plan, Feedback results to relevant units and pursue reforms
- Implementation of strategic plans with state law
- The new management approaches such as knowledge management
- Promotion of capacity building and planning skills
- Systematic design of a strategic committee meetings To engage those involved in planning
- Training courses to improve necessary skills

Finally, Models of strategic planning for educational institutions such as universities and educational need assessment for preparing staff to participate in the strategic planning process should be studied more in the future. The relationship exists between management styles and effectiveness of the strategic plan also should be considered as an important factor especially in universities which senior managers have a severe impact on research and educational performance that most be improved through the qualified strategic plan.

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References

1. *Mc Namara C. strategic planning in nonprofit or for profit organization(2000), Available at: http://www.mapn.p.org/library/plan-dec/str-plan*
2. *Asefzadeh S, Rezapour A. Health planning. 1 st ed. Qazvin: Qazvin University of Medical Sciences; 2007; 164 [In Persian]*
3. *Tabibi SJ, Maleki M, Delgoshai B. Sterategic Planning. 1st ed. Tehran: Ministery of Health and Medical Education; 2004; 90 [In Persian]*
4. *Greenley, G.E., Hooley, G.J., Broderick, A.J. and Rudd, J.M. "Strategic Planning Differences Among Different Multiple Stakeholder Orientation Profiles", Journal of Strategic Marketing, (2004); Vol. 12, No. 3: pp 163-182.*
5. *Lombardi DJ. Health care management. London: John wiley & son Inc; 2007. 60-75*
6. *Visseosy J. Health operations management. London: routledge; 2005; 39- 51*
7. *Rudd JM, Greenley AT. Strategic planning and performance: Extending the debate. Journal of Business Research 2008 Feb; 61(2): 99-10*

8. *Vila J, Canales JI. Can Strategic Planning Make Strategy More Relevant and Build Commitment Over Time. The case of RACC. Long Range Planning 2008 Jun; 41(3): 273*

9. *Hamid Tohidi, Aida Jafari, Aslan Azimi. Strategic planning in Iranian educational organization.procedia social and Behavioral sciences 2 (2010) 3904-3908*

10. *Vafaeezadeh M,R. effective factore of strategic planning in Iran's marine industry. Tehran University of medical sciences. (1998).*

11. *Gordon J, Hazlett C & et all. Strategic planning in medical education: enhancing the learning environment for students in clinical settings. Med Educ. 2000; 34(10): 841-50. Available at: www.ncbi.nlm.nih.go*

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The prevalence of Parvovirus B19 in patients with Sickle cell disease in southern Turkey

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Abstract

Objective: Human parvovirus B19 (HPV B19) has a remarkable tropism for erythroid progenitor cells and this infection leads to important morbidity in patients with sickle cell disease. The virus can induce transient aplastic crisis. The patients with sickle cell disease are great importance to epidemiological surveillance programs and disease prevention strategies. The aim of the present study was to investigate the prevalence of HPV B19 in patients with sickle cell disease by using PCR and ELISA techniques in southern Turkey.

Materials and Methods: All the serum samples were collected from 113 patients with sickle cell disease and 64 healthy subjects. Antibodies to HPV B19 were measured by ELISA method. Viral DNA was extracted by using a Real-time PCR LightCycler Parvovirus B19 Quantification kit.

Results: Of the total participants, 46 (40.7%) of patients and 18 (28.1%) of control group were found to IgG positive (P = 0.094), whereas 13 (11.5%) of patients and 9 (14.1%) of control group were found to IgM positive (P = 0.620). HPV B19 DNA were detected in 20 (17.7%) of patients and 9 (14.1%) of control group (P = 0.530). Good degree correlation was found when the comparison B19 DNA and specific IgM antibodies (r=0.666, P<0.001). Transient aplastic crisis was found in 47.8% of patients with HPV B19 infection.

Conclusions: The data in the present study document that HPV B19 is a common pathogen for both children and adults with sickle cell disease and healthy persons in Turkey.

Key words: Human parvovirus B19, Sickle cell disease, aplasia, prevalence.

Introduction

Human parvovirus B19 (HPV B19), which was identified in by Cossart et al., is a small, single-stranded, nonenveloped DNA virus and a member of the *Erythrovirus* genus (1, 2). Infections with this virus are occur worldwide and can result in a wide range of clinical findings (3, 4). Manifestations of parvovirus B19 infection changes from asymptomatic, nonspecific, cold like symptoms or subclinical infection for most people (5, 6). On the other hand, clinical presentations associated with the infection contain erythema infectiosum, arthropathy, transient aplastic crisis (TAC), chronic red cell aplasia, and hydrops fetalis (6).

The virus has a remarkable tropism for erythroid progenitor cells and thus can cause eventually triggering a transient arrest in erythropoiesis (7). Patients with chronic hemolytic disorders, including sickle cell disease (SCD), thalassemia, hereditary spherocytosis, red cell enzymopathies such as pyruvate kinase deficiency, and autoimmune hemolytic anemia are at risk of violent clinical illness (8-12). The virus induces a cessation of erythropoiesis and acute erythroblastopenia. This condition is often called as transient aplastic crisis (13, 14).

HPV B19 infection leads to important morbidity in patients with SCD (9, 15). The patients are pretty much infectious during aplastic crisis and usually become highly viremic. They should be isolated to prevent contamination of the virus (13).

The patients with sickle cell disease are should be followed closely terms of HPV B19 infection. For this reason, it is great importance to epidemiological surveillance programs and disease prevention strategies. The aim of the present study was to investigate the prevalence of HPV B19 in patients

with sickle cell disease by using polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) techniques in southern Turkey.

Materials and methods

Serum samples were collected from 113 patients with SCD and 64 healthy subjects were taken as controls. All the serum samples were selected from Mustafa Kemal University Medical School internal medicine and pediatrics polyclinics. The patients (58 males and 55 females) with SCD were recruited ages ranging from 2 to 51 years (mean ± SD: 18.4 ± 9.1 years). The control subjects (29 males and 35 females) were recruited with ages ranging from 1 to 41 years (mean± SD: 17.9 ± 10.4 years).

The serum levels of anti HPV B19 IgG and IgM were measured by ELISA method according to instructions of the manufacturer (NovaTec Immundiagnostica GmbH®, Germany).

Extraction of DNA

Viral DNA was extracted from 200 µl of sample by using a Real-time PCR LightCycler Parvovirus B19 Quantification kit (Roche®, Germany) according to instructions of the manufacturer. Nucleic acids were eluted from the filter column with 50 µl of nuclease distilled water. Isolated DNA samples were stored at -80°C until further use.

Primer and probes

PCR primers and a TaqMan probe were used to amplification of 123 bp region of NS1 gene in the study. Primary sequences of the primers and probe were as follows:

NS1-F (Forward): 5'-GAAGACTGGATGATGACAGATCCA-3',

NS1-R (Reverse): 5'-TGCTGTTTTTGTTCTTGCTAGAGTAA-3'.

NS1-P (Probe): FAM-AATGATGGCTCAAACCGGAGGAGA-BHQ1.

The fluorogenic probe was 5' labeled with the 6-carboxyfluorescein (FAM) and 3' labeled with the Black Hole Quenchers (BHQ1).

Preparation of standard plasmid DNA

NS1 region was amplified in a 25 µl reaction mixture containing 3 µl deoxynucleoside triphosphates (dNTP), 16 µl bidistilled sterile water, 2.5 µl buffer, 1 µl of each primer (NS1-F and NS1-R), 1 µl of BQ, and 0.5 µl of DNA polymerase. Amplification reactions were performed with 1 cycle at 94°C for 5 minute followed by 30 cycles of 94°C for 30 seconds, 58 °C for 45 seconds, and 72 °C for 30 seconds. A final extension 7 minute was added after the last cycle. This PCR product is produced by inserts to a vector (pMD18-T) in the host bacterium. It is purified recombinant plasmid by using a commercial kit from this bacterium. These products were stored at -20 ° C for later use.

Real-time PCR

NS1 gene was amplified with a thermocycler in a 25 µl reaction mixture containing 2.5 µl PCR buffer, 3.5 µl dNTP, 3 µl $MgCl_2$, 1 µl of each primer (NS1-F and NS1-R), 1 µl recombinant plazmid, 0.5 µl probe (PPV-P), 0.5 µl hotstart Taq DNA polimerase, and 12 µl bidistilled sterile water. PCR amplification reactions of NS1 gene were performed with 1 cycle at 95°C for 30 seconds followed by 40 cycles of 95°C for 10 seconds, 58 °C for 20 seconds, and a final extension at 72°C for 20 seconds. The statistical significance of observed differences was assessed by means of the chi-square test for categorical variables; Mann-Whitney *U* test for continuous variables; nonparametric correlation (Spearman's twotail) for association. All tests were performed using SPSS (version 15.0, USA). *P* less than 0.05 were considered to be statistically significant.

Results

The serological and PCR data of the both groups tested are shown in Table 1. Forty six (40.7%) of patients with sickle cell disease and 18 (28.1%) of control group were found to have detectable levels of anti parvovirus B19 IgG (χ^2 = 2.8, d.f. = 1, *P* = 0.094). Thirteen (11.5%) of patients with the sickle cell disease and 9 (14.1%) of control group were found to have detectable levels of parvovirus B19 IgM (χ^2 = 0.246, d.f. = 1, *P* = 0.620). HPV B19 DNA were detected in 20 (17.7%) of patients with the sickle cell disease and 9 (14.1%) of control group (χ^2 = 0.394, d.f. = 1, *P* = 0.530). Good degree correlation was found when the comparison B19 DNA and specific IgM antibodies (r=0.666, *P*<0.001).

Confirmation of HPV B19 infection was found in 23 (20.4%) of 113 patients by showing parvovi-

Table 1. Serological and PCR data of the patients with sickle cell disease and control group

Group tested	B19-IgM antibodies positive n (%)	B19-IgG antibodies positive n (%)	B19 DNA n (%)
Sickle cell anemia n=113	13 (11.5)	46 (40.7)	20 (17.7)
Control n=64	9 (14.1)	18 (28.1)	9 (14.1)

Table 2. Results of B19 DNA and specific IgM antibodies detected in serum samples of patients with B19 infection according to clinical manifestations

Clinical manifestations	Only B19 DNA +	B19 DNA + IgM +	B19 DNA + IgG +	Only IgM +
Not clinical signs	1	4	4	2
Transient aplastic crisis	-	6	4	-
İnfection	-	-	-	1
Transient aplastic crisis + İnfection	1	-	-	-
Total	2	10	8	3

Table 3. Prevalence of HPV B19 IgG antibodies in patients with SCD and control group according to age groups

Age	Patients with sickle cell disease for IgG positive			Control group for IgG positive		
	n	Number	%	n	Number	%
0-10	20	2	10	19	3	15.8
11-20	51	28	54.9	17	5	29.4
21-30	31	12	38.7	20	8	40
31-40	9	4	44.4	7	2	28.6
41-51	2	0	0	1	0	0
Total	113	46	40.7	64	18	28.1

Table 4. The comparison pediatric and adult patients and control group according to serology and PCR

Age	Patients with sickle cell disease			Control group			*P*		
	IgM n (%)	IgG n (%)	B19 DNA n (%)	IgM n (%)	IgG n (%)	B19 DNA n (%)	IgM	IgG	B19 DNA
Pediatric group (0-16) 58 patient, 30 control	7 (12.1)	20 (34.5)	10 (17.2)	8 (26.7)	7 (23.3)	9 (30)	0.086	0.285	0.170
Adult group (≥ 17) 55 patient, 34 control	6 (10.9)	26 (47.3)	10 (18.2)	1 (2.9)	11 (32.4)	0 (0)	0.177	0.168	0.009
Total	13	46	20	9	18	9	0.620	0.094	0.530

rus B19 DNA and/or specific IgM antibody. Two patients only viral DNA could be detected, as 10 patients were tested positive for both parvovirus B19 DNA and specific IgM. Eight patients were found positive for both viral DNA and specific IgG. Other 3 patients, only specific IgM could be detected. Results of B19 DNA and specific IgM antibodies detected in serum samples of patients with HPV B19 infection according to clinical manifestations are summarized in Table 2.

Prevalence of HPV B19 IgG antibodies in patients with sickle cell disease and control group according to age groups are shown in Table 3. The rate of positive identification of HPV B19 IgG antibody in young patients with the sickle cell disease was higher than other groups. The comparison pediatric and adult groups are shown in Table 4. There was statistical difference between the adult patients with sickle cell disease and adult control group in terms of HPV B19 DNA (*P*=0.009).

Discussion

SCD is common illness in Southern Turkey. The incidence of SCD is 10.0% in the Cukurova region of Southern Turkey (16, 17). HPV B19 infection can leads to repress erythropoiesis and acute erythroblastopenia in these group patients. The patients usually extremely viremic and risk of virus transmission is increased (18). Tight following of these patients with SCD terms of HPV B19 infection are necessary for the formulation of epidemiological surveillance and disease prevention. This is the first study evaluating the prevalence of HPV B19 in patients with SCD in Turkey.

Infections with HPV B19 are occurring worldwide and situations of infection have been reported in all seasons. Seroprevalence increases with age and more than 70% of the adult population is seropositive. Children are the master resource of contamination and outbreaks can persist for months in schools and child care centers (19). In a study among Korean plasmapheresis donors, it was reported that prevalence of IgG antibodies to HPV B19 is 59.38% (20). In the other study, it was shown that anti HPV B19 IgG antibodies are 32.1% in patients with chronic hemolytic anemia is majority of the SCD (21). Smith-Whitley et al found the serology of HPV B19 IgG 29.5% in children with SCD while the same group of patients, Zimmerman et al that 53% (15, 22). In another study, it was reported that HPV B19 IgG antibodies were detected 56.5% of the sickle cell anemia, 15.2% of the control patients. There was statistical difference between the sickle cell anemia and control patients in terms of HPV B19 IgG seropositivity (18). In the present study, there was found to have detectable HPV B19 IgG 40.7% of patients with the SCD and 28.1% of control group. There was no statistical difference between the patients and control subjects in terms of HPV B19 IgG seropositivity.

In a study of 12 years in Ireland, it was reported that prevalence of IgM antibodies to HPV B19 is 4.5% (23). In the other report, it was shown that HPV B19 IgM in 4 of 19 patients with TAC (21). Us et al found 12.6% were positive for specific IgM in patients with hematological disorders such as acute and chronic leukemia, malignant lymphoma, myelodysplastic syndrome, and multiple myeloma. But in this patient group, 70.9% were received multiple blood transfusions or blood products (24). In the present study, there was found to have detectable HPV B19 IgM 11.5% of patients with SCD and 14.1% of control group. Also 17.7% of patients with SCD and 14.1% of control group were found to contain HPV B19 DNA. Although 2 patients only viral DNA and other 3 patients only specific IgM could be detected, good degree correlation was found when the comparison B19 DNA and specific IgM antibodies.

It was discovered in 1981 that HPV B19 caused TAC in children with sickle-cell anemia and it is found from further studies that nearly 70% of HPV B19 infections in TAC [19]. In the present study, TAC was found in 11 (47.8%) of 23 patients with HPV B19 infection by showing HPV B19 DNA and/or specific IgM antibody. In a study, the opposite anti B19 IgM could be detected in only 4 (21.1%) of 19 patients with TAC and none of them had HPV B19 DNA (21).

The data in the present study document that HPV B19 is a common pathogen for both children and adults with SCD and healthy persons in Turkey. Although seroprevalence increases with age, it could not be shown in this study in full. The reason for this may be less the number of cases studied according to age groups.

In conclusion, there was no statistical difference between the patients and control subjects in terms of HPV B19 IgG and IgM seropositivity and HPV B19 DNA. But while the comparison pediatric and adult groups; there was statistical difference between the adult patients with SCD and adult control group in terms of HPV B19 DNA. Well degree correlation was found when the comparison HPV B19 DNA and specific IgM antibodies. TAC was found in 47.8% of patients with HPV B19 infection. This is the first study evaluating the prevalence of HPV B19 in patients with SCD in Turkey.

References

1. *Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet 1975; 1(7898): 72-73.*

2. *International Committee on Taxonomy of Viruses (ICTV). Virus taxonomy: 2009 release. Virology division, IUMS. Available at http://www.ictvonline.org*

3. *Katta R. Parvovirus B19: a review. Dermatol Clin 2002; 20: 333-342.*

4. *Plummer FA, Hammond GW, Forward K, et al. An erythema infectiosum-like illness caused by human parvovirus infection. N Engl J Med 1985; 313: 74-79.*

5. *Brown KE. Human Parvoviruses, Including Parvovirus B19 and Human Bocavirus. In: Mandell GL, Bennet JE, Dolin R, (eds). Principles and Practice of Infectious Diseases, 7th edn. Churchill Livingstone, Philadelphia, 2010.*

6. *Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev 2002; 15: 485-505.*

7. *Ozawa K, Kurtzman G, Young N. Replication of the B19 parvovirus in human bone marrow cell cultures. Science 1986; 233: 883-886.*

8. *Pattison JR, Jones SE, Hodgson J, et al. Parvovirus infections and hypoplastic crisis in sickle-cell anaemia. Lancet 1981; 1(8221): 664-665.*

9. *Serjeant BE, Hambleton IR, Kerr S, Kilty CG, Serjeant GR. Haematological response to parvovirus B19 infection in homozygous sickle-cell disease. Lancet 2001; 358: 1779-1780.*

10. *Lefrère JJ, Couroucé AM, Bertrand Y, Girot R, Soulier JP. Human parvovirus and aplastic crisis in chronic hemolytic anemias: a study of 24 observations. Am J Hematol 1986; 23: 271-275.*

11. *Davidson RJ, Brown T, Wiseman D. Human parvovirus infection and aplastic crisis in hereditary spherocytosis. J Infect 1984; 9: 298-300.*

12. *Young NS. Hematologic manifestations and diagnosis of parvovirus B19 infections. Clin Adv Hematol Oncol 2006; 4: 908-910.*

13. *Servey JT, Reamy BV, Hodge J. Clinical presentations of parvovirus B19 infection. Am Fam Physician 2007; 75: 373-376.*

14. *Chorba T, Coccia P, Holman RC, et al. The role of parvovirus B19 in aplastic crisis and erythema infectiosum (fifth disease). J Infect Dis 1986; 154: 383-393.*

15. *Smith-Whitley K, Zhao H, Hodinka RL, et al. Epidemiology of human parvovirus B19 in children with sickle cell disease. Blood 2004; 103: 422-427.*

16. *Guler E, Garipardic M, Dalkiran T, Davutoglu M. Premarital screening test results for β-thalassemia and sickle cell anemia trait in east Mediterranean region of Turkey. Pediatr Hematol Oncol 2010; 27: 608-613.*

17. *Cürük MA, Zeren F, Genç A, Ozavci-Aygün S, Kilinç Y, Aksoy K. Prenatal diagnosis of sickle cell anemia and beta-thalassemia in southern Turkey. Hemoglobin 2008; 32: 525-530.*

18. *Regaya F, Oussaief L, Bejaoui M, Karoui M, Zili M, Khelifa R. Parvovirus B19 infection in Tunisian patients with sickle-cell anemia and acute erythroblastopenia. BMC Infect Dis 2007; 7: 123.*

19. *Corcoran A, Doyle S. Advances in the biology, diagnosis and host-pathogen interactions of parvovirus B19. J Med Microbiol 2004; 53: 459-475.*

20. *Oh DJ, Lee YL, Kang JW, Kwon SY, Cho NS. Investigation of the prevalence of human parvovirus B19 DNA in Korean plasmapheresis donors. Korean J Lab Med 2010; 30: 58-64.*

21. *Sant'Anna AL, Garcia Rde C, Marzoche M, et al. Study of chronic hemolytic anaemia patients in Rio de Janeiro: prevalence of anti-human parvovirus B19 IgG antibodies and the development aplastic crises. Rev Inst Med Trop S Paulo 2002; 44: 187-190.*

22. *Zimmerman SA, Davis JS, Schultz WH, Ware RE. Subclinical parvovirus B19 infection in children with sickle cell anemia. J Pediatr Hematol Oncol 2003; 25: 387-389.*

23. *Nicolay N, Cotter S. Clinical and epidemiological aspects of parvovirus B19 infections in Ireland, January 1996-June 2008. Euro Surveill 2009; 14: 19249.*

24. *Us T, Ozune L, Kasifoglu N, Akgun Y. The investigation of parvovirus B19 infection in patients with haematological disorders by using PCR and ELISA techniques. Braz J Infect Dis 2007; 11: 327-330.*

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Detecting and reporting child abuse by health workers in Serbia

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Abstract

Introduction: The detecting and reporting cases of child abuse in Serbia today is closer determined by Special protocol provided by the special health care system for protection of children from abuse and neglecting, by Ministry of Health of the Republic of Serbia. This protocol was published in April 2009. However, it should be noted that this is yet an unexplored area in Serbia.

Aim: The aim of this study was to collect knowledge about detection and willingness to report child abuse, as well as how well health workers and whether they are educated at all for these procedures.

Methods: In this phenomenological study a quantitative method was used and as a technique for data collection a survey was used. As a research instrument a questionnaire with mostly closed questions was used. The collected data were analyzed quantitatively by the methods of descriptive statistics (analysis), while the answers to the open questions were analyzed qualitatively. The sample included doctors and nurses of both sexes from the two largest, and by organization similar child health institutions in Serbia: from the University Children Hospital (UDC) and the Institute for Health Protection of Mother and Child "Dr Vukan Cupic" (IMD). The study was conducted on a sample of 135 health professionals from different disciplines (specialization) of pediatric health care institutions previously mentioned.

Results: The study identified that only 20% of health workers recognized and reported cases of child abuse, and that out of the total number of health workers who had reported child abuse case, 69.4% were trained for it.

Conclusion: The research data indicate that only one fifth of health care workers who reported child abuse case, revealed them only by recognizing the symptoms of abuse. On the other hand, out of the total number of health workers who have reported child abuse cases, most of them were trained for it.

Key words: Detection, reporting, child abuse, health care workers.

Introduction

Health workers at any level of health care play an important role in the protection, primarily in the detection and reporting of child abuse [1,2]. Patronage nurse during home visits can detect cases of physical child abuse. They are a vital link between the child at risk or already abused child and the access to services and resources for protection from abuse [3]. Health workers from the emergency services are often the first medical experts who interact with the child that comes to the examination. Therefore, the observation of nurses in the waiting room can play an important role in detecting physical abuse [4]. Missed opportunity to recognize the abuse victim means that the proper diagnosis has not been made and that the child will return to the environment in which the abuse was made. Since the recognition (detection) of the abuse is one of the skills of health workers, many articles on child abuse intended for health professionals (Spencer, 2002; Joughin, 2003; Mulryan et al., 2004) have been published. The aforementioned sources are educational, informative for health workers how to recognize (detect) symptoms and signs of all kinds of abuse, including emotional abuse [5]. Contact and intervention of health professionals with children and their parents can minimize the trauma after sexual abuse [6].

The empathy of health workers for the problems of abused children in Serbia begins to increase during the 20-ies in twentieth century. The reasons for

this are numerous, and among them is the immediate encounter of a large number of health workers with children who have been exposed to violence in the war zone. Another very important reason is the activities of nongovernmental organizations that during the years of war and crisis strengthened [7].

Today the relationship of health workers and abused children in Serbia is closely determined by Special protocol provided by the special health care system for protection of children from abuse and neglecting, by Ministry of Health of the Republic of Serbia. This protocol was published in April 2009, and it was intended for health workers and health associates engaged in health care of children. The general goal of this Protocol is to protect children from all kinds of abuse [8].

By this Special protocol of the health care system for child protection from abuse and neglecting the health workers and health associates at all levels should be more included. Health workers are among the first where a child or a child's family members ask for help when a child is injured. Therefore, health workers are in a unique position to detect and report cases of child abuse [9].

Detection of cases of child abuse cases is the first and very important step for the further course and outcome of the health of the child. The role of health workers in detecting cases of abuse is great because practically all children come into contact with health workers. Health workers at all level of health care have an important role in detecting cases of abuse. Detection of child abuse can happen in two ways: by identifying violations or changes in child`s behavior, or by confession of the child, which can be directly (when abused children confide in health worker) and indirectly (when someone else confides in health worker and provides information about child abuse) [9].

Reporting cases of child abuse is the most important step in the process of protecting children from abuse. Beside the ethical, health workers have also the legal right and obligation (Article 253 of the Code of Criminal Procedure) to report child abuse case. Failure to report cases of abuse is punishable. The detected case of child abuse the health worker reports to the police and the competent social work center. A child whose life or health are at risk should not be left to the person suspected of being an abuser or conceals the abuser, but must be hospitalized until the competent authorities (police and experts from the Center for Social Work) arrive [9].

The research conducted in Serbia, which dealt with child abuse was oriented to the: extent, causes and manifestations of child abuse (Stojakovic, 1984, Svenson, 2007), the visibility of violence against children and the role of the individual services in the treatment of abused children (Milosavljevic, 1998), knowledge about the doctor's attitudes towards their own contribution to protecting children from abuse and position on mandatory reporting of abuse (Engineering, 2000), long-term consequences that violence has on victims of violence (Nikolic-Future, 2002), the level of protection of abused children and social work centers in the detection, assessment and treatment (Zegarac, 2004). Until this very day Serbia lacks in experienced research on detection and reporting of cases child abuse by health care workers [8]. This fact influenced the goal of our research.

Aim of paper

The aim of this study was to collect knowledge about detection and willingness to report child abuse, as well as education of health professionals for these procedures.

Methods

This phenomenological study used a quantitative method and a survey as a technique for data collection. As a research instrument a questionnaire with mostly closed questions was used. The collected data were analyzed quantitatively by the methods of descriptive statistics (analysis), while the answers to open questions were analyzed qualitatively. Some open questions were closed after the research, that is, the responses to the questions were grouped in categories.

The statistical methods applied in this study are:

- Descriptive analysis, which involves the application of statistical methods that deal with collection, assorting and analysis of the statistical data. Its aim is to describe the measured phenomenon.
- Chi-Squared test, which task was to establish a statistically significant difference

or relationship between two or more categorical variables that had normal levels of measurement.
- Data processing was performed by the computer statistical program PASW 18.0.

The sample included doctors and nurses of both sexes from the two largest, and by organization similar child health institutions in Serbia, from the University Children Hospital (UDC) and the Institute for Health Protection of Mother and Child "Dr Vukan Cupic" (IMD). The sample included a total of 94 doctors from the UDC and the IMD (which accounted for 51% of permanent staff physicians) and a total of 41 nurses from the emergency services including radiology technicians from the UDC and the IMD (which constitute 82% of total nurses employed in the aforementioned services). Total of 135 health professionals from different disciplines (specialization) of pediatric health care institutions mentioned. Collection of empirical data on the UDC and the IMD was conducted in the period from 8 April 2010 to 12 May 2010.

Results

Ways in which health workers detected reported cases of child abuse

Research data indicate that most respondents, that is 20% of them (27) that have reported cases of child abuse detected them by recognizing the symptoms of abuse. Only 2.2% of respondents (3) used as a way of discovering that the heard from a person escorting child that he/she was abused. There were no respondents who have used as a way of discovering the child`s confession about abuse. The results also revealed that there were respondents who had reported cases of child abuse detecting them by combination of two or all three ways of detecting cases of child abuse. The highest percentage of respondents 11.1% (15) detected cases combining all three modes of detection. Then, 4.4% (6) in detection combined the recognized symptoms with an information obtained by the child`s companion. It was 2.9% of respondents (4) that combined the recognized symptoms and the confession of the child. The least, that is 1.5% of respondents (2) in detection combined the story told by the child and his/hers companion.

It is important to note that 57.7% (78) of respondents did not discover cases of child abuse.

The relation of detection modes in cases of child abuse was analyzed in accordance to occupation of the respondent. This comparison reveals that over 25.5% of physicians (24) than nurses 7.3% (3) reported cases of child abuse detected by recognizing the symptoms of abuse. The above-mentioned reveals also that more physicians 14.9% (14) than nurses 2.4% (1) that had reported cases of child abuse revealed them in combination of all three modes of detection.

The relation between detecting modes of child abuse was analyzed, according to whether respondents were systematically trained to protect abused children. This comparison revealed the following data:
- in the group with the most respondents who, as a mode of detecting child abuse, used recognizing symptoms, 11.8% (16) of respondents were educated versus 8.2% (11) of respondents who were not educated,
- in the group of subjects who used combination of all three detection modes, 7.4% (10) of respondents were educated versus 3.7% (5) of the respondents who were not educated.
- in the group which as a detection mode used a statement of the child`s companion, there were less, that is 1.5% (2) respondents who were educated versus 3% (4) respondents who were not educated,
- in the group which as a detection mode used a statement of the child`s companion, there were more, that is 1.5% (2) educated respondents in relation to 0.7% (1) non-educated respondents,
- in the group which as a detection mode used a child`s statement there were more, that is 2.1% (3) educated respondents in relation to 0.7% (1) non-educated respondents,
- in the group which as a detection mode used both statement from a child and from a companion there were 1.5% (2) educated respondents while there were no non-educated respondents,
- there were no respondents who have used the child's statement as a detection mode.

Experience of health workers who have reported cases of child abuse

By researching we have come to the information and experiences of health workers who have reported suspected child abuse. Less, that is 57 respondents (42.2%) reported the suspicion about child abuse, compared to 78 subjects (57.8%) who did not report this suspicion.

The relation between occupation and the fact whether the respondents reported suspected child abuse was analyzed. Based on the results ($\chi^2 = 8.918$, df = 1, p = 0.002), significant statistical differences occur. It is obvious that more doctors (51.1%) than medical technicians and nurses report (22%) suspected child abuse. Then, the relation between the facility where respondents work and gender of the respondents and the fact whether the respondents reported suspected child abuse was analyzed. Based on the results, none of the cases marked statistically significant differences. The relation of respondents who reported suspected child abuse and the mode by which they detected reported cases was analyzed. Based on the results ($\chi^2 = 130.953$, df = 2, p = 0.000), significant statistical differences occur. Based on these results it is obvious that a small number, that is 27 respondents (20%) detected cases of child abuse by recognizing, and then reporting them.

A statistically significant difference was observed in relation between subjects who detected and reported cases of abused children compared to how many of these subjects were educated and trained for protection of abused children. Table 1, reveals that among respondents who reported cases of child abuse were more, that is 69.4% of those who have had training and education for protection of abused children, versus 26.7% of respondents who were not trained.

Most of the respondents, precisely 72 (54.1%) answered the question "Have you or your colleagues had a bad experience while reporting child abuse cases?" that they were not familiar with such cases. Then, 48 respondents (36.1%) answered that they or their colleagues had a bad experience in these cases. The least, that is, 13 subjects (9.8%) responded that neither they nor their colleagues had a bad experience in the mentioned cases. This question was not answered by two respondents (1.5%).

The relation between the fact whether the respondents reported cases of abused children and the answers to the question of whether the respondents

Table 1. Respondents who discovered and reported cases of abused children according to how they were trained for and protection of abused children

			Have you reported cases of child abuse?		Total
			yes	not	
Is he/she education by	he/she education by	Count	34	15	49
		%	69.4%	30.6%	100.0%
	had no education	Count	23	63	86
		%	26.7%	73.3%	100.0%
Total		Count	57	78	135
		%	42.2%	57.8%	100.0%

χ^2=23,268; df = 1; p = 0,000

Table 2. Report cases of abused children and the answer to the question of whether the respondents or their colleagues had a bad experience when logging

			Did they have a bad experience		Total
			there were no adverse experiences	negative experiences	
Are the reported cases	yes	Count	45	12	57
		%	78.9%	21.1%	100.0%
	not	Count	75	1	76
		%	98.7%	1.3%	100.0%
Total		Count	120	13	135
		%	90.2%	9.8%	100.0%

χ^2=14,387; df = 1; p = 0,000

or their colleagues had a bad experience when reporting cases of child abuse was analyzed. Based on the results, the statistically significant differences occur. The table 2, presents that the majority, that is 78.9% (45) of respondents who reported cases of abuse, had positive experiences while reporting it. Only 21.2% (12) of respondents who reported cases of abuse had a negative experience while reporting it.

The respondents were asked to, if they had or their colleagues had a bad experience while reporting cases of abuse, to state with whom this negative experience had happened. To this question that was left open, we received responses that the negative experience occurred with child`s companion or the Court. No one responded that they had a negative experience with health workers, employees in the centers for social work or police.

To the question whether you or one of your colleagues at work were exposed to parental violence (companion) for attempting to discover and / or report the case of child abuse, most respondents, that is 79 (59.4%) were not familiar with such cases; 35 subjects (26.3%) responded "no." The least, that is, 19 patients (14.3%) responded "yes." This question was not answered by 2 patients (1.5%).

The relation between the fact whether the respondents reported cases of child abuse and the fact that respondents or their colleagues were exposed to parental violence (companion) for attempting to detect and / or report the case of child abuse was analyzed. Based on the results, the statistically significant differences occur. Based on the results presented in Table 3, 68.4% of respondents who reported cases of child abuse had been exposed to some form of violence by parents (companion) for attempting to detect and / or report the case of child abuse. The same experience share 31.6% of respondents who did not report these cases and they have tried to do so.

The reasons why health workers did not report specific cases of child abuse

The majority, 106 respondents (78.5%) said they did not avoid reporting suspicions of child abuse, while 29 subjects (21.5%) stated that they have avoided, or that failed to report all suspected cases of child abuse.

By the research found out the data on the reasons why health workers did not report specific cases of child abuse. Uncertainty in the diagnosis of child abuse as a reason for not reporting was the most cited, that is 11 respondents (8.1%). Then there were 10 respondents (7.4%) who gave the following answers: "The case should be reported to the director," "Surgeon receives them, not me", "I note to the doctor", "To whom should I report, my job description does not allow that". Four respondents indicated the negative consequences that the child may have as a reason for not reporting (3%). Personal security as a reason for not reporting is also indicated in 4 respondents (3%). None of the respondents as a reason for not reporting cases of child abuse stated "I felt / I thought someone else would report it".

Discussion

Research data indicate that the majority of health workers, who have reported cases of child abuse, detected them by recognizing symptoms of abuse. It is also obvious that in almost all groups of health professionals who have discovered (no matter which way), and reported cases of child abuse there is a higher percentage of those who were trained for protection of abused children versus those who were not. The only exception is the group which as a detection mode used a statement of a companion, because it groups a smaller percentage of educated respondents compared to those who were not.

Table 3. Reporting cases of child abuse and exposure to some form of violence by parents

			Have you reported cases of child abuse?		Total
			yes	not	
Whether they were exposed to some form of violence	yes	Count	13	6	19
		%	68.4%	31.6%	100.0%
	not	Count	43	71	114
		%	37.7%	62.3%	100.0%
Total		Count	56	77	133
		%	42.1%	57.9%	100.0%

$\chi^2=6,297;\ \ df = 1;\ p = 0,012$

In practice, none of the respondents only used the child's statement (confession of a child) that was abused as a way to detect reported cases of child abuse. Based on this analysis we conclude that the detection of cases of child abuse is an important factor in training health professionals in that field.

By researching we have come to realize that almost half of health workers reported suspected child abuse. However, based on the analysis it is obvious that a small number of health workers discovered cases of child abuse by recognition after which the report followed.

Based on the analysis that included half of the respondents we can conclude that gender of the respondent had no influence on the work with abused children (reporting suspected abuse). The same result was revealed by some similar researches in the world [10, 11]. On the other hand, we have found that reporting of cases of child abuse depends on, that is - it is correlated with education of health workers in this field. Our results show that a high percentage of health workers who have reported cases of abuse were trained for protection of abused children.

This research showed that a high percentage of health workers who have reported cases of child abuse had a positive experience while reporting it. Only one fifth of health workers had negative experiences when reporting such cases, particularly with child`s companion/parent or the Court. These results seem encouraging, if we take into account that the detection and reporting of cases of child abuse by health workers depends on previous experience in the detection and reporting of such cases.

Among respondents who were exposed to some kind of violence by parents (companion) for attempting to detect and report cases of abuse, there were more than twice of those who reported case of abuse than those who did not.

Based on the results of research we found that about a fifth of health workers avoided to report suspicions of child abuse, that is did not report all suspected cases of child abuse. Research by Delerond conducted in 2000 in America showed data that 53% of doctors and 58% of nurses did not report all suspected cases of child abuse [12]. This means that we received data that are not in accordance with results of similar studies, that is, in our study, the reporting rate is higher than in other countries.

Most health workers as a reason for not reporting alleged the uncertainty of the diagnosis of child abuse. By comparison study that was conducted in Salzburg 1985 revealed data that 38% of doctors did not want to report a case of child abuse before being sure in the diagnosis [13]. This means that the data we have received are similar to this research study, with the exception of Austria, where the reporting rate is four times higher than in our country.

Conclusion

The research data indicate that only one fifth of health care workers who reported child abuse case, revealed them only by recognizing the symptoms of abuse. Even some studies [12, 14, 15] in the world have shown that a small number of health employees detects and reports cases of child abuse to the child protection services. On the other hand, out of the total number of health workers who have reported child abuse cases, most of them were trained for it. Our results support the results of research [16] which found that the health workers trained theoretically and practically for the protection of abused children successfully discovered and reported cases of abused children in relation to health care workers who werre not trained in this area. Then, studies conducted in the United Kingdom [17, 18] have shown that educational programs can overcome barriers related to the detection and reporting of cases of abused children. This knowledge suggests that the current training of health workers about protection of molested children would be more useful for reporting than for detecting cases of child abuse.

References

1. *Vines S, Williams-Burgess C. Effects of a community health nursing Parent-Baby (Ad) Venture Program on depression and other selected maternal-child health outcomes. Public Health Nursing, 1994; 11(3): 188-195.*

2. *Olds D, Robinson J, O'Brien R. and others, "Home Visiting by Paraprofessionals and Nurses: A Randomized, Controlled Trial," Pediatrics 100, 2002; 486-496.*

3. *Adams BL. Assessment of child abuse risk factors by advanced practice nurses. Pediatr Nurs, 2005; 31: 498–502.*

4. *Powell C. Protecting children in the accident and emergency department. Accident and Emegency Nursing, 1997; 5: 76-80*

5. *Keane C, Champan R. Evaluating nurses knowledge and skills in the detection of child abuse in the Emergency Department. International Emergenci Nursing, 2008; 16: 5-13*

6. *Mulvhill D. Nursing care of children after a traumatic incident. Comprehensive Pediatric Nrsing, 2007; 30: 15-28*

7. *Ишпановић-Радојковић В. Одакле смо пошли, докле смо стигли и куда идемо у заштити деце од злостављања и занемаривања. У Срна, Ј. уредник (2001) Од групе до тима. Београд: Центар за брак и породицу. 2001.*

8. *Мишић М. Злостављање деце и здравствени радници. Београд: Факултет за специјалну едукацију и рехабилитацију (магистарска теза). 2011.*

9. *Special protocol of health care system to protect children from abuse and neglecting (2009) Belgrade: Ministry of Health, Republic of Serbia.*

10. *Ashton V. The effect of personal characteristics on reporting child malltreatment. Child Abuse & Neglect, 2004; 28: 985-997*

11. *Срна Ј. Од групе до тима. Београд: Центар за брак и породицу. 2001.*

12. *Vieth V. A Call to End Child abuse in the United States Within 120 Years.in Gafner, R. (2006) Journal of Aggression, Maltreatment & Trauma. Binghamton: The Haworth Maltretment & Trauma Press. 2006.*

13. *Милосављевић М. Насиље над децом. Београд: Факултет политичких наука. 1998*

14. *Feng JY, Levine M. Factors associated with nurses' intention to report child abuse: a national survey of Taiwanese nurses. Child Abuse and neglecht, 2005; 29: 783-789*

15. *Garrusi B, Safizadeh H, Bahramnejad B. Physicians' Perception Regarding Child Maltreatment In Iran (IR) . The Internet Journal of Health. 2007; Volume 6 Number 2*

16. *Palusci V, McHugh M. Interdisciplinary training in the evaluation of child sexual abuse. Child Abuse & Neglect, 1995; 19(9): 1031-1038*

17. *Benger JR, McCabe SE. Burns and scalds in preschool children attending accident and emergency: accident or abuse? Emergency Medicine Journal, 2001; 18: 172-174*

18. *Benger JR, Pearse AV. Simple intervention to improve detetcion of child abuse in emergency departmens. British Medical Journal 324, 2002; 780-782*

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Do serum Zinc levels and oxidative status change in familial Mediterranean fever patients during attack and attack-free periods?

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Abstract

Objective: Familial Mediterranean fever (FMF) is a chronic autoinflammatory disease, and thus there may be an abnormality in the oxidative stress level of FMF patients. We aimed to determine Zinc (Zn) and oxidative stress levels in patients with familial Mediterranean fever (FMF) and compare these characteristics with those in healthy controls.

Method: Fifty patients with FMF during the attack period (AP) and 38 patients with FMF during attack-free periods (AFP) and 30 healthy controls were enrolled in the study. We determined oxidative status, including total antioxidant capacity (TAC), total oxidant status (TOS) and calculation of oxidative stress index (OSI), in the sera of FMF patients and healthy controls.

Results: Levels of Zn and TAC were found to be significantly lower ($p < 0.001$) in both the serum and whole blood of the FMF-AP group and FMF-AFP group when compared with the HC group. The Zn and TAC levels in the FMF-AP group were found to be markedly lower ($p<0.001$) compared with the FMF-AFP group. Levels of TOS and OSI were found to be significantly higher ($p < 0.001$) in both the serum and whole blood of FMF-AP group and FMF-AFP group compared with the HC group. The TOS levels and OSI values were found to be significantly higher ($P < 0.001$) in the FMF-AP group compared to the FMF-AFP group. In the FMF patient group, a significant positive correlation was observed between serum Zn levels and TAC.

Conclusion: Our study demonstrated that Zn levels were reduced in serum and oxidative stress was increased during AP in patients with FMF.

Key words: Familial Mediterranean fever, Zinc, oxidative stress, antioxidant status, attack.

Introduction

Familial Mediterranean feve (FMF) is an autosomal recessive disorder characterized by recurrent acute attacks of fever and inflammatory reactions of the serosal membranes. The most common clinical findings are peritonitis, articular complaints, and pleurisy accompanied by fever. Several non-specific immunological abnormalities and elevations in acute phase reactant levels have been observed during FMF attacks, but these usually return to normal in attack-free periods (AFP).[1-3]

Zn (Zn) is an intracellular signaling molecule and it plays an important role in cell-mediated immune functions and inproecting against oxidative stress. Zn is also an anti-inflammatory agent.[4] These unique properties mean that Zn may have significant therapeutic benefits in several diseases in humans, where concurrent Zn deficiency may complicate clinical features, adversely affect immunological status, increase oxidative stress, and increase the generation of inflammatory cytokines.[5] Oxidative stress and chronic inflammation may play important causative roles in many chronic diseases. Zn protects the cell from oxidation damage by free radicals. This may be due to several factors: its by stabilizing the cell membrane structure, maintaining an adequate level of metallothioneins (which are free radical scavengers), acting as an essential component of superoxide dismutase (SOD), acting as a protective agent for thiols, and in preventing the interaction between chemical groups with iron

to form free radicals, as well as by acting as an inhibitor of nicotinamide adenine dinucleotide phosphate oxidase (effective scavenger of radicals).[6-8]

FMF is a chronic autoinflammatory disease, and persistent respiratory burst caused by activated neutrophils may generate reactive oxygen species (ROS) in patients with this disease. Alteration in the oxidant-antioxidant profile is known to occur in rheumatic diseases. There have been studies in the literature on the possible role of FR/ROS in the pathogenesis of some rheumatologic conditions such as rheumatoid arthritis (RA), Behcet's disease, systemic lupus erythematosus etc.[9-13] We aimed to determine Zn and oxidative stress levels in patients with FMF and compare these characteristics with those in healthy controls.

Materials and methods

The study was conducted in the Faculty of Medicine of Cumhuriyet University, Departments of Internal Medicine-Rheumatology and Department of Biochemistry between December 2010- September 2011. The study protocol was approved by the Ethics Committee of the University. Written informed consent was obtained from all patients and controls.

The study groups consisted of 38 FMF patients in the attack-free period (AFP) (mean age: 29.9 ± 12.4 years; male/ female: 12/26), and 50 patients with FMF in the attack period (AP) (mean age: 32.8 ± 13.6 years; male/female: 21/29). The diagnosis of FMF was established according to the Tell-Hashomer criteria.[14] The disease durations were 5.5 ± 3.5 years and 5.6 ± 3.9 years for FMF-AFP patients and FMF-AP patients, respectively. Age and sex distributions and disease durations were similar in both groups. All patients were being treated with colchicine at the time. All FMF patients and age/ sex matched healthy controls (HC) were in the age range of 20 to 40 years. The HC group consisted of 30 individuals including 12 males and 18 females with no history of other potential health problems. Exclusion criteria were as follows: presence of systemic diseases, including chronic renal failure, diabetes mellitus, ischemic heart disease, and malignancy; trauma; heavy exercise; and use of drugs with potential effects on biochemical parameters.

In total, an 8 ml sample of venous blood was taken in the morning before breakfast from each patient who applied during AP or AFP to the Rheumatology Clinic. The blood samples of patients during FMF attack were obtained after 24-96 hours from acute attack initiation. Venous blood was taken from the HC, in the same way as in the FMF patients. The serum samples were obtained by centrifuging blood samples at 3,000 rpm for 15 min at 4°C. Afterwards serum samples were stored at -70°C until analysis.

High sensitivity C-Reactive Protein (hs-CRP), erythrocyte sedimentation rate (ESR), fibrinogen, albumin and white blood cell (WBC) count were measured on the same day as the venous blood samples were taken. Serum hs-CRP level was determined by the nephelometric method (Beckman Array 360 Protein System, Minnesota, Brea, CA, USA). The ESR was measured by the Westergreen method, and ESR was recorded within one hour. Fibrinogen levels were measured by the clotting time method (Beckman Coulter, Inc., Fullerton, CA, USA), and leukocytes were determined with an automatic hematology analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). Albumin was measured on a Synchron® LX 20 analyzer (Beckman Coulter, 95942 Villepinte–Roissy-CDG, France). Assay of serum Zn concentration was performed by direct colorimetric determination (Sentinel Diagnostics, Milani Italy). The total antioxidative capacity of plasma (TAC) levels and the total oxidative stress (TOS) were determined with a spectrophotometric kit (Rel Assay Diagnostics, Gaziantep, Turkey). The levels of TAC and TOS were assayed in an autoanalyzer (Beckman Coulter LX 20, Inc., Fullerton, CA, USA). The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit to TAC was converted to mmol/l, and the OSI value was calculated according to the following formula;[15] OSI (arbitrary unit) = TOS (μmol H2O2 Eq/l)/TAC (μmol trolox Eq/l)

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) 15.0 Package (SPSS Inc., Chicago, IL, USA). Values were expressed as means ± SD. The significance of the mean differences between groups was assessed by Student's t-test. Differences were assessed by Chi-squared test for categorical variables. Relationships between variables were tested using Pearson's correlation analysis. P values of less than 0.05 were regarded as significant.

Results

Demographic characteristics and acute-phase reactants values of the study groups are presented in Table 1. The mean age of FMF subjects in the FMF-AP and FMF-AFP groups were 32.86 ± 13.67 and 29.94 ± 12.45 years respectively while the mean age of those in the HC group was 30.2 ± 8.9 years. The disease durations were 5.5±3.5 and 5.6±3.9 years for the FMF-AFP and FMF-AP groups respectively. The groups were similar in terms of age and gender. Serum ESR levels were 14.48 ± 27.00 and 26.83 ± 27.75 mm/hr for FMF patients in AFP and FMF patients during acute attack. Serum hs-CRP levels were 3.12 ± 2.62 and 38.05 ± 43.93 mg/L for FMF patients in the attack-free period and FMF patients during acute attack (normal range for hs-CRP: 0-6). The ESR, hs-CRP and fibrinogen levels were significantly higher in the FMF-AP group than in the FMF-AFP and HC groups ($p<0.001$).

The serum Zn levels and TAC, TOS, and OSI values of the study groups are presented in Table 2. The Zn and TAC levels were found to be significantly lower ($P <0.001$) in the FMF-AP and FMF-AFP groups compared to the HC group. The TOS levels and OSI values were found to be significantly higher ($P <0.001$) in the FMF-AP and FMF-AFP groups compared to the HC group. The Zn levels were found to be significantly lower ($P <0.001$) in the FMF-AP group compared to the FMF-AFP group. However, the TAC values were not fount to be significantly different ($P > 0.05$) in the FMF-AP group compared to the FMF-AFP group. The TOS levels and OSI values were found to be significantly higher ($P <0.001$) in the FMF-AP group compared to the FMF-AFP group.

For the FMF patient group, significant positive correlations were observed between serum Zn levels and TAC and albumin levels ($r = 0.486$, $p<0.001$ and $r = 0.397$, $p<0.001$, respectively). Zn was observed to be negatively correlated with TOS, OSI, and hs-CRP level ($r = - 0.312$, $p < 0.001$; $r = - 0.345$, $p<0.001$ and $r = - 0.185$, $p<0.05$, respectively).

Discussion

In order to investigate the antioxidant status in FMF disease, we measured TAC, TOS and OSI. The serum TAC levels were significantly lower in patients with FMF compared to healthy controls;

Table 1. Demographic characteristics and acute-phase reactants of the groups. Results are expressed as mean ± SD of study groups

	FMF-AP group n=50	FMF-AFP group n=38	HC group n=30
Sex (male/female)	21/29	12/26	12/18
Age (years)	32.8 ± 13.6	29.9 ± 12.4	30.2 ± 8.9
Disease duration (years)	5.6 ± 3.9	5.5 ± 3.5	-
ESR (mm/hr)*	26.8 ± 27.7 [a,b]	14.4 ± 27.0	13.4 ± 22.6
hs-CRP (mg/dl)	38.0 ± 43.9 [a,b]	3.1 ± 2.6	2.8 ± 2.1
Fibrinogen (mg/dl)	324.7 ± 72.5 [a,b]	262.7 ± 45.5	282.7 ± 33.7
Leukocytes (/mm^3)	8746.0 ± 3170.7	7726.4 ± 4104.2	7604 ± 2845.2
Albumin (g/dl)	4.0 ± 0.6 [a,b]	4.4 ± 0.4[a]	4.7 ± 0.3
[a] Significantly different from HC at p<0.001 level, [b]Significantly different from FMF-AFP at p<0.001 level			

Table 2. Serum Zn levels and TAC, TOS, OSI values in different groups. Results are expressed as mean ± SD

	FMF-AP group n=50	FMF-AFP group n=38	HC group n=30
Zn (μg/dl)	58.26 ± 15.64 [a,b]	68.78 ± 13.58 [a]	95.20 ± 17.61
TAC	1.22 ± 0.24 [a]	1.17 ± 0.21 [a]	1.54 ± 0.18
TOS	17.46 ± 13.87 [a,b]	9.43 ± 5.79 [a]	3.34 ± 1.18
OSI	1.56 ± 1.45 [a,b]	0.83 ± 0.52 [a]	0.21 ± 0.08
[a] Significantly different from HC at p<0.001 level, [b]Significantly different from FMF-AFP at p<0.001 level			

also, the serum TOS levels and OSI values were significantly higher than those of the healthy controls. This confirms other reports of fluctuated or unchanged oxidant/antioxidant status in FMF disease.[16,17] In the literature there is evidence of increased oxidative stress in FMF patients both in remission and attack periods.[18-20] In the present study we observed increased oxidative stress in FMF patients both in remission and in attack periods.

In order to investigate Zn deficiency in FMF disease, we measured serum Zn levels. We found that FMF patients had significantly lower serum Zn levels than healthy controls. There are conflicting data in the literature about a lower serum Zn in inflammatory diseases such as Behçet's disease, RA and SLE.[21-23] Low serum Zn in FMF disease is probably due to increased consumption of Zn because the antioxidant activity of Zn may act as a free radical scavenger and/or decreased serum albumin levels and/or malnutrition.[24,25]

In the present work, in a comparison of findings in FMF patients during attack and remission periods, the TOS level and OSI were found to be significantly increased and showed no significant differences in TAC level in the attack period. Similar to our findings, Ediz et.al.[16] reported that FMF patients had significantly higher GSH, MDA and PC levels and no significant differences were found in activities of CATand GSH-Px in the acute attack period compared to the findings in the AFP. Guzel et. al.[17] reported that the T-SH level was found to be significantly increased and CuZn SOD activity significantly decreased in the attack period. The results of previous studies about oxidative stress in other inflammatory rheumatic diseases, such as RA and Behçet's disease, are in goodagreement with our findings for FMF. Noyan et al.[26] reported that MDA levels were significantly higher in active period patients compared Behçet's disease patients in the inactive period. In another study, a comparison of RA patients grouped according to disease activity revealed enhanced lipid, protein, DNA oxidation status and exhaustion of the antioxidant defence system in those with greater disease activity.[27] In the present study we observed increased oxidative stress in FMF patients in attack periods.

In a comparison of the findings in FMF patients during attack and remission periods, the Zn level was found to be significantly decreased in attack periods. In the present study, a correlation between serum Zn levels and oxidative stress parameters and hs-CRP levels was observed. The low level of Zn in patients with AP-FMF was probably due to the increase in inflammation like that seen in Behçet's Disease (21). In particular, increased expressions of intracellular metallo-thioneins following oxidative damage can sequester plasma Zn[28], and up-regulation of Zn-importing proteins by pro-inflammatory cytokines can also reduce the level of plasma Zn.[29] A negative correlation was observed between Zn levels and TOS, OSI and hs-CRP levels. A positive correlation observed between Zn levels and TAC and albumin levels. Guo et al.[30] reported that the Cu/Zn ratio was strongly correlated with oxidative stress and inflammation in patients on peritoneal dialysis.

In recent years, there has been great interest in the role of oxidative stress in the pathogenesis of inflammatory disease such as FMF, RA, Behçet's and Crohn's disease.[21,27,30] In many diseases concurrent Zn deficiency may complicate clinical features, adversely affect immunological status, increase oxidative stress and increase the generation of inflammatory cytokines.[4] Consequently, it is important that the status of Zn and oxidative stress is assessed and that Zn deficiency and oxidative stress are corrected in these inflammatory diseases. We conclude that plasma Zn, TAC, TOS levels and OSI values may be favorable markers of oxidative stress, inflammation, and immune status in FMF patients. Further studies are needed to determine the preventive and therapeutic effect of Zn and antioxidant supplementation in FMF patients.

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References

1. *Samuels J, Aksentijevich I, Torosyan Y, Centola M, Deng Z, Sood R, et al. Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. Medicine (Baltimore). 1998; 77: 268–97.*

2. *Chae JJ, Aksentijevich I, Kastner DL. Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. Br J Haematol. 2009; 146: 467-78.*
3. *Korkmaz C, Ozdogan H, Kasapcapur O, Yazici H. Acute phase response in familial Mediterranean fever. Ann Rheum Dis, 2002; 61: 79–81.*
4. *Ananda S. Prasad. Impact of the discovery of human Zn deficiency on health. Journal of the American College of Nutrition, 2009; 28: 257–265.*
5. *Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokines production and T cell subpopulations in experimentally induced Zn-deficient humans. Am J Physiol, 1997; 272: 1002–1007.*
6. *Ananda S. Prasad. Zn: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care, 2009; 12: 646–652*
7. *Davis SR, McMahon RJ, Cousins RJ. Metallothionein knockout and transgenic mice exhibit altered intestinal processing of Zn with uniform Zn-dependent Zn transporter-1expression. J Nutr 1998; 128: 825–831*
8. *Tapiero H, Tew KD. Trace elements in human physiology and pathology: Zn and metallothioneins. Biomed Pharmacother, 2003; 57: 399–411*
9. *Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am J Med, 1967; 43: 227–53.*
10. *Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford: Clarendon Press; 2007.*
11. *Rahman K. Studies on free radicals, antioxidants, and cofactors. Clin Interv Aging, 2007; 2: 219–236.*
12. *Ben-Chetrit E, Touitou I. Familial Mediterranean fever in the world. Arthritis Rheum, 2009; 61: 1447-53.*
13. *Tunca M, Akar S, Onen F. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine (Baltimore), 2005; 84: 1–11.*
14. *Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum, 1997; 40: 1879–85.*
15. *Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry, 2005; 38(12): 1103–1111.*
16. *Ediz L, Ozkol H, Tekeoglu I, Tuluce Y, Gulcu E, Koyuncu I. Increased oxidative stress in patients with familial Mediterranean fever during attack period. African Health Sciences, 2011; (S1): 6 – 13*
17. *Guzel S, Andican G, Seven A, Aslan M, Bolayirli M, Guzel EC, Hamuryudan V. Acute phase response and oxidative stress status in familial Mediterranean fever (FMF). Mod Rheumatol 2011. DOI 10.1007/s10165-011-0517-5*
18. *Kirkali G, Tunca M, Genc S, Jaruga P, Dizdaroglu M. Oxidative DNA damage in polymorphonuclear leukocytes of patients with familial Mediterranean fever. Free Radic Biol Med, 2008; 44: 386-93.*
19. *Karaguezyan KG, Haroutjunian VM, Mamiconyan RS, Hakobian GS, Nazaretian EE, Hovsepyan LM, et al. Evidence of oxidative stress in erythrocyte phospholipid composition in the pathogenesis of familial Mediterranean fever (periodical disease). Clin Pathol, 1996; 49: 453-5.*
20. *Sarkisian T, Emerit I, Arutyunyan R, Levy A, Cernjavski L, Filipe P. Familial Mediterranean fever: clastogenic plasma factors correlated with increased O2(-) production by neutrophils. Hum Genet, 1997; 101: 238-42.*
21. *Najim RA, Sharquie KE, Abu-Raghif AR. Oxidative stress in patients with Behcet's disease: I correlation with severity and clinical parameters. Journal of Dermatology, 2007; 34: 308–14.*
22. *Serce AF, Yilma MI. Trace elements and antioxidant enzymes in Behçet's disease. Rheumatol Int, 2002; 2: 93-96.*
23. *Ala S, Shokrzadeh M, Pur Shoja AM, Saeedi Saravi SS. Zn and copper plasma concentrations in rheumatoid arthritis patients from a selected population in Iran. Pak J Biol Sci. 2009; 15: 1041-4.*
24. *Maureen MB. Zn deficiency and child development. Am J Clin Nutr, 1998; 68: 464-9.*
25. *Michael H. Human Zn deficiency. J Nutr, 2000; 130: 1344S-1349S.*
26. *Noyan T, Sahin I, Sekeroglu R, Dulger H. The serum vitamin C levels in Behcet's disease. Yonesi Med J 2003; 44: 771–778.*
27. *Seven A, Guzel S, Aslan M, Hamuryudan V. Lipid, protein, DNA oxidation and antioxidant status in rheumatoid arthritis. Clin Biochem. 2008; 41: 538–43.*
28. *Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, Knutson MD, et al. Interleukin-6 regulates the Zn transporter Zip14 in liver and contributes to the hypoZnemia of the acute-phase response. Proc Natl Acad Sci USA, 2005; 102: 6843–8.*
29. *Haase H, Rink L. The immune system and the impact of Zn during aging. Immun Ageing, 2009; 6: 9.*
30. *Guo CH, Chen PC, Yeh MS, Hsiung DY, Wang CL. Cu/Zn ratios are associated with nutritional status, oxidative stress, inflammation, and immune abnormalities in patients on peritoneal dialysis. Clin Biochem, 2011; 44: 275-80*
31. *Kruidenier L, Kuiper I, Lamers CB, Verspaget HW. Intestinal oxidative damage in inflammatory bowel disease. Semi quantification, localization and association with mucosal antioxidants. J Path, 2003; 201: 28–36.*

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Cardiac diseases prediction and rule extract with data mining - Classification techniques

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Abstract

Objective: In this study data mining classification techniques Decision Trees, Artificial neural networks (ANNs), and Support Vector Machine (SVM) are analyzed on Coronary artery Disease (CAD) dataset. Finding interesting rules from a coronary artery disease data set is one of the objectives of this topic.

Background: CAD is one of the major causes of disability in adults and is one of the main causes of death in the developed countries that result in several illnesses, disability, and death as well. There is a wealth of data available within the healthcare systems. The aim of this research is to find useful information from coronary artery disease data set.

Methods and material: The data we study was collected from patients with coronary heart disease. 4948 patients (from 2005 to 2010) who had suffered from coronary artery disease were included in the analysis. The technique used in this paper is classification with three techniques namely, Decision Trees, SVM and Neural Network. Coronary artery disease is the target variable and 24 inputs or predictor variables are used for the classification. Performance of these techniques is compared through sensitivity, specificity, and accuracy. In our study results were achieved using average value of tenfold cross-validation for each algorithm.

Results: The mean age of the 2064 patients was 58.22±13.006 SD (values between15-94) and 54-64 years old was the most. The overall accuracy of the SVM was 96.41% in the training set and 81.5% in the validation set. The most significant factor influencing CAD is chest pain.

Discussion: All three algorithms have the ability to predict coronary artery disease with various degrees of confidence. SVM algorithm is the most useful for evaluation and prediction of CAD patients with a non-CAD. Elderly males (age>53) have high probability to be diagnosed with CAD.

Conclusion: Classification efficiency of different algorithms was evaluated. Although extracting rules could aid to early prediction of disease, further investigation with more features and larger data sets is still required.

Key words: Data mining, classification, coronary artery disease, rule extract, knowledge discovery, prediction.

Introduction

The term heart disease used to describe a number of illnesses that affect the circulatory system, which including heart and blood vessels(1). Heart disease was the major cause of deaths in the different countries including Iran. Coronary heart diseases and Cardiovascular disease are some categories of heart diseases (2). Cardiovascular disease (CVD) results in several illness, disability, and death. IT is one of the most important causes of death in the world especially in developed countries (3-5). The World Health Organization has estimated that 12 million deaths occur worldwide, every year due to the cardiovascular diseases (3). According to the Iranian Ministry of Health, 39.9 percent causes of deaths are due to coronary heart disease, which is considered the most common cause of death in the country so that occur one of every three deaths is due to cardiovascular disease (6). The cost of coronary artery disease in one year has imposed to the society has been estimated more than three hundred billion Rials ($30 million) (7).

Prevention and treatment of heart disease are becoming an essential and increasing global concern (3, 8). Dramatic increase in CVD and their complications, effects and high costs that enter on society, caused the medical community following plans for further evaluation, prevention, early detection and effective treatment. In the last decades, an impressive data amount about the cardiovascular factors and diseases have been stored in very large

databases. Many extensive epidemiological studies have been performed with the intention to detect factors that increase the risk of this cardiovascular disease (9).

Medical data mining has great potential for exploring the hidden patterns in the data sets of the medical domain. These patterns can be utilized for clinical diagnosis(2). Most of the time, the clinical decisions are made by physician's experience. All doctors are not experienced or expert. Systems with diagnosis support for unskilled and experienced doctors would be a guideline for clinical decision making(10). The aim of this study was to compare different data mining methods to evaluate the relationships between coronary artery disease variables and find the most important risk factors in coronary artery diseases.

Introduction to data mining

Today's, we see abundant data collected about the different disease with various intentions. During the last decade, a large volume of data and information have been stored electronically in databases. The results of this accumulation and integrating of data is that the organizations in data-rich and in the knowledge creation derived from them have become powerless. Integrating data in a wide spectrum rapidly have expanded and its rate is rapidly increasing, while practical use of the data resources are limited (11). The volume of medical data which stored electronically is growing every day but huge collections of these raw data by themselves are worthless which they should be analyzed and converted into information and knowledge. Enormous rapid growth of databases almost in every area of human efforts has created great demand for new powerful tools for turning data into useful, task-oriented knowledge. In efforts to satisfy this need, researchers have explored ideas and methods developed in machine learning, pattern recognition, statistical data analysis, data visualization, neural nets, etc. These efforts have emerged a new research area, often called data mining and knowledge discovery (12).

Finding undiscovered information and useful patterns in a database is often referred to as data mining (3). The process of knowledge discovery in databases (KDD) consists of sequenced steps, including problem understanding, data understanding and preparation, data mining, result interpretation and evaluation, and finally the use of induced knowledge (9). The basic step of knowledge extract is converting data into knowledge to help in decision making, called data mining(4). Data mining has a wide use in the healthcare domain such as diagnoses and patient management (13). Due to the wide availability of huge amounts of data and the imminent need for turning such data into useful information and knowledge, data mining has attracted a great deal of attention in the information industry (14). Extracting effective rules from a database is a beneficial but a challenging effort which would help physicians administer the therapeutic process (15). Relationships, rules and essential information about or from the data cannot be easily extracted because of database size and more features. Data mining is focused on discovering these rules and relationships (16). Important variables and significant rules identification also classification techniques are used in this study.

Data Mining Algorithms

There are various data mining techniques available with their suitability in health care domain. DM techniques have its own advantages and disadvantages when applied to a variety of problems. Several DM algorithms such as classification which performed successfully in the medical field are used in this research. Classification is one of the important techniques of data mining. Assign a class to find previously unseen records as accurately as possible is the aim of classification (17). We used some of the most common predictive data mining methods (18) for our goals.

Artificial Neural Networks (19)

Duo to their predictive good performance, are most popular in various area of medicine(20, 21) and lead to appropriate decisions (22). ANN consists of many connected processing elements including multiple input nodes and weighted interconnections. "Connections may be from input to hidden/output layer (feed-forward) or in both directions (feedback)"(23).

Support Vector Machines (SVM)

A category of classification which has been paid attention to in recent years is called SVM. It's a new method for the classification of both linear

and nonlinear data (24) and in terms of predictive accuracy is a powerful algorithm(18). In fact, SVM is a linear learning machine constructed through an algorithm that uses an optimization criterion (25).

Decision Tree

It is a graphic representation of obtained knowledge in the form of a tree where each non leaf node denotes a test on an attribute and each branch indicates an output of a test (29). Due to nodes and branches organized hierarchically, they are easy to construct, to interpret. They are reliable and have a better accuracy in clinical decision making (22, 30). Besides this structure, it produces an exclusive rule set. C5 and CART decision tree are most current decision tree algorithms.

Related research

Automated diagnosis of CAD based on decision support system (DSS) was done by Tsipouras et al. Four-stage methodology (fuzzy model and optimization, decision tree induction and crisp model formulation) was proposed for the automated creation of the DSS. Of 199 subjects, 89 were normal and CAD was present in the other 110 subjects and 19 features selected. Results show gender is the most important feature in the produced set of rules and optimized fuzzy model DSS improves by 15% in terms of accuracy the results of the crisp rule-based classifier and finally the proposed DSS is automatically generated using the proposed data-driven methodology; only an initial annotated data set is needed in order to create a DSS for a specific domain of application (31).

A model Intelligent Heart Disease Prediction System (IHDPS) built with the aid of data mining techniques like Decision Trees, Naïve Bayes and Neural Network was proposed by Sellappan Palaniappan et al. IHDPS was capable of answering queries that the conventional decision support systems were not able to. It facilitated the establishment of vital knowledge, e.g. patterns, relationships amid medical factors connected with heart disease. IHDPS subsists well-being web-based, user-friendly, scalable, reliable and expandable (32).

Karaolis et al developed a data mining system based on decision trees for the assessment of CHD related risk factors targeting in the reduction of CHD events. The events investigated through this study were: MI, PCI, and CABG. Three classification models were developed based on decision trees for classifying these patients (5).

Ordonez using association rules and the C4.5 decision tree algorithm for the prediction of cardiac disease based on 25 medical attributes documented that decision trees are not as powerful as association rules to exploit a set of numeric attributes manually binned and categorical attributes and several related target attributes. Constrained association rules can be an alternative to other statistical and machine learning techniques applied in medical problems (33).

Diagnosis of Coronary Heart disease (CHD) using Bayesian networks is performed by Twardy et al. Bayesian networks (BNs) and their application to Medical domains were investigated. They evaluated and compared these BNs, comparing them to each other and to a suite of other machine learning methods (34).

Logistic regression with backward stepwise elimination has been used to develop a predictive model for coronary artery disease in patients presenting to a chest pain unit with a normal or non-diagnostic ECG. Variables independently associated with the presence of coronary diseases were typical pain, aspirin use, diabetes and age with odds ratios of 1.9, 3.2, 1.7 and 2.1 respectively. A risk score obtained by combining these 4 factors was related to the occurrence of a clinical event during the patient's stay in the chest pain unit, to coronary artery disease prevalence (which varied from 3.9% in those with a score of 0 to 66.7% in those with a score of 4), to all-cause mortality, and to the development of acute coronary syndrome during the 6-month follow up period(35).

Artificial Neural network methods based on presenting clinical data have been applied in the diagnosis of myocardial infarction. Heir use was purported to avoid the delay in treatment associated with current practice of assessing serial ECG and biomedical data. Neural network derived model were shown to be no more effective than appropriately optimized logistic regression models(36). In many study artificial neural networks have been used to predict cardiovascular diseases (36-42).

Table 1. Coronary Artery Disease data set

Attribute	Description	Range Mean ± Stdv
P rate	Pulse rate in beats per minute (BPM)	Numerical(30-150) 75.9±10.2
age	Age in years	Numerical(15-94) 58±3
creat	Serum Kreatinine in mg/dL	Numerical(0.2-11.6) 1.2±0.55
Attribute	**Description**	**Class code (1=calss1; 2= class2, …)**
sex	gender	1= male; 0=female
fbs	Fasting blood sugar in mg/dl	1=70-100; 2=101-126; and 3= more than>= 127
chol	Serum cholesterol in mg	1=<= 200; 2=between200-239; and 3= above240
Hgb(50)	Hemoglobin gm/ml	1= (Hb >= 13.5 and Hb <= 18 and age>17 and sex=1) or (Hb >= 12 and Hb <= 16 and age>17 and sex=0) or (Hb >= 11 and Hb <= 16 and age < 17)= normal; 2=(Hb< 13.5 and age>17 and sex=1) or (Hb < 12 and age>17 and sex=0) or (Hb <= 11 and age < 17)= low level; and 3= (Hb >18 and age>17 and sex=1) or (Hb > 16 and age>17 and sex=0) or (Hb > 16 and age < 17)= high level
HDL	high-density lipoprotein in mg/dL	1= best>=60; 2= poor(HDL <= 40 and sex=1) or (HDL <= 50 and sex=0); and 3= better level (between 40-59 for men and 50-59 for women)
LDL	low-density lipoprotein in mg/dL	1= optimal<=100; 2= near optimal100-129; 3= border line high130-159; 4= high160-189; and 5= very high>=190
trig	Triglyceride in mg/dl	1=less than 150; 2=150-199; 3= 200-499; and 4= >= 500
Marital s	Marital status	0=Single 1=married
dm	Diabetes mellitus	1= history of diabetes; 0= no such history
hyp	Hypertension in mmHg	0= no; 1= yes
FH	Family History of coronary disease	0= no; 1= yes
PH	Past history of heart disease	0= no; 1= yes
disld	Dyslipidemia	0= no; 1= yes
smoking	Smoker or not	2=current 3= past 4= recent 5= never
EF	Ejection fraction	1=good(50-75) 2=fair(30-49) 3=poor(<30)
CP	Chest Pain	2= yes; 3=no
Sys bp	Systolic blood pressure Mm/Hg	1=hypotension =<90; 2=desirable =90-119; 3=border line hypertension =120-139; and 4=hypertension >=140
Dia bp	Diastolic blood pressure Mm/Hg	1= hypotension <60=; 2=desirable =61-79; 3=border line hypertension =80-89; and 4= hypertension >=90
EET	Exercise Stress Test	0= normal; 1= abnormal
comorbidity	Absence or presence of one or more disorders as well as a primary disease	0= no; 1= yes
ST and T Change	ST segment and T wave of ECG changes	0= normal; 1= having ST-T wave abnormality
Diagnosis (dependent variable)	Coronary artery disease diagnosed by physicians through ECG	0= no; 1=yes

Materials and Methods

Patient Population

For this study, the population consisted of 4,890 patients from the University and Teaching Hospital, Rajaei Cardiovascular Center in Tehran, Iran who had admissions for heart disease-related diagnoses during the period from July 18, 2006 to December 30, 2011. The data set was stored in the structured query language (SQL) database. Patients with coronary artery disease were included in the study. In order to diagnose the presence or absence of CAD, Coronary angiography or fluoroscopy was performed. All coronary angiograms were visually assessed by experienced angiographers. Significant CAD was defined as at least one 50% or greater diameter stenosis in at least one coronary artery vessel. This medical data set contains 25 attributes that have been taken corresponding to the numeric and categorical attributes. Exactly, 93% of patient data was extracted from these databases because patient record's data such as Identification were unavailable from the data set. Table 1 demonstrates features with acceptable class and values. A list of variables thought to be associated with CAD was produced. A checklist was developed from this list. And these variables were reviewed by a cardiovascular specialist to decide which of those held for predicting CAD. Then, they were organized into two groups including (1) categorical and (2) numerical. The method used in this approach includes crisp.

Data pre-processing

The data set is highly noisy due to the diversity of patients' history, physical, and clinical classes then, we have tried to efficiently preprocess the data set using data mining preprocessing techniques, and obtained good experimental results. Generally, pre-processing of input variables is a vital step in any data mining task. The first task of pre-processing is handling patient records with missing and outlier data. For cleaning data base, we did following tasks: first we deleted repeated features records, records cleaned that have spelling errors, and additional tokens and other irregularities and irrelevancies are excluded. Scaling and coding features are given in Table 1. Data coding were provided by the participating two cardiovascular specialists and are as coded by valid resources such as heart disease associations. Then, several policies have been adopted to detect outlier and filling the missing values.

Dealing with Missing value

The hospital data set is faced with many features that have missing values. The missing data accounts for a lot in this data set. Several replacement strategies have been adopted to fill the missing values; First strategy, If a feature was encountered in more than 50% of records with missing values, in this situation, characteristic cannot be an effective feature in the analysis; As a result such feature was removed, e.g. Weight and job features.

Table 2. Attribute with missing and alternative value

Attribute	missing data (%)	method; alternative value(s)
Age	0	-
Sex	0	-
Marital status	0	-
EET	0	-
Diabetes	0	-
Hypertension	0	-
Dyslipidemia	0	-
Family history	0	-
Comorbidity	5.24	Mode; 0
EF	4.36	Mode; 1
Diagnosis	2.17	Mode; 1
HgB	8.86	Mode; 2 (for class1)
		Mode; 2 (for class2)
		Mode; 2 (for class3)
Kreatinine	11.2	Mean; 1.9(for class1)
		Mean; 1.25(for class2)
		Mean; 1.18(for class3)

Second policy; if a feature was encountered in less than 12% of records with missing values, Mean values of records was replaced instead of the missing values in the numeric features, such as kreatinine with 11.2% missed that mean value was replaced according to its accepted class (table 2). If the feature is a nominal or ordinal type, Mode values are replaced. The comorbidity, EF, HgB and diagnosis features followed mentioned rule (table 2).

Third strategy; Classification algorithms such as C5.0 with 10fold cross validation and 20 boost rap applied to estimate missing values that fea-

tures containing missing values in more than 10% of records. Table 3 demonstrates these features with the accuracy of the estimated model.

Table 3. Number of features with missing data values and accuracy result

Features	missed data (%)	accuracy result (%)
HDL	32.73	96.77
LDL	16.58	94.15
Pulse rate	25.38	mean error 0.7*
Systolic BP	24.65	97.89
Diastolic BP	24.65	96.86
Chest pain	21.08	97.61
Cholesterol	19.68	97.89
FBS	18.08	91.7
Triglyceride	16.97	95.81
Smoking	26.59	97.24

**Was calculated by regression*

Dealing with Outlier

In data set may be some features have outlier in some of the records. Outlier is desired as a real value for a feature in the record. Outlier in the data set has a negative effect on the accuracy of the algorithms, so it is necessary to find these values and resolve them. Outlier values investigated for each feature separately. First we transformed data to excel and SQL format and detected outlier data that was clear using sorting methods. At later stage, analysis has been done over the numerical characteristics. If attribute values follow normal distribution; And values are located between the range of (3σ, 5σ) and interval (-5σ,-3σ), in which the σ is standard deviation of attribute values in existing records, these values are recognized as outlier. And if the value is greater than 5σ-5σ or less, this case is recognized as extreme amounts.

If the values do not follow a normal distribution; the Box Chart is used to identify extreme values and outlier. These points are recognized based on the quartiles concept. The values that are between 25% and 75%, in fact, being located between the first and third quartile, are not considered as an outlier.

Normality distribution values of these characteristics have been investigated using Minitab14. The results showed that features are not normally distributed. The records included in the extreme values are excluded from the analysis. To determine the outlier values in any feature, it has been replaced with the nearest acceptable value of the features.

After cleaning and preprocessing, 2064 completed records were obtained for data mining tasks.

Separating data into training and testing sets is an important part of evaluating data mining models. We partitioned data set into a training set and testing set, 80% of the data is used for training, and 20% of the data is used for testing.

Statistical Analysis

The mean age of the 2064 patients was 58.22±13.006 SD (values between15-94) and 54-64 years old was the most. The sample was composed of 1266 (61.34 %) men and 798 (38.66 %) women. 1264 (61.24%) of population was patient with CAD and 800 (38.76) was patient without CAD. Appendix A demonstrates more detail about statistical results of the data set.

Results

These data mining models were developed using data mining classification tools. Once we evaluated the model created using the training data then its results was compared with the results using test data.

The performance of a diagnostic method is usually described as classification accuracy, sensitivity and specificity. These measures are based on the number of true positive (TP), true negatives, false positive (FP) and false negative (FN) and are defined as follows:

Accuracy = TP + TN / All patients
Sensitivity = TP / patients with the disease
Specificity = TN / patients without the disease

The true positives are all patients with the disease and positive test result, whereas the true negatives are all patients without the disease and negative test result (43).

Table 3 shows the sensitivity, specificity and accuracy for different classification techniques. The overall accuracy of the SVM was 96.41% in the training set; it was 81.5% in the validation set as shown in Table 4.

Table 4. Analysis of data with coronary artery disease data set

Algorithm	Accuracy %	Specificity %	Sensitivity %
Decision Tree (C5.0)	84.54	65	97
C&RT	70.36	53	82
QUEST	67.92	52	78
CHAID	70.24	64	71
Neural Network	68.05	65	72
SVM	96.41	95	96
Designed algorithm	95.92	93	98

Table 5. Variable importance for classification of the CAD

Algorithms	Important factors
C&RT tree	1-chest pain 2- past history 3-comorbidity 4-hypertension 5- age 6-sex 7-EF 8- pulse rate 9- systolic blood pressure 10- LDL 11- triglyceride 12-Hgb
QUEST	1-Chest pain 2- comorbidity 3-age 4-EF 5-kreatinine 6-pulse rate 7- HgB 8- systolic blood pressure 9- FBS 10-dyslipidemia
CHAID	1-chest pain 2- past history 3-EF 4-comorbidity 5- age 6- sex 7-ST 8- diabetes 9-dyslipidemia 10-hypertension 11- cholesterol
C5.0	1-chest pain 2- age 3- past history 4-comorbidity 5-FBS 6-EF 7- triglyceride 8-hgb 9- family history 10- LDL 11-ST 12-sex
SVM	1-Chest pain 2- past history 3- FBS 4-EF 5-HB 6- marital status 7- cholesterol 8-HDL 9-diabetes 10- diastolic BP 11-smoking 12- sex
Neural network	1-Chest pain 2- comorbidity 3-age 4-smoking 5- FBS 6- EF 7- triglyceride 8- HgB 9- dyslipidemia 10- past history 11- ST 12-Sex

The most significant factor influencing CAD that obtained from all algorithms is chest pain and was included in all induced techniques. As shown in table 4, other important risk factors are comorbidity, pass history, hypertension, age, sex, smoking and ejection fraction.

Based on the decision tree model, sample rules were extracted from this predictive model and confirmed by two cerebrovascular doctors. However, more investigation with more features and larger data sets is still required.

Diagnosis classification rules are interpreted in table 5. Rules are presented in two parts: 1) those which express the absence of CAD disease (with 5 rules) and 2) those that shows there exists CAD disease (with 4 rules). The most significant or interesting rules are set in order by number.

Table 6. Selected Rules by cardiovascular Specialists

Rule No.	Rules for CAD subjects
1.	if Chest Pain = 2.000 and comorbidity = 1.000 then 1.000
2.	if age>53 and Chest Pain=2.000 and CEF=1.000 and CST=1.000 and sex=1.00 then 1.000
3.	if Pulse Rate <= 88 and CST = 1.000 then 1.000
4.	if age > 53 and Family History = 0.000 and Chest Pain = 2.000 and CHB = 2.000 then 1.000
5.	if age > 70 and Pass History = 1.000 and Smoking = 2.000 and Pulse Rate <= 100 then 1.000
Rule No.	**Rules for healthy subjects**
1.	if Hypertension = 0.000 and comorbidity = 0.000 and Cholesterol = 2.000 and CEF = 1.000 and CST = 0.000 then 0.000
2.	if age > 42 and age <= 53 and Smoking = 5.000 and comorbidity = 0.000 and CLDL = 1.000 then 0.000
3.	if sex = 1.000 and age <= 44 and comorbidity = 0.000 and Cholesterol = 1.000 and Diastolic B.P = 2.000 then 0.000
4.	if Hypertension = 0.000 and Pulse Rate <= 88 and comorbidity = 0.000 and Cholesterol = 1.000 and HDL = 3.000 and ST = 0.000 then 0.000

Discussion

There are different data mining techniques that can be used for the prediction of cardiovascular disease. Using data mining in the analysis of medical data is a good opportunity to investigate the relationships between variables. All three algorithms have the ability to predict coronary artery disease with various degrees of confidence. The present

study demonstrates that the SVM algorithm is the most useful for evaluation and prediction of CAD patients with a non-CAD. Similar study confirms this finding (44). This algorithm can be adopted as a model, which helps the physicians in evaluating CAD. On the other hand, we can apply this model to pre-diagnose the possibilities of a CAD if a person has risk factors. Despite the simplicity of decision tree, results with acceptable accuracy presented in the field of data mining in relation to coronary artery disease and came out to be second best. The neural network did not correctly predict CAD subjects with disease diagnosis or healthy. The same study confirms this subject (45).

There have been many studies of risk-factors in cardiac disease (5, 19, 45-48). Most were derived from the UCI date set and may not necessarily apply to local or regional practice. Many risk factors have been associated with coronary artery disease. Although different risk factors were obtained from the algorithms investigated, chest pain and past history were the major influence factors. In other words CP variable has the most effect in CAD presence or absence. Some of the features identified as important in other multivariate analysis, such as age and sex. The absence of comorbidity, hypertension (45) and smoking too is supported by other studies.

Application of data mining in analyzing the medical data is a good method for considering the existing relationships between the variables. Accurate data, suitable preprocessing and suitable data mining technique will offer reasonable results in medical data mining (46).

From the obtained results, it is clear that the elderly males (age>53) have a high probability to be diagnosed with CAD. It is consistent with Tsipouras study(31). Another important risk factor for CAD is the smoking consumption. It has also been demonstrated that smoking has on effect on the CAD prediction in men; 61% of nonsmoker men did not suffer from CAD (105/171) and 64% of smokers had CAD (469/730).

The absence of hypertension, moderate cholesterol, not smoking and appropriate levels of LDL and HDL were very important factors that had a major role in staying subjects healthy. So controlling and bringing down the blood pressure, appropriately and relatively low levels of LDL, non-smoking and tobacco consumption and regular daily activities can help to reduce the amount of risk for coronary heart disease. In investigating the results, it is interesting to note that patients having chest pain were assumed to have as CAD subjects in all algorithms. This represents the highly important influence of chest pain to early diagnosis of cardiac patients. However, As it was shown, It is noteworthy that the the absence of chest pain cannot be an indicative of the health of coronary arteries. Obviously, in the medical field the diagnoses are basically dependent on physician's experience; hence some extracted rules may be not accepted easily. However, by taking the knowledge of medical treatment, more effective knowledge and rules can be obtained (15).

Conclusion

In this paper, we have presented several algorithms for coronary artery disease diagnosis and prediction using data mining techniques. Firstly, we have extracted significant rules from the coronary artery disease data set for the efficient prediction of disease based on the sensitivity and accuracy indicators.

As we mentioned earlier, the coronary artery diseases database consisting of 2064 cases has been used in this study. For predicting CAD, 25 attributes are listed, which included their demographic data, clinical results, and diagnoses. Three classification efficiency indicators commonly used in the medical field, sensitivity, specificity and accuracy, were employed in this study to evaluate the efficiency of classification models. We think extracted rules could aid, as useful knowledge for physicians, to early prediction of disease and consequently decrease CAD morbidity and perhaps, mortality. If risk factor such as Hypertension, EF, LDL, Cholesterol, smoking and HDL was controlled, CAD risk of a subject may be reduced significantly.

Limitation

Few medical databases are made available to researchers. Medical data are generally not structured and it is spread in various locations. This is a reason confirms the fact that multiple scaled medical databases need much effort during earlier phases of the KDD process. In these circumstances, some DM techniques manifest difficulties in

data handling or the results generate poor performances in one or more applied techniques (49). It should be noted that the results of our study based on a partly small population and its generalization to the larger population need to more studies and large samples with more variables.

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Reference

1. *Rajkumar1 A, Reena GS. Diagnosis Of Heart Disease Using Datamining Algorithm Global Journal of Computer Science and Technology 2010; 10(10): 39-43.*
2. *Soni J, Ansari U, Sharma D. Predictive Data Mining for Medical Diagnosis: An Overview of Heart Disease Prediction. International Journal of Computer Applications2011; 17(8): 43-8.*
3. *Jilani TA, Yasin H, Yasin M, Ardil C. Acute Coronary Syndrome Prediction Using Data Mining Techniques-An Application. International Journal of Computational Intelligence2009; 5(4).*
4. *Patil BS, Kumaraswamy YS. Intelligent and Effective Heart Attack Prediction System Using Data Mining and Artificial Neural Network. European Journal of Scientific Research2009; 31(4): 642-56.*
5. *Karaolis MA, Moutiris JA, Hadjipanayi D, Pattichis CS. Assessment of the Risk Factors of Coronary Heart Events Based on Data Mining With Decision Trees. IEEE Transactions on information technology in biomedicine 2010; 14(3).*
6. *Cardiovascular disease is the most common cause of death in the country. Tehran: Ministry of Health and Medical Education; 2009; Available from: http: // www.behdasht.gov.ir/index.aspx?siteid=1&pageid=24979&newsview=12090.*
7. *Soltanian AR, Mahjub H, gudarzi S, Nabipur I, Jamali M. Five year survival rate in patients with acute myocardial infraction in Bushehr. Scientifin journal of Hamadan university of medical science2007; 16(3): 33-7.*
8. *Limbu YR, AMaskey, Bahadur M, Malla R, Sharma D, Shrestha NK. A study on cardiovascular disease pattern of admitted cases in newly emerged national heart centre. Journal of Nepal Medical Association2002; 41: 284-8.*
9. *Gamberger D, Lavrac N, Krstacic G. Active subgroup mining: a case study in coronary heart disease risk group detection. Artificial Intelligence in Medicine 2003; 28(1): 27-57.*
10. *Anbarsi.M, Aanupria.E., N.CH.S.N.iyengra. Enhanced Prediction of Heart Disease with Feature Subset Selection using Genetic Algorithm. International Journal of Engineering Science and Technology 2010; 2(10): 5370-6.*
11. *Shahrabi J, Shakurniaz V. data mining in Sql Server. first ed. Tehran: AmirKabir University brabch; 2009.*
12. *S. Sumathi, Sivanandam SN. Introduction to Data Mining and its Applications. In: Kacprzyk J, editor. Studies in Computational Intelligence, Volume 29: Springer; 2006.*
13. *Liu P, Lei L, Yin J, Zhang W. Healthcare data mining: predicting inpatient length of stay. 3rd International IEEE Conference Intelligent Systems, September 2006; 2006. p. 261-6.*
14. *Han J, Kamber M. Data mining: concepts and techniques: Morgan Kaufmann; 2006.*
15. *Hara A, Ichimura T, Yoshida K. Discovering multiple diagnostic rules from coronary heart disease database using automatically defined groups. Journal of Intelligent Manufacturing2005; 16(6): 645-61.*
16. *Markuzon N, Welsch R, Flietstra BC. A data mining approach for acoustic diagnosis of cardiopulmonary disease. The Department of the Air Force: Massachusetts Institute of Technology; 2008.*
17. *Kaur H, Wasan S. Empirical Study On Applications Of Data Mining Techniques In Healthcare. Journal of Computer Science2006; 2(2): 194-200.*
18. *Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. international journal of medical informatics2008; 77(2): 81-97.*
19. *Wilson P, D'Agostino R, Levy D, Belanger A, Silbershatz H, Kannel W. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97(18): 1837.*
20. *Schwarzer G, Vach W, Schumacher M, Universitet S. On the misuses of artificial neural networks for prognostic and diagnostic classification in oncology. Statistics in medicine2000; 19(4): 541-61.*
21. *Rani KU. Analysis of Heart Diseases Dataset using Neural Network Approach. Arxiv preprint arXiv: 111026262011.*
22. *Kaur H, Wasan SK. Empirical study on applications of data mining techniques in healthcare. Journal of Computer Science2006; 2(2): 194-200.*

23. *Chang KC, Tseng MC, Weng HH, Lin YH, Liou CW, Tan TY. Prediction of length of stay of first-ever ischemic stroke. Stroke2002; 33(11): 2670-4.*
24. *Kamath C. Scientific data mining: a practical perspective: Siam; 2009.*
25. *Wang J. Encyclopedia of data warehousing and mining: Idea Group Reference; 2006.*
26. *Sumathi S, Sivanandam S. Introduction to data mining and its applications: Springer-Verlag New York Inc; 2006.*
27. *Berner ES. Clinical decision support systems: theory and practice: Springer Verlag; 2007.*
28. *Witten IH, Frank E, Hall MA. Data Mining: Practical machine learning tools and techniques: Morgan Kaufmann; 2011.*
29. *Kajabadi A, Saraee MH, Asgari S, editors. Data mining cardiovascular risk factors2009: IEEE.*
30. *Sitar-Taut DA, Sitar-Taut AV. Overview on How Data Mining Tools May Support Cardiovascular Disease Prediction. Journal of Applied Computer Science 2010; 4.*
31. *Tsipouras MG, Exarchos TP, Fotiadis DI, and, et, al. Automated Diagnosis of Coronary Artery Disease Based on Data Mining and Fuzzy Modeling. IEEE Transactions on information technology in biomedicine2008; 12(4): 447-58.*
32. *Palaniappan S, Awang R. Intelligent heart disease prediction system using data mining techniques. IJCSNS2008; 8(8): 343.*
33. *Ordonez C. Comparing association rules and decision trees for disease prediction. ACM; 2006. p. 24.*
34. *Twardy C, Nicholson A, Korb K, McNeil J. Data mining cardiovascular bayesian networks. Monash University, School of Computer Science & Software Engineering, Melbourne2004; 165.*
35. *Martínez-Sellés M, Ortiz J, Estévez Á, Andueza J, de Miguel J, Bueno H. A new risk score for patients with a normal or non-diagnostic ECG admitted to a chest pain unit. Revista Espanola de Cardiologia2005; 58(7): 782-8.*
36. *Harrison RF, Kennedy RL. Artificial Neural Network Models for Prediction of Acute Coronary Syndromes Using Clinical Data From the Time of Presentation. Annals of Emergency Medicine2005; 46(5): 431-9.*
37. *Baxt W. Use of an artificial neural network for data analysis in clinical decision-making: the diagnosis of acute coronary occlusion. Neural Computation 1990; 2(4): 480-9.*
38. *Das R, Turkoglu I, Sengur A. Diagnosis of valvular heart disease through neural networks ensembles. Computer Methods and Programs in Biomedicine2009; 93(2): 185-91.*
39. *Das R, Turkoglu I, Sengur A. Effective diagnosis of heart disease through neural networks ensembles. Expert Systems with Applications2009; 36(4): 7675-80.*
40. *Lapuerta P, Azen SP, LaBree L. Use of neural networks in predicting the risk of coronary artery disease. Computers and biomedical research1995; 28(1): 38-52.*
41. *Shen Z, Clarke M, Jones R, Alberti T. A new neural network structure for detection of coronary heart disease. Neural Computing & Applications 1995; 3(3): 171-7.*
42. *Turkoglu I, Arslan A, Ilkay E. An intelligent system for diagnosis of the heart valve diseases with wavelet packet neural networks. Computers in biology and medicine2003; 33(4): 319-31.*
43. *Anamika G, Naveen K, Vasudha B. Analysis of Medical Data using Data Mining and Formal Concept Analysis. World Academy of Science, Engineering and Technology2005; 11: 61-4.*
44. *Kumari M, Godara S. Comparative Study of Data Mining Classification Methods in Cardiovascular Disease Prediction. International Journal of Computer Science and Technology2011; 2(2): 304-8.*
45. *Catherine F. Risk factors for coronary artery disease and the use of neural networks to predict the presence or absence of high blood pressure. BMC Genetics 2003; 4: 1-6.*
46. *Kajabadi A, Saraee MH, Asgari S. Data mining cardiovascular risk factors. Application of Information and Communication Technologies; Baku: IEEE; 2009. p. 1-5.*
47. *Beaglehole R, Magnus P. The search for new risk factors for coronary heart disease: occupational therapy for epidemiologists? International journal of epidemiology2002; 31(6): 1117-22.*
48. *Ellen P. application of data mining techniques in the prediction of coronary artery disease: use of anesthesia time series and patient risk factor data Australia: Queesland university of technology; 2009.*
49. *Andrei D, Viviana A. Overview on How Data Mining Tools May Support Cardiovascular Disease prediction. Journal of Applied Computer Science & Mathematics: 2010; 8 (4).*
50. *Lundin M, Lundin J, Burke H, Toikkanen S, Pylkkonen L, Joensuu H. Artificial neural networks applied to survival prediction in breast cancer. Oncology2000; 57(4): 281-6.*

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Appendix A. Statistical result of data set features

variable	value	Proportion %	Feature count with diagnosis class (0,1)	variable	value	Proportion %	Feature count with diagnosis class (0,1)
Sex	0 1	38.66 61.34	354= 0, 44= 1 446= 0, 820= 1	HDL	1 2 3	2.47 81.64 15.89	18=0, 33=1 649=0, 1036= 1 133= 0, 195= 1
Marital status	0 1	8.62 91.38	72= 0, 106= 1 728= 0, 1158= 1	FBS	1 2 3	42.34 31.06 26.6	346= 0, 528= 1 254= 0, 387= 1 200= 0, 349= 1
Pass history	0 1	72.38 27.62	518= 0, 976= 1 288= 0, 570= 1	Systolic BP	1 2 3 4	.34 26.07 58.91 14.68	4=0, 3= 1 242=0, 296= 1 426=0, 790= 1 128=0, 175=1
Diabetes	0 1	71.51 28.49	597= 0, 879= 1 203= 0, 385= 1	Diastolic BP	1 2 3 4	.82 37.94 53.25 7.99	10=0, 7= 1 323=0, 460= 1 396=0, 703= 1 71=0, 94= 1
Hypertension	0 1	55.23 44.77	441= 0, 699= 1 565= 0, 924= 1	EF	1 2 3	47.96 41.71 10.31	413=0, 577= 1 278= 0, 574= 1 100=0, 113= 1
Family history	0 1	89.05 10.95	723= 0, 1115= 1 77= 0, 149= 1	ST change	0 1	64.43 35.56	563= 0, 767= 1 237= 0, 497= 1
Dyslipidemia	0 1	58.82 41.18	511= 0, 703= 1 289= 0, 561= 1	HgB	1 2 3	25.29 74.42 .29	232= 0, 290= 1 563=0, 973= 1 -
Smoking	2 3 4 5	33.14 19.67 5.14 42.05	26=0, 422= 1 139= 0, 267= 1 31= 0, 75= 1 368= 0, 500= 1	Triglyceride	1 2 3 4	63.71 17.15 18.27 .87	526= 0, 789= 1 132= 0, 222= 1 137=0, 240= 1 5=0, 13= 1
Chest pain	2 3	74.95 25.05	495= 0, 4052= 1 305= 0, 212= 1	LDL	1 2 3 4 5	64.78 18.9 9.74 4.8 1.79	534= 0, 803= 1 150= 0, 240=1 73=0, 128= 1 30= 0, 69= 1 13= 0, 24= 1
Comorbidity	0 1	58.19 41.81	540= 0, 661= 1 260= 0, 603= 1	Cholesterol	1 2 3	79.46 13.52 7.03	647= 0, 993= 1 106= 0, 173= 1 47= 0, 98= 1
EET	0 1	96.61 3.39	772= 0, 1222= 1 28= 0, 42= 1				

The compare of effect of exhaustive exercise on MDA, ADA, GSH and SOD activity in women

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Abstract

The purpose of this study was to determine the changes of Malondialdehyde (MDA), Glutathione (GSH), Adenosine Deaminase (ADA) and Superoxide Dismutase (SOD) levels with pre and post acute exercise in obese middle-aged women (body mass index, BMI ≥30.0). Twenty four middle-aged women participated in this study. Subjects were divided as obese and non-obese group. The descriptive statistics of the participants were computed and the significance between the pre and post exercise levels of MDA, GSH, ADA and SOD values was determined. The results showed that obesity and acute exercise causation oxidative stress in obese women. Especially in obese women the increased levels of MDA and ADA levels may pose serious health problems. Obesity breaks down the anti oxidant defense mechanism of the body by effecting the GSH and SOD activities. That is why sudden and exhaustive exercises in obese people may have adverse effects on health.

Key words: Adenosine deaminaze, acute exercise, obese women, anti-oxidant.

Introduction

Obesity is gradually becoming a major heath concern all over the world. Obesity is an independent atherosclerosis risk factor [1]. The anomalies in body weight, body fat percentage and body mass index (BMI) cause difficulty in physical activity during exercise and increase the amount of oxygen consumed [2,3]. During a strenuous exercise the rate of metabolism increases almost 100 times of the resting condition depending upon intensity of the muscle activity [4,5]. The increased amount of oxygen increases the amount of superoxide anion formed in mitochondria. This anion forms reactive oxygen species known as free radicals. Free radicals may causation oxidative stress depending upon intensity and the duration of exercise [6-8] It is thought that lipid peroxidation occurs if amount of free radicals surpasses the amount of anti oxidants responsible for immunity of cell [9]. Malondialdehyde (MDA), an important lipid peroxidation product which occurs as a result of oxidative injury is used as indicator of oxidative stress [5]. Oxidative stress is known to cause many diseases as well as DNA and cell damage [10-13].

Regular moderate exercise has been proposed due to health benefits, including an increase of maximum oxygen consumption and decreased risk of obesity, cardiovascular disease and metabolic syndrome. However, acute exercise increases oxidative stress because, during exercise, increased strain of transporting oxygen to active muscles may damage cellular components. Especially, oxidative stress by induced exercise in obese higher than non-obese following exercise [14]. Numerous reports have documented increases in by-products of lipid peroxidation, especially malondialdehyde (MDA) following exercise. Saiki et al. reported that obese individuals have greater oxidative stress following acute treadmill exercise than non-obese counterparts. Santos-Silva reported that resting MDA levels were higher in trained adolescent swimmers compared to control group. However, not all studies have shown increases in MDA levels especially in response to exercise training. Miyazaki et al. [15] reduction of indices of oxidative stress following strenuous endurance training. These beneficial effects in response to exercise training imply a link between oxidative stress and increased antioxidant defense. Increased levels of blood antioxidant activity at rest were found in trained subjects. In addition, this antioxidant activity was further increased following exercise training.

Physical exercise has been associated with a re-

duction in cardiovascular morbidity and mortality [16-18]. One mechanism that may underlie this beneficial effect involves an up regulation of endothelial nitric oxide synthase (eNOS), increasing local production of nitric oxide [18]. Paradoxically, exercise also increases total body oxygen uptake, increasing production of reactive oxygen species [19]. The manner in which vasculature adapts to this oxidant stress remains unclear. In view of the important role of superoxide dismutase (SOD) in modulation of nitric oxide bioactivity, we examined the hypothesis that nitric oxide itself might modulate SOD expression. Thus, controlling amount of O_2^- is critically important for preserving nitric oxide bioactivity in vessel wall. SOD represent a major cellular defense against O^{2-} and formation of peroxynitrite [20,21]. Adenosine is known to regulate myocardial and coronary circulatory functions. Adenosine not only dilates coronary vessels, but attenuates beta-adrenergic receptor-mediated increases in myocardial contractility and depresses both sinoatrial and atrioventricular node activities [22].

The purpose of this study was to determine MDA, Glutathione (GSH), Adenosine Deaminase (ADA) and Superoxide Dismutase (SOD) levels in the blood acute exercise in obese (body mass index, BMI ≥30.0) and non-obese women. Based on priority studies, we hypothesized that acute exercise not only would augment oxidant parameters but also would decreased antioxidant status in obese women compare to non-obese women.

Material and method

Participants

The study was participated 24 women. Participants were divided into 2 groups: obese group (OG n= 12) BMI of 32.99±1.83 (kg/cm^2), age 36.70±4.59 (years) and height of 164.60±4.29 (cm) and control group women (NOG) BMI of 23.30±1.25 (kg/cm^2), age of 34.20±3.40 (years) and height 161.40±3.90 (cm). Participants' descriptive data are presented in table 1.

Including criteria

The dietary habits of groups were similar. The economic and socio-cultural status was the same. The participants were chosen according to the following criteria a being a non-smoker and non-drinker b) having no apparent health defect c) using no medication or anti-oxidant supplementation such as vitamins. The participants were adequately briefed and motivated about importance of the study. The participants signed a volunteering form warning them about conditions to be complied. The blood samples of the participants were taken by a doctor. The study was approved by İnönü University Ethical Committee.

Exercise Protocol

Exercise was performed on a treadmill regulated between 07.30 and 10.30 a.m. after an overnight fast. All participants were done Bruce Exhaustive Test Protocol by changing the slope and the velocity of the treadmill in three minutes intervals.

Blood collection and Biochemical parameters analyses

There were 10 cc venous blood samples from left arms of samples at sitting position with use of plastic syringes before and after exercise. All blood samples were allowed to clot room temperature and centrifuged at 3,500 rpm (4°C) for 15 minutes, resultant serum was then harvested. Serum samples were frozen and stored at -80°C until finally analyses.

ADA activity was measured in μmol/l using method developed by Ellis and Goldberg [23]. GSH determination was made in μmol/l by use of the method developed [24]. The plasma MDA level was measured nmol/l with use Uchiyama and Mihara method [25]. The method is based upon conver-

Table 1. All participants of descriptive data

Parameters	Obese Group (12)		Non-obese Group (12)	
	X	Sd	X	Sd
Age (years)	36.70	4.59	34.20	3.40
Height (cm)	164.60	4.29	161.40	3.90
Body weight (kg)	89.55	3.27	60.10	3.50
Body Mass Index (BMI) (kg/cm^2)	32.99	1.83	23.30	1.25

sion of superoxide radicals, formed by total (Cu–Zn and Mn) SOD (EC 1.15.1.1) via xantin oxidase, into H_2O_2 which reduces the nitrolue tetrazolium salt into a blue colored formazan which gives a maximum absorbance at 560 nm [26]. The data were given in u/l.

Statistical analyze

Before statistical analyses were done, the normality of distribution of data was evaluated by kolmogorof-smirnov test. Whole values were indicated as mean ± Standart Deviation (Sd). Biochemical parameters were compared difference between pre and post exercise using paired sample t-test. All data were measured by use SPSS 17.0 version for Windows and $p \leq 0.05$ was accepted as level of statistically significant.

Results

In this study the descriptive statistics of some of physical and physiological parameters of participants were computed and significance between pre and post exercise levels of MDA, GSH, ADA and SOD values was determined

For the differences between MDA, GSH, ADA and SOD values of OG before and after acute exercise are shown with paired t-test in Table 2. It was found that difference between MDA, GSH and ADA values pre-test and post-test significantly different ($p<.05$). Furthermore, there was no significant in serum SOD enzyme activity pre- and post-test in obese women ($p>.05$).

The values of MDA, ADA, GSH and SOD enzyme activity pre- and post-test on does not affect acute exercise in non-obese group ($p>.05$).

Discussions

In this study were investigated the effects of acute exercise on oxidant and antioxidant status in obese and non-obese women. The result of this study indicated that MDA concentrations significantly increased immediately after acute exercise in obese women. Whereas, non-obese women MDA concentrations not significant. It is known that MDA causes oxidative stress and increases lipid peroxidation depending on the obesity that increased lipid peroxidation has been reported in obese individuals following acute submaximal [27-29] and maximal exercise [30,31]. When the oxygen consump-

Table 2. Analyses of pre and post exercise MDA, GSH, ADA and SOD levels of obese group

Parameters		N	X	Sd	X_1-X_2 %	p
MDA	Pre-test (X_1)	12	19.63	2.93	15.20	
	Post-test (X_2)	12	22.61	3.51		**.029***
GSH	Pre-test (X_1)	12	31.38	1.33	4.63	
	Post-test (X_2)	12	29.93	1.48		**.042***
ADA	Pre-test (X_1)	12	15.98	4.63	16.35	
	Post-test (X_2)	12	18.58	5.17		**.013***
SOD	Pre-test (X_1)	12	1.61	.31	0.25	
	Post-test (X_2)	12	1.59	.44		.894

($p<0.05$)

Table 3. Analyses of pre and post test MDA, GSH, ADA and SOD levels of non-obese group

Parameters		N	X	Sd	X_1-X_2 %	p
MDA	Pre-test (X_1)	12	18.98	3.27	8.6	
	Post-test (X_2)	12	20.61	3.48		.165
GSH	Pre-test (X_1)	12	27.38	1.98	0.1	
	Post-test (X_2)	12	26.93	2.11		.843
ADA	Pre-test (X_1)	12	16.38	4.79	0.9	
	Post-test (X_2)	12	17.88	4.85		.582
SOD	Pre-test (X_1)	12	1.54	.45	0.2	
	Post-test (X_2)	12	1.50	.43		.774

($p<0.05$)

tion is low superoxide radicals and its derivatives are eliminated by anti oxidant immunity reaction. However, in case of excessive oxygen consumption defensive mechanism may not cope with increased free radical concentration which may result in cell damage [12,13]. Some of studies claim that acute exercise has no effect on serum MDA concentration value [32,33] and the acute exercise has no effect on MDA concentrations and oxidant conditions of obese women [34]. The data obtained in this study contradict with literature results. Özçelik et al. emphasize that acute exercises have a significant effect on MDA values [35]. It was also stated that obesity increases oxidative stress which shows parallelism with this study [36].

GSH, is an anti oxidant molecule in mammalians which provides protection against oxidative stress. The GSH value was reported to increase as a result of acute exercise in literature, because this seems to be one of most reliable shows exercise-induced oxidant production [7,37,38]. Revan et al. [39] noted a decrease in serum blood GSH after acute exercise. Di-Renzo et al. [2] reported a significant decrease in blood decreased GSH level immediately after acute exercise in obese women [2]. The findings of the present study indicate that after acute exercise significant decrease in obese women.

The studies in literature state that SOD value does not change with acute exercises [40,41]. These results show a good accordance with our data. However, it was indicated that acute exercise causes a significant increase in SOD values which contradicts with results obtained in this study [7,42].

Our study revealed that acute exercises increased ADA values in obese women ADA increases activity of rate determining enzyme purine nucleotide cyclus and decreases activity of adenosine. This change contributes the increase in post exercise activity adenosine activity of obese women. Adenosine was reported to have a cardio protective effect [43]. According to the literature survey there are no studies where ADA activity and oxidative stress were investigated together. In this context the ADA activity is very important to show the effect of exercise in future studies.

Conclusion

In conclusion acute exercise may causation oxidative stress more in obese women than non-obese women. Especially in obese women increased levels of MDA and ADA levels may pose serious health problems. The obesity breaks down anti oxidant defense mechanism of body by effecting GSH activities. That is why sudden and exhaustive exercises in obese people may have adverse effects on health. Therefore, obese women should be done rather than acute exercise as much as possible regular exercise. However there are further studies with larger groups needed to clarify this.

References

1. *Hen K, Bogdanski P, Szulinska M, Jablecka A, Pupek-Musialik D (2010) Influence of regular physical activity on oxidative stress in women with simple obesity. Pol Merkur Lekarski 28(166): 284-88*

2. *Di-Renzo L, Galvano F, Orlandi C, Bianchi A, Di-Giacomo C, La-Fauci L, Acquaviva R, De-Lorenzo A (2010) Oxidative stress in normal-weight obese syndrome. Obesity 18(11): 2125-30*

3. *Karaouzene N, Merzouk H, Aribi M, Merzouk SA, Berrouiguet AY, Tessier C, Narce M (2011) Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: A comparison of older with young men. Nutr Metab Cardiovasc Dis 21: 792-799*

4. *Urso ML, Clarkson PM (2003) Oxidative stress, exercise and antioxidant supplementation. Toxicology 189: 41-54*

5. *Karacan S, Çetin F, Çolakoğlu FF (2010) The effect of calisthenic exercise bout on oxidant and anti-oxidant status in middle aged an postmenopausal women. Isokinetics and Exercise Science 18: 39-44*

6. *Naziroğlu M, Kilinç F, Uğuz AC, Celik O, Bal R, Butterworth PJ, Baydar ML (2010) Oral vitamin C and E combination modulates blood lipid peroxidation and antioxidant vitamin levels in maximal exercising basketball players. Cell Biochem Funct 28(4): 300-05.*

7. *Aguilo A, Tauler P, Fuantespina E, Tur JA. Cordova A, Pons A (2005) Antioxidant response to oxidative stress induced by exhaustive exercise. Physiol Behav 84: 1-7*

8. *Sirmali M, Uz E, Sirmali R, Kilbaş A, Yılmaz HR, Altuntaş I, Naziroğlu M, Delibaş N, Vural H (2007) Protective effects of erdosteine and vitamins C and E combination on ischemia-reperfusion induced lung oxidative stress and plasma copper and zinc levels in a rat hind limb model. Biol Trace Elem Res 118(1): 43-52*

9. *Silva LA, Pinho CA, Silveira PC, Tuon T, De Souza CT, Dal-Pizzol F, Pinho RA (2009) Vitamin E supplementation decrease muscular and oxidative damage but not inflammatory response induced by eccentric contraction. J Physiol Sci 60: 51-57*

10. *Fisher-Wellman K, Bell HK, Bloomer RJ (2009) Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid Med Cell Long 2(1): 43-51*

11. *Antoncic-Svetina M, Sentija D, Cipak A, Milicic D, Meinitzer A, Tatzber F, Andrisic L, Zelzer S, Zarkovic N (2010) Ergometry induces systemic oxidative stress in healty human subjects. Tohoku J Exp Med 221(1): 43-8*

12. *Elahi MM, Kong YX, Matata BM (2009) Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev 2(5): 259-69*

13. *Essick EE, Sam F (2010) Oxidative stress and autophagy in cardiac disease neurological disorders, aging and cancer. Oxid Med Cell Longev 3(3): 167-77*

14. *Vincent HK, Innes KE, Vincent KR (2007) Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes Metab 9(6): 813-39*

15. *Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Haga S, Ji LL, Ohnı H (2001) Strenuous endurance training in human reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 84: 1-6*

16. *Spector SL. Surette ME (2003) Diet and asthma: Has the role of dietary lipids been overlooked in the managemen of asthma? Ann Allergy Asthma Immunol 90(4): 371-377*

17. *Ellwood P, Asher MI, Björkstén B, Burr M, Pearce N. and Robertson CF (2001) Diet and asthma, allergic rhino conjunctivitis and atopic eczema symptom prevalence: An ecological analysis of the International Study of Asthma and Allergies in Childhood (ISAAC) data. Eur Respir J 17: 436-443*

18. *Cheng J, Peng G, Zhang Q. Deng H (2002) Preliminary clinical study on the correlation between allergic rhinitis and food factors. Journal of Clinica Otorhinolaryngology 16: 393-396*

19. *Allen S, Britton JR. Leonardi-Bee JA (2009) Association between antioxidant vitamins and asthma outcome measures: Systematic review and meta-analysis. Thorax 64(7): 610-912*

20. *De Batlle J, Garcia-Aymerich J, Barraza-Villarreal A. and Antó JM, RomieuI I (2008) Mediterranean diet is associated with reduced asthma and rhinitis in Mexican children. Allergy 63(10): 1310-1316*

21. *Woods RK, Thien FC. Abramson MJ (2003) Dietary marine fatty acids (fish oil) for asthma in adults and children. Cochrane Database Syst Rev 3: CD001283*

22. *Kaur B, Rowe BH. Arnold E (2009) Vitamin C supplementation for asthma. Cochrane Database Systematic Reviews 21(1): CD000993*

23. *Ellis G, Goldberg DM (1970) A reduced nicotinamide adenine dinucleotide-linked kinetic assay for adenosine deaminase activity. J Lab Clin Med 76(3): 507-17*

24. *Fairbanks V, Klee GG (1986) Biochemical aspects of hematology. In: N. W. Tietz Editor Ttextbook of clinical chemistry. W. B. Saunders, Philadelphiz*

25. *Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal Biochem (34): 271-8*

26. *Sun Y, Larry W, Oberley W, Ving U (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34: 497-500*

27. *Vincent HK, Morgan JW, Vincent KR (2004) Obesity exacerbates oxidative stress levels after acute exercise. Med Sci Sports Exerc 36: 772-9*

28. *Vincent HK, Bourguignon CM, Vincent KR, Weltman AL, Bryant M, Taylor AG (2006) Antioxidant supplementation lowers exercise-induced oxidative stress in young overweight adults. Obesity 14(12): 2224-35*

29. *Lwow F, Dunajska K, Tworowska U, Jedrzejuk D, Laczmanski L, Milewicz A, Szmigiero L (2007) Post-exercise oxidative stress and obesity in postmenopausal women: the role of beta3-adrenergic receptor polymorphism. Gynecol Endocrinol 23(10): 597-603*

30. *Vincent HK, Vincent KR, Bourguignon C, Braith RW (2005) Obesity and postexercise oxidative stress in older women. Med Sci Sports Exerc 37(2): 213-9*

31. *Vincent HK, Bourguignon C, Vincent KR (2006) Resistance training lowers exercise-induced oxidative stress and homocysteine levels in overweight and obese older adults. Obesity 14(11): 1921-30*

32. *Bloomer JR, Cole JB (2009) Relationship between blood lactate and oxidative stress biomarkers following acute exercise. Open Sports Medicine Journal 3: 44-48*

33. *Munoz-Marin D, Olcina G, Tımon R, Robles RC, Caballero MJ, Maynar M (2010) Effect of different*

exercise intensities on oxidative stress markers and antioxidant response in trained cyclists. J Sports Med Phys Fitness 50(1): 93-8

34. *Melton CE, Tucker PS, Fisher-Wellman KH, Schiling BK, Bloomer RJ (2009) Acute exercise does not attenuate postprandial oxidative stress in prediabetic women. Phys Sportsmed 37(1): 27-36*

35. *Özçelik O, Karataş F (2008) Effects of Incremental Exercise Test on Serum Malondialdehyid and Vitamin A E C Levels in Obese Subjects. Fırat Univ J Health Sci 22(6): 337-341*

36. *Codoner-Franch P, Tavarez-Alonso S, Murria-Estal R, Tortajada-Girbes M, Simo-Jorda R, Alonso-Iglesias E (2012) Elevated advanced oxidation protein (AOPPs) indicate metabolic risk in severely obese children. Nutr Metab Cardiovasc Dis 22(3): 237-43*

37. *Sen CK (2001) Update on thiol status and supplements in physical exercise. Can J Appl Physiol 26: 4-12*

38. *Deminice R, Sicchieri T, Payao PO, Jardao AA (2010) Blood and salivary oxidative stress biomarkers following an acute session of resistance exercise in humans. Int J Sports Med 31(9): 599-603*

39. *Revan S, Balci SS, Pepe H, Kurtoğlu F, Erol AE, Akkuş H (2010) Short duration exhaustive running exercise does not modify lipid hydroperoxide glutathione peroxidase and catalase. J Sports Med Phys Fitness 50(2): 235-40*

40. *Skarpanska-Stejnborn A, Basta P, Pilaczynska-Szczesnizak L, Horoszkiewicz-Hassan M (2010) Black grape extract supplementation attenuates blood oxidative stress in response to acute exercise. Biol Sport 27(1): 41-46.*

41. *McClean MC, McNeilly A, Trinick T, Duly Ellie, Murphy HM, McLaughlin AJ, Burge G, Davison WG (2009) Acute exercise and impaired glucose tolerance: Effects on glycaemic control, oxidative stress and arterial stiffness. Med Sci Sports Exerc 41(5): 36-41*

42. *Pinho RA, Silva LA, Pinho CA, Scheffer DL, Souza CT, Benetti M, Carvalho T, Dal-Pizzal F (2010) Oxidative stress and inflammatory parameters after an ironman race. Clin J Sport Med 20(4): 306-11.*

43. *Kinugawa T, Fujita M, Ogino K, Kato M, Osaki S, Igawa O, Shigemasa C, Hisatome I, Kitakaze M (2006) Catabolism of adenine nucleotidesfavors adenosine production following exercise in patients with chronic heart failure. J Card Fail 12(9): 720-5*

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Association of *PPARG* gene polymorphism (Pro12Ala) with type 2 diabetes in the Iranian population

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Abstract

Purpose: The present study aimed to investigate the relationship between Pro12Ala polymorphism and T2DM occurrence in Iranian population.

Methods: Totally, 80 cases and 80 healthy matched controls were included in the study. PCR-RFLP technique was applied to investigate the polymorphism. Data were analyzed by $\chi 2$ test using SPSS software.

Results: The G allele (Ala 12) may has a modest and protective association with the development of T2DM in Iranian population (OR=2.117; 95% CI: 1.016-4.455; *P*= 0.022) while, Pro allele may consider as a risk factor to make individual prone to T2DM (OR=0.472; 95% CI: 0.224-0.984; *P*= 0.022). There was a 1.9-fold increase in the risk of T2DM associated with the CC allele (adjusted OR: 1.94, 95% CL 0.92–4.01). In contrast, there was no association between the CG polymorphism and the risk of T2DM (adjusted OR: 0.51, 95% CL 0.24– 1.03)

Conclusions: The *PPARG* gene polymorphism has a modest association with the development of T2DM in Iranian patients.

Key words: *PPARG*, Pro12Ala polymorphism, PCR-RFLP technique, Type 2 diabetes.

Introduction

Diabetes is the most prevalent non-infectious disorder of endocrine glands with an increasing rate of incidence [1]. According to the international health organization's report, frequency of diabetic patients is expected to rise up to 333 million people in 2025 [2].

Middle Asia has been regarded as the epidemic center of Type 2 diabetes mellitus (T2DM) [3, 4]. T2DM is the most common metabolic disorder with complicate interplays between genetic and environmental factors. The disorder is the end-stage of chronic and progressive deficiency owing to insulin resistance, functional decrease of pancreatic β cells and the increased production of glucose by the liver [5,6]. Diabetic high blood sugar can negatively affect small arteries (such as neuropathy, nephropathy and retinopathy) and large arteries (such as cardiovascular disease) and may bring about outcomes including kidney insufficiency, blindness and non-thrombosed amputate of body limbs [7]. Over 1.5 million diabetic patients live in Iran [8].

Due to the high mortality and morbidity rate of diabetes and the resultant shorter life expectancy in diabetes patients [9], knowledge about the incidence and affecting factors are remarkable. Although some genetic and environmental factors involved in the risk of developing diabetes have been identified with certainty [10], there is still conflicting data on the roles of other factors.

The peroxisome-proliferator-activated receptor γ gene (*PPARG)* is a member of the nuclear hormone receptor superfamily [11]. This transcription regulator PPARγ2 isoform (PPARγ2*)* is expressed selectively and at higher level in adipose tissue where it modulates the expression of target genes implicated in adipocyte differentiation and glucose homeostasis [12]. Therefore, the gene is regarded as a major candidate for such complex phenotypes as T2DM or obesity. However, evidence in this regard is contradictory [10,13,14].

A polymorphism involving C to G substitution at nucleotide 34 in the PPARγ2 isoform which causes proline to alanine in position 12 of the PPARγ2 protein (Pro12Ala) [15] was selected since it has been associated with a reduced risk of type 2 diabetes mellitus development [16].

We launched the present study for first time on an Iranian population to investigate the relationship between Pro12Ala polymorphism and T2DM occurrence.

Method and materials

Subjects

Totally, 80 T2DM patients selected based on their blood sugar data (over 200 mg/ml) (mean age±SD, 53.83±7.54, 25 male and 55 female) referred to Golestan hospital, Ahvaz, Iran were recruited in this study. A total of 80 age, sex and ethnic origin matched unrelated healthy volunteers were sampled.

Having being explained the purpose and method of the study, all the subjects signed the written consent form. This study was approved by the ethics committee of the School of Medicine, Ahvaz Jundishapur University of Medical Sciences.

Genotyping

Blood samples were taken from both groups in EDTA tubes. DNA isolation was performed by Genomic DNA extraction kit (Cat. No. K-3032, Bioneer co., Ltd. Korea). Genotyping was performed by the PCR-RFLP method: DNA was amplified by polymerase chain reaction (PCR) using a pair of specific primers: forward primer TGTCTTGACTCATGGGTGTATTC Reverse primer: ATCAGTGAAGGAACCGCTTT designed by using Primer3Plus website (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi), generated a 185 bp fragment. PCR reactions were carried out in a thermocycler instrument (Applied Biosystems 2720) under the following conditions: 95 ^{o}C for 5 min, 35 cycles including denaturing at 95 ^{o}C for 30 sec, annealing for 30 sec at 55.2 ^{o}C and extension at 72 ^{o}C for 20 sec. A final extension was followed at 72 ^{o}C for 5 min. PCR products were digested by BslI restriction enzyme which identifies and cuts the restriction site found in the product from C allele.

RFLP products were loaded on 8% polyacrylamide gel electrophoresis (PAGE) and the sizes of DNA bands were distinguished using 100 bp ladder (GeneOn GmbH, Germany). The 185bp PCR product creates162 and 23bp DNA fragments after restriction by BslI enzyme (60 minute at 37 ^{o}C). Individuals with G/G genotype should only show the 185bp band, while C/C and C/G genotypes should show 23/162bp and 185 and 23/162bp bands, respectively. Some of the genotyping was confirmed by direct DNA sequencing.

Statistical analysis

The resulted date was analyzed by using SPSS statistical software (SPSS Inc., Chicago, IL, USA). Deviations from Hardy-Weinberg equilibrium were considered using $\chi 2$ statistics. Allele and genotype frequencies were compared using $\chi 2$ and Fisher exact tests. Univariate analyses were first performed to calculate the crude odds ratios (OR) and their 95% confidence limits (CL) for the cases vs. control and various genotypes. Odds ratios and 95% CL adjusted for age, were then estimated using logistic regression analysis as an estimate of the relative risk and strength of association. For the logistic regression analysis the dichotomous response variable for the PPARG genotype was regressed against case status and age (continuous).

P value of less than 0.05 was considered statistically significant.

Results

Table 1 summarizes the demographics of the study population with regard to age and gender. The male/female ratio in the case and control groups was not equal (case 25/ 55, control 35/45). The mean ages of patients and controls (±SD) were 53.8 ± 7.54 and 51.2 ± 10.06 years, respectively. However, no statistically significant difference was observed in mean ages. There were a statistically significant higher number of controls under the age of 45 years compared to cases. The genotype distributions of patients and control subjects were within the values expected from Hardy-Weinberg equilibrium for each SNP. We observed three bands 185bp,162bp and 23bp in two groups of patients and controls (Figure1).

We found a statistically significant difference between patients and controls in the frequencies of Ala 12 allele which revealed protective effect against T2DM (OR=0.472; 95% CI: 0.224-0.984; *P*= 0.022) whereas, Pro allele plays as a risk factor to make individual prone to T2DM (OR=2.117; 95% CI: 1.016-4.455; *P*= 0.022). Also, we found signi-

ficant susceptible result for individuals genotyped CC (OR=2.402; 95% CI: 1.081-5.387; *P*= 0.015) and a protective role endowed by genotype CG (OR=0.416; 95% CI: 0.186-0.925; *P*= 0.015) (Table 2).

Table 1. Demographic characteristics of the study population

Variables	Case	Control
Age, Mean ± SD	53.8 ± 7.54	51.2 ± 10.06
≤45 years, No. (%)	10 (12.5)	25 (31.2)
>45 years, No. (%)	70 (87.5)	55 (68.8)
Gender, No. (%)		
Male	25 (31.2)	45 (56.2)
Female	55 (68.8)	35 (43.8)
BMI, Mean ± SD	28.2 ± 4.1	26.1 ± 3.7
FBS, Mean ± SD	162.8 ± 59.4	88.2 ± 9.66
BUN, Mean ± SD	13.4 ± 3.6	14.08 ± 5.73
Cr, Mean ± SD	0.92 ± 0.28	0.99 ± 0.37
TG, Mean ± SD	183.4 ± 92.1	150.0 ± 74.6
Cholesterol, Mean±SD	172.3 ± 43.2	168.5 ± 42.5
HDL, Mean ± SD	52.7 ± 11.2	41.2 ± 11.8
LDL, Mean ± SD	86.3 ± 34.1	37.35 ± 51.8
HbA1, Mean ± SD	8.5 ± 2.01	6.25 ± 0.35
Nephropathy, No. (%)	1 (1.2)	0
Retinopathy, No. (%)	10 (12.5)	0

BMI: Body Mass Index; FBS: Fasting Plasma Glucose; BUN: Blood Urea Nitrogen; CR: creatinine; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

There was a 1.9-fold increase in the risk of T2DM associated with the CC allele (adjusted OR: 1.94, 95% CL 0.92–4.01). In contrast, there was no association between the CG polymorphism and the risk of T2DM (adjusted OR: 0.51, 95% CL 0.24– 1.03) (Table 2).

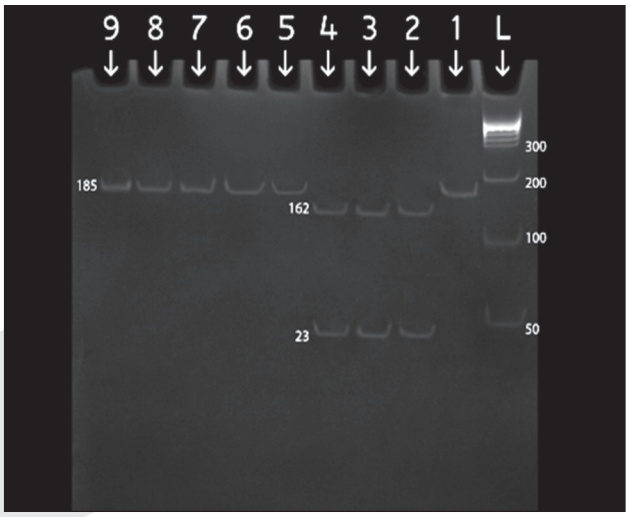

Figure 1. Pro12Ala polymorphism in PPARG gene. L) The marker (50bp) DNA, Rows 1 and 5-9 show a 185 bp PCR product remaining uncut after restriction digestion and represents the CC genotype, Rows 2, 3 and 4 are cut products (162 and 23 bp fragments) representing the genotype CG

Discussion

In this case-control study, we explored the effect of *PPARG* gene polymorphism for type 2 diabetes mellitus (T2DM), southwest Iran, consisting of Arabs in over half of its population. However, since the gene (*PPARG*) is located on an autosomal chromosome (3p25), the ratio difference may have minimal influence on the results. Single–locus analysis showed significant influence of Pro12Ala polymorphism on susceptibility to T2DM. The role of the *PPARG* gene in glucose and fat metabolism has been previously clarified which would possibly affect obesity, insulin sensitivity and T2DM occurrence [13,14]. Pro12Ala, caused by replacement of C by G at the level of DNA, is one of the main studied polymorphisms in *PPARG* and could have a direct effect on T2DM symptoms [13,15]. Several association studies have demonstrated that the al-

Table 2. PPARG allelic frequencies and the genotype distribution among type 2 diabetic patients and controls and estimates of risk associated with CC and CG genotypes

Genotypes and alleles	Cases (n = 80)	Controls	Crude OR (95% CL)	Adjusted OR (95% CL)b
CC	66 (82.5%)	53 (66.2%)	2.40 (1.08-5.38)[d]	1.94 (0.92-4.01)[d]
CG	14 (17.5%)	27 (33.8%)	0.41 (0.18-0.92)[d]	0.51 (0.24-1.03)[d]
C	146 (91.2%)	133 (83.1%)	2.11 (1.01-4.45)[d]	1.52 (1.12-2.23)[d]
G	14 (8.8%)	27 (16.9%)	0.47 (0.22-0.98)	0.53 (0.19-1.01)

Adjusted for age (continuous variable), [d]: Statistically significant difference

lele is negatively associated with the development of T2DM, suggesting a protective role for it. In contrast, other investigators have failed to confirm any independent role for the *PPARG* association with T2DM and have rather suggested its interaction with other genes [10].

Similar results which suggest a susceptibility role for Pro have been observed in Japanese [17], Caucasian American[18], Finnish[19] and Danish populations [20]. Conversely, a deleterious effect of Ala 12 allele has been shown in Canadian Oji-Cree individuals [21], Germans [22,23], French [24], Polish [25], Korean [26] or Pima Indians [27].

Many investigators have put forth the idea that this polymorphism, which is within the domain enhancing ligand independent activation, reduces the risk of T2DM through producing a PPARγ2 protein in adiposities which is less efficient in transcription stimulation of PPARγ2 target [28]. This would likely lead to increased suppression of lipolysis by insulin and the resultant decrease in free fatty acid secretion that could in turn cause a greater insulin sensitivity [28,29]. In summary, this study suggested that *PPARG* gene is likely to be related to the development of T2DM in an Iranian population. However, more studies with more SNPs in the gene, enough sample size and on different ethnic groups are required for clarification of the issue. Functional studies on *PPARG* action will be needed to obtain a deeper insight into the mechanism (s) through which its common variant exert its role in T2DM susceptibility

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References

1. *Qiao, Q., Hu, G., Tuomilehto, J., Nakagami, T., Balkau, B., Borch-Johnsen, K., Ramachandran, A., Mohan, V., Iyer, S.R., Tominaga, M., Kiyohara, Y., Kato, I., Okubo, K., Nagai, M., Shibazaki, S., Yang, Z., Tong, Z., Fan, Q., Wang, B., Chew, S.K., Tan, B.Y., Heng, D., Emmanuel, S., Tajima, N., Iwamoto, Y., Snehalatha, C., Vijay, V., Kapur, A., Dong, Y., Nan, H., Gao, W., Shi, H., Fu, F.: Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes Care 26(6), 1770-1780 (2003).*
2. *Simpson, R.W., Shaw, J.E., Zimmet, P.Z.: The prevention of type 2 diabetes--lifestyle change or pharmacotherapy? A challenge for the 21st century. Diabetes Res Clin Pract 59(3), 165-180 (2003). doi:S0168822702002759 [pii]*
3. *King, H., Aubert, R.E., Herman, W.H.: Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 21(9), 1414-1431 (1998).*
4. *Amos, A.F., McCarty, D.J., Zimmet, P.: The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med 14 Suppl 5, S1-85 (1997).*
5. *Bloomgarden, Z.T.: Insulin resistance: causes and consequences. Int Rev Neurobiol 65, 1-24 (2005). doi:S0074-7742(04)65001-X [pii] 10.1016/S0074-7742(04)65001-X*
6. *Hegele, R.A., Pollex, R.L.: Genetic and physiological insights into the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol 289(3), R663-669 (2005). doi:00275.2005 [pii] 10.1152/ajpregu.00275.2005*
7. *Camacho, P., Pitale, S., Abraira, C.: Beneficial and detrimental effects of intensive glycaemic control, with emphasis on type 2 diabetes mellitus. Drugs Aging 17(6), 463-476 (2000).*
8. *Azizi, F.: Diabetes in the Islamic Republic of Iran. IDF bulletin 41, 38-39 (1996).*
9. *Morgan, C.L., Currie, C.J., Peters, J.R.: Relationship between diabetes and mortality: a population study using record linkage. Diabetes Care 23(8), 1103-1107 (2000).*
10. *Wang, F., Han, X.Y., Ren, Q., Zhang, X.Y., Han, L.C., Luo, Y.Y., Zhou, X.H., Ji, L.N.: Effect of genetic variants in KCNJ11, ABCC8, PPARG and HNF4A loci on the susceptibility of type 2 diabetes in Chinese Han population. Chin Med J (Engl) 122(20), 2477-2482 (2009).*
11. *Spiegelman, B.M.: PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes 47(4), 507-514 (1998).*
12. *Latruffe, N., Vamecq, J.: Peroxisome proliferators and peroxisome proliferator activated receptors (PPARs) as regulators of lipid metabolism. Biochimie 79(2-3), 81-94 (1997). doi:S0300-9084(97)81496-4 [pii]*
13. *Celi, F.S., Shuldiner, A.R.: The role of peroxisome proliferator-activated receptor gamma in diabetes and obesity. Curr Diab Rep 2(2), 179-185 (2002).*
14. *Yang, J., Zhang, D., Li, J., Zhang, X., Fan, F., Guan, Y.: Role of PPARgamma in renoprotection in Type 2 diabetes: molecular mechanisms and therapeutic potential. Clin Sci (Lond) 116(1), 17-26 (2009). doi:CS20070462 [pii] 10.1042/CS20070462*

15. *Yen, C.J., Beamer, B.A., Negri, C., Silver, K., Brown, K.A., Yarnall, D.P., Burns, D.K., Roth, J., Shuldiner, A.R.: Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochemical and biophysical research communications 241(2), 270-274 (1997).*

16. *Altshuler, D., Hirschhorn, J.N., Klannemark, M., Lindgren, C.M., Vohl, M.C., Nemesh, J., Lane, C.R., Schaffner, S.F., Bolk, S., Brewer, C., Tuomi, T., Gaudet, D., Hudson, T.J., Daly, M., Groop, L., Lander, E.S.: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26(1), 76-80 (2000).*

17. *Mori, H., Ikegami, H., Kawaguchi, Y., Seino, S., Yokoi, N., Takeda, J., Inoue, I., Seino, Y., Yasuda, K., Hanafusa, T., Yamagata, K., Awata, T., Kadowaki, T., Hara, K., Yamada, N., Gotoda, T., Iwasaki, N., Iwamoto, Y., Sanke, T., Nanjo, K., Oka, Y., Matsutani, A., Maeda, E., Kasuga, M.: The Pro12 -->Ala substitution in PPARgamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes 50(4), 891-894 (2001).*

18. *Li, W.D., Lee, J.H., Price, R.A.: The peroxisome proliferator-activated receptor gamma 2 Pro12Ala mutation is associated with early onset extreme obesity and reduced fasting glucose. Mol Genet Metab 70(2), 159-161 (2000). doi:10.1006/mgme.2000.2999 S1096-7192(00)92999-8 [pii]*

19. *Douglas, J.A., Erdos, M.R., Watanabe, R.M., Braun, A., Johnston, C.L., Oeth, P., Mohlke, K.L., Valle, T.T., Ehnholm, C., Buchanan, T.A., Bergman, R.N., Collins, F.S., Boehnke, M., Tuomilehto, J.: The peroxisome proliferator-activated receptor-gamma2 Pro12A1a variant: association with type 2 diabetes and trait differences. Diabetes 50(4), 886-890 (2001).*

20. *Poulsen, P., Andersen, G., Fenger, M., Hansen, T., Echwald, S.M., Volund, A., Beck-Nielsen, H., Pedersen, O., Vaag, A.: Impact of two common polymorphisms in the PPARgamma gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both? Diabetes 52(1), 194-198 (2003).*

21. *Hegele, R.A., Cao, H., Harris, S.B., Zinman, B., Hanley, A.J., Anderson, C.M.: Peroxisome proliferator-activated receptor-gamma2 P12A and type 2 diabetes in Canadian Oji-Cree. J Clin Endocrinol Metab 85(5), 2014-2019 (2000).*

22. *Ringel, J., Engeli, S., Distler, A., Sharma, A.M.: Pro-12Ala missense mutation of the peroxisome proliferator activated receptor gamma and diabetes mellitus. Biochem Biophys Res Commun 254(2), 450-453 (1999). doi:S0006-291X(98)99962-4 [pii] 10.1006/bbrc. 1998.9962*

23. *Zietz, B., Barth, N., Spiegel, D., Schmitz, G., Scholmerich, J., Schaffler, A.: Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 (PPARgamma2) is associated with higher levels of total cholesterol and LDL-cholesterol in male caucasian type 2 diabetes patients. Exp Clin Endocrinol Diabetes 110(2), 60-66 (2002). doi:10.1055/s-2002-23487*

24. *Clement, K., Hercberg, S., Passinge, B., Galan, P., Varroud-Vial, M., Shuldiner, A.R., Beamer, B.A., Charpentier, G., Guy-Grand, B., Froguel, P., Vaisse, C.: The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. Int J Obes Relat Metab Disord 24(3), 391-393 (2000).*

25. *Malecki, M.T., Frey, J., Klupa, T., Skupien, J., Walus, M., Mlynarski, W., Sieradzki, J.: The Pro12Ala polymorphism of PPARgamma2 gene and susceptibility to type 2 diabetes mellitus in a Polish population. Diabetes Res Clin Pract 62(2), 105-111 (2003). doi:S0168822703001645 [pii]*

26. *Oh, E.Y., Min, K.M., Chung, J.H., Min, Y.K., Lee, M.S., Kim, K.W., Lee, M.K.: Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects. J Clin Endocrinol Metab 85(5), 1801-1804 (2000).*

27. *Muller, Y.L., Bogardus, C., Beamer, B.A., Shuldiner, A.R., Baier, L.J.: A functional variant in the peroxisome proliferator-activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. Diabetes 52(7), 1864-1871 (2003).*

28. *Deeb, S.S., Fajas, L., Nemoto, M., Pihlajamaki, J., Mykkanen, L., Kuusisto, J., Laakso, M., Fujimoto, W., Auwerx, J.: A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20(3), 284-287 (1998).*

29. *Li, D., Kang, Q., Wang, D.M.: Constitutive coactivator of peroxisome proliferator-activated receptor (PPARgamma), a novel coactivator of PPARgamma that promotes adipogenesis. Molecular endocrinology (Baltimore, Md 21(10), 2320-2333 (2007).*

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Anti-Toxoplasma antibody prevalence, primary infection rate, and risk factors in a study of toxoplasmosis in 1.026 pregnant women southeastern Turkey

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Abstract

Purpose: In this study it is aimed to determine seropositivity of *Toxoplasma gondii (T. gondii)* in pregnant women who were recruited to Adiyaman Obstetric and Children Hospital and identify the risk factors related to it.

Materials and Methods: Between October 2010 and October 2011, 1026 pregnant women were examined prospectively for toxoplasmosis. After a questionnaire applied to the pregnant women, anti-*Toxoplasma* IgG and IgM antibodies were investigated with ELISA. Univariate and multiple logistic regression analyses were used to determine the risk factors related to *Toxoplasma gondii.*

Results: Seroprevalences of IgG and IgM against *T. gondii* were 35.5% and 3.2% respectively. The mean age of the women was 28±5 years. The mean of gestation week was 10±7 (from 2 to 37). We found that the risk of *T. gondii* was 2.02 times higher between ages 33-40 than that of 17-24, after adjusting for education level, drinking water, eating raw meat and current cat ownership. Among the environmental variables, the effect of drinking water (adjusted OR=2.40, 95% CI=1.25-4.60, p<0.001), eating raw meat (adjusted OR=2.27, 95% CI=1.67-3.09, p<0.001) and current cat ownership (adjusted OR=5.92, 95% CI=3.50-10.04, p<0.001) on positivity of *Toxoplasma* IgG antibody was found to be statistically significant. Regarding to the results of multiple logistic regression analyses, no significant relationship were observed between anti-*Toxoplasma* IgG antibodies and residential address, monthly household income, gestational age, milk consumption and touching raw meat.

Conclusion: Childbearing age women in Adiyaman province living under poor socioeconomic conditions and education level are at risk for acquiring infection with *T. gondii*. We suggest pregnant women who live in Adiyaman where general network water is mostly used (94.4%) to drink boiled water, not to consume raw meat and keep away from cats.

Key words: Pregnancy, Anti-*Toxoplasma* antibodies, risk factors, Adiyaman.

Introduction

Toxoplasmosis is a worldwide zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*), which infects humans and most warm-blooded animals. Felids are the definitive host of this protozoan parasite being the only species able to excrete sporulated oocysts into the environment (1). Oocysts, remarkably stable environmentally, are transmitted to other hosts through inadvertent ingestion. Humans acquire *T. gondii* through ingestion of undercooked meat, contact with feline faeces and rarely through drinking contaminated water or through transplantation of a contaminated organ (2,3). However, within immunocompetent humans, 90% of *T. gondii* infections are asymptomatic. Vertical transmission of toxoplasmosis from an acutely infected pregnant woman could cause serious damage and malformation. Toxoplasmosis during pregnancy can cause congenital infection and manifest as mental retardation and blindness in the infant. Other findings are diminished visual acuity and neurological sequelae such as hydrocephalus, calcification in the brain, paresis, epilepsy and schizophrenia (4,5).

Early diagnosis of the mother, the fetus and the newborn is necessary to prevent human toxoplasmosis. Monitoring the immune response by measuring the titer and affinity of specific antibodies makes a major contribution to diagnosis of congenital toxoplasmosis and evaluation of time of infection (6). The serological screening of pregnant women for toxoplasmosis and the follow-up until delivery are not routine procedures in Turkey. In a few studies performed in our country, seroprevalence of *T. gondii* infection in women at childbearing age was found to be between 19.2% and 85%; and it is estimated that incidence of congenital toxoplasmosis is 0.1% (7). The results of some studies indicated that the factors associated with increased risk were ingestion of raw or undercooked meat, use of kitchen knives that had not been sufficiently washed, and ingestion of unwashed raw vegetables or fruits. A recent case-control study from Europe examined the risk factors that predisposed pregnant woman to infection with *T. gondii*. In this study, approximately 30% to 63% of the infections were due to exposure to inadequately cooked or cured meat, which was interpreted to be the main risk factor for pregnant women in Europe. The association might be due to oocyst contamination by dirty production techniques or to confounding by other lifestyle (for example, eating undercooked organically produced meat) (1,8).

Detailed knowledge on the prevalence and risk factors of infection with *T. gondii* are required to design appropriate prevention measures against infection during pregnancy and congenital transmission. In the present study, we therefore investigated seroprevalence of infection with *T. gondii* and associated risk factors in a large number of pregnant women attending a public tertiary care obstetric hospital in Adiyaman.

Materials and Methods

Study design and population

Adiyaman has a semi-arid climate. Summers are very hot and very dry. Temperatures reach 45°C at the height of summer for the majority of the time. Winters are cool/cold and snowy. The average annual precipitation is 400-800mm. The population of the central district of the province was 170.075 including counties and villages. However due to recent migration, mostly caused by the displaced villagers due to the Atatürk dam (part of southeastern Anatolian project, called GAP), the population is estimated to be over 590,000. The economical level of the population is very low and the cat population is high, they stroll freely around the community.

A total of 1026 pregnant women were included in the study, between (17-40 years). Inclusion criteria for the study were attendance for delivery at the Obstetrics and Children's Hospital, residency in Adiyaman or in the rural region surrounding the city, between October 2010 and October 2011. Exclusion criteria from data were absence of a questionnaire, lack of a serum samples, or an insufficient quantity of serum. To determine the seroprevalence of infection with *T. gondii* venous blood was obtained from study participants at the Hospital.

Questionnaires

A short questionnaire interview for pregnant women was carried out to obtain information associated with *T. gondii* infection. There were questions eliciting socio-demographic data including age, education level, residential address (urban, rural), monthly wage, gestational age and environmental factors; drinking water, eating raw meat, milk consumption (yes or no) touching raw meat and current cat ownership. Additional information about ocular and neurological damage in children was collected.

Serologic analysis for Toxoplasma

The sera were sent to the serology and Microbiology laboratory for investigation of the anti-*Toxoplasma gondii* IgG, and IgM antibodies by the EIA (Cobas Core, Roche, Germany and ETI-TOXOK-A, DiaSorin, Germany) technique. In the evaluation of the results, as recommended by the manufacturer, for anti-*Toxoplasma* antibodies <1 IU/mL was considered non-reactive, ≥1-<30 IU/mL indeterminate, ≥30 IU/mL and reactive. Samples with concentrations ≥30 IU/mL are considered positive for IgG antibodies to *T. gondii* and indicate either acute and or latent infection. For all samples with concentrations ≥30 IU/mL a *Toxoplasma* IgM test was performed to exclude early *Toxoplasma* infection. Women with positive IgG titers but negative IgM titers were considered latently infected. Women with positive IgG and IgM titers were considered to have a possible recent infection.

Statistical Analysis

Statistical analyses were performed by using SPSS 11.5 for Windows package program. Continuous data were summarized as mean±standard deviation, whereas frequency (percentage) was used for categorical data. Univariate logistic regression analysis was used to evaluate the association between IgG and socio-demographic factors and environmental factors as univariately. Also, multiple logistic regression was performed to determine the risk factors on IgG positivity. Forward elimination (likelihood ratio) method was used for the selection of variables. The entry and removal criteria used were p values of 0.05 for entry and p values of 0.10 for variable removal in multiple logistic regression analysis. Odds ratio (OR) and its 95% confidence interval (CI) were given. $p<0.05$ was considered as statistically significant.

Ethics

The study was performed in accordance with the Helsinki Declaration was approved by the local ethics committee of Adiyaman University. All participants were informed about the study, and informed written consent was obtained from all study participants

Table 1. Univariate logistic regression of socioeconomic factors associated with infection with Toxoplasma gondii in 997 pregnant women

Socio-demographic factors		Toxoplasma IgG positivity	OR(95%CI)	p
Age	17-24 (n=305)	100 (32.8%)		<0.001
	25-32 (n=509)	161 (31.6%)	0.95(0.70-1.29)	
	33-40 (n=183)	92 (50.3%)	2.07(1.42-3.02)	
Education level	Illiteracy (n=93)	37 (39.8%)		0.007
	Primary school (n=517)	189 (36.6%)	0.87(0.55-1.37)	
	Secondary school (n=133)	29 (21.8%)	0.42(0.24-0.76)	
	High school (n=180)	65 (36.1%)	0.86(0.51-1.43)	
	University (n=74)	33 (44.6%)	1.22(0.66-2.26)	
Residential address	Urban (n=736)	248 (33.7%)		0.058
	Rural (n=261)	105 (40.2%)	1.32(0.99-1.77)	
Monthly household income, MW	250TL (n=345)	105 (30.4%)		0.057
	375-750TL (n=413)	156 (37.8%)	1.39(1.02-1.88)	
	≥750TL (n=239)	92 (38.5%)	1.43(1.01-2.02)	
Gestational age	1st trimester (n=723)	241 (33.3%)		0.072
	2nd trimester (n=234)	94 (40.2%)	1.34(0.99-1.82)	
	3rd trimester (n=40)	18 (45.0%)	1.64(0.86-3.11)	

*OR: Odds Ratio, CI: Confidence Interval, * MW = minimum wage of 250TL = U.S. $180*

Table 2. Univariate Logistic Regression of eating and drinking habits associated with infection with Toxoplasma gondii in 997 pregnant women

Environmental factors		Toxoplasma IgG positivity	OR(95%CI)	p
Drinking water	General network (n=942)	327 (34.7%)		0.061
	Bottled (n=55)	26 (47.3%)	1.69(0.98-2.91)	
Eating raw meat	No (n=354)	95 (26.8%)		<0.001
	Yes (n=643)	258 (40.1%)	1.83(1.38-2.43)	
Milk consumption	No (n=336)	122 (36.3%)		0.671
	Yes (n=661)	231 (34.9%)	0.94(0.72-1.24)	
Touching raw meat	No (n=539)	178 (33.0%)		0.088
	Yes (n=458)	175 (38.2%)	1.25(0.97-1.63)	
Cat ownership	No (n=918)	299 (32.6%)		<0.001
	Yes (n=79)	54 (68.4%)	4.47(2.73-7.33)	

OR: Odds Ratio, CI: Confidence Interval

Results

A total of 1,300 pregnant women were enrolled, and data of 1026 women (from age 17, to 40) were analyzed. The datasets of 29 women were excluded because of absence of a questionnaire, an incomplete questionnaire, lack of serum samples, or insufficient quantity of serum**.** The mean age of the women was 28±5 years. The mean of gestation week was 10 ±7 (from 2 to 37). The seropositivity of *Toxoplasma* specific IgG was 388 (35.5%) among pregnant women (chronic phase). 36 (3.2%) pregnant women were IgM positive (acute phase). 602 (61.3%) had no detectable IgM or IgG against. While there were no statistically significant relationship between residential address, monthly household income, gestational age and *Toxoplasma* seropositivity (p>0.05), age and education level were found to be significant (p<0.001 and p=0.007, respectively) in univariate analyses (Table 1). Among the environmental variables, only the relation between drinking water (general network or bottled) (p=0.061) and current cat ownership (p<0.001) with positivity of *Toxoplasma* IgG antibody was found to be statistically significant (Table 2).

After univariate analyses, the multiple logistic regression was performed in order to determine the risk factors on IgG positivity. Among the socio-demographic and environmental factors age, education level, drinking water, eating raw meat and current cat ownership were found to be statistically significant in multivariate analyses (Table 3). Being a cat owner increases the risk of IgG positivity almost 6 times after adjusted for age, education level, drinking water (bottled or general network) and eating raw meat. Also, in the questionnaire, pregnant women were asked if their children had neurological disturbances and visual defects. Of the 1026 pregnant women, 756 had children and of their children four was found to have Down syndrome, four epilepsy, six visual defects and five mental retardations. It was established that the mother of the child with visual defect was *Toxoplasma* IgG antibody.

Discussion

Infection with *T. gondii* has a high prevalence in the Southeastern Anatolia Region of Turkey. Whereas acute infection in most persons is asymptomatic or mild, infection during pregnancy may cause transmission to the fetus with severe signs and symptoms (9). The infection is commonly acquired by the oral route and thus represents a preventable infection. However, to develop appropriate prevention programs, detailed knowledge on prevalence and risk factors is needed.

Toxoplasmosis is a common disease in our country as all over the world. As the incidence of this infection varies largely with geographic location and nutritional habits, it is important to determine regional prevalence. Seroprevalence of *T.gondii* infection range between 15%-77% in different countries (10).

In the present study, we screened more than 1026 pregnant women attending a tertiary care obstetric hospital in Adiyaman. The seropositivity of *Toxoplasma* specific IgM and IgG was 3.2%, 35.5%, respectively. It was found that *Toxo-*

Table 3. Results of multiple logistic regression analysis of socio-demographic and environmental factors with Toxoplasma gondii in 997 pregnant women

Socio-demographic and environmental factors		Adjusted OR (95% CI)	p
Age	25-32 vs. 17-24	0.83(0.60-1.17)	<0.001
	33-40 vs. 17-24	2.02(1.33-3.06)	
Education Level	Primary school vs. Illiteracy	1.18(0.73-1.91)	0.002
	Secondary school vs. Illiteracy	0.50(0.26-0.94)	
	High school vs. Illiteracy	1.13(0.75-2.30)	
	University vs. Illiteracy	1.11(0.75-2.30)	
Drinking Water	Bottled vs. General network	2.40(1.25-4.60)	0.008
Eating raw meat	Yes vs. No	2.27(1.67-3.09)	<0.001
Cat ownership	Yes vs. No	5.92(3.50-10.04)	<0.001

Adjusted OR: Odds Ratio adjusted for age, education level, drinking water, eating raw meat and cat ownership, CI: Confidence Interval

plasma seropositivity was higher in the pregnant women between the ages of, 33-40 (50.3%) and it was found to be significant. the seropositivity of *Toxoplasma* specific IgG was 33.3%, 40.2%, and 45.0% in pregnant women at the first, second and third trimester respectively. It was found that seropositivity of *T. gondii* infection increases during pregnancy. The increase in prevalence with age is consistent with results of previous studies (11-13).

Previously, seroprevalence in pregnant women was investigated in Adiyaman, Urfa and Malatya (Provinces which share borders with Adiyaman) and found to be 48.4%, 60.4% and 37.6%, respectively (14,15). It was found to be quite higher in Adiyaman in the study of Kolgelier *et al*. compared to ours (16). It is thought that this difference may arise from the age difference between patient populations.

It is well documented that lamb and goat meat are sources of *T. gondii*. Finding beef as a source of infection is unexplained because *T. gondii* has never been isolated from edible beef in Europe or North America (17). Bradyzoites can be found in up to 8% of beef, 20% of pork, and 20% of lamb (18). In Turkey, beef and lamb are commonly used, especially mixed together. Pork is never used due to religious ban. Southeastern regions of Turkey, a special food prepared with raw meat and called raw meatball is consumed more than in other regions. Although this food is prepared without meat when it is served in restaurants, raw meat is used when it is prepared at home. Meatball made of raw meat is consumed commonly in Adiyaman and Urfa provinces. While the most consumed meat is lamb in Urfa, beef is the most consumed meat in Adiyaman. We suppose that Urfa has higher *Toxoplasma* IgG seropositivity compared with Adiyaman provinces.

Several authors have emphasized the influence of urban versus rural settings in toxoplasmosis (19). No relation was found residential address, monthly household income and *Toxoplasma* infectious. The fact that significant increase did not occur in *Toxoplasma* prevalence in pregnant women in urban areas may be attributed to the high number of street cats in urban areas as in rural areas. It was found that *Toxoplasma* seropositivity was higher in pregnant women graduated from university (44.6%) while the lowest *Toxoplasma* seropositivity frequency was observed in in pregnant women graduated from secondary school.

Direct contact with a cat was associated with *T. gondii* seropositivity in the univariate analysis in accordance with other studies. Cats often spread oocysts outside the home, and feral cats may be responsible for much of the environmental contamination with oocysts because sporulated oocycts maintain infectivity for a long time in water or soil (20-22). Surprisingly, significant relation was found between having cat at home (OR=5.92) and *Toxoplasma* seropositivity.

Most people (94.4%) use general network water, followed by bottled (5.5%). Relation was found between using drinking water and toxoplasmosis seropositivity (OR=2.40, 95%CI=1.25-4.60, $p<0.001$). Interestingly, We found that the risk of *T. gondii* was 2.40 times higher between users general network. It is thought that tap water is contaminated with oocysts and plays a role in transmission.

T. gondii tachyzoites have been isolated from goats' milk and cows' colostrums. The connection between the infection and tasting meat dishes during cooking, as well as consuming unpasteurized milk and its products, was demonstrated. *T. gondii* tachyzoites were isolated from cow milk and udders (23-26). In our study, there were no statistically significant relationships between milk consumption.

A newborn exposed to *T. gondii* in utero may develop congenital toxoplasmosis with major ocular and neurological consequences (5). In the questionnaire, pregnant women were asked whether their children had any neurological or ocular disturbances. Of their children four was found to have Down syndrome, four epilepsy, six visual defects and five mental retardations. Since we couldn't reach clinical and serologic data of pregnant women and children, we can not claim that diseases found in children emerge from *Toxoplasma* infection. It shouldn't be ignored that diseases found in children could be related to seropositivity of *T. gondii* in pregnant women.

Due to its long term complications and the fact that *T. gondii* is omnipresent, epidemiological studies on its seropositivity help shape health policies in individual countries. The aim of this is to collectively evaluate available epidemiological data on the worldwide *Toxoplasma* seropositivity, particularly focusing on pregnant women of childbearing age (15-45 years). To our knowledge, this study is the first to implicate the risk factors as a source of *T. gondii* infection in this area.

In our province where the information on the diseases is scant, women become pregnant at young ages and the number of children is high, especially pregnant women should be informed on the disease and ways of protection should be explained. Women in childbearing age in Adiyaman, where the frequency of consumption of raw meat is high, should be monitored for *T. gondii* in their first antenatal examination. The majority of the people use drinking water from the municipality as drinking water. It has been established that drinking water is contaminated by oocysts. It should be stressed that, in this region in which infrastructure has not been completed yet, pregnant women may run the risk of the contamination of tap water with oocysts.

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References

1. *Dubey JP. Toxoplasmosis a waterborne zoonosis. Vet Parasitol. 2004; 126(1-2): 57-72.*
2. *Tenter AM, Heckerroth AR, Weiss LM. Toxoplasma gondii: from animals to human. Int J Parasitol. 2000; 31(2): 217-20.*
3. *Hill D, Dubley JP Toxoplasma gondii: transmission, diagnosis and prevention. Clin Microbiol Infect. 2002; 8(10): 634-40.*
4. *Akyar I. Seroprevalence and Coinfections of Toxoplasma gondii in Childbearing Age Women in Turkey. Iranian J Publ Health.2011;40(1):63-67.*
5. *Henriquez SA, Brett R, Alexander J, Pratt J, Roberts CW. Neuropsychiatric disease and Toxoplasma gondii infection. Neuroimmunomodulation. 2009; 16(2): 122-33.*
6. *Macones GA, McNamara J, Wallenstein M, Squires K. 'Congenital toxoplasmosis' by Berrébi et al. Am J Obstet Gynecol. 2010; 203(6): e1-3.*
7. *Ertug S, Okyay P, Turkmen M, Yuksel H. Seroprevalence and risk factors for Toxoplasma infection among pregnant women in Aydin province, Turkey. BMC Public Health. 2005; 5:66. doi:10.1186/1471-2458-5-66.*
8. *Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA., et al. Sources of Toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ. 2000; 321(7254): 142-147.*
9. *Montoya JG, Liesenfeld O Toxoplasmosis. Lancet. 2004; 363(9425): 1965-76.*
10. *Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. Am J Epidemiol. 2001; 154(4): 357-65.*
11. *Sroka S, Bartelheimer N, Winter A, Heukelbach J, Ariza L, Ribeiro H.,et al. Prevalence and risk factors of toxoplasmosis among pregnant women in Fortaleza, Northeastern Brazil. Am J Trop Med Hyg. 2010; 83(3): 528-33*
12. *Rey LC, Ramalho IL. Seroprevalence of toxoplasmosis in Fortaleza, Ceara, Brazil. Rev Inst Med Trop Sao Paulo. 1999; 41:171-174 .*
13. *Varella IS, Wagner MB, Darela AC, Nunes LM, Muller RW. Seroprevalence of toxoplasmosis in pregnant women. J Pediatr. 2003; 79: 69-74.*

14. *Harma M, Gungen N and Demir N. Toxoplasmosis in pregnant women in Sanliurfa, Southeastern Antolia City, Turkey. J Egypt Soc Parasitol. 2004; 34(2): 519-25.*

15. *Kafkasli A, Uryan D, Buhur A, Koroglu M, Durmaz R Toxoplasma gondii screening amoung pregnant women in an outpatient clinic. Turkish Journal of Perinatolog. 1996; 4: 94-96.*

16. *Kolgelier S, Demiraslan H, Katas B, Guler D Seroprevalence of Toxoplasma gondii in pregnant women. Dicle Med J. 2009;36:170-172.*

17. *Dubey JP. Sources of Toxoplasma gondii infection in pregnancy. Until rates of congenital toxoplasmosis fall, control measures are essential. BMJ. 2000; 321(7254): 127-8.*

18. *Beazley DM and Egerman RS. Toxoplasmosis. Semin Perinatol.1998; 22(4): 332-8.*

19. *Dubey JP, Miller NL, Frenkel JK. Toxoplasma gondii life cycle in cats. J Am Vet Med Assoc. 1970; 157(11): 1767-1770.*

20. *Ambroise-Thomas P, Petersen E. Congenital Toxoplasmosis:Scientific Background, Clinical Management and Control. Paris: Springer-Verlag. 2000*

21. *Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A. Eng J Risk factors for Toxoplasma gondii infection in pregnancy. Results of a prospective case-control study in Norway. Am. J. Epidemiol. 1996; 144(4): 405-412.*

22. *Kravetz JD, Federman DG. Toxoplasmosis in pregnancy. Am J Med. 2005; 118(3): 212-6.*

23. *Dubey JP. Toxoplasmosis in sheep, goats, pigs and cattle. In: Dubey J, Beattie C eds. Toxoplasmosis in animals and man. Boca Raton, Florida, CRC Press. 1998; pp:61114.*

24. *Bonametti AM, Passos JN, Koga da Silva EM, Macedo ZS. Probable transmission of acute toxoplasmosis through breast feeding. J Trop Pediatr. 1997; 43(2): 116.*

25. *Dubey JP, Thulliez P. Persistence of tissue cysts in edible tissues of cattle fed Toxoplasma gondii oocysts. Am J Vet Res. 1993; 54(2): 270-3.*

26. *Jones JL, Dubey JP. Foodborne toxoplasmosis. Clin Infect Dis. 2012; 55(6): 845-51.*

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Psychophysical status of human trafficking victims

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Abstract

Human trafficking causes a number of consequences. Among them especially significant are grave consequences for the physical and mental health of victims. Victims are traumatized, physically and mentally worn out, exposed to HIV infections, venereal diseases, with severe consequences resulting from long-term abuse and torture. The level of physical and mental damage is so high. In this paper we analyzed some of the most common consequence sof human trafficking that harm mental and physical health of victims.

Key words: Human trafficking, victims of trafficking, HIV infections, venereal diseases, mental and physical health of human trafficking victims.

Introduction

Human trafficking is a global phenomenon that intensively affects states in economic and political transition and post-conflict stress. It is a phenomenon with deep social and economic implications. Victims of human trafficking can be everyone: women, men, girls, and boys. Data associated to this phenomenon are alarming. Even though research and estimations differ, it can be stated with certainty that on a yearly basis, hundreds of thousands of human beings are trafficked globally. The forms of exploitation used to accumulate large profits mutually differ, but they all imply violent exploitation and abuse of human beings and their rights.

There is a wide range of potential forms of exploitation, from sexual, labor exploitation, criminal involvement, arrangement of marriages, organ donation, to forced military service. Exploitation may undertake different forms, such as: prostitution and other forms of sexual exploitation, force labor, slavery or actions similar to slavery and removal of organs [1].

Human trafficking represents gross violation of human rights, and it should therefore be recognized as the most extreme form of human rights violation [2]. Victims are denied the right to life, work, education, dignity, safety, equality, freedom of movement, right to health, etc [3].

Female victims of human trafficking during sexual exploitation are not able to perform normal health protection measures, so the prevalence of HIV/AIDS has the greatest percent, which denies the right to life and health, as well as the concept of human dignity, in contrast to international norms and set civilization standards [4,5].

In most of the cases, the level of physical and mental harm caused to victims is so serious and long-lasting that it is often not possible to medically re-balance and enable victims to reach satisfactory level of physical and mental health [6,7].

Method

From the period of March 2002. until December 2011., ASTRA SOS telephone service in Republic Serbia has identified 391 victims of trafficking [8].

When the countries of destination for trafficking victims in 2011. year, the most common destination is, as in previous years, Serbia. Final destination of trafficking victims are recruited for the purpose of labor exploitation were Germany, Russia and Chechnya.In individual cases, the destinations were Italy, Slovenia and Bosnia. Of the 40 identified victims in 2011. year, 17 were from Serbia and the Serbian territory are exploited. Serbia is a country of origin and destination for nine out of ten children who were identified in 2011. year. Internal trade was in 2011. year represented 42.5% of identified cases of trafficking (2010-51%, 2009-42%, 2008-25%).

The majority oftrafficking victims in ASTRA study were vulnerable to trafficking because they suffered from maltreatment and tragic home lives. They also had problems in theirfamily dysfunctions as the one source of creating vulnerability in children who fall under the influence of

traffickers. In this study we also noticed that the standard dysfunctional family patterns are present in this group.

We did not investigate other strong influences one can usually find in the literature, such as individual and peer factors, along with larger environmental factors often presented that play a significant role in child trafficking.

Our intention was not to advocate that those most probable to trafficking victims may be those that are poor, neglected, and abused.

We come to conclusion that family dynamics and socioeconomic status are important risk factors, but we recognize that those interviewed for the study were youth that had been arrested and incarcerated. We admit that numerous risk factors create vulnerability, not just poor children who come from substance abusing and neglectful or abusive parents.

Vulnerability can be also found and created in children who come from wealthy, two-parent, seemingly healthy familie.

Results

Among them are 299 persons had citizenship of the Republic of Serbia, while three people have dual citizenship. Of the total number of identified victims, 144 people are children (36.83%). In 2011. year, 40 people were identified as victims of trafficking, and another 10 (25%) were children. Among the 40 newly identified victims, 13 people were male and all were of legal age when recruited and when mining began in the trafficking chain, while all the children identified in the previous year, and uposlednje three years were female.

Sexual exploitation is the most common form of exploitation to which victims were exposed (23). Labor exploitation have been exposed to all the males identified in this period (13), while in one case were present both types of exploitation. Through the forced marriage of two people being exploited, and one person was forced to commit crimes. Victims were most often drawn to trafficking through business deals (14).

Recruitment was conducted by Macro (4), by the very people close - close and extended family, Jack (6) or a person the victim knew a relatively shallow (8). In two cases the trade were foreign nationals (Bosnia and Herzegovina and Russia), while other victims, 38 of them (95%) were from Serbia. Human trafficking is sanctioned by the Criminal Code Articles 184, 388 and 389, on the basis of which is described territorial extent of the problem.

*Table 1. Territorial distribution of criminal charges in relation to criminal offenses and perpetrators in Serbia (PD- Police Department, CCRS- The Criminal Code of Republic Serbia, *- Directorate of Immigration, **- Directorate for Public Order) [8]*

Number of criminal charges in relation to criminal offenses				Number of perpetrators		
Department of the Interior RS	**Article No. 388. CCRS**	**Article No. 389. CCRS**	**Article No. 184. CCRS**	**Article No. 388. CCRS**	**Article No. 389. CCRS**	**Article No. 184. CCRS**
PD Belgrade*	7	1	5	20	5	7
PD Belgrade**	1	0	12	1	0	14
PD Pirot	/	/	/	/	/	/
PD Kragujevac	1	0	2	1	/	3
PD Nis	/	/	1	/	/	2
PD Novi Pazar	1	/	/	2	/	/
PD Novi Sad	15	0	3	32	/	3
PD Uzice	/	/	/	/	/	/
PD Smederevo	/	/	/	/	/	/
PD Leskovac	/	/	/	/	/	/
PD Vranje	4	/	/	8	/	/
PD Pancevo	1	/	/	3	/	/
PD Zrenjanin	2	/	/	6	/	/

Table 2. Territorial distribution of persons injured by these offenses and damaged by crime in Serbia (M-Male, F-Female, Sr-Serbian citizen, Fr- Foreign citizens, nd- no data) [8]

Number of persons injured by these offenses							Citizenship damaged by crime					
Department of the Interior RS	**Article No. 388. CCRS**		**Article No. 389. CCRS**		**Article No. 184. CCRS**		**Article No. 388. CCRS**		**Article No. 389. CCRS**		**Article No. 184. CCRS**	
Sex/Nationality	M	F	M	F	M	F	Sr	Fr	Sr	Fr	Sr	Fr
PD Belgrade,* Directorate of Immigration	1	14	1	/	/	6	13	2	1	/	6	/
PD Belgrade,** Directorate for Public Order	1	/	/	/	/	14	1	/	/	/	14	/
PD Pirot	/	/	/	/	/	/	/	/	/	/	/	/
PD Kragujevac	/	1	/	/	/	2	1	/	/	/	2	/
PD Nis	/	/	/	/	/	1	/	/	/	/	/	1
PD Novi Pazar	/	1	/	/	/	/	1	/	/	/	/	/
PD Novi Sad	3	30	/	/	nd	nd	33	/	/	/	nd	nd
PD Uzice	/	/	/	/	/	/	/	/	/	/	/	/
PD Smederevo	/	/	/	/	/	/	/	/	/	/	/	/
PD Leskovac	/	/	/	/	/	/	/	/	/	/	/	/
PD Vranje	/	3	/	/	/	/	3	/	/	/	/	/
PD Pancevo	/	1	/	/	/	/	1	/	/	/	/	/
PD Zrenjanin	/	2	/	/	/	/	2	/	/	/	/	/

Discussion

Some of the PhysicalConsequences of Human Trafficking Victims

Human trafficking victims in all phases and processes of this crime suffer physical and sexual abuse, and are forced to live in the conditions of physical and mental imprisonment. It is not rare that victims are also murdered as a message and warning to other victims in order to prevent testifying or seeking help, as well as ensuring constant control and surveillance for prolonged abuse and exploitation of the victims. Human traffickers constantly use violence and threats of violence. Victims are often beaten, raped, closed or limited in movement, denied food or water, tortured by knives and cigarettes, drugged and stoned, in order to provide absolute obedience. The sum of these abuses the victim experiences as the following: she is alone in a foreign country, isolated from the contact with countrymen, unable to communicate in her maternal language, confiscated personal and travel documents, unable to contact family, disoriented due to constant transfer and movement, subjected to constant physical and sexual abuse, unable to contact police for help due to fear of consequences; demanded to engage in physically dangerous and unprotected sexual activities on a daily basis with maximum number of hours, with male clients with whom she can't communicate; live under the regime of threats and repression towards her and her family [5].

Human trafficking victims are often ill due to difficult unsanitary conditions of life where they are forced to engage in prostitution and provision of sexual services. In addition, female victims of trafficking, working in various brothels often get pregnant, so their oppressors force them to abortions, while others are solely trafficked for the purpose of baby delivering that are later also subject of trafficking. Consequences of modern slavery refer to numerous psycho-physical and sexual traumas. Concurrently, as a consequence of forced sexual exploitation, a variety of sexually transmitted diseases appear, the most difficult one being the AIDS, as well as a variety of psychological consequences, such as different forms of depression, phobias, post-traumatic stress disorder, neuroticism.

Additionally, there are other diseases caused by unsanitary living conditions such as TBC, plague and other contagious diseases. Victims of modern slavery are daily exposed to large number of unprotected sexual relations with clients of diverse pro-

files and health status. Evaluations of the magnitude of the problem of AIDS presence in girls and women victims of human trafficking and estimations of their access to effective treatment, protection and support, during rescue or imprisonment, could improve the programs focused on victims' aid [6].

Alarming data indicate that there is a trend of significant decrease of the average age of the victims, based on new perceptions and requests of the clients who believe that sexual relation with children minimizes the risk of sexually transmitted diseases. It is commonly known that any form of prostitution represents a source of spreading various, even deadly diseases. It should be emphasized that prostitutes transmit diseases even when they are tested for them. Tests for HIV infection and other infections cannot detect the disease once it is captured, even though causes of the disease exist, live, and multiply in that person, and easily pass to another person during sexual contact, which subsequently transmits them to others, who may also transmit to others, and so on. Each minute inside the mouth, a million new lymphocytes appear, while in case of the presence of an infection, this number reaches ten million. In case individual is infected, lymphocytes contain multiple AIDS viruses. Often dentist are the first ones to suspect that a person suffers from AIDS once they see fertilization and mouth bleeding, white material and similar changes. Even a healthy individual almost always has small wounds in mouth after tooth brushing, rotten teeth, gingivitis, orsharp food, through which viruses easily enter. Today we know that an individual in blood and secretionshas diverse amount of viruses in different phases of disease. There is certainly a difference in types of kisses. However, from the abovementioned, it is clear that the possibility of getting infected by certain kisses cannot be disregarded [5].

Condyloma (genital warts) are a disease caused by the HPV virus i.e. *Humani Papilloma Virus*. HPV virus causes cervical cancer in women and treatment must be initiated straight after detection. The HPV virus has around 100 variations and not all of them cause condyloma, but the most prevalent types do. Only 4 types of virus cause cervical cancer, while over 30 causecondyloma. The most prevalent types of virus cause condyloma as a consequence, or the danger of cervical cancer. A number of documents confirm that the use of barrier contraception methods like condoms does not prevent spreading the HPV (*Consensus Statement on Cervical Cancer* of the US national institute for health in 1996). This institute, the National Institute of Allergy and Infectious Diseases and the Department of Health and Human Services of the US government, in the document *Workshop Summary* in 2000 indicate that there are no clinical evidence that condom would be a safe means of protection from diseases of Chlamydia (*Chlamydia Trachomatis)*, herpes, or syphilis. Chlamydia causes uterus infections, which ultimately results in infurtility, and in case infected woman is pregnant, child often dies even before birth.

„Human trafficking victim, an Ukrainian Olena Popik, died from AIDS in a hospital in Mostar. She was illegally in Serbia in the beginning of the 2004. The doctor, who performed autopsy, concluded that she was infected by AIDS and a number of other dangerous diseases including hepatitis C, miliary tuberculosis, syphilis and others. Throughout the last year, the Ukrainian represented an extreme epidemiological risk for everyone with whom she had any contact, and particularly those with whom she had sexual contact" [5].

SomeofthePsychologicalConsequences of Human Trafficking Victims

Human trafficking victims enter the circle of terror and cruelty, due to extremely brutal psychophysical treatment performed by the human traffickers. As a result of such exposure, the victims suffer from chronic trauma that tends to be long-term. Physical exhauster, confusion, phobias, obsessive-compulsive ideation, disorientation, amnesia, strong emotions, grief and inability to communicate represent determining psychophysical traits of an individual who has been liberated from the 'claws' of human traffickers during the first contact.

Post-traumatic stress disorder is the one of the common consequences of trauma [9]. Common symptoms of the post-traumatic stress disorder involve: episodes of repeated experience of trauma in imposed memories (flashbacks–Sudden revival of memories of past events), dreams or nightmares that appear on a persisting base of 'numbness' (Reduced emotional response associated with exposure to trauma), disregard of environment, emotio-

nal flatness (Persistent depressive mood of minor extent or in case the degree of emotional response is significantly reduced but is not lacking.), isolation from others, anhedonia (Inability to enjoy, loss of interest for pleasurable activities), avoidance of activities and situation that remind trauma. Rarely, dramatic attacks of fear, panic, or aggression may occur as stimulus causing rapid memory and/or revival of trauma and primary reactions to it [10].

Basic PTSD symptoms become chronic in case recovery does not provide favorable outcome. The most common symptoms refer to: intrusion, avoidance and hyper-sensitivity. Intrusion implies symptoms as flashbacks. Avoidance in case of victims of violence often causes disengagement from certain forms of social contacts avoiding situations that remind traumatic event. Hyper-sensitivity is the third key element of psycho-trauma or PTSD, and involves strong feelings of fear, isolation, rage, anger, excitement, insomnia and lack of concentration. Children as victims of human trafficking are often submitted to sexual abuse in family, and subsequently in the community, and traffic for the purpose of sexual exploitation represents the third step in the circle of violence that these children are exposed to. Likewise, they are often substance and alcohol dependent. Additionally, they exhibit significant behavioral disorders, while due to the long-term repetitive sexual trauma, they are unable to develop mature defense mechanisms, but commonly exhibit serious psychological disturbances associated with mental disorders. Due to the long-term exposure to psychological, physical, sexual and economic abuse and variety of manipulations by adults, these children, compared to other victims of sexual trauma, mainly demonstrate loss of trust in others, further complicating the process of the establishment of trust and safety with professional and assistants.

Intensity and range of sexual, physical and psychological violence that children victims of human trafficking suffer satisfy two basic criteria for diagnosing psychological trauma and its consequences. That involves exposure to threat to life and repeated sexual abuse during youth. Exposure to psychological trauma in these cases causes serious consequences in terms of physiological functioning of the organism, cognitive (thoughts and comprehension) and emotional behavior [11].

Conclusions

Human trafficking victims are exposed to numerous risks that may directly or indirectly impact their health. The risks may result from physical, psychological abuse (exploitation), enforced alcohol and drug abuse, social boundaries and manipulation, economic exploitation, legal insecurity related to victims' labor exploitation and inadequate working conditions and marginalization. Each of these risks involves heavy consequences to human trafficking victims' health [12]. It should be noted that the victims are almost never exposed to only one of the mentioned risks; rather they are often interrelated and multiplied. Impact of the aforementioned risks on health depends on their duration and intensity, as well as personal ability to tackle these problems. For instance, risk of physical abuse may result in violent taking of life (murder), self-harm (acute and chronic physical injuries) in the form of bruises, scratches, abrasions, head injuries, damages in muscles, nerves, senses, and all these consequences may result in minor or severe forms of disability.

Sexual abuse may result in damages of reproductive and sexual health, urinary infections, inability of birth, amenorrhea, dysmenorrhea, acute and chronic pain during sexual contact, occurrence of the cancer of uterus, incontinence, unwanted pregnancy, impact of insecure abortions (septic shock), etc. Psycho-physical risks and consequences of the victims, among other things demand interventions of medical institutions, doctors of different specializations and other medical staff, whose main goal is to evaluate health state, health support, and subsequently planning and realization of the program of therapy for chronic diseases and injuries. Data on victims' psycho-physical status are confidential and cannot be publicly exposed. Medical staff is only authorized to share information on victims' psycho-physical status, based on informed consent, to other professionals who provide direct assistance to human trafficking victims [13].

Exceptions are provided in Article 20. Codex of Professional Ethics. That is, doctor is acquitted of professional confidentiality or dismissed from the obligation of professional silence in case of client's informed consent or for his/her benefit, his family or society, or this has been decided in

accordance with law. In case of clients consent, doctor will evaluate which data should be kept confidential if their release could harm the client. In case of court order, the doctor needs to inform the client, except if otherwise provided by the court decision. In most cases, victims require medical assistance, to resolve acute health issues, such as injuries, diseases and abortions resulting from human trafficking, treatment of sexually transmitted diseases and substance addiction. Concurrently, the need for psychological support and assistance is considered crucial. Mental state examination and psychological treatment are performed on the basis of needs' hierarchy. Primary, organic health is addressed, then physical safety and daily routine, stabilization of physiological functions of sleep and alimentation, establishment of a trusting relationship between the victim of human trafficking, professionals and assistants, and finally in-depth psychological evaluation. Each victim and children in particular, has specific health and psychological status, which requires individualized treatment.

References

1. *UN Protocol to prevent, suppress and punish trafficking in persons, especially women and children, supplementing the United Nations convention against transnational organized crime. /2000/, New York, United Nations. Available:www.uncjin.org/Documents/Conventions/dcatoc/ final_documents_2/convention_%20traff_eng, 10.06.2012.*

2. *Vertamatti M. A. F., de Abreu L. C., Otsuka F. C., da Costa P. R. F., Ferreira J. D., Tavares C., Santos M. E., Barbosa C. P., Factors Associated to Time of Arrival at the Health Service after Sexual Violence, HealthMed Journal, ISSN1840-2291, Vol. 6. No. 1., 2012.*

3. *Wheaton E. M., Schauer E. J., Galli T. V.,Economics of Human Trafficking, International Migration, ISSN 0020-7985, Vol. 48., No. 5., pp. 114-141, 2010.*

4. *Moossy R., Sex Trafficking: Identifying Cases and Victims, National Institute of Justice Journal, U.S. Department of Justice, Office of Justice Programs, ISSN: 1067-7453, Iss. 262, pp. 1-10, 2010.*

5. *Rahman M. A., Human Trafficking in the era of Globalization: The case of Trafficking in the Global Market Economy, Transcience Journal, ISSN2191-1150, Vol 2., No1., 2011.*

6. *Kara S., Designing More Effective Laws Against Human, Northwestern Journal of International Human Rights, ISSN 1549- 828X, Vol. 9, No. 2 p. 123-147, 2011.*

7. *Williamson S., Prior M., Domestic Minor Sex Trafficking: A Network of Underground Players in the Midwest,Journal of Child & Adolescent Trauma, Taylor & Francais Group, ISSN: 1936-1521, Vol. 2, Iss. 1, pp. 1-16, 2009.*

8. *Trafficking in Serbia, Report for the Period 2000-2010, ASTRA - Action against Trafficking in Human Beings - Belgrade, ISBN:978-86-84889-14-2, pp. 364-381.*

9. *The UN manual on justice for victims, Available:www.uncjin.org/Standards/9857854.pdf,pp.7-8, 10.06.2012.*

10. *The ICD-10 classification of mental and behavioural disorders, World Health Organization, Geneva, 1992.*

11. *Zimmerman C, Yun K, Shvab I, Watts C, Trappolin L, Treppete M, et al. The health risks and consequences of trafficking in woman and adolescent - Findings from a European study. London, London School of Hygiene and Tropical medicine, 2003; Available:www.lshtm.ac.uk/php/ghd/docs/traffickingfinal.pdf, 10.06.2012.*

12. *Alempijevic Dj., Jecmenica D., Pavlekic S, Savić S, Aleksandric B., Forensic medical examination of victims of trafficking in human beings, Torture, 2007, vol. 17/2.*

13. *Banovic B., Bjelajac Z., Traumatic experiences, psychophysical consequences and needs ofhuman trafficking victims, Vojnosanitetski Pregled: Military Medical and Pharmaceutical Journal of Serbia, ISSN: 0042-8450, Vol. 69, No. 1, pp. 94-97, 2012.*

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Efficacy of folk medicinal plant extract Ankaferd Blood Stopper on full-thickness skin wound healing

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Abstract

Objective: Several haemostatic agents are available for clinical use. Ankaferd Blood Stopper (ABS), a mixture of five medicinal plant extracts, has been used historically as a haemostatic agent. The aim of this in vivo study was to investigate the effects of ABS on early full-thickness skin wound healing using a rat dorsal defect model.

Methods: Eighteen male albino wistar rats were used in this study and were divided into two groups: ABS group (Group 1) and the shame group (Group 2). After deep anesthesia with ketamine and xilosin, standard full-thickness skin excision wounds (1,5cm diameter and 2 mm deep) were created on the dorsal area of the all animals. The tampon form of the ABS was locally delivered at the wound site every day with the 2.5 X 7 cm^3 mL. The shame group underwent a daily tampon form of saline. The wound closure percentage is measured on 0, 3 and 14 days. The wound tisue samples were collected from the dorsal area on 14 day and were examined histopathologically for inflamatosis, granulocytosis and Fibrosis. The anatomical measurement and histopathological results were analyzed statistically by the Mann-Whitney U test. The Statistical Package for the Social Sciences (SPSS) version 11.5 was used for the data analysis. The analyses were conducted using a 0.05 confidence level. **Results:** Anatomically, the measurement of the wound closure percentage were significantly different on 3rd and 14th day between the ABS and the shame group ($p < 0.001$, $p= 0.031$ respectively). Histopathologically, also significant differences were found in both groups in terms of granulocytosis ($p = 0.019$) at the 14 the day. Decreased inflammation and increased fibrosis were found in the ABS group, but there were no statistically significant differences from the shame group ($p = 0.065$, $p = 0.088$; respectively).

Conclusions: This study revealed that ABS product can provide an efficacious to accelerate wound healing. Further in vitro and in vivo studies are necessary for evaluating the benefits and possible adverse effects of the application of ABS product on wound healing.

Key words: Ankaferd Blood Stopper, wound healing, full-thickness skin excision.

Introduction

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue (1). Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues (2). In mammals, wound healing is a result of three overlapping processes: hemostasis and inflammation, granulation tissue formation, re-epithelialization and remodelling, which enable wound closure and the restoration of a functional barrier (3). Wound healing normally develops without complications, but the resolution of inflammation is critical. Indeed, several conditions like ageing, obesity and many other disorders can compromise the normal resolution of the inflammation and resulted in chronic wound and ulcer development, leading to tissue damage (4).

Literally millions of traumatic wounds are treated each year in emergency departments. Wound management is an area of still ongoing research, much of which supports traditionally performed techniques, however some of which also is still trying to resolve controversial topics. Plants have the immense potential for the management and treatment of wounds. A large number of plants are

used in many countries for the treatment of wounds and burns (5-9). Ankaferd Blood Stopper® (ABS; Ankaferd Health Products Ltd., Istanbul, Turkey) is a traditional folk medicinal plant extract product that has been approved in the management of external hemorrhage and dental surgery bleedings in Anatolia. ABS comprises a standardized mixture of the plants Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica. Several studies have shown that each of these plants has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators (10-15).

Several animal studies of different experimental models including incisional or excisional traumas have revealed its efficacy in stopping the bleeding in vivo (16-18). However, few study has been investigated on the anti-inflammatory activities of Ankaferd Blood Stopper® (19). To our knowledge previosly no study has reperted on skin wound healing activities of ABS. The aim of this in vivo study was to evaluate the effects of Ankaferd Blood Stopper® on early wound healing using a rat dorsal full-thicness skin wound model.

Methods

Ankaferd Blood Stopper

Ankaferd Blood Stopper (Trend Teknoloji Ilac AS, Istanbul, Turkey) is a licensed pharmaceutical plant extract that is applied directly to injured skin and mucosa as a liquid (solution) or spray or in a dressing (tampon) (20). It produces active hemostasis for the management of external, postsurgical, and dental hemorrhage (21). ABS contains a standardized mixture of 5 medicinal plant extracts. The active ingredients in the tampon form of ABS (2.5 X 7 cm^3 mL) are as follows: 0.18 mg of dried root of Urtica dioica, 0.24 mg of dried leaf of Vitis vinifera, 0.27 mg of dried leaf of Glycyrrhiza glabra, 0.21 mg of dried leaf of Alpinia officinarum, and 0.15 mg of dried leaf of Thymus vulgaris (22). This study used the tampon form of ABS supplied in 2.5 cm x 7 cm – 3 ml to the full-thickness skin wound healing in animal model.

Animals

Eighteen male rats weighing between 180 and 200 g were used in this study. The animals were purchased locally from the Research Institute of Physiology (Gaziantep, Turkey). Twenty wistar albino rats were maintained in the Physiology Department animal house at a constant temperature of 22 ± 4°C with a 12-hour light/dark cycle and fed Standard pellet chow and water ad libitum. The animals of each group were sacrificed with an overdose of pentobarbital (40 mg / kg) on the 14th day after surgery. The experimental protocol was in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC), and was approved by the Harran University Medical School Ethics Committee.

Experimental Design

The rats randomly assigned to two groups of 9 animals each. Group 1, study group, with full skin-thickness excisions, treated daily with tampon form of ABS. Group 2, shame, daily irrigated with saline and closed with a tampon. Prior to surgery, the animals were anesthetized with a 0.7 mL intramuscularly injection of a solution containing xylazine hydrochloride (Rompun®, Bayer, Leverkusen, Germany) and ketamine hydrochloride (Ketalar; Pfizer, New York, NY USA) at 1 / 0.5 proportion, 0.1 mL / 100 g body weight. Surgery was performed under sterile conditions. After surgery, dressing (tampon) form of the ABS® was applied to Group 1 (study group) at the first day, and this application was repeated everyday, while Group 2 received no treatment and served as the shame group. No postoperative complications were noticed during the postsurgical course. All animals survived throughout the study period.

Macroscopic skin wound-healing assay

A standardized ellipsoid round full thickness sckin tissue exicision (epidermis, dermis, and hypodermis) defect (1.5 cm in diameter, 1 mm in depth) was created on the dorsal area of rats (Figure 1A). The wound closure rats were measured on the day 3, and day 14 by the tape measure.

Tissue preparation and histopathological examination

On day 14 the rats were anesthetized and the part of the wounds were taken from animals for histopathological examination. The wound specimens at 14 day postwounding including full

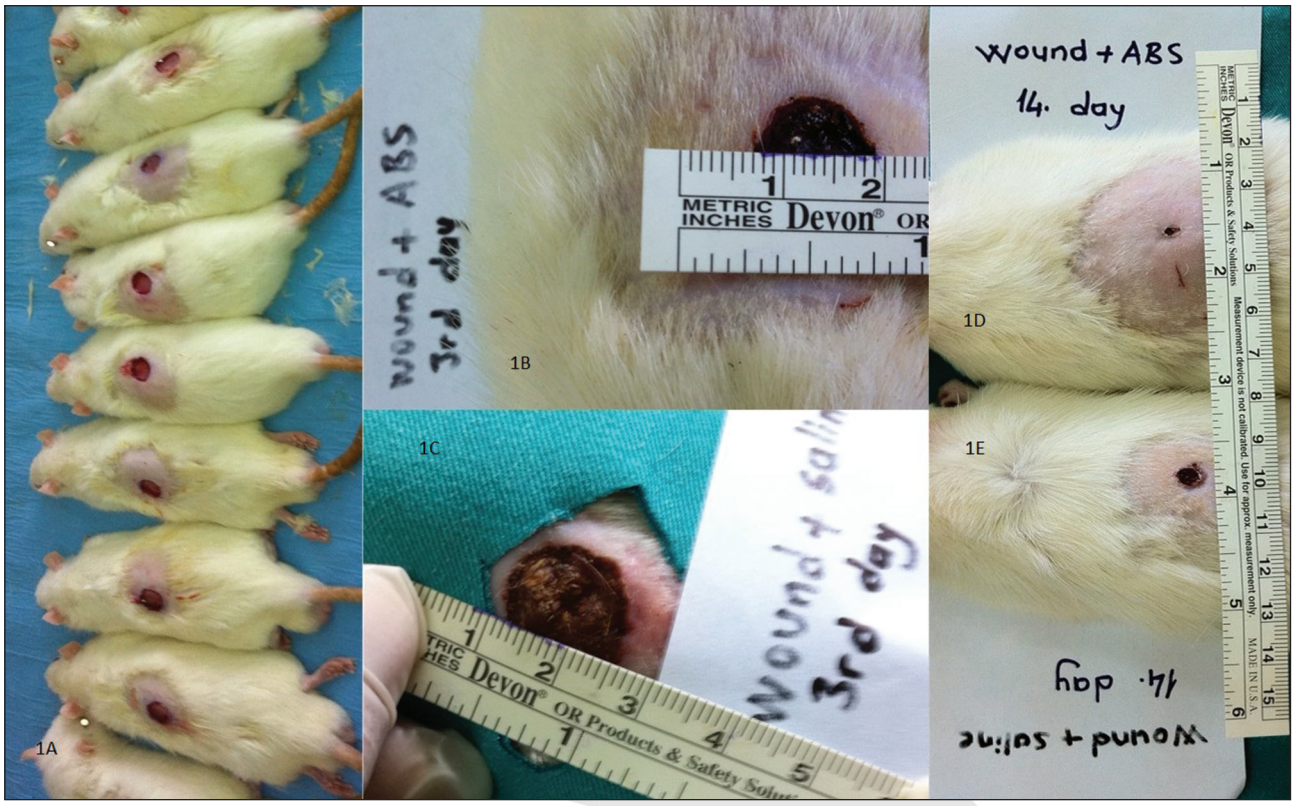

Figure 1. Wound healing effect in rats. A standardized ellipsoid round full thickness sckin tissue exicision defect (1.5 cm in diameter, 1 mm in depth) were created on the dorsal area of rats (1A) at the 0 day. ABS significantly contributed to the wound closure percentage compared with the control group at 3th (1B: ABS group, 1C: shame group) and 14th (1D: ABS group,1E:shame group) postwounding day

thickness skin layers (epidermis, dermis, and hypodermis) were fixed in 10 % neutral buffered formalin overnight at 4 °C and processed according to the routine light microscope tissue processing methods. The specimens were embedded in paraffin and routine hematoxylin and eosin (H&E) staining and Masson Trichrome (MT) staining were performed. The sections were examined with light microscope under 100 x magnification (Olympus BX51 TF, Tokyo, Japan).

A histomorphological review was performed by a single blinded pathologist to evaluate the presence of inflamatosis, granulocytosis and fibrosis. The scores for inflamatosis, granulocytosis and fibrosis were determined by counting the associated cells and their ratio to the total cell count in a standardized area at 100 x magnification (Figure 2A, 2B, 2C, 2D,2E) . As shown in table 1, the ratio of cells between 0-25% was scored as none, 25-50 % as slight (+), 50- 75 % as moderate (++), and 75-100 % as advanced (+++).

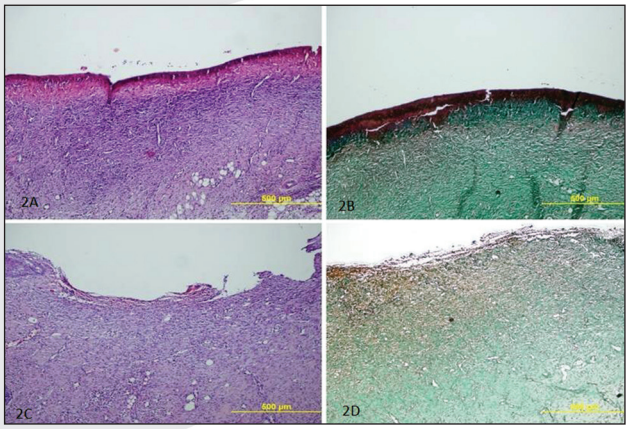

Figure 2. Photomicrograph of full thickness wounds in rats at 14th day (magnification, 100x). Shame group: advanced presence of inflammation stained with H&E (2A), slight granulation and fibrosis with MT (2B). ABS group: slight inflammation, moderate granulation and moderate fibrosis stained with H&E (2C), and Moderate granulation, moderate fibrosis with MT (2D)

The wound specimens at 14 day postwounding including full thickness skin layers (epidermis,

dermis, and hypodermis) also were observed under Olympus DP71 image analyzing system. 5 µm tissue sections with H&E and MT staining were examined and photographed.

Data analysis

Statistical analyses were carried out using the SPSS statistical package, version 11.5 (SPSS, Inc, an IBM Company, Chicago, Illinois) for Windows. The data on percentage anti-inflammatory and wound healing was compared among groups using the Mann- Whitney U test. The mean and standard deviation or median, minimum-maximum data were calculated for each group. All data are expressed as the mean and 95 % confidence intervals. The values of $p \leq 0.05$ were considered statistically significant.

Results

Anatomically wound of the all animals were measured on 3rd and 14th days (Table 1). On the 3rd day, median of the wound diameters were 1 ± 0.15 (0.9 - 1.2) in the ABS group and 1.4 ± 0.1 (1.3 - 1.4) in the shame group. Median of the wound closure also were significantly higher in the ABS group on the 14th day. Comparisons between the ABS and shame groups indicate a significant variability in the wound closure on the 3rd and 14th days ($p < 0.001$, $p = 0.031$ respectively). The appearance of the repaired wounds sites is shown in Figure 1B,1C, 1D and 1E.

As shown in the table 2, in the study group, 89 % of the specimens show moderate granulositosis, while in the shame moderate granulositosis was observed in 33.3 % of the specimens. Statistically significant differences were found between the ABS and shame groups as for the granulositosis ($p<0.019$). In the ABS group, 33.3 % of the specimens was show moderate inflamatosis and 44 % moderate fibrosis, while in the shame group moderate inflamatosis was observed in 56 % and moderate fibrosis in 11.1 % of the specimens, respectively (Figure 2A,2B,2C and 2E). Decreased inflammation and increased fibrosis were found in the ABS group, but there were no statistically significant differences from the shame group ($p=0.065$, $p=0.088$; respectively).

Discussion

In this study, we tested the effects of ABS tampon form on the full tickness skin wound healing in a rat model. According to our findings, fibrosis,

Table 1. The percentage of inflamatosis, granulocytosis and fibrosis between the ABS and shame group*

Characteristic	ABS group [n (%)]	Shame group [n (%)]	Statistical significance [p]¥
Inflamatosis			
None	33.3	0	
Slight(+)	33.3	33.3	0.065
Moderate(++)	33.3	56	
Advanced(+++)	0	11.1	
Granulocytosis			
None	0	0	
Slight(+)	11	66.7	0.019
Moderate(++)	89	33.3	
advanced(+++)	0	0	
Fibrosis			
None	0	11.1	
Slight(+)	55	78.8	0.088
Moderate(++)	44	11.1	
advanced(+++)	0	0	

**Trademark: Ankaferd Health Products Ltd., Istanbul, Turkey.*

¥From independent samples t-test. Values are Median ± IR (min - max). The ratio of cells between 0-25% was scored as none, 25-50 % as slight (+), 50- 75 % as moderate(++), and 75-100 % as advanced (+++).

Table 2. Excision wound closure rate between the ABS and shame group*

Characteristics	Day 3 wound diameters [cm]		Day 14 wound diameters [cm]	
No. of Animals	**ABS**	**Shame**	**ABS**	**Shame**
1	1.1	1.3	0.11	0.16
2	1.0	1.3	0.15	0.24
3	0.9	1.4	0.12	0.21
4	0.9	1.4	0.20	0.21
5	1.2	1.3	0.05	0.20
6	1.2	1.4	0.20	0.22
7	1.0	1.4	0.22	0.10
8	1.0	1.4	0.16	0.40
9	1.1	1.3	0.11	0.30
¥Median (Min-Max)	1±0.15(0.9-1.2)	1.4±0.1(1.3-1.4)	0.15±0.09(0.05-0.22)	0.21±0.09(0.10-0.40)
p	< 0.001		0.031	

**Trademark: Ankaferd Health Products Ltd., Istanbul, Turkey.*

¥ From independent samples t-test. Values are Median ± IR (min-max)

granulositosis and wound contraction percentage were increased, while inflammation decreased.

Each ingredient of ABS mixture has specific characteristics. T. vulgaris has been shown to exhibit varying levels of anti-oxidant activity, which may help to prevent in vivo oxidative damage, such as lipid peroxidation, associated with atherosclerosis (12). Inoculation experiments on detached leaves of V. vinifera exhibited enhanced resistance towards pathogens (10,11). A. officinarum inhibits nitric oxide production in lipopolysaccharide activated mouse peritoneal macrophages (13). G. Glabra inhibits angiogenesis, decreases vascular endothelial growth factor production and cytokineinduced neovascularization (14). U. dioica can produce hypotensive responses through a vasorelaxation effect mediated by the release of endothelial nitric oxide and the opening of potassium channels, and through a negative inotropic action (15).

Generally wound-healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibers (23). The wound healing of the skin defect was determined by the percentage of wound surface covered by regenerating epidermis. The wound closer percentage of the ABS treated group was found to be which was significantly higher than that of shame group of animals on 14th post wounding day. These wound closure rates made a good match to the results of wound scratch assay in vitro and demonstrated the accelerating effect of ABS on the wound healing. It was seen that the wounds treated by ABS recovered much more quickly with better skin appearance.

During the proliferative phase, a granulation tissue (new stroma) is formed. Fibroblasts constitute the predominant cell type in granulation tissue. They start to proliferate and produce matrix components (hyaluronan, fibronectin, proteoglycans and type I and III procollagen), which are then deposited locally (24). The histopathological results of the our study showed that the wound treated with ABS showed more intense granulositosis which is associated with new tisue formation activity of the components of the ABS. This indicates a positive role of the extract on the cutaneous wounds healing process. In addition, the ABS treated group showed increased fibrosis rate. But occurrence of fibrosis was statistically similar in both groups.

Wound healing is a physiological process necessary for repair and regeneration of injured skin tissue. This process greatly depends on the crosstalk between inflammation, oxidative stress and angiogenesis (25). Inflammatory cells appear very rapidly in a wound after injury, as reported by Kagawa and collaborators (26). In their study, isler et al showed that ABS decreased the inflammation and necrosis process and increased the new bone formation in early bone healing period without causing any foreign body reaction in a rat model (19). Accordingly, in our study decreased inflammation was found in the ABS treated group,

which is probably related to the antiinflammatory activity of some components of the haemostatic agent. Also we did not see any adverse effects during the observation and could not make any conclusion on wound healing.

Conclusion

This experimental study revealed that ABS product can provide an efficacious to accelerate wound healing. It is decreases the inflammation and increase the granulositosis and fibrosis in early full ticknes wound healing period. Further in vitro and in vivo studies are necessary to evaluating the benefits and possible adverse effects of the application of ABS on wound healing.

Authors' contributions

MTG: Protocol, development and manuscript writing, HK, OS and SK: Data Collection & data entry, TD and MC: Statistical Support and data analysis. All authors read and approved the final manuscript.

References

1. *Jalalpure SS, Agrawal N, Patil MB, ChimkodeR, Tripathi A:Antimicrobial and wound healing activities of leaves of Alternanthera sessilis Linn. International Journal of Green Pharmacy 2008, 2: 141–144.*

2. *Martin P: Wound healing-aiming for perfect skin regeneration. Science 1997, 276: 75–81.*

3. *Tan W, Bailey AP, Shparago M, Busby B, Covington J, Johnson JW, et al.: Chronic alcohol consumption stimulates VEGF expression, tumor angiogenesis and progression of melanoma in mice. Cancer Biol Ther 2007,6: 1211-1217.*

4. *Pond CM: Paracrine interactions of mammalian adipose tissue. J Exp Zool A Comp Exp Biol 2003,295: 99-110.*

5. *Nayak BS,. Pinto Pereira LM: Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine 2006, 6: 41.*

6. *Parente LM, Lino Junior Rde S, Tresvenzol LM, Vinaud MC, de Paula JR, Paulo NM: Wound Healing and Anti-Inflammatory Effect in AnimalModels of Calendula officinalis L. Growing in Brazil. Evidence-Based Complementary and Alternative Medicine 2012, 2012:375671. doi:10.1155/2012/375671*

7. *Kosger HH, Ozturk M, Sokmen A, Bulut E, Ay S: Wound healing effects of Arnebia densiflora root extracts on rat palatal mucosa. European Journal of Dentistry 2009, 83: 96–99.*

8. *Garg VK, Paliwal SK: Wound healing activity of ethanolic and aqueous extract of Ficus benghalensis. J Adv Pharm Technol Res 2011, 2:110-114.*

9. *Nayak S, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A: Evaluation of wound healing activity of Allamanda cathartica. L. and Laurus nobilis. L. extracts on rats. BMC Complementary and Alternative Medicine 2006, 6: 12.*

10. *Barka EA, Belarbi A, Hachet C, Nowak J. Audran JC: Enhancement of in vitro growth and resistance to gray mould of Vitis vinifera co-cultured with plant growth-promoting rhizobacteria. FEMS Microbiol Lett 2000, 186: 91-95.*

11. *Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A: Inhibitory effect of endophyte bacteria on Botrytis cinerea and its influence to promote the grapevine growth. Biol Control 2002, 24: 135-142.*

12. *Lee SJ, Umano K, Shibamoto T, Lee KG: Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chem 2007, 91:131-7.*

13. *Matsuda H, Ando S, Kato T, Morikawa T, Yoshikawa M: Inhibitors from the rhizomes of Alpinia officinarum on production of nitric oxide in lipopolysaccharide-activated macrophages and the structural requirements of diarylheptanoids for the activity. Bioorg Med Chem 2006,14:138-142.*

14. *Sheela ML, Ramakrishna MK, Salimath BP: Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by Glycyrrhiza glabra. Int Immunopharmacol 2006,6: 494-498.*

15. *Testai L, Chericoni S, Calderone V, Nencioni G, Nieri P, Morelli I, et al.: Cardiovascular effects of Urtica dioica L. (Urticaceae) roots extracts: in vitro and in vivo pharmacological studies. J Ethnopharmacol 2002, 81: 105-109.*

16. *Bilgili H, Kosar A, Kurt M, Onal IK, Goker H, Captug O, et al.: Hemostatic Efficacy of Ankaferd Blood Stoper in a Swine Bleeding Model. Medical Principles and Practice 2009, 18: 165-169.*

17. *Huri E, Akgül T, Ayyildiz A, Ustün H, Germiyanoğlu C: Hemostatic Role of a Folkloric Medicinal Plant Extract in a Rat Partial Nephrectomy Model: Controlled Experimental Trial. Journal of Urology 2009,181: 2349-2354.*

18. *Ismail Iynen, Ozgur Sogut, Rustu Kose: The Efficacy of Ankaferd Blood Stopper in Heparin-Induced Hemostatic Abnormality in a Rat Epistaxis Model. Otolaryngology –Head and Neck Surgery 2011; 145: 840-844.*

19. *Isler SC, Demircan S, Cakarer S, Cebi Z, Keskin C, Soluk M, et al.: Effects of folk medicinal plant extract Ankaferd Blood Stopper® on early bone healing. J Appl Oral Sci 2010, 18:409-414.*

20. *Kurtaran H, Ark N, Ugur KS, Sert H, Ozboduroglu AA, Kosar A, et al: Effects of a topical hemostatic agent on an epistaxis model in rabbits. Current Therapeutic Research 2010; 71: 105-110.*

21. *Singer AJ, McClain SA, Katz A: A porcine epistaxis model: hemostatic effects of octyl mcyanoacrylate. Otolaryngol Head Neck Surg 2004,130: 553-557.*

22. *Goker H, Haznedaroglu IC, Ercetin S, Kirazlı S,Akman U,Ozturk Y, et al.: Haemostatic actions of the folkloric medicinal plant extract, Ankaferd blood stopper. J Int Med Res 2008, 36: 163-170.*

23. *Gupta N, Jain UK: Investigation of wound healing activity of methanolic extract of stem bark of mimusops elengi linn. Afr J Tradit Complement Altern Med 2011, 8: 98-103.*

24. *Robson MC, Steed DL, Franz MG: Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg 2001, 38: 72–140.*

25. *Soares R, Azevedo I: Inhibition of S1P by polyphenols prevents inflammation and angiogenesis: NFkappaB, a downstream effector? Free Radic Biol Med 2007, 42: 311.*

26. *Kagawa S, Matsuo A, Yagi Y, Ikematsu K, Tsuda R, Nakasono I: The time-course analysis of gene expression during wound healing in mouse skin. Leg Med (Tokyo) 2009, 11: 70-75.*

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Impact of Iron-folic acid supplementation on passive avoidance memory in adult male Wistar rats

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Abstract

Background: Iron imbalance in the brain, including excess accumulation and deficiency, is associated with neurological disease and cognitive dysfunction. On the other hand, folic acid is a water-soluble B-vitamin that may have roles in prevention of central nervous system development, mood disorders, dementia and Alzheimer's disease. So, in this study we assessed the effects of co-administration of iron and folic acid supplementation on passive avoidance memory in adult male Wistar rats.

Methods: Animals were divided into four groups randomly with 8 in each: 1) Fe30: Rats that received iron 30 mg/kg/day via gavage for one week, 2) FA15: Rats that received folic acid 15 mg/kg/day single daily intra peritoneal for one week, 3) Fe+FA: rats that received coadministration iron 30mg/kg/day and folic acid 15 mg/kg/day for one week. 4) Sham (saline & sorbitol): received co-administration folate and iron vehicles for one week then all groups were tested in shuttle box for memory.

Results: Data showed that co-administration Fe and FA (Fe+FA) significantly improved short term ($P<0.001$) and long term ($P<0.05$) memory with compare to each iron or folic acid group alone.

Conclusion: It appears that iron-folic acid supplementation co-administration had better effect than iron supplementation alone.

Key words: Folic acid, iron, avoidance memory, rat.

Introduction

Iron is an essential trace metal in the human diet due to its obligate role in a number of metabolic processes (1). It is essential for nearly all living organisms as it is involved in fundamental processes such as transport and exchange of oxygen, enzyme action, and DNA, RNA and protein syntheses. The brain metabolism depends on its balanced iron concentrations in various its regions (2, 3). In the brain, iron is an important co-factor for the generation of dopamine and several cellular and intracellular processes e.g. tyrosine hydroxylase (3). Iron is found predominantly in oligodendrocytes in the brain and required for myelin production (4) as well as in the production of several neurotransmitters such as norepinephrine and serotonin and generation of GABAergic activity (5). Iron imbalances in the brain, including excess accumulation and deficiency, are associated with neurological disease and dysfunction; yet, their origins are poorly understood (6). It is now generally accepted that iron accumulation in selective regions of the brain may acts as free radicals, thereby possessing implication for the etiology of neurodegenerative disorders (7). Although excess Iron appears to be one of the main factors in the metal induced neurodegeneration. Evidence suggests that brain iron deficiency at any time in life may disrupt metabolic processes and subsequently change the cognitive and behavior functions (8) On the other hand, however, total Fe losses vary from 0.17 to 1.05 mg/day, according to age and gender, a process resulting mainly from elimination of intestinal epithelial death cells. For this reason the losses necessarily must be compensated by nutritional supplementation (9). Even so, until 1974 little or no attention was paid to brain iron metabolism and brain function. Since then there has been an active interest in brain iron metabolism, not only as a consequence of its deficiency with an effect on cognitive processes, but also the role of excess brain iron accumulation and its involvement in neurodegeneration and progressive neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington chorea, and Haller Voren Spatz disease (10). In contrast to many of the dietary supplements now flooding the shelves of grocery stores and pharmacies, research on dietary folate and its links to human disease began

decades ago, well prior to the dietary supplement craze that has recently descended upon us (11). Folic acid (folate) is a water-soluble B-vitamin which present in food such as dried beans, peas, lentils, oranges, whole-wheat products, liver, asparagus, beet, broccoli, Brussels sprouts, and spinach (12). Folate participates in the transfer of 1-carbon unit (such as methyl, methylen, and formyl groups) to the essential substrates involved in the synthesis of DNA, RNA, and proteins. Folic acid plays a role in the methylation of homocysteine providing the methyl group for the conversion of methionine to s-adenosyl methionine (13, 14). An increase in homocysteine (Hcy) levels is a major consequence of folate deficiency that may have adverse effects on multiple organ systems during aging Recent studies of cell culture and animal models of neurodegenerative disorders have provided evidence that folate deficiency and elevated Hcy levels can indeed neurons vulnerable to dysfunction and death. Exposure of cultured rat hippocampal neuron to folate medium and Hcy promotes apoptosis and increase the vulnerability of the neurons to being killed by amyloid β-peptide, a protein believed to be responsible for nerve cell death in Alzheimer's disease (14) There is evidence to implicate Hcy in increased oxidative stress, DNA damage, the triggering of apoptosis and excitotoxicity, all important mechanisms in neurodegeneration (15). During the past decade data have accumulated that support roles for folate and Hcy in modifying risk of Alzheimer's and Parkinson's diseases (16, 17). Some studies showed that, the B vitamins, specifically folate, have been implicated in neurological disorders, including those associated with cognitive disorders (18). More recently, folate deficiency may also contribute to the declines in cognitive and other neurological functions that occur during normal aging (19). Conversely, high folate intake was associated with lower risk of Alzheimer's diseases (20). The adverse effects of folate deficiency and elevated Hcy levels on the developing brain have been well documented. But, relatively little research has investigated the effects of impact iron-folic acid supplementation on cognitive function in normal aging (21). So, purpose of this study was to assess the potential effects of impact of iron- folic acid supplementation (IFA) on passive avoidance memory in adult subjects.

Materials and Methods

Animals: Forty adult male albino rats of Wistar strain (250±20g, 3-4 months) were used in this study that obtained from Ahvaz Jundishapur University of Medical Sciences (AJUMS) laboratory animal center. Animals were housed in standard cages under controlled room temperature (20±2 ◦C), humidity (55-60%) and light exposure conditions 12:12 h light–dark cycle (lighted on 07:00 am). All experiments carried out during the light phase of the cycle (8:00 am to 6:00 pm). Access to food and water were ad libitum except during the experiments. Animal handling and experimental procedures performed under observance of the University and Institutional legislation, controlled by the Local Ethics Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used. Prior to the onset of behavioral testing, all rats were gentle handled for 5 minutes daily. Animals were divided randomly into four groups, consisting of 8 animals in each: 1) Fe30; rats that received iron 30 mg/kg/day via gavage for one week (5) , 2) FA15; rats that received folic acid 15 mg/kg/day single daily intra peritoneal for one week (22) , 3) Fe+FA; rats that received coadministration iron 30mg/kg/day and folic acid 15 mg/kg/day, rats recived folic acid two hours after recived iron supplementation for one week, 4) Sham; received coadministration of vehicle of folic acid (normal saline) and vehicle of iron supplementation (%5 sorbitol in water), rats recived normal saline two hours after received %5 sorbitol in water for one week.

Then, all groups were trained for passive avoidance learning and short- and long-term memories with using two-way shuttle box apparatus.

Passive avoidance task: The apparatus used for evaluation the passive avoidance task was two-way shuttle box (Borj Sanaat Co. Iran), which consisted of two adjacent Plexiglas compartments of identical dimensions (27×14.5×14 cm). For the experimental procedure, on the first day (adaptation) each rat was allowed a 3 minutes adaptation period prior and free access to either the light or dark compartment of the box to avoidance training and after being placed in a shuttle-box. Following this adaptation period, on the second day (training

phase) rats were placed into the illuminated compartment and 30 seconds later the sliding door was raised. Upon entering the dark compartment the door was closed and a 1.5 mA constant-current shock was applied for 3 seconds. After 20 seconds the rat was removed from the dark compartment and placed into home cage. In order to test short-term and long term memories, 48 hours and 30 days after receiving foot shock, the rats were placed in illuminated chamber and 30 seconds later the sliding door was raised and the latency of entering the dark compartment (step-through latency) and the time spent there during 5 min were recorded again. The maximum time that considered in this procedure were 300 seconds (24, 25).

*Statistics***:** Data were expressed as mean ±S.E.M. of values for memory test. Statistical analysis was performed by one-way ANOVA followed by LSD post hoc test. A *P-value* less than 0.05 were assumed to denote a significant difference and levels of significance are indicated by symbols: * for difference between groups vs. control and # for difference between treated groups with iron group.

Result

Analyze data in step-through latencies 48 hour after training between FA15, Fe30, Fe+FA and sham groups showed that there was significant (P<0.001) difference between rats received iron supplementation and Fe+FA groups (Fig. 1) and so, 30 days after training, in step-through latencies there was significant (P<0.05) difference between rats which were received Fe+FA and iron group (Fig. 2). On the other hand, statistically analysis in the time spent in the dark chamber 48 hour after training, there was no significant difference between Fe+FA and iron groups in this step (Fig. 3). Also 30 days after training, in this step there was no significant difference between any of groups (Fig. 4). Then, our results showed that Fe+FA supplementation improved short-term and long-term memory with compare to iron supplementation alone but there was no difference between Fe+FA supplementation group and folic acid group with 15mg/kg/day dose.

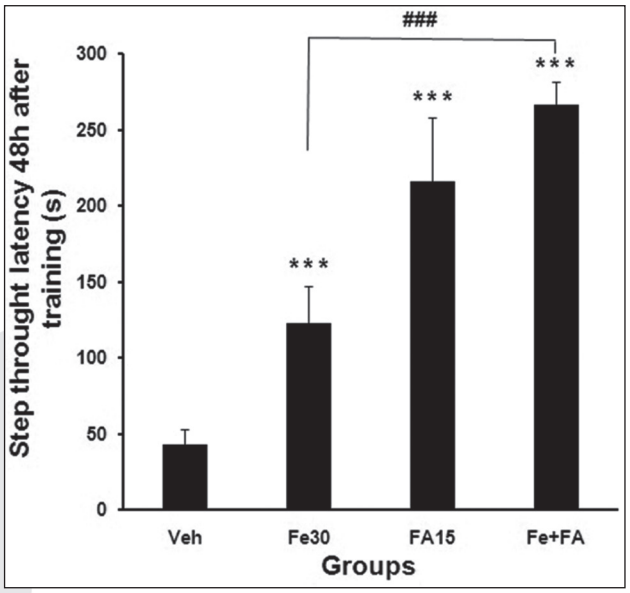

*Figure 1. Comparing groups on step-through latency 48 h after training, (***P<0.001, n=8), (Veh: Sham treated, FA15: Folic acid 15mg/kg/day, Fe30: iron 30mg/kg/day, Fe+FA: iron 30mg/kg/day+ Folic acid 15mg/kg/day)*

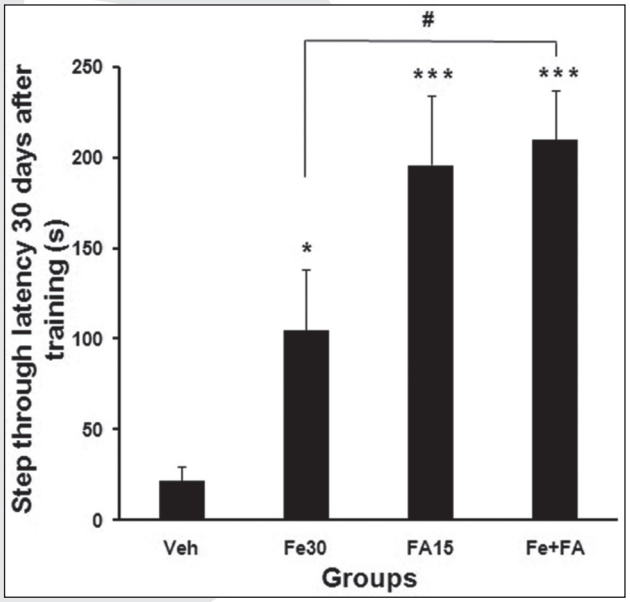

*Figure 2. Comparing groups on step-through latency 30 days (long-term memory) after training (*P<0.05, ***P<0.001, n=8), (Veh: Sham treated, FA15: FA15: Folic acid 15mg/kg/day, Fe30: iron 30mg/kg/day, Fe+FA: iron 30mg/kg/day+ Folic acid 15mg/kg/day)*

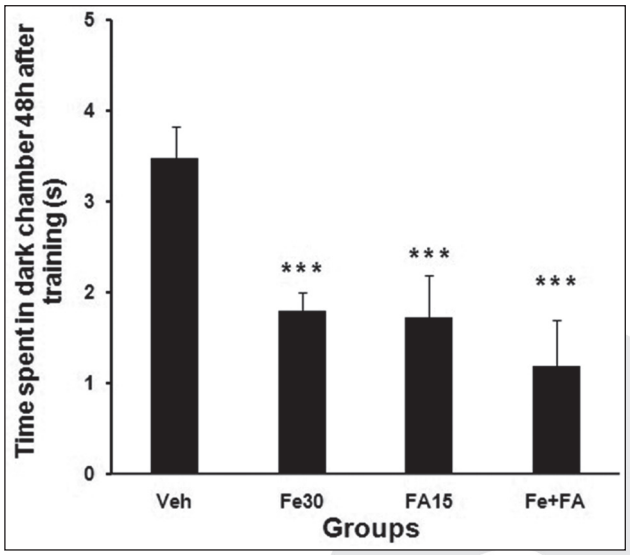

*Figure 3. Comparing groups on time spent in the dark chamber 48 h after training, (*** P<0.001, n=8), (Veh: Sham treated, FA15: Folic acid 15mg/kg/day, Fe30: iron 30mg/kg/day, Fe+FA: iron 30mg/kg/day+ Folic acid 15mg/kg/day)*

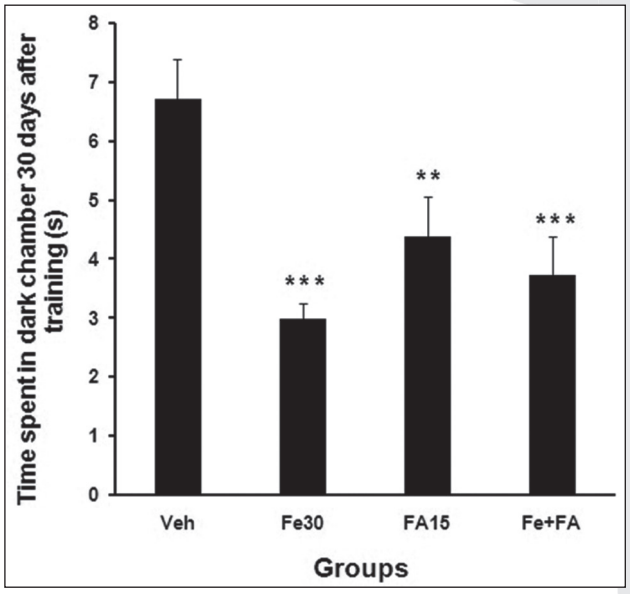

Figure 4. Comparing groups on time spent in the dark chamber 30 days after training (n=8), (Veh: Sham treated, FA15: Folic acid 15mg/kg/day, Fe30: iron 30mg/kg/day, Fe+FA: iron 30mg/kg/day+ Folic acid 15mg/kg/day)

Discussion

The present study has investigated possibility that Fe+FA administration could increase passive avoidance memory comparative with iron administration alone. In a previous reports we had demonstrated that iron supplementation whit 30 mg/kg/day or folic acid whit 15 mg/kg/day increased passive avoidance memory in separately. But in this study we assessed the potential effects of Fe+FA supplementation on passive avoidance memory in adult subjects. Passive avoidance condition was assessed in particular since the relation between this behavior and Fe+FA in adult is not well documented in the literature. The data shown here demonstrated for the first time that IFA supplementation significantly improved short term (P<0.001) and long term (P<0.05) memory compare to iron group. The prevalence of iron, folic acid and vitamin B12 deficiencies and their role in the pathophysiology of recurrent aphthous stomatitis is not well known, although several reports have considered their importance and relevance (26). In the brain, sufficient iron supply is critical to a wide variety of biochemical pathways, ranging from basic cellular metabolism to catecholamine synthesis and myelination. Iron is also involved in the production of reactive oxygen species and can be toxic in excess. Thus, both iron overload and deficiency must be avoided, yet imbalances do occur in some individuals for reasons that are yet unknown. Iron overload in relevant brain regions is linked to Parkinson's disease and Alzheimer's disease, while iron deficiency is associated with restless legs syndrome and attention deficit hyperactivity disorder. Iron deficiency also leads to specific cognitive impairment and emotional effects, which vary across developmental stages (6). However, it has been proposed that iron accumulation in the brain mediates oxidative damage, and neuronal death associated with neurodegenerative disorders (27). It is interesting to note, however, after a long period of understanding role of folic acid in the pathogenesis of megaloblastic anemia, increase attentions were given to folic acid in the last decade due to its role in the prevention of the development of various diseases, including neural tube defect, atherosclerosis heart disease, and cancer. Despite their important role in cognitive function, the value of B vitamin supplementation is unknown. The evidence does not yet provide adequate evidence of an effect of vitamin B6 or B12 or folic acid supplementation, alone or in combination, on cognitive function testing in people with either normal or impaired cognitive function (28). B vitamins such as thiamin,

riboflavin, vitamin B6, and folate are known to be essential for the maintenance of normal metabolic functions in the brain (29). Among Other B Vitamins, Folte has received much attention recently as its low serum level is found to be closely associated with structural and functional abnormalities in the brain. Low serum folate levels have been related to atrophy of the cerebral cortex, dementia cerebrovascular diseases and to specific domains of cognitive functioning such as episodic recall and recognition (29, 30). In addition to this, folate supplementation has shown a positive effect on cognitive functions and memory deficits (16, 31). Folate, along with vitamin B12, is known to be important for cognitive function (29). The mechanisms by which chronic folate deficiency adversely affects CNS function are incompletely understood. Folic acid plays an essential role in one-carbon metabolism: it is required both in the remethylation of homocysteine to methionine and in the synthesis of *S*-adenosyl-methionine, the principal biological methyl donor in numerous methylation reactions. Reduced DNA methylation during folate deficiency results in altered gene expression and thereby may disrupt genome integrity Dietary folate also has a major impact on homocysteine levels, which may exert direct neurotoxic and pro-oxidative actions with an inverse relationship between plasma folate and homocysteine concentrations (32). One of the most recent reviews on folic acid clearly states its importance in neuropsychiatric disorders. Dietary folate is required for the normal development of the nervous system, playing important roles in regulating neurogenesis and programmed cell deathRecent epidemiological and experimental studies have linked folate deficiency and resultant increased homocysteine levels with several neurodegenerative conditions, including stroke, AD, and Parkinson's disease. Folate deficiency sensitizes mice to dopaminergic neurodegeneration and motor dysfunction caused by neurotoxin MPTP. Additional experiments indicate that this effect of folate deficiency may be mediated by homocysteine. These findings suggest that folate deficiency and hyperhomocysteinemia might be risk factors for Parkinson's disease (33). But Literature relating to functional benefits of Fe+FA interventions (such as cognitive abilities) among young adolescents is limited (34). Sen and kanani was assess impact of daily Fe+FA supplementation on cognition of underprivileged primary schoolgirls in vardodara that those eividence indicated that a higher uptake of iron is needed to lead to cognitive improvement; and the encouraging finding is that twice IFA was consistently comparable to daily Fe+FA in this regard. This finding has important program implications as twice weekly Fe+FA supplement will cost less and be more feasible to deliver to beneficiary girls than daily Fe+FA. As regards the absorption of iron and reduction of anemia from a physiological perspective (34). Studies reviewed by Hallberg on daily as well as intermittent Fe+FA supplementation reported that six times more iron was absorbed when Fe+FA was given daily than when given weekly and concluded that there was no mucosal block during oral iron therapy in humans. However, he further stated that, if relatively high iron doses were given for a long time to subjects with low grade anemia, then all the doses of iron, all dosage schedules, and all iron preparations will give a similar Hb response. Thus, in the long run, intermittent iron-folate therapy will perhaps have a satisfactory impact on reducing anemia at lower cost and greater compliance (35.)

Conclusion

However, from our study and other literature reviewed it appears that iron or folate supplementation could improve memory significantly, but co-administration of iron with folate (Fe+FA) was more effective. The effect of co-administration of iron with folate (Fe+FA) hadn't any difference with folate alone.

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References

1. *Sharp P, Srai SK. Molecular mechanisms involved in intestinal iron absorption. Word Journal Gastroenterology. 2007; 13(35): 4716-4724.*

2. *Maaroufi K, Ammari M, Jeljeli M, et al. Impairment of emotional behavior and spatial learning in adult wistar rats by ferrous sulfate. Physiolo & Behav. 2009 96: 343-349.*

3. *Meinecke Ch, Morawski M, Reinert T, et al. Cellular distribution and localisation of iron in adult rat brain (Substantia nigra). Nucl. Instr. and Meth inPhys. Res. B. 2006; 249: 688-691.*

4. *Takeda AT amanoHb T, Tochigi M. Zinc homeostasis in the hypocampus of zinc-dependent young adult rats. Neurochem Int. 2005; 46: 221-225.*

5. *Moazedi AA, khombi shooshtari M, Parham GA. Dose dependent effects of iron supplementation on short-term and long-term memory in adult male wistar rats. J. Biol. Sci 2010; 10: 648-652.*

6. *Jellen LC, Beard JL, Jones BC. Systems genetics analysis of iron regulation in the brain. Biochimie. 2009; 91: 1255-1259.*

7. *De Lima M.M, Pieta Dias C, Presti Torres J, Dornelles A, Garsia VA, Scalco FS, et al.Reversion of age-related recognition memory impairment by iron chelation in rats. Neurobiol. Agi. 2008; 29:1052-1059.*

8. *Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive function in young woman. American Journal of Clinical Nutrition. 2007; 85(3): 778-787.*

9. *Otero GA, Pliego-River FB, Porcayo-Mercado R. Working Memory impairment and recovery in iron deficient children. Clinical Neurophysiology. 2008; 119: 1739-1746.*

10. *Youdim MBH. Brain iron deficiency and excess; cognitive impairment and neurodegeneration with involvement of striatum and hippocampus. Neurotoxicity Research. 2008; 14(1): 45-56.*

11. *Mattson MP, Kruman I, Duan W. Folic acid and homocysteine in age-related disease. Aging Research Reviews. 2002; 1: 95-111.*

12. *Meshkin B, Blum K. Folate nutrigenetics: A convergence of dietary folate metabolism, folic acid supplementation, and folate antagonist pharmacogenetics. Drug Metab Lett. 2007; 1:55-60.*

13. *Brocardo PS, Budni J, Kaster MP, Santos AR, Rodriques AL. Folic acid administration produces an antidepressant-like effect in mice: Evidence for the involvement of the serotonergic and noradrenergic systems. Neuropharmaco. 2008; 54: 464-473.*

14. *Gregory S, Kelly ND. Folates: Supplemental forms and therapeutic application. Altern Med Rev. 1998; 3(3): 208-220.*

15. *Sachdev PS. Homocysteine and brain atrophy. Prog Neuro-Psychopharmacol & Bio psychiatry. 2005; 29: 1152-1161.*

16. *Durga J, Van Boxtel MP, Schouten EG, Kok FJ, Katan MB, Verhoef P. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomized, Double blind, controlled trial. Lancet. 2007; 369:208-216.*

17. *Chen H, zhang SM, Schwarzschild MA, Hernan MA, Logroscino G, Willett WC, et al. Folate intake and risk of Parkinson's disease. Am. J. Epidemiol. 2004; 160(4): 368-375.*

18. *Lalonde R, Joyal CC, Botez MI. Effects of folic acid and folinic acid on cognitive and motor behaviors in 20-month-old rats. Pharmacol Biochem and Behav. 1993; 44: 703-707.*

19. *Mattson MP, Shea TB. folate and homocysteine metabolism in neuoral plasticity and neurodegenerative disorders. Trand Neurosci. 2003; 26: 137-146.*

20. *Martignoni E, Tassorelli C, Nappi G, Zangaglia R, Pacchetti C, Blandini F. Homocysteine and Parkinson's disease: A dangerous liaison? J Neurol Sci. 2007; 257: 31-37.*

21. *Wahlin TBR., Wahlin BW., Backman L., The influence of serum vitamin b12 and folate status on cognitive functioning in very old age. Biological Psychology. 2001; 56:247-265*

22. *Khombi Shooshtari M, Moazedi AA, Parham GA. Memory and motor coordination improvement by folic acid supplementation in healthy adult male rats. Iranian Journal of Basic Medical Science. 2012 (in press).*

23. *Moazedi AA, Ehsani Vostacolaee S, Chinipardaz R. Effect of oral aluminum chloride administration during lactation on short and long-term memory of their offspring. Bio. Sci. 2008; 4: 676-722.*

24. *Hugh E, Criswell& George R B. Similar effects of ethanol and flumazenil on acquisition of a shuttlebox avoidance response during withdrawal from chronic ethanol treatment. Br. J. Pharmacol. 1993; 110: 753-760.*

25. *Takeda A, T amanoH, Tochigi M. Zinc homeostasis in the hypocampus of zinc-dependent young adult rats. Neurochem Int. 2005; 46: 221-225.*

26. *Piskin S, Sayan C, Durukan N, Senol M. Serum iron, ferritin, folic acid , and vitamin B12 levels in recurrent aphthous stomatitis. European Academy of Department Dermatology and Venerology. 2002; 16, 66-67.*

27. *De Lima MNM, Presta-Torrest J, Caldana F, Grazziotin M, Scalco FS, Guimaraes MR. Desferoxamine reverse neonatal iron-induced recognition memory impairment in rats. European Journal of Pharmacology. 2007; 570: 111-114.*

28. *Balk EM, Raman G, Tatsioni A, Chung M, Lau J, Rosenberg IH. Vitamin B6, B12, and folic acid supplementation and cognitive function. Arch Intern Med. 2007;167:21-30.Epub 2007/06/17.*

29. *Chang N, Kim EJ, Kim KNHyesook Kim H, Kim SY, Jeong BS. Nutrition Research and Practice .2009; 3(1), 43-48.*

30. *Hassing L, Wahlin A, Winblad B, Backman L. Further Evidence on effects of vitamin B12 and folate levels on episodic memory functioning: a population- based study of healthy very old adults. Society of Biological Psychiatry. 1999; 45: 1472-14.*

31. *Tettamanti M, Garri MT, Nobili A, Riva E, Lucca U. Low folate and the risk of cognitive and functional deficits in the very old: The monzino 80-plus study. J Am Coll Nutr. 2006; 25(6): 502-508.*

32. *Kronenberg G, Harms C, Sobo RW, Cardozo-Pelaez F, Linhart H, WinterB, et al. Folate deficiency induces neurodegeneration and brain dysfunction in mice lacking uracil DNA glycosylase. The Journal of Neuroscience. 2008 ; 28(28):7219 –7230.*

33. *Moretti R, Torre P, Antonello RM, Cattruzza T, Cazzato G. Vitamin B12 and folate depletion in cognition: A Review. Neurology India. 2004; 52: 310-318.*

34. *Sen A, Kanani J. Imoact of iron-folic acid supplementation on cognitive abilities of school girls in vadodara. Indian Pediatrics.2009; 46:137-143.*

35. *Hallberg Z. Combating iron deficiency: daily administration of iron is far superior to weekly administration. Am J Clin Nutr. 1998; 68: 213-217.*

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Knowledge, attitudes and practices of parents regarding circumcision in Trabzon

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Abstract

Background: Circumcision is very widespread in Turkey, and male children are mainly circumcised for religious reasons, since the practice is widely in both Islam and Judaism, at ages and under circumstances that vary according to custom.

Objective: This study was intended to determine the circumcision status of primary school children in the provincial capital of Trabzon, Turkey, as well as parents' knowledge, attitudes and practices.

Methods: The subjects of this descriptive study consisted of male students progressing to the 4th and 5th years of primary schools in Trabzon. Parents of students at 10 primary schools thought to be representative of the 76 primary schools in the provincial capital and chosen on the basis of settlement location and socioeconomic levels constituted the study sample. Data were evaluated on the basis of questionnaires completed by 843 participating families.

Results: The families taking part had a total 1424 male children, of whom 1333 (93.6%) had been circumcised. These children had most commonly (37.4%) been circumcised at 13-35 months. In terms of age at circumcision, 28.8% of families said they had their children circumcised because they had reached an appropriate age and 21.0% because of existing health problems such as urinary tract infection, phimosis and hernia. Although only 3 (0.3%) families said that circumcision was unnecessary, these had also had their sons circumcised. Families believing in the need for circumcision most frequently cited religious reasons (59.1%), followed by circumcision permitting greater male reproductive organ hygiene (43.0%), it being beneficial in general health terms (3.8%), traditional reasons (2.4%) and assisting sexual development (1.1%).

Conclusions: All families had their children circumcised, the most common reasons being religious ones, as well as considering the medical benefits of circumcision.

Key words: Circumcision, attitudes, traditions, ethic, child.

Introduction

Circumcision is one of the earliest known surgical procedures, the history of which goes back some 4000 years. It is widely practiced in many countries, especially in the Muslim world and the United States of America[1-5]. Half of all males in the USA and Canada are circumcised, and one in six of all males in the world[6]. It is widespread in Africa, the Middle East and Australia, less common in China, Japan, Taiwan and other Far Eastern countries such as North Korea that share the same cultural values, less common again in India and Europe, and comparatively rare in New Guinea and South America[3,7]. Circumcision is very common in Turkey, with a prevalence approaching 100% [3, 8].

While circumcision is practiced for religious reasons in Muslim and Jewish societies, in European countries it is performed for the treatment of conditions such as phimosis, paraphymosis and recurrent balanitis. Newborn babies are widely circumcised in the USA and Canada because of its protective effect against penis and ureteral carcinoma, urinary tract infection and diseases originating from sexual relations. In Korea, it is widely performed at primary school age and in early adolescence, for non-religious reasons [2, 7]. In Turkey, children are circumcised for religious reasons, at ages and under conditions that vary according to custom. Although there have been many studies regarding the medical benefits of circumcision, it is essentially a social phenomenon and to a large extent based on religious foundations[3].

Although circumcision appears to be a simple surgical procedure, it is essential that it be performed by specially trained doctors and assistant

health personnel, with a good knowledge of and experience of applying basic principles of surgery. Circumcision is a surgical intervention, and as with all such procedures there is a risk of complications. Incidences of 0.2% to 5% for complications such as infection in the wound site, hemorrhage and gangrene have been reported, and as some of these are irreversible they can result in serious problems[3-5,8,9]. In ethical terms, the medical practitioner must receive permission from one of the parents in order to perform circumcision[10,11]. The medical world has for a long time used this protocol as the basis for receiving authorization for non-therapeutic circumcisions. Yet there is article in law to support this. Circumcision, whether of a newborn or not, is a procedure that is neither diagnostic nor therapeutic[12, 13]. Therefore, a non-therapeutic intervention is a contravention of the law. No parent can give permission for a contravention of the law. Supposed "parental permission" cannot justify a non-therapeutic or non-diagnostic intervention. Moreover, it is unclear how a non-therapeutic procedure can be in a child's interests. A British court has issued a ruling on this subject[10]. British Medical Council rules declare that a non-therapeutic procedure, or one whose benefits are uncertain, can only be performed through a judicial ruling[13].

Article 24.3 of the Declaration of Children's Rights refers to circumcision as traditional procedures harmful to children's health. Therefore, child circumcision consistently violates various human rights laws and needs to be examined under the heading of an unethical medical procedure.

In the absence of any health problem, it is generally families in Turkish society that decide when a child should be circumcised. Circumcision is therefore influenced by parental beliefs and attitudes. There have been few studies in Turkey examining family attitudes and behavior. This study was therefore intended to determine the circumcision status of primary school students in the provincial capital of Trabzon and also parental knowledge, attitudes and practices regarding it.

Subjects and methods

This descriptive study was performed between October 2007 and December 2008. The study population consisted of boys progressing to the 4th and 5th years in primary schools in the provincial capital of Trabzon. The number of male students in these years in the 76 primary schools concerned is 10,040. The number of students to be included in the study was calculated using the formula $n= Z^2_{1-\alpha/2}(p\ (1-p)/d^2$. Estimating that 75% (p) of the boys in this age group would have been circumcised, an appropriate figure of 800 was determined, with a 95% Confidence Interval and 0.3 deviation. Firstly, a list of all the classes in the schools in the provincial capital was established. Sampling was performed among parents of students at 10 primary schools randomly selected on the basis of being considered representative of the province and of settlement locations and socioeconomic levels. Although we calculated that at least 800 families would be required, we planned to administer questionnaires to 950 families, on the basis that some would probably decline to participate. Questionnaires were collected from school administrations at the end of the study, and 843 families had finally completed them. Data were evaluated on the basis of these 843 questionnaires.

Date was collected by questionnaire. This form was drawn up using the format employed by Oh et al. and Lee et al. [2, 7]. Questionnaires were forwarded to the principals of the schools included in the sampling. The study was described to the teachers of the classes chosen by the schools, and the questionnaires were forwarded to student's families by those teachers. The requisite written permission, No. 39386 dated 15 October, 2007, for the study to be performed with primary school students was obtained from the Trabzon Governor's Office Provincial Education Directorate.

Results

Eight hundred forty-three families participated; 57.5% of the parents completing the questionnaire were mothers. Mothers' average age was 36.6±5.6, and that of fathers 41.0±5.7. Of the mothers responding to the questionnaire, 38.6% were primary school graduates and 72.0% housewives, while 33.0% of fathers were university graduates and 33.7% small traders. Of the families, 45.1% had one male child, and 43.3% had 2. The total number of male children of the families participating was 1424, of which 1333 (93.6%) had been

circumcised. The great majority of these (37.4%) had been circumcised at 13-35 months (Table 1).

When families were asked the reason for the age at which they had their children circumcised, one was cited in the case of 1036 of the total 1333 subjects circumcised; 28.8% of families stated that the age was appropriate and 21.0% said they had their children circumcised because of health problems such as urinary tract infection, phimosis and hernia (Table 2).

Table 1. Various Characteristics of the Parents Participating and Distribution of Children by Age at Circumcision

		No.	%*
Mother's educational level (n=843)			
	Illiterate	16	1.9
	Literate	17	2.0
	Primary school	325	38.6
	Middle school	77	9.1
	High school	227	26.9
	University	181	21.5
Father's educational level (n=843)			
	Illiterate	2	0.2
	Literate	5	0,6
	Primary school	202	24.0
	Middle school	95	11.3
	High school	261	31.0
	University	278	33.0
Mother's occupation (n=843)			
	Housewife	607	72.0
	Civil servant	94	11.2
	Health personnel	49	5.8
	Self-employed/small trader	43	5.1
	Worker	39	4.6
	Retired	11	1.3
Father's occupation (n=843)			
	Self-employed/small trader	360	42.7
	Civil servant	209	24.8
	Worker	195	23.2
	Health personnel	34	4.0
	Retired	33	3.9
	Unemployed	12	1.4
Total Monthly Family Income (TL) (n=843)			
	<500	111	13.2
	500-999	188	22.3
	1000-1499	187	22.2
	1500-1999	106	12.6
	≥2000	251	29.8
Ages at circumcision (n=1333)			
	≤ 12 months	116	8.7
	13-35 months	498	37.4
	36-71 months	480	36.0
	≥ 72 months	239	17.9

**Percentages calculated on the basis of "n" figures.*

Table 2. Reasons Cited by Families for the Age at Which They Had Their Children Circumcised

Reasons	No.	%
The age was thought to be appropriate	298	28.8
Recommended by a physician due to existing health problems (urinary tract infection, phimosis, hernia)	217	21.0
Family circumstances being most appropriate	140	13.5
Concern that health problems might arise in the future, even though none existed at the time	89	8.6
The child being unaware of circumcision, unable to understand its significance and incapable of remembering it	60	5.8
So two existing male children could be circumcised at the same time	46	4.4
Religious reasons	43	4.1
In order to have it done before the child started school	37	3.6
In order to avoid psychological effects and fear	34	3.3
The idea that parents neglected the time of circumcision	20	1.9
The idea of early healing if the child was still in diapers	17	1.6
The child had not yet developed a sense of pain	17	1.6
Decision taken by family elders	6	0.6
The child's own desire to be circumcised	5	0.5
Traditional reasons	3	0.3
Since the procedure was not performed in babyhood	2	0.2
Mass circumcision ceremony	2	0.2
Total	**1036**	**100.0**

** Percentages are based on the 1036 responses setting out a reason for age at circumcision.*

Asked "at what age should circumcision be performed?", 1.8% of families said under the age of 1 year, 32.0% between the ages of 1 and 3, 27.8% between 3 and 6, 26.4% after 6 and 11.9% said they did not know. In terms of the place that families considered appropriate for circumcision, 759 (95.5%) of the 803 families who responded to that question selected health institutions such as hospitals, health clinics or health kiosks, while 30 (3.7%) selected a location considered appropriate by the person performing it, so long as this was someone trained, and 14 (1.7%) said at home. Of the families with circumcised children, 1312 stated where they had had their children circumcised, 557 (42.5%) said at home, 500 (38.1%) in hospital, 137 (10.4%) in a health kiosk, 68 (5.2%) in a private surgery and 50 (3.8%) in a health clinic. Asked about the persons performing the circumcision, the most common answers given were professional 'circumcisers' and health personnel (713; 54.3%) followed by doctors (596; 45.4%) and barbers (3; 0.3%).

A health problem had developed in 522 (39.6%) circumcised children; these included pain in 183 (34.9%), swelling in 121 (23.2%), fever in 93 (17.9%), difficulty in urinating in 58 (11.1%), bleeding in 52 (10.0%) and other problems in 2.8%. A health problem was determined to have occurred in 304 (42.6%) circumcisions performed by professional circumcisers and health technicians, 217 (36.4%) performed by doctors and 1 of the 3 children circumcised together at a barber's shop. Nine (1.6%) of the children developing a health problem were hospitalized and required long-term treatment programs. Seven of these nine children were circumcised by a doctor and 2 by professional circumcisers or health technicians.

Although only 3 (0.3%) families said that circumcision was unnecessary, they all had their children circumcised (Table 3).

The families that believed in the need for circumcision most frequently cited religious reasons (495; 59.1%), followed by circumcision allowing the male reproductive organ to be kept cleaner (360; 43.0%), it being beneficial to health (32; 3.8%), traditional reasons (20; 2.4%) and the idea that it assists sexual development (9; 1.1%).

Seven hundred fifty-two participants (89.2%) thought that circumcision is beneficial to health in some way, while 91 (10.8%) thought it entails no health benefits.

Table 3. Families' Opinions Regarding Whether Circumcision Is Necessary

Question Whether circumcision is necessary (n=828)	No.	%
Definitely necessary	795	96.0
Maybe necessary	19	2.3
Don't know	7	0.9
May not be essential	4	0.5
Definitely unnecessary	3	0.3

Table 4. Families' opinions regarding the benefits of circumcision

Belief in the benefits of circumcision	Yes		No		Don't know	
	No.	%	No.	%	No.	%
Prevents penile cancer	536	63.6	169	20.0	138	16.4
Prevents urinary tract and kidney infections	628	74.5	88	10.4	126	14.9
Circumcision prevents cervical cancer in women	410	48.6	236	28.0	196	23.3
Circumcision prevents genital infections in women	465	55.2	189	22.4	189	22.4

When asked about the benefits of circumcision, families stated that it prevents bladder and kidney infections, as well as cervical cancer and genital infections in women (Table 4).

Discussion

Circumcision in Turkey is performed more for religious and traditional reasons than for its medical benefits, generally between the ages of 5 and 7, after children have acquired the ability to reason. The reason for this is that in Turkish tradition, circumcision occupies an important place in the lives of the mother, father and child, that families celebrate the occasion together with their children and want their children to remember it in later life[3,14,15].

The question by whom, where and when healthy and problem-free circumcision is to be performed is of considerable importance[3,9].

The reasons behind circumcision may vary from one society to another. Religious and traditional reasons are the most important in Turkey, as well as others such as avoiding medical problems, celebrating the event and it being a step toward manhood[8,16,17]. In Korean society, by contrast, there is a common idea that it will enhance hygiene and future sexual functions, and also assist the child's sexual development and endow him with a larger penis. The level of religious reasons is very low (<0.5%) [2, 7]. In our study, religious reasons were the most important factors behind circumcision (59.1%).

While there are traditional, religious and medical aspects to circumcision, there is also a very serious anti-circumcision movement today. This movement, first initiated by atheists, then supported by Christians and more recently also by a group of Jews, initially sought to base its objections on scientific grounds. They then attempted to broaden the movement by raising various ethical objections. The movement even objects to circumcision by trying to undermine the religious grounds on which it is based, claiming that in the event of the child converting to a different faith or believing in no religion at all in the future, being circumcised might eliminate individuals' right to freely choose their own religion[17]. In one study performed in Korea, 2.1% of participants said they regarded circumcision as unnecessary[7]. In our study, 3 families (0.3%) stated that circumcision was unnecessary, though they still had their children circumcised. This does, however, show that there are opponents of circumcision even in Turkey.

Are there any health benefits to circumcision even though it is performed for religious and traditional reasons? Oh et al. reported that families believed it prevents genital infections (80.6%), cervical cancer (64.7%) and urinary tract (62.7%) and kidney infections (53.4%) that might develop in the future[7]. Şahin et al. also reported that families believed that circumcision protects against infections[8]. In our study, the majority of families believed that circumcision is necessary and that

it prevents urinary tract and kidney infections (87.6%) and penile cancer (76.0%). These results show that medical benefits are also considered among the factors making circumcision necessary.

The debate over the best age for circumcision continues, although avoiding circumcision as much as possible between the ages of 3 and 6 is recommended in order to avoid negativities arising from castration complex. Circumcision can be performed before these ages, during which psychological injury may develop, or after [9, 18]. Since urinary system infection is more commonly seen in the first year of life, and particularly in the first 6 months, circumcision as early as possible is recommended, especially in neonates with urinary system abnormalities, since circumcision in the newborn period prevents urinary system infections. It has also been emphasized that circumcision in this period produces better esthetic results[3, 8, 19-21].

In a study performed in South Korea, Oh et al. reported that children were circumcised more in earlier adolescence (≈ 11 years) than neonatally[7].

In our study, a not insignificant number of families (36.0%), a level comparable to that cited in other research, had their children circumcised at 36-71 months, outside the ideal ages (Table 5).

Very few children born in hospital in Turkey are circumcised immediately after birth, and generally at the age of 5-7 [3]. In our study, only 8.7% of children were circumcised before the age of 1, and neonatal circumcision was determined to be very rare indeed. The families in our study stated that they had not considered having newborn children circumcised (65.4%). Among the reasons for not considering circumcision in the neonate period was that it was risky because the organ concerned was very small and that the baby would experience pain. This suggests that families are unaware of the advantages posed by early circumcision.

The difference among the justifications for age at circumcision in different societies is striking. In Oh et al.'s study, the period they preferred and when families had their children circumcised, elementary school or slightly earlier, was described by families as when children would feel the least pain[7]. In studies in Turkey, families have emphasized that children younger than 6 months heal faster, that they think that children younger than 2 do not feel pain and that children circumcised at 6 and over "will not be afraid" as they now regard themselves as men[3,16]. In this study, 28.7% of families described the ages they had their children circumcised as "the right age," while 21.0% stated that they had had to have their children circumcised for medical reasons.

Circumcision is not just the removal of a piece of skin from the penis. Medical problems that may be encountered are sometimes irreversible and have a negative impact on the individual's life, and even that of his family. For that reason, circumcision, a surgical procedure, must be performed under hygienic conditions and by expert physicians. In Turkey, circumcision is sometimes performed by people such as barbers or butchers, especially in rural areas, as well as by the health officials known as 'circumcisers.' In their study, Sivaslı et al. reported that 74.5% of children had been operated on by such a 'circumciser," and Şahin et al. cited a figure of 13.3% [3,8]. Even in our study, performed in the provincial capital, 54.3% of children had been circumcised by such officials.

In addition, the level of circumcisions at health institutions in Turkey is also low. For instance, in the study by Sivaslı et al. 17.3% of circumcisions were performed in hospitals and 8.2% in clinics[3]. In the present study, 42.5% of circumcisions were performed at home and 38.1% in hospital.

The most common complications tend to be minor and treatable: pain, bleeding, swelling or inadequate skin removal, excess bleeding and amputation of the glans penis, the penile shaft and the glans, infection, urinary retention, meatal ulcer, meatal stenosis, fistulas, loss of penile sensitivity, sexual dysfunction and edema of the glans penis[22-24]. A health problem developed in 39.6% of children circumcised, 9 (1.6%) of whom were hospitalized and had to be enrolled in long-term treatment programs. Circumcision is a small operation, but must be regarded as an important one in terms of outcome, and the need for it to be performed under hygienic conditions by physicians with expertise on the subject should not be forgotten.

Table 5. Frequencies of age at circumcision according to various studies in the literature

Study authors	Year of study	Place	Age /age group	Incidence (%)
Şahin et al.	*2003*	Ankara	<1 year 13-35 months 3-6 years >6 years	14.8 7.7 35.9 41.6
Top et al.	*2008*	Giresun/Turkey	<3 years 3-6 years >6 years	13.5 42.2 44.3
Sivaslı et al.	*2003*	Gaziantep/ Turkey	<1 month 2-3 months 4-6 months 7-12 months 1-2 years 2-6 years 7-13 years	9.6 8.0 5.6 35.2 13.4 21.3 6.9
Sang et al.	*2003*	Busan/ Korea	7 years 8 years 9 years 10 years 11 years 12 years	18.7 23.4 32.7 41.9 49.9 64.8
Kahriman et al.	*2010, present study*	Trabzon/Turkey	≤ 12 months 13-35 months 36-71 months ≥ 72 months	8.7 37.4 36.0 17.9

Conclusion

The limitation of this study, the study is done in primary schools in urban area, but not included rural area. We established that all families had their children circumcised, that the main reason cited was religious and that the medical benefits of circumcision were also considered. Families were not sensitive to the issue of the age at which circumcision should be performed. Yet circumcision is the first and a serious procedure impinging on the body and sexuality of a male child. Society perceives circumcision as a ritual. There is also a need for social education programs stressing the medical importance of the procedure, which also affects the male child's later life in terms of problems that are or may be encountered.

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References

1. *Atikeler MK, Geçit İ, Bodakçi MN, Ergin E. Complications of the circumcision performed within and outside the hospital. Fırat University Medical Journal of Health Sciences.2001; 15: 477-479.*

2. *Lee SD, Park E, Choe BM. Parental concerns on the circumcision for elementary school boys. A Questionnaire Study J Korean Med Sci. 2003; 18: 73-9.*

3. *Sivaslı E, Bozkurt Aİ, Ceylan H, Çoşkun Y. Knowledge, attitude and behaveior of parents regarding circumcision in Gaziantep. Journal of Pediatrics. 2003; 46: 114-118.*

4. *Yazıcı M, Etensel B, Gürsoy H. Complications of circumcision. Journal of Adnan Menderes University Medical Faculty. 2003; 4: 5-7.*

5. *Ceylan H, Topçu K, Özokutan BH at al. Circumcision and complications. Ege Pediatri Bulletin. 2004; 11: 1-5.*

6. *Dunsmuir WD and Gordon EM. The history of circumcision. BJU International. 1999; 83: 1-12.*

7. *Oh SJ, Kim KD, Kim KM, Kim KS, Kim KK, Kim JS et al. Knowledge and attitudes of Koreans towards their sons' circumcision: a nationwide questionnaire study. BJU International. 2002; 89: 426-32.*

8. *Şahin F, Beyazova U, Aktürk A. Attitudes and practices regarding circumcision in Turkey. Child: Care, Health and Development. 2003; 29: 275-280.*

9. *Balkan E. & Kılıç N. Circumcision and complications. Güncel Pediatri. 2005; 3: 22-23.*

10. *Committee on Medical Ethics The law and ethics of male circumcision - guidance for doctors. British Medical Association, London: 2003.*

11. *College of Physicians and Surgeons of British Columbia Policy Manual: Infant Male Circumcision. Vancouver, BC: College of Physicians and Surgeons of British Columbia: 2004.*

12. *American Academy of Pediatrics Committee on Bioethics. Informed consent, parental permission, and assent in pediatric practice. Pediatrics. 1995; 95: 314-317.*

13. *Seeking Patients Consent: The Ethical Considerations (1998) General Medical Council, London.*

14. *Tekgül S. Circumcision. Çocuk Sağlığı ve Hastalıkları Dergisi. 2000; 43: 297-302.*

15. *Yurdakök M. Kutsal Kitaplarda Çocuk Hekimliği Bilgileri. Alp Ofset Matbaacılık Makine San. ve Tic. Ltd Şti, Ankara 2001. 65-92.*

16. *Top FÜ, Üstüner F, Esüntimur Y, Uykan U, Pekdemir EA. The Knowledge, Behaviour and Attitude About of Circumcision in Families in Giresun. Çocuk Dergisi 2008; 8: 166-171.*

17. *Aksoy Ş. Sünnet, Sünnet mi? Medimagazin. 2010; 11: 470, 4.*

18. *Öztürk MO, Uluşahin A. Ruh Sağlığı ve Bozuklukları 11. Baskı, Ankara 2008.*

19. *Wiwell TE, Tencer HL, Welch CA, Chamberlain JL. Circumcision in children beyond the neonatal period. Pediatrics 1993; 92: 791-3.*

20. *Schoen EJ, Wiswell TE, Moses S. New policy on circumcision – cause for concern. Pediatrics. 2000; 105: 620–623.*

21. *Lerman SE, Liao JC. Neonatal circumcision. Ped Clin North Am, 2001; 48: 1539-1557.*

22. *Weiss HA, Larke N, Halperin D, Schenker I. Complications of circumcision in male neonates, infants and children: a systematic review. BMC Urology. 2010; 10: 1-13.*

23. *Ademuyiwa O, Bode CO. Complications of neonatal circumcision: Avoiding common pitfalls in a common procedure. African Journal of Paediatric Surgery, 2009; 6: 134-136.*

24. *Söylemez H, Burcu B. Habits and results of circumcısion in Turkey. The New Journal of Urology. 2009; 5: 13-18.*

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Use of the QIDS-C_{16}, QIDS-SR_{16} and the 17-item Hamilton rating scale for depression (HAM-D_{17}) in a Turkish college student sample

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Abstract

Objective: To evaluate the psychometric properties and relations among the Quick Inventory of Depressive Symptomatology (QIDS-C_{16}), clinical version, the Quick Inventory of Depressive Symptomatology, self-report version (QIDS-SR_{16}), and the 17-item version of the Hamilton Rating Scale for Depression (HAM-D_{17}) in a Turkish student sample

Materials and methods: Slightly modified versions of the three scales were administered to 114 outpatients at the Uludağ University campus-based family health center. SAS, MPlus, Multilog, and SS-IRT were used to provide descriptive statistics, classical psychometric analyses, including factor analysis, test informations, and equatings of the three scale scores.

Results: The internal consistencies (Cronbach's a) values of the QIDS-$C_{16,}$ QIDS-SR_{16}, and HAM-D_{17} were 0.81, 0.80, and 0.89. Both versions of the QIDS were unidimensional, but the HAM-D_{17} required two dimensions according to a parallel analysis criterion. The pattern of item-total correlations for the two versions of the QIDS was similar. The correlation between the QIDS-C_{16} and QIDS-SR_{16} was 0.70. Similarly, the correlation between the QIDS-C_{16} and HAM-D_{17} was 0.79, and the correlation between the QIDS-SR_{16} and the HAM-D_{17} was 0.59. These respective correlations increased to 0.87, 0.94 and 0.70 when disattenuated (corrected for unreliability). Scores on the three measures were also equated.

Discussion: All three measures have good psychometric properties and convergent validity. In particular, this extends the utility of the QIDS-SR_{16}, the object of a previous study. One important limitation in the present case is the small sample size (N = 114) Use of the QIDS-SR_{16} is recommended when a self-reported instrument is appropriate even though this measure has slightly less in common with the QIDS-C_{16} and HAM-D_{17} when clinical judgment is possible.

Key words: Major depressive episode, screening scale, validity, reliability.

Introduction

In a previous study, Mergen, Bernstein, Tavli et al. (1) compared the self-report version of the Quick Inventory of Depressive Symptomatology, in a Turkish student sample (QIDS-SR_{16}-T) to (a) the American version (QIDS-SR_{16}-US) and (b) the Turkish version of the Beck Depression Inventory (BDI-II-T). Turkish data were obtained from 626 outpatients at the Uludağ University campus-based family health center, and American data were obtained from 584 respondents at an American University as administered to a class in introductory psychology.

Internal consistencies (Cronbach's a) of the two versions of the QIDS were in the .70s and were higher for the longer BDI-II-T at .89. However, the two versions of the QIDS were both unidimensional whereas the BDI-II-T was not. Item-total correlations of the two versions of the QIDS were similar, and the correlation between the QIDS-SR_{16}-T and BDI-II-T was .90 when disattenuated (corrected for unreliability). Multiple group item response theory analysis (2) analysis indicated that the two versions of the QIDS had different intercepts but the same factor loadings. The difference in intercepts was not surprising given that participant selection in the Turkish sample favored those with depressive pathology.

It was concluded that the psychometric properties of the QIDS-SR_{16}-T and its convergent validity with the BDI-II-T is at least satisfactory for use when a self-reported instrument is appropriate.

This study compared the clinical version of the QIDS (QIDS-C_{16}), the QIDS-SR_{16}, and the 17-item version of the Hamilton Rating Scale for Depression (HAM-D_{17}) in a Turkish student sample. Since all data were gathered in a Turkish sample, "-T" in the abbreviations of the three tests is redundant and will not be used.

Methods

The three scales, i.e., the QIDS-C_{16}, the QIDS-SR_{16}, and HAM-D_{17}, were administered to 114 outpatients who participated at the Uludağ University campus-based family health. As previously noted (1), the QIDS-C_{16} and QIDS-SR_{16} were obtained from the www.ids-qids.org website (3). A few minor changes were made to accommodate actual Turkish usage, e.g., references to weight in pounds were replaced by weight in kilograms. The HAM-D_{17} was as originally published, in contrast to variants with more or fewer items. The translations are available from the first author.

The sample consisted of students, who presented consecutively to the outpatient clinic of the university. The study conformed to the Helsinki declaration requirements and had approval from the University ethics committee. The students gave informed consent and voluntarily participated in the study. They completed the QIDS-SR_{16} and HAM-D_{17} in 7-10 minutes.

Statistical Analyses

Descriptive analyses, classical test theory (CTT), exploratory factor analysis and IRT analyses were all employed. However, only the test information functions and test-equation results of the latter will be presented because the limited sample size makes the item-specific results somewhat unstable. The classical test theory analyses generated item means, item standard deviations, item-total correlations (r_{it}), scale means, and scale standard deviations. This analysis also included intercorrelating the scale scores. The dimensionality of the three tests was evaluated using exploratory factor analysis. Parallel analysis (4, 5, 6, 7) was used to decide upon the number of factors (dimensionality). The version used in this study consisted of generating a series of 50 matrices of random normal deviates. Each matrix had 9 columns and 114 rows for the two versions of the QIDS and 17 columns and 114 rows for the HAM-D_{17}. Thus, the number of columns equaled the number of items on the actual test and the number of rows equaled the number of participants. Each of the resulting matrices was then subjected to principal component analysis and the eigenvalues averaged within each of the three sets. A test may be considered unidimensional if (a) its first eigenvalue exceeds its simulated counterpart and (b) all of its subsequent obtained eigenvalues are smaller than the corresponding simulated eigenvalues. All of these analyses used SAS 9.2.

The test information function derived from the Samejima (8,9) IRT model describes the sensitivity of the latent variable, depression in this case (symbolized q and measured in standard deviation units) to changes in score. In essence it is a reliability-like measure, but one that is a function of q rather than a constant for all test scores. Finally, test equating involves finding scores on two or more tests with equivalent or nearly equivalent scores on q. Thus, a score of 3 on test A and a score of 5 on test B are considered equated if they produce similar values of θ. In the present case, this means that a patient getting a score of 3 on test A is as depressed as a patient who gets a score of 5 on test B. This equating employed the SS-IRT program developed by Orlando, Sherbourne, and Thissen (10).

Results

Demographic results

Table 1 contains the relevant demographic information. Although patients with a family history of depression did not score significantly different from those without a family history on the two versions of the QIDS, they did score lower on the HAM-D_{17}. The respective means were 13.3 and 8.5, $t(112) = 2.39$, $p < .02$. Not surprisingly, patients on antidepressant medication scored higher on all three tests than those not on antidepressant medications. The respective means for the QIDS-C_{16}, QIDS-SR_{16}, and HAM-D_{17} were 9.6, 13.3, and 20.3 for the former group and 5.8, 7.9, and 8.2 for the latter group, $t(112) = 2.93$, 2.70, and 4.71, $p< .01$.

The QIDS-SR_{16} scores were higher in the present study than in that reported previously by Mergen, Bernstein, Tavli et al. (1). The present and earlier means were 8.2 and 6.9, $t(738) = 2.46$, $p < .02$. Be-

cause the present sample was slightly more variable than the earlier sample (s = 5.2 vs. 4.9), Cronbach's α was also slightly larger in this sample (.80 vs.78)

None of the remaining group differences (age, city vs. non-city residence, gender, income, or number of siblings approached significant.

Table 1. Demographic Characteristics of Sample

Item	Est.
Mean Age	20.6
St. Dev. Age	3.9
Income: <500 TL* (%)	33
Income: 501-1000 TL (%)	37
Income: 1001-2000 TL (%)	22
Income: >2000 TL (%)	8
No. Siblings = 0 (%)	6
No. Siblings = 1 (%)	29
No. Siblings = 2 (%)	34
No. Siblings = 3 (%)	13
No. Siblings = 4+ (%)	18
City Dwelling (%)	81
Family History of Depression (%)	17
Female Gender (%)	44
On Antidepressants (%)	9

TL= Turkish money unit (1$=1.77 TL on 4/12/2012)

CTT Analysis

Table 2 contains the relevant CTT results. The correlation between the QIDS-C_{16} and QIDS-SR_{16} was .70; the correlation between the QIDS-C_{16} and HAM-D_{17} was .79, and the correlation between the QIDS-SR_{16} and HAM-D_{17} was .59. Correcting for attenuation (unreliability) by dividing these respective correlations by the square root of the product of the coefficients a yields respective estimates of the error-free correlations of .87, .94, and .70. In other words, the two clinical versions correlated more highly with one another than did either with the self-report even though one of the clinical versions was identical in content to the self-report version.

Exploratory Factor Analysis (Dimensionality)

Both versions of the QIDS were unidimensional by the parallel analysis criterion. Specifically, the first eigenvalues of the QIDS-C_{16} were 3.82 and 1.13 and the QIDS-SR_{16} were 3.71 and 1.04. In contrast, the first two simulated eigenvalues were 1.44 and 1.29. This first factor accounted for 42.5% and 41.2% of the variances in the respective measures. However, the HAM-D_{17} con-

Table 2. Item means, item standard deviations, item-total correlations (r_{it}), scale means, scale standard deviations, and Cronbach's a

Domain	QIDS-C_{16}			QIDS-SR_{16}			HAM-D_{17}		
(Item)	Mean	S. D.	r_{it}	Mean	S. D.	r_{it}	Mean	S. D.	r_{it}
1	1.39	.93	.40	1.80	.96	.41	.69	.83	.71
2	.65	.75	.65	.74	.85	.59	.33	.67	.62
3	.70	.81	.23	1.01	.97	.47	.10	.38	.24
4	.85	.76	.62	1.08	.89	.67	1.03	1.01	.66
5	.40	.70	.60	.80	1.21	.35	.38	.77	.25
6	.12	.36	.33	.20	.57	.50	.73	1.06	.45
7	.43	.65	.55	.61	.91	.53	.76	.97	.53
8	.75	.59	.61	.67	.76	.63	.51	.90	.57
9	.83	.77	.65	1.25	1.15	.42	.93	.98	.55
10							.54	.73	.53
11							.17	.42	.43
12							1.01	1.03	.70
13							.93	.93	.68
14							.15	.50	.36
15							.18	.47	.62
16							.39	.77	.53
17							.47	.81	.64
Scale	6.12	4.05		8.35	6.24		9.30	8.19	
a	.81			.80			.89		

tained two factors (barely) by this criterion as its first three eigenvalues were 6.41, 1.60, and 1.39 whereas the first three simulated eigenvalues were 1.74, 1.58, and 1.46. A promax (oblique) rotation of the HAM-D_{17} item data provided a factor correlation of .54. Using a criterion of .4 to define a salient variable, items 1, 2, 6-8, 12, and 15-17 loaded on factor I, which accounted for 32.0% of the variance. Items 1, 3-5, and 9-14 loaded on factor II, which accounted for 30.4% of the variance, items 1 and 12 thus crossloaded. Collectively, the two factors accounted for 47.1% of the variance. Factor 1 could be considered as depressive content while Factor2 could be considered as anxious content.

Test Information Functions

Figure 1 contains the test information functions (TIF) for the two versions of the QIDS and the HAM-D_{17}. The points to note are: (a) all three measures peak between slightly above 0 and around +1.0 on the q axis (between average and moderate levels of depression for this sample), denoting the region of maximum sensitivity, (b) the HAM-D_{17} has greater sensitivity than either QIDS measures except at the extremes of the distribution, and (c) the difference between the two QIDS measures are fairly small and possibly reflective of sampling error.

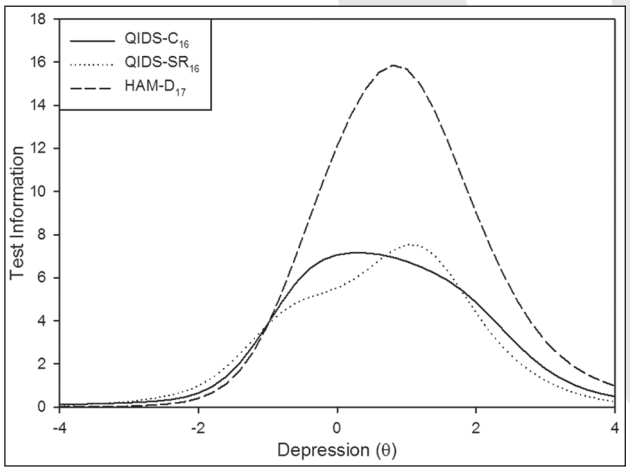

Figure 1. Test information functions

Test Equating

Table 3 contains the results of equating the three tests. Thus, a raw score of 1 on each of the tests corresponds to a depression level (q) of 1.40 to 1.30. Not all values equate exactly so expected *a posteriori* (EAP) values within ± .1 q units were accepted as matching.

Table 3. Results of equating the QIDS-C_{16}, QIDS-SR_{16}, and HAM-D_{17}, and

QIDS-C_{16}	QIDS-SR_{16}	HAM-D_{17}	θ
0	0	0	-1.80 to -1,70
1	1	1	-1.40 to -1.30
2	2 to 3	2	-1.20 to -.88
3	4	3 to 4	-.69 to -.52
4	5	5	-.42 to -.40
5	6	6 to 7	-.29 to -.17
6	7 to 8	8	-.06 to .10
7	9	9 to 10	.14 to .26
8	10 to 11	11 to 12	.40 to .56
9	12	13	.65 to .70
10	13	14	.67 to..83
11	14 to 15	15 to 16	.94 to 1.10
12	16	17	1.20
13	17 to 18	18 to 19	1.30 to 1.40
14 to 15	19	20 to 21	1.50 to 1.60
16	20 to 21	22 to 23	1.70 to 1.80
17	22	24 to 26	1.90 to 2.00
18	23	27	2.10
19	24	28	2.30
20	25	29 to 31	2.30 to 2.50
21	26	32 to 33	2.60 to 2.70
22		34	2.80
23	27	35	2.90 to 3.00
24		36	3.10
25		37	3.30
26		38 to 39	3.40 to 3.60
27		40 to 45	3.70 to 3.90

Discussion

The main findings are that the Turkish versions of these three tests are psychometrically adequate in terms of their item characteristics, overall reliability, and convergence upon one another. As previously noted (1), the Turkish version of the QIDS-SR_{16} also converges upon the results obtained from an American sample, so it is reasonable to assume depressive symptomatology is being measured in all cases. An additional finding is that the two clinical versions correlate more highly with one another than either does with the self-report version despite the identical content of the clinical and self-report versions of the QIDS.

The HAM-D_{17} is slightly more sensitive to differences in depression than either version of the QIDS, and all three measures are most sensitive to somewhat above average levels of depression (roughly the 60th to the 85th percentile) within the present group. However, the greater sensitivity of the HAM-D_{17} is somewhat offset by its multidimensionality, i.e., its apparent tendency to confound depressive and anxious symptomatology, certainly a familiar problem in the psychiatric literature. This confounding seems important given the different medications most appropriate for the two conditions. One can of course use only the Hamilton's depression items, but that will tend to reduce its sensitivity. As was noted in our previous study, the QIDS-SR_{16} seems appropriate for the evaluation of depressive symptomatology whenever a self-descriptive measure is appropriate. One benefit of our approach is that we have furnished a QIDS-HAM-D equating for users of one test who are relatively unfamiliar with the alternative(s).

Rush et al. (11) compared the 30 item Inventory of depressive symptomatology (IDS-SR_{30}), the QIDS-SR_{16}, the QIDS-$C_{16,}$ the HAM-D_{17}, and two of the latter's variants differing as to number of items (HAM-D_{21} and HAM-D_{24}) in a sample of 596 patients being treated for depression. The internal consistency Cronbach's α of the QIDS-SR_{16} was 0.86, and the HAM-D_{17} was 0 .83. Thus, their QIDS-SR_{16} was marginally higher than the present and their HAM-D_{17} was marginally lower.

Rush et al. (12) is similar to the present study in that they administered the QIDS-C_{16}, QIDS-SR_{16}, and HAM-D_{17}, using a sample of 582 patients. The internal consistency (Cronbach's α) of the three scales were respectively 0.87, 0.87 and 0.89 in their study as opposed to 0.81, 0.80, 0.89 in this study. Again, these differences are marginal.

Brown et al. (13) administered the QIDS-SR16, IDS-SR30, HAM-D17 and Mini Asthma Quality of Life Questionnaire to 73 asthmatic patients at treatment exit. Their primary interest was in the apparent co-occurrence of asthma and depression. The internal consistency Cronbach's α values of QIDS-SR_{16} and HAM-D_{17} were both .87 and their respective standard deviations were 4.8 and 4.7. In our study, the internal consistency Cronbach's α values of QIDS-SR_{16} and HAM-D_{17} were respectively .80 and .89. In addition, the standard deviations were 6.24 and 6.19. The slight differences in coefficient a are thus not readily explained by differences in variability, as they often can be.

The marginal differences in reliability among the studies, including the present may reflect two things beyond simple random error. First, greater reliabilities tend to be associated with greater variabilities. For example, the variabilities of the QIDS-C_{16} and HAM-D_{17} (but not the QIDS-SR_{16}) were greater in Rush et al. (2006) than in this study (12). Reliability, being a ratio of true variance to total variance, increases as total increases since the remaining component, error, is, by definition, independent of true variance and remains constant. As a result, increasing total variance normally also increases true variance by that same amount (14). Second, the data in some studies, e.g., Rush et al. (12) were obtained from an exit interview following prior assessments. We typically find that reliabilities increase with number of assessments as respondents learn to treat items as a group rather than as separate items.

This paper adds to the number of scales that measure depressive symptomatology available in Turkish, which have at least some supporting psychometric data. These data could be found in the book of Aydemir & Köroğlu(15). The others include Akdemir, Örsel, Dağ et al. (16) who first adapted the HAM-D_{17}, which is a clinical scale. They noted a Cronbach's α of .75 and a test-retest (stability) reliability of 0.85. The scale correlated .48 with a translation of the Beck Depression inventory. In turn, Hisli (17) adapted the Beck Depression Inventory, a self-report scale. The study reported a Cronbach's α was .80. and a correlation of .50 with MMPI scale 2 (depression).

Özer, Demir, Tuğal, et al. (18) adapted the Montgomery-Asberg depression rating scale (MADRS), a clinical scale.. They reported pairwise interrater reliabilities among four psychiatrists/raters ranging from .66 to .86. Cronbach's α ranged from .74 to .84, and the correlations between this scale and the Beck Depression Inventory scores ranged from .64 to .68.

Tatar and Saltukoğlu (19) adapted the Center for Epidemiological Studies Depression Scale (CES-D), a self-report scale. Its Cronbach's α was .89. The test-retest correlation was .69. It correlated .87 with the Beck Depression Inventory.

Finally, Aydemir, Güven, Küey, et al. (20) adapted the Hospital Anxiety and Depression Scale, which is a self-report scale. Cronbach's α was .78 for the depression sub-scale and it correlated .72 with the Beck Depression Inventory.

Limitations

One major limitation of the study is the relatively small number of observations, especially as compared to its predecessor. As before, the study sample consists of university students seen at an outpatient clinical setting, so it might not apply it to a more general Turkish population. A third important limitation is that we did not have repeated test data to evaluate stability and sensitivity to change of the Turkish QIDS-SR_{16}, which would be necessary if the scale were to monitor therapeutic effects. This was not the goal of this study nor its predecessor. Finally, the degree to which the test equating will be stable depends upon the relevant sample size. Ours can only provide highly tentative estimates until a much larger sample can be obtained.

References

1. *Mergen H, Bernstein IH, Tavli V, Ongel K, Tavlı T, Tan Ş. Comparative Validity and Reliability Study of The QIDS-SR16 in Turkish and American College Student Samples. Klinik Psikofarmakoloji Bülteni 2011; 21(4): 289-301.*
2. *De Ayala, RJ. The theory and practice of item response theory. New York: The Guilford Press, 2009.*
3. *IDS/QIDS Instruments in English and Multiple Translations. www.ids-qids.org accessed on 4/12/2012.*
4. *Horn JL. An empirical comparison of various methods for estimating common factor scores. Educational and Psychological Measurement 1976; 25: 313-322.*
5. *Humphreys LG, Ilgen D. Note on a criterion for the number of common factors. Educational and Psychological Measurement 1969; 29: 571-578.*
6. *Humphreys LG, Montanelli RGJr. An investigation of the parallel analysis criterion for determining the number of common factors. Multivariate Behavioral Research 1975; 10: 193-206.*
7. *Montanelli RGJr, Humphreys LG. Latent roots of random data correlation matrices with squared multiple correlations on the diagonal: a Monte Carlo study. Psychometrika 1976; 41: 341-348.*
8. *Samejima, F. Estimation of latent ability using a response pattern of graded scores. Psychological Monographs 1969; 4: 2.*
9. *Samejima, F. Graded response model. In: van Linden, W. and Hambleton, R.K. (Eds.), Handbook of Modern Item Response Theory. Springer-Verlag, New York, 1997. p.85-100.*
10. *Orlando, M., Sherbourne, C.D. and Thissen, D. Summed-score linking using item response theory: application to depression measurement. Psychological. Assessessment 2000; 12, 354-359.*
11. *Rush AJ, Trivedi MH, İbrahim HM, Carmody TJ, Arnow B, Klein DN et al..The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), Clinician Rating (QIDS-C), and Self-Report (QIDS-SR): A Psychometric Evaluation in Patients with Chronic Major Depression. BioIogical Psychiatry 2003; 54: 573-583*
12. *Rush AJ, Bernstein IH, Trivedi MH et al. An evaluation of the quick inventory of depressive symptomatology and the Hamilton rating scale for depression: a sequenced treatment alternatives to relieve depression trial report. Biological Psychiatry 2006; 59(6): 493-501.*
13. *Brown ES, Murray M, Carmody TJ et al. The Quick Inventory of Depressive Symptomatology-Self-report: a psychometric evaluation in patients with asthma and major depressive disorder. Annals of Allergy, Asthma, and Immunology 2008; 100(5): 433-8.*
14. *Nunnally, JC, Bernstein IH.. Psychometric Theory. 3rd ed. New York: McGraw-Hill, 1994.*
15. *Aydemir Ö, Köroğlu E. Psikiyatride Kullanılan Klinik Ölçekler. Ankara: HYB Yayıncılık, 2012.*
16. *Akdemir A, Örsel S, Dağ İ, Türkçapar H, İşcan N, Özbay H. Hamilton Depresyon Değerlendirme Ölçeği (HDDÖ)'nin Geçerliği, Güvenilirliği ve Klinikte Kullanımı. Psikiyatri Psikoloji Psikofarmakoloji Dergisi 1996; 4(4): 251-259.*
17. *Hisli N. Beck Depresyon Envanterinin Üniversite Öğrencileri İçin Geçerliliği, Güvenilirliği. Psikoloji Dergisi 1989; 7: 3-13.*
18. *Özer S, Demir B, Tuğal Ö, Kabakçı E, Yazıcı MK. Montgomery-Asberg Depresyon Değerlendirme Ölçeği: Değerlendiriciler Arası Güvenilirlik ve Geçerlilik Çalışması. Türk Psikiyatri Dergisi 2001; 12(3): 185-194.*

19. *Tatar A, Saltukoglu G. CES-Depresyon Ölçeği'nin Doğrulayıcı Faktör Analizi ve Madde Cevap Kuramı kullanımı ile Türkçe'ye uyarlanması ve Psikometrik Özelliklerinin İncelenmesi. Klinik Psikofarmaloji Bülteni 2010; 20: 213-222.*

20. *Aydemir Ö, Güvenir T, Küey L, Kültür S. Hastane Anksyete ve Depresyon Ölçeği Türkçe Formunun Geçerlilik ve Güvenilirliği. Türk Psikiyatri Dergisi 1997; 8(4): 280-287.*

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The prevalence of carotid artery calcification or atherosclerotic plaque in end-stage renal disease being treated with hemodialysis and peritoneal dialysis patients detected on panoramic radiography

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Abstract

Objective. The aim of this study is to determine the presence of carotid artery calcifications (CACs) detected on panoramic radiographs (PR) in end-stage renal disease patients (ESRD) with hemo and peritoneal dialysis and to analyze the relationship between this calcification prevalence and dialysis period.

Design. Subjects and Methods. A random sample of 170 panoramic radiographs belonged to ESRD patients were investigated.

Results. Of the 155 ESRD patients included in the data analysis, 41 (26 %) were detected as having CACs on PRs (29 PD &12 HD patients). There was a statistically significant difference in terms of mean age between the patients with CACs and those without CACs. A statistical difference was found in PD and HD period between the patients with CACs and those without CACs.

Conclusions. Suggesting the results of the study the incidental finding of CACs in the area of the carotid arteries on PRs should be followed up with carotid screening due to detect asymptomatic patients in terms of f stroke. Therefore, it could give us the life-saving information.

Key words: panoramic radiograph, carotid artery calcification, end-stage renal disease

Introduction

Atherosclerosis is a chronic and progressive inflammatory disease, which takes place in the intima-media layer of the arterial wall. Many general and systemic factors are revealed to play an important role in the etiology of the process, including age, blood pressure, diabetes, hyperlipidemia, smoking, and radiotherapy (1, 2). In the general population, atherosclerotic disorder is known as an important etiology of morbidity and mortality (3). The risk is also known to increase in patients with chronic diseases. End-stage renal disease (ESRD) patients have a much higher prevalence of atherosclerosis in comparison to the normal population (4, 5).

Cardiovascular disease and stroke are known to be the leading cause of mortality in ESRD patients. These patients need dialysis and have a ten- to twenty-fold risk in comparison to the age and sex of matched normal subjects in terms of having atherosclerotic heart and vascular disease (6, 7). Vascular calcifications are commonly seen in these patients due to ESRD (8). Although the etiology of vascular calcification in patients with ESRD is not clear, the process is most probably multi-factorial. In some reports, older age and a longer time on dialysis have been revealed as the major risk factors in vascular calcification (7, 9, 10). In addition, the process results in increased stiffness of the elastic-type, capacitive, large arteries, such as common carotid and the aorta artery (11). Arterial stenosis due to carotid atheromas is one of the most important risk factors leading to stroke (3). Approximately 80% of all strokes are ischemic due to atherosclerotic disorder in the carotid bifurcation region (12-14).

Panoramic radiographs (PRs), which commonly display the existence of calcifications in the region of the 2nd, 3rd, and 4th cervical vertebrae, are gene-

rally necessary during routine dental examination (15-18). Early in the 1980s, Friedlander and Lande first reported the incidence of carotid artery calcification (CAC) on PRs, and they suggested that these radiographs were able to play an important role in the early diagnosis of CAC, which are known to cause more serious stroke and heart disease (19, 20).

Many reports have investigated this further to confirm the ability of PRs to detect CACs in the carotid bifurcation region through correlation with medical history, Doppler spectral analysis, or cervical spine radiographs (20-24). In these studies, the overall prevalence rate of CAC was revealed to range from 2% to 11% of the adult population, and the incidence increases remarkably in samples with specific chronic diseases (23, 25-28). In our previous study, which was first performed with peritoneal dialysis (PD) patients, the incidence of CAC was found to be 27.3%, and this was the highest reported prevalence in the literature (19).

The aim of the current study is to investigate the prevalence of CAC by using standard PRs in ESRD patients (the largest sample), who need dialysis, in order to evaluate the predictive value of the radiographs in the diagnosis of atherosclerotic heart and vascular disease.

Material and methods

Patients

For the study, patients with ESRD being treated with PD and HD were referred for a dental examination, and the PRs taken for routine radiographic examination were investigated for the presence of CAC. All the PRs were performed at the Department of Oral Diagnosis and Radiology, Erciyes University Faculty of Dentistry. Since the radiographs had originally been taken for routine dental examination and not for the investigation of CAC, it was not necessary to seek ethical approval for the study.

PR processing and evaluation

All radiographs were obtained by the same radiology technician using either a conventional (Orthopantomograph® OP 100, Instrumentarium Corp., Tuusula, Finland) or digital (Orthopantomography® OP 200D, Instrumentarium Corp., Tuusula, Finland) device. Conventional radiographs were processed in an automatic film processor according to the manufacturer's recommendations, and digital radiographs were printed with a laser printer at the same magnification factor (1: 1.3). Radiographs were viewed on a standard viewbox in subdued ambient light.

PRs were evaluated by the same authors (ETE & YS), and any radiographs that showed questionable evidence of CAC were excluded from the study. Radiographs were also excluded if they did not include vertebrae C3 and C4, or if the patient had moved during the radiograph exposure. Each PR was viewed in subdued ambient light using transmitted light from a standard viewbox. The presence of a radiopaque nodular mass (or masses) adjacent to the cervical vertebrae at or below the intervertebral space between C3 and C4 was diagnosed as a CAC. Unilateral and bilateral CACs on the radiographs were recorded separately. For a differential diagnosis of CACs, other cervical calcifications, such as calcified triticeous cartilage, calcified thyroid cartilage, hyoid bone, and submandibular salivary gland sialoliths, were excluded according to Carter's study (26). To check intra-observer variation, all of the PRs were re-evaluated by the same authors (ETE & YS) after one month. The dialysis period of all the patients was recorded. The patients diagnosed with CACs in the PRs were contacted by phone for further Doppler ultrasound confirmation. Of the 14 patients, four cases had passed away due to stroke. The other patients with CACs diagnosed by PRs were referred to the Erciyes University School of Medicine's Radiology Department for color Doppler ultrasonography (CDUS) examination to determine the location and extent of calcification. The presence of CAC was confirmed by an ultrasound examination. Figure 1 shows the PR of a PD patient displaying calcifications on both cervical regions. Figure 2a demonstrates a color Doppler image of the right, while Figure 2b shows the left internal carotid artery of the same patient in Figure 1, but revealing the extent of the CACs. Figure 3 shows the PR of a HD patient displaying calcifications on both cervical regions. Figure 4a demonstrates color Doppler images of the right, and Figure 4b shows the left internal carotid artery of the same patient in Figure 3, but reveals the extent of the CACs.

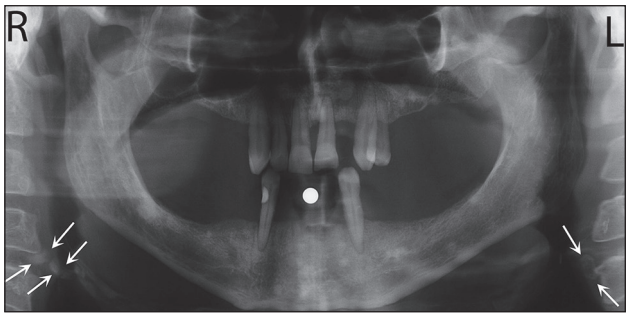

Figure 1. Shows the PR of a PD patient displaying calcifications on both cervical regions

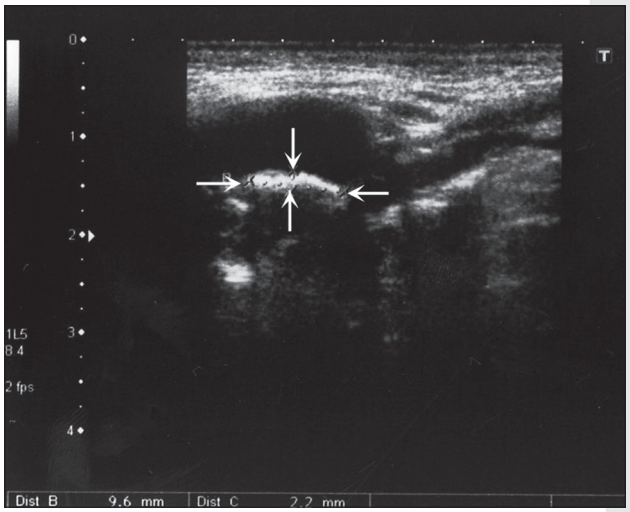

Figure 2a. Demonstrates color doppler image of the right

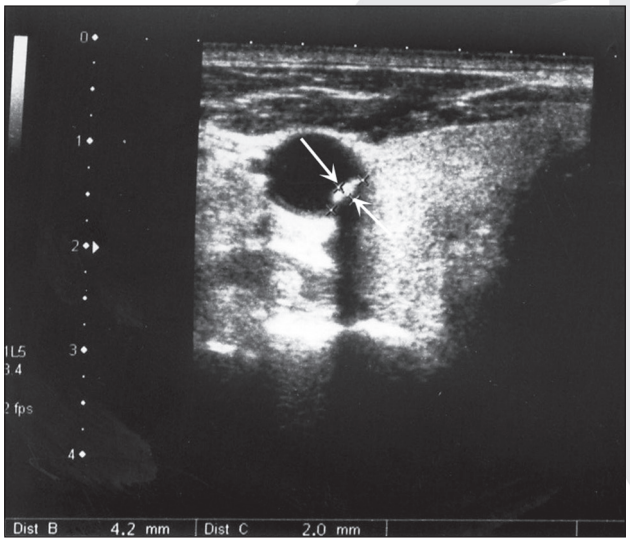

Figure 2b. Shows the left internal carotid artery of the same patient in Figure 1 showing the extent of CACs

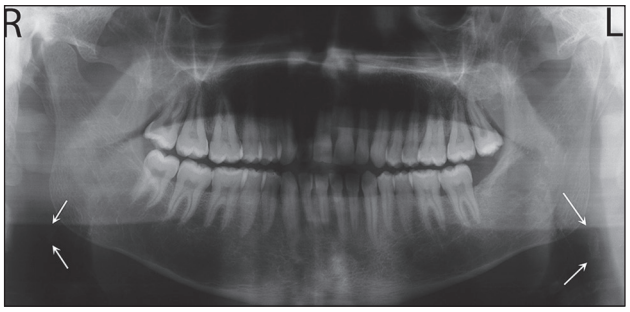

Figure 3. Shows the PR of a HD patient displaying calcifications on both cervical regions

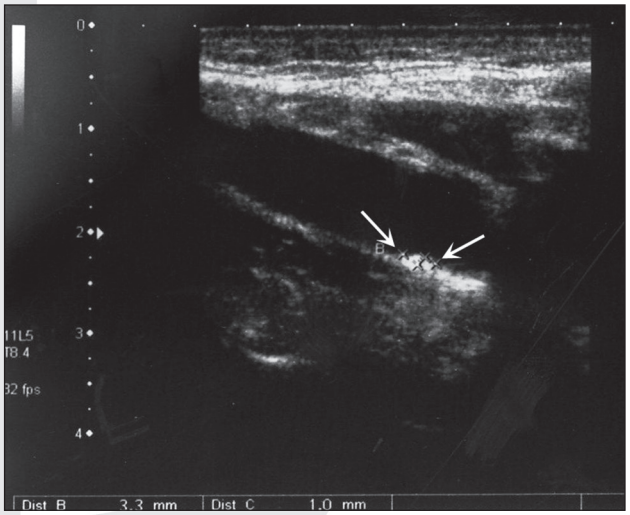

Figure 4a. Demonstrates Color Doppler images of the right

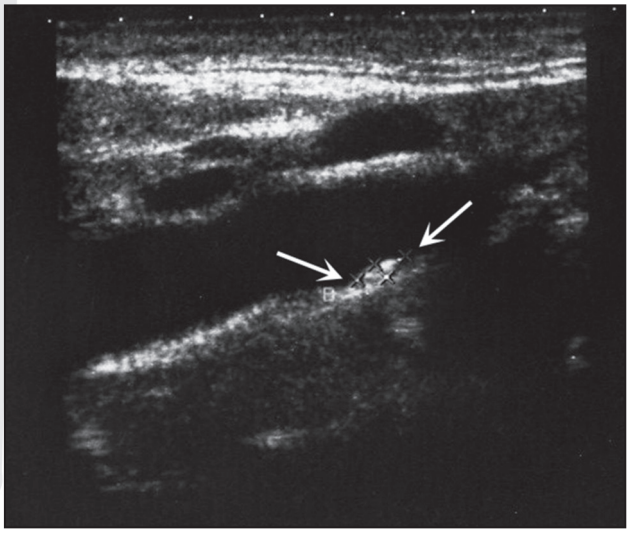

Figure 4b. Shows the left internal carotid artery of the same patient in Figure 3 showing the extent of CACs

Statistical analysis

Statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 13.0; SPSS Inc., Chicago,

IL, USA). The differences between the categorical variables were analyzed using chi-square, Fisher's exact test, the Mann-Whitney U test and the Spearman correlation test. P-values < 0.05 were considered to be statistically significant.

Results

A total of 170 patients with ESRD being treated with PD and HD were included in the study. The PRs of 15 patients who had questionable CACs were excluded; hence the PRs of 155 ESRD patients with PD (116) and HD (39) were included in the data analysis. This population consisted of 85 males and 70 females, with an overall mean age of 44.4 ± 14.2 years. ESRD patients being treated with PD included 58 males and 58 females. The mean age was 44.9 ± 13.7 years. The ESRD population being treated with HD comprised 27 males and 12 females. The mean age was 42.8 ± 15.5 years.

The mean age of ESRD patients was 44.4 ± 14.2 years, and there was no statistically significant difference in respect of mean age between males and females (P = 0.526). Of the 155 ESRD patients included in the data analysis, 41 (26 %) had CACs on the PRs, and of those, 17 (11%) were male and 24 (15%) were female. There was a significant difference in terms of mean age between the patients with CACs (49.9 ± 15.1 years) and those without CACs (42.5 ± 13.4 years) (P = 0.004). Calcifications were unilateral in 31 (76%) and bilateral in 10 (24%) patients with CACs. A total of 51 CAC cases were therefore detected in the 41 patients and, of these, 12 (29%) were located on the right side and 19 (46%) were located on the left side.

For patients being treated with PD, the mean age of patients was 44.9 ± 13.7 years, and there was no statistically significance in the mean age between males and females (P = 0.134, $P > 0.05$). Of the 116 PD patients who had the data analysis, 29 (25%) were detected as having CACs on PRs, and of these, there were 12 (10%) males and 17 (15%) females. The mean age was statistically different between the patients with CACs (50.5 ± 13.2 years) and those without CACs (43.1 ± 13.6 years) (P = 0.012). Calcifications were unilateral in 22 (76%) patients and bilateral in 7 (24 %) patients. A total of 36 CAC cases were therefore detected in the 29 patients and, of these, 6 (21%) were located on the right side and 16 (55%) were located on the left side.

For patients being treated with HD, the mean age of the patients was 42.8 ± 15.5 years, and there was no significant difference in respect of the mean age between males and females (P = 0.338). Of the 39 HD patients who had the data analysis, 12 (31%) were detected as having CACs on PRs, and of these, there were 5 (13%) males and 7 (18%) females. There was a statistical difference between the mean age of the patients with CACs (48.4 ± 19.5 years) and those without CACs (40.4 ± 13.1 years) (P = 0.140). Calcifications were unilateral in 9 (75%) patients and bilateral in 3 (25%) patients. A total of 18 CAC cases were therefore detected in the 12 patients, and of these, 6 (50%) were located on the right side and 3 (25%) were located on the left side.

There was not a statistically significant difference between PD and HD patients in terms of CAC distribution (P = 0.120), and Table 1 shows the distribution of CAC localization according to dialysis methods. The difference between CAC percentages in PD and HD patients was not significant (P = 0.480). Figure 5 shows the distribution of CAC prevalence in the investigated cohort.

The mean dialysis period for all ESRD patients (both PD and HD) was 4.3 ± 3.5 years (range 1 – 15 years). There was no significant difference between the dialysis periods of males and females (P = 0.411). There was, however, a statistical difference in terms of the dialysis periods between the patients with CACs (5.9 ± 4.0 years) and those without CACs (3.6 ± 3.0 years) (P = 0.0001). The mean age and dialysis period of ESRD patients with CACs are shown in Table 2.

Table 1. Shows the distribution of CAC localization according to dialysis methods

All ESRD (n=155)			PD (n=116)			HD (n=39)			P (HD vs PD)
Right	Left	Bilateral	Right	Left	Bilateral	Right	Left	Bilateral	Fischer's Exact Test
12 (29 %)	19 (46 %)	10 (24 %)	6 (21 %)	16 (55 %)	7 (24%)	6 (50 %)	3 (25 %)	3 (25 %)	0,120

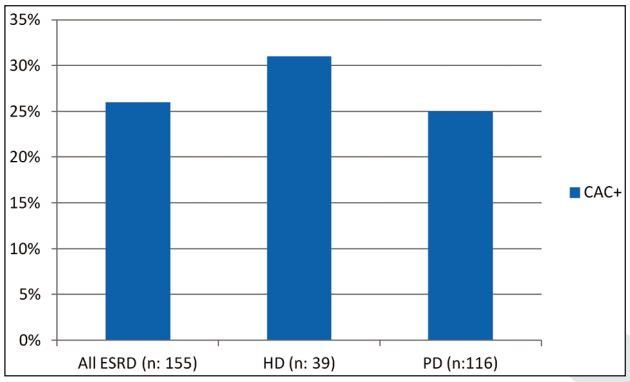

Figure 5. Shows the distribution of CAC prevalence in the investigated cohort

The mean dialysis period for PD patients was 5.4 ± 4.4 years (range 1 – 14 years). No significant difference in the dialysis period was detected between males and females (P = 0.472). There was, however, a statistical difference in terms of the PD period between the patients with CACs (5.1 ± 3.2 years) and those without CACs (3.5 ± 2.9 years) (P = 0.014). The distribution of the PD period in CAC-positive patients according to sex is shown in Table 2.

The mean dialysis period for HD patients was 5.4 ± 4.4 years (range 1 – 15 years). No significant difference in the dialysis period was found between males and females (P = 0.237). There was, however, a statistically significant difference in the HD period between the patients with CACs (8.1 ± 5.2 years) and those without CACs (4.2 ± 3.4 years) (P = 0.009). The distribution of the HD period in CAC-positive patients according to sex is shown in Table 2. In the evaluation of CAC presence in PD or HD patients, there was no significant difference between the types of dialysis methods (n = 12 HD patients with CACs; n = 29 PD patients with CAC) (P = 0.48).

Discussion

Most patients with ESRD need some form of dialysis during their lifetime (29). Therefore, while the activators of calcification are increased, the inhibitors of calcification are reduced in patients with ESRD, and these cause metastatic vascular calcification as an important etiology of vascular injury (30). In a previous study, there was a very high prevalence of cardiovascular calcification in ESRD patients receiving long-term PD. These results reveal that the presence of vascular calcification may have prognostic implications for PD patients (8). It was reported that the presence of these calcifications in ESRD patients causes increased stiffness of the large, capacitive, elastic-type arteries, such as the aorta and common carotid artery (31). Several observational cohort studies have demonstrated the prognostic importance of cardiovascular calcification in ESRD patients (8). Furthermore, in a follow-up study of 57 HD patients, all of the patients had an increased calcification score during the 1 – 2 years following the study (32). In addition, a significant relationship was reported between the extent of coronary artery disease and the extent of atherosclerosis in the thoracic aorta and carotid arteries (33).

PRs are used routinely in the evaluation of patients with dental problems. Since, calcification is used as a clinical indicator of atherosclerosis (34), according to Friedlander and Cohen (35), the incidental finding of CAC on PRs is an important marker for vascular events. Because of other radiopacities, such as anatomical entities (hyoid bone, epiglottis, thyroid cartilage, the stylohyoid, and the stylomandibular ligament) and pathological entities (submandibular gland sialolith, phleboliths, tonsillolith, calcified acne, and calcified lymph nodes) in this area (36), advanced diagnostic examinations, such as ultrasonography, magnetic resonance imaging (MRI), or angiography might be required to confirm the presence and extent of vascular calcification (37, 38). Although, PRs are not as useful as ultrasonography, MRI, or angio-

Table 2. The mean age, gender and dialysis vintage (years) according to CAC positive and negative groups are shown for all ESRD, HD and PD patients.

	All ESRD (n=155)			PD (n=116)			HD (n=39)		
	CAC +	CAC -	p	CAC +	CAC -	P	CAC +	CAC -	P
Age (years)	49.9± 15.1	42.5±13.4	0.004	50.5±13.2	43.1±13.6	0.012	48.4±19.5	40.4±13.1	0.140
Male (n %)	17 (11%)	68 (44%)	<0,001	12 (10%)	46 (40%)	<0,001	5 (13%)	22 (56%)	0.001
Dialysis vintage (years)	5.9± 4.0	3.6± 3.0	<0,001	5.1±3.2	3.5±2.9	0.014	8.1±5.2	4.2±3.4	0.009

graphy for detecting atherosclerotic plaque in the carotid arteries, and especially the stenosis of the vessels (3), because it is cheap and non-invasive in comparison to other imaging methods, PRs taken as part of a routine dental examination should be carefully examined for evidence of CAC. Dentists should therefore refer patients with suspected CAC for further medical examination (39).

In the literature, there are many articles on incidental CAC detected by PRs in other populations, and the reported prevalence of CAC on PRs ranges between 2 – 5% in the dental outpatient population (26, 27, 38, 40). Notwithstanding, patients with a history of certain medical problems, including a history of cerebrovascular accidents (41), dilated cardiomyopathy (42), type II diabetes mellitus (36), elevated cholesterol levels (43), and renal disease (4, 19), have a higher prevalence of calcification detected in PRs due to the underlying disorders causing atherosclerosis.

The present study showed an increased prevalence of CAC in the majority of ESRD patients. These findings are in agreement with the claim, which is accepted by many authors, and there is consistency between an acceleration of both vascular calcification and atherosclerosis in ESRD patients (44, 45). In the current study, the presence of CAC was found in 26% of the ESRD patients. Kansu et al., using the PRs of 69 adult patients with renal disease (35 renal transplant recipients and 34 HD patients), found a similarly high prevalence of CAC in 15.7% of renal transplants patients and 17.6% in patients with HD (4). An even higher prevalence of 27.3% was reported in our previous study, which evaluated the prevalence of CAC in the PRs of PD patients (19).

In the literature, although there is a greater male preponderance of calcification compared to females (4, 46), in both our previous and current studies, females had a higher CAC prevalence compared to males (19). However, the present study had the largest sample for investigating CAC prevalence in ESRD patients being treated with HD and PD. In addition, it is the only study comparing the commonalities and the efficacy of the two dialysis methods in these patients in terms of CAC presence and the dialysis period. Therefore, the detection of CAC in patients with ESRD is very important for the determination of atherosclerosis in relation to coronary heart disease and stroke. Mortality and morbidity in these patients are generally due to these disorders.

The extent of arterial calcification increases with the dialysis period and age (31). Ectopic (metastatic) calcification due to an abnormality in calcium and phosphate metabolism, which is related to the duration of dialysis, is also very important for arterial calcification, and it is very common in patients with ESRD (4, 31). In this study, the relation between the presence of CAC and the dialysis periods of all ESRD patients was investigated. It was found that there is a statistically significant difference in the PD period between patients with CACs and those without CACs ($P = 0.014$). Furthermore, the HD period was higher in patients with CACs than in patients without CACs ($P = 0.009$). As expected, there was statistical difference in the mean age between the patients with CACs and those without CACs ($P = 0.012$) for PD patients. In addition, a significant difference in mean age between patients with CACs and those without CACs ($P = 0.140$) was observed for HD patients. However, no significant difference between the presence of CAC and the type of treatment was found when comparing the dialysis methods ($P = 0.48$).

Conclusion

PR is an indispensable diagnostic tool routinely used in dental examinations, and clinicians usually focus on the teeth and jaws only. But the same radiographs may be used for other purposes, such as detecting CAC. Carotid artery calcifications found as incidental findings in routine PRs may be important prognostic markers for future coronary artery disease, strokes, and death in patients with ESRD. The current study comprised the largest sample investigating CAC prevalence in ESRD patients being treated with HD and PD. Also, it is the only study comparing the commonalities and the efficacy of the two dialysis methods in these patients in terms of CAC presence and the dialysis period. Therefore, the detection of CAC in patients with ESRD is very important for the determination of atherosclerosis in respect of coronary heart disease and stroke. Mortality and morbidity in these patients are generally due to these disorders. We believe that dentists caring for patients with dental problems should carefully evaluate their PRs for evidence of CAC,

and refer them for further medical evaluation since detecting asymptomatic patients at risk of stroke could impart life-saving information.

References

1. *Friedlander AH, Garrett NR, Chin EE, Baker JD. Ultrasonographic confirmation of carotid artery atheromas diagnosed via panoramic radiography. J Am Dent Assoc. 2005 May; 136(5): 635-40; quiz 82-3.*
2. *Nadareishvili ZG, Rothwell PM, Beletsky V, Pagniello A, Norris JW. Long-term risk of stroke and other vascular events in patients with asymptomatic carotid artery stenosis. Arch Neurol. 2002 Jul; 59(7): 1162-6.*
3. *Madden RP, Hodges JS, Salmen CW, Rindal DB, Tunio J, Michalowicz BS, et al. Utility of panoramic radiographs in detecting cervical calcified carotid atheroma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Apr; 103(4): 543-8.*
4. *Kansu O, Ozbek M, Avcu N, Genctoy G, Kansu H, Turgan C. The prevalence of carotid artery calcification on the panoramic radiographs of patients with renal disease. Dentomaxillofac Radiol. 2005 Jan; 34(1): 16-9.*
5. *London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC. Arterial calcifications and bone histomorphometry in end-stage renal disease. J Am Soc Nephrol. 2004 Jul; 15(7): 1943-51.*
6. *Cheung AK, Sarnak MJ, Yan G, Dwyer JT, Heyka RJ, Rocco MV, et al. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. Kidney Int. 2000 Jul; 58(1): 353-62.*
7. *Moe SM, O'Neill KD, Reslerova M, Fineberg N, Persohn S, Meyer CA. Natural history of vascular calcification in dialysis and transplant patients. Nephrol Dial Transplant. 2004 Sep; 19(9): 2387-93.*
8. *Wang AY. Vascular and other tissue calcification in peritoneal dialysis patients. Perit Dial Int. 2009 Feb; 29 Suppl 2: S9-S14.*
9. *London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant. 2003 Sep; 18(9): 1731-40.*
10. *Moe SM, O'Neill KD, Fineberg N, Persohn S, Ahmed S, Garrett P, et al. Assessment of vascular calcification in ESRD patients using spiral CT. Nephrol Dial Transplant. 2003 Jun; 18(6): 1152-8.*
11. *Friedlander AH, Garrett NR, Norman DC. The prevalence of calcified carotid artery atheromas on the panoramic radiographs of patients with type 2 diabetes mellitus. J Am Dent Assoc. 2002 Nov; 133(11): 1516-23.*
12. *Tarim Ertas E ME, Sisman Y, Sahman H, Etoz M, Sekerci AE. . Incidental Findings Of Carotid Artery Stenosis Detected By Calcifications On Panoramic Radiographs: Report Of Three Cases. Oral Radiology. 2010; 26(2): 116-21.*
13. *Almog DM, Tsimidis K, Moss ME, Gottlieb RH, Carter LC. Evaluation of a training program for detection of carotid artery calcifications on panoramic radiographs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000 Jul; 90(1): 111-7.*
14. *Anderson CS, Jamrozik KD, Burvill PW, Chakera TM, Johnson GA, Stewart-Wynne EG. Determining the incidence of different subtypes of stroke: results from the Perth Community Stroke Study, 1989-1990. Med J Aust. 1993 Jan 18; 158(2): 85-9.*
15. *Sisman Y, Gokce C, Sipahioglu M. Bilateral Elongated Styloid Process in an End-stage Renal Disease Patient with Peritoneal Dialysis: Is there Any Role for Ectopic Calcification? Eur J Dent. 2009 Apr; 3(2): 155-7.*
16. *Sisman Y, Ertas ET, Gokce C, Menku A, Ulker M, Akgunlu F. The Prevalence of Carotid Artery Calcification on the Panoramic Radiographs in Cappadocia RegionPopulation. Eur J Dent. 2007 Jul; 1(3): 132-8.*
17. *Romano-Sousa CM, Krejci L, Medeiros FM, Graciosa-Filho RG, Martins MF, Guedes VN, et al. Diagnostic agreement between panoramic radiographs and color Doppler images of carotid atheroma. J Appl Oral Sci. 2009 Jan-Feb; 17(1): 45-8.*
18. *Lewis DA, Brooks SL. Cartoid artery calcification in a general dental population: a retrospective study of panoramic radiographs. Gen Dent. 1999 Jan-Feb; 47(1): 98-103.*
19. *Gokce C, Sisman Y, Sipahioglu M, Ertas ET, Akgunlu F, Unal A, et al. The prevalence of carotid artery calcification on the panoramic radiographs of end-stage renal disease patients with peritoneal dialysis: do incidental findings provide life-saving information? J Int Med Res. 2008 Jan-Feb; 36(1): 47-53.*
20. *Cohen SN, Friedlander AH, Jolly DA, Date L. Carotid calcification on panoramic radiographs: an important marker for vascular risk. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Oct; 94(4): 510-4.*
21. *Yoon SJ, Yoon W, Kim OS, Lee JS, Kang BC. Diagnostic accuracy of panoramic radiography in the detection of calcified carotid artery. Dentomaxillofac Radiol. 2008 Feb; 37(2): 104-8.*
22. *Carter LC, Haller AD, Nadarajah V, Calamel AD, Aguirre A. Use of panoramic radiography among an ambulatory dental population to detect patients at risk of stroke. J Am Dent Assoc. 1997 Jul; 128(7): 977-84.*
23. *Friedlander AH, Baker JD. Panoramic radiography: an aid in detecting patients at risk of cerebrovascular accident. J Am Dent Assoc. 1994 Dec; 125(12): 1598-603.*
24. *Friedlander AH, Friedlander IK. Identification of stroke prone patients by panoramic radiography. Aust Dent J. 1998 Feb; 43(1): 51-4.*

25. *Friedlander AH, Lande A. Panoramic radiographic identification of carotid arterial plaques. Oral Surg Oral Med Oral Pathol. 1981 Jul; 52(1): 102-4.*

26. *Carter LC. Discrimination between calcified triticeous cartilage and calcified carotid atheroma on panoramic radiography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000 Jul; 90(1): 108-10.*

27. *Pornprasertsuk-Damrongsri S, Thanakun S. Carotid artery calcification detected on panoramic radiographs in a group of Thai population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Jan; 101(1): 110-5.*

28. *Haller AD, Calamel AD, Carter LC. Association of calcified carotid atheromas on panoramic radiographs with risk factors with stroke. J Dent Res. 1997; 76: 249.*

29. *Noshad H, Sadreddini S, Nezami N, Salekzamani Y, Ardalan MR. Comparison of outcome and quality of life: haemodialysis versus peritoneal dialysis patients. Singapore Med J. 2009 Feb; 50(2): 185-92.*

30. *Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. Circulation. 2007 Jul 3; 116(1): 85-97.*

31. *Guerin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant. 2000 Jul; 15(7): 1014-21.*

32. *Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC. Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. Am J Kidney Dis. 1996 Mar; 27(3): 394-401.*

33. *Rohani M, Jogestrand T, Ekberg M, van der Linden J, Kallner G, Jussila R, et al. Interrelation between the extent of atherosclerosis in the thoracic aorta, carotid intima-media thickness and the extent of coronary artery disease. Atherosclerosis. 2005 Apr; 179(2): 311-6.*

34. *Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. Arterioscler Thromb Vasc Biol. 2004 Jul; 24(7): 1161-70.*

35. *Friedlander AH, Cohen SN. Panoramic radiographic atheromas portend adverse vascular events. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Jun; 103(6): 830-5.*

36. *Friedlander AH, Maeder LA. The prevalence of calcified carotid artery atheromas on the panoramic radiographs of patients with type 2 diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000 Apr; 89(4): 420-4.*

37. *Almog DM, Horev T, Illig KA, Green RM, Carter LC. Correlating carotid artery stenosis detected by panoramic radiography with clinically relevant carotid artery stenosis determined by duplex ultrasound. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Dec; 94(6): 768-73.*

38. *Ohba T, Takata Y, Ansai T, Morimoto Y, Tanaka T, Kito S, et al. Evaluation of calcified carotid artery atheromas detected by panoramic radiograph among 80-year-olds. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Nov; 96(5): 647-50.*

39. *Ertas ET, Sisman Y. Detection of incidental carotid artery calcifications during dental examinations: Panoramic radiography as an important aid in dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. Jun 8.*

40. *Tamura T, Inui M, Nakase M, Nakamura S, Okumura K, Tagawa T. Clinicostatistical study of carotid calcification on panoramic radiographs. Oral Dis. 2005 Sep; 11(5): 314-7.*

41. *Friedlander AH, Manesh F, Wasterlain CG. Prevalence of detectable carotid artery calcifications on panoramic radiographs of recent stroke victims. Oral Surg Oral Med Oral Pathol. 1994 Jun; 77(6): 669-73.*

42. *Sung EC, Friedlander AH, Kobashigawa JA. The prevalence of calcified carotid atheromas on the panoramic radiographs of patients with dilated cardiomyopathy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Mar; 97(3): 404-7.*

43. *Friedlander AH. Identification of stroke-prone patients by panoramic and cervical spine radiography. Dentomaxillofac Radiol. 1995 Aug; 24(3): 160-4.*

44. *Krasniak A, Drozdz M, Pasowicz M, Chmiel G, Michalek M, Szumilak D, et al. Factors involved in vascular calcification and atherosclerosis in maintenance haemodialysis patients. Nephrol Dial Transplant. 2007 Feb; 22(2): 515-21.*

45. *Amann K, Gross ML, Ritz E. Pathophysiology underlying accelerated atherogenesis in renal disease: closing in on the target. J Am Soc Nephrol. 2004 Jun; 15(6): 1664-6.*

46. *Johansson EP, Ahlqvist J, Garoff M, Karp K, Jaghagen EL, Wester P. Ultrasound screening for asymptomatic carotid stenosis in subjects with calcifications in the area of the carotid arteries on panoramic radiographs: a cross-sectional study. BMC Cardiovasc Disord.11: 44.*

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

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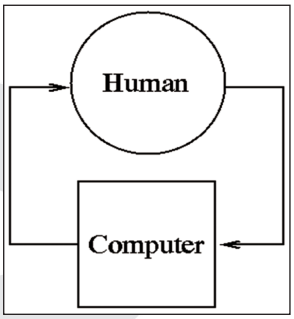

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

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References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

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