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The impact of hypochromic anemia on the pregnancy outcome

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Abstract

Anemia is a condition that is defined by a reduced number and volume of red blood cells in the circulation and / or a reduced amount of hemoglobin concentrations, regardless of etiology. According to the WHO diagnosis of anemia in pregnancy is set when the baseline level of hemoglobin (below 6.8 mmol / L), and this is also the boundary between the real physiological and anemia in pregnancy.

The aim is to give a complete picture of the effects of iron deficiency anemia on pregnancy outcomes, according to which the following objective are set: determine the weight, length and Apgar score of newborns, of women in control and test groups, determine the values of haematological parameters of control and test groups by trimesters, and determine the correlation of haematological parameters in relation to the trimester of pregnancy. The research of this study is prospective, clinical, descriptive and controlled, and was conducted at the Department of Gynecology and Obstetrics, at the Clinical Center of the University of Sarajevo in the year 2010. Of all pregnant women that were „controlled", monitored, either ambulatory or hospitalized at the Clinic of Obstetrics and Gynecology, the study included a total of 200 pregnant women older than 18 years of age. Subjects were chosen at random and divided into two groups: experimental, in which 100 pregnant women who are diagnosed with iron deficiency anemia and a control group which included the same number of pregnant women selected at random however without anemia. Iron deficiency anemia is a pathological condition often accompanying pregnancy, and pregnant women who have not started timely treatment of this type of anemia. As a result of inadequate treatment many complications related to the fetus, the weight and length, Apgar score occur. In addition, pregnant women with untreated iron deficiency anemia are considered to be of high-risk pregnancy.

Introduction

Anemia is a condition that is defined by a reduced number and volume of red blood cells in the circulation and / or a reduced amount of hemoglobin concentrations, regardless of etiology. (1)

The consequence is a reduced ability of the blood to transports oxygen to the tissues.(2)

Williams defines anemia as a condition that occurs due to the concentration of hemoglobin in the blood to levels below the normal (7.5 mmol / L) for women (8.3 mmol / L) for men. (3) Due to iron deficiency, anemia (iron deficiency anemia - IDA) is characterized by small red blood cell production. Microscopic - erythrocytes are pale or bright colors. That is why this is called anemia and hypochromic microcytic anemia. It can occur due to excessive loss of insufficient intake or poor absorption of iron. It is also known as anemia caused due to malnutrition. (4) The definition of anemia can be based on hemoglobin or hematocrit. (5)

According to the WHO diagnosis of anemia in pregnancy is set when the baseline level of hemoglobin (below 6.8 mmol / L), and this is also the boundary between the real physiological and anemia in pregnancy. (6) Lack of iron is very common in the human population, particularly in women of reproductive age. According to some data 1/5 up to 1/2 of healthy women of reproductive age has reduced iron stores, while 10% had anemia caused by lack of iron (7). The etiology of iron deficiency anemia is different and manifold. It occurs when the amount of iron in the body is lower than normal, due to a lack of iron in the diet, poor transport

in the body, or inadequate exploitation of iron and blood loss due to prolonged long, more or less occasional bleedings. (8)

In women of "generative age", excessive bleeding during the menstrual cycle is the most common cause of sideropenia, while in men and postmenopausal women, sideropenia is mostly caused by bleeding in the digestive tract. Iron from food is absorbed in the initial part of the small intestine, the duodenum. Various diseases that affect the mucous membranes of the upper small intestine influence the acceptance of iron which can lead to iron deficiency anemia. Also, the surgical procedures by which e the initial part of the small intestine is removed, cause a decreased absorption of iron, such as a subtotal gastrectomy. Sideropenia in such patients can be treated with oral iron preparations. (9)

Reduced dietary iron intake caused by sideropenia is common in infants and children. Due to their growth their needs are thus greater. With older children, milk is a poor source of iron, so in addition to low dietary iron intake, intestinal parasitic infestation is often seen, along with bleeding from the gastrointestinal tract. It is important to stress that the disadvantaged group are definitely pregnant and lactating women - on average in pregnancy 900 mg of iron transfers to the fetus and placenta, while breastfeeding takes up to 30 mg per month. Multiple pregnancies and excessive vomiting are possible factors that cause development of anemia. (10)

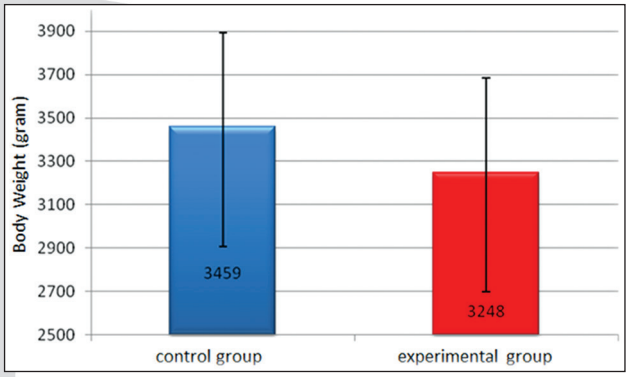
Research objectives

The aim is to give a complete picture of the effects of iron deficiency anemia on pregnancy outcomes, according to which the following objective are set: determine the weight, length and Apgar score of newborns, of women in control and test groups, determine the values of haematological parameters of control and test groups by trimesters, and determine the correlation o f haematological parameters in relation to the trimester of pregnancy.

Research subjects and methods

The research of this study is prospective, clinical, descriptive and controlled, and was conducted at the Department of Gynecology and Obstetrics, at the Clinical Center of the University of Sarajevo in the year 2010. Of all pregnant women that were „controlled", monitored, either ambulatory or hospitalized at the Clinic of Obstetrics and Gynecology, the study included a total of 200 pregnant women older than 18 years of age. Subjects were chosen at random and divided into two groups: experimental, in which 100 pregnant women who are diagnosed with iron deficiency anemia and a control group which included the same number of pregnant women selected at random however without anemia. Data were collected from ambulatory and laboratory results, patient histories, birth protocols and protocol of newborns at the Clinic for Gynecology and Obstetrics within the Clinical Center of the University of Sarajevo.

Survey results

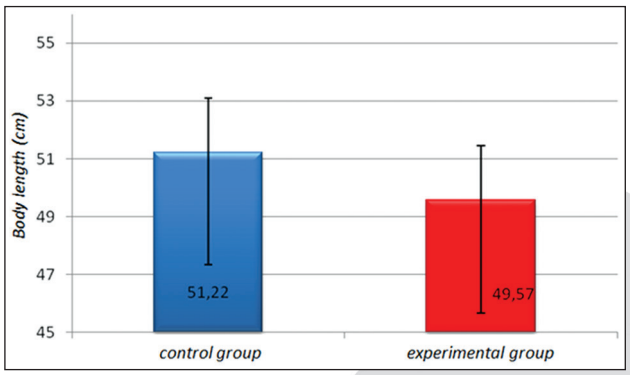


Graph 1. Body weight of infants of subjects in control and experimental group

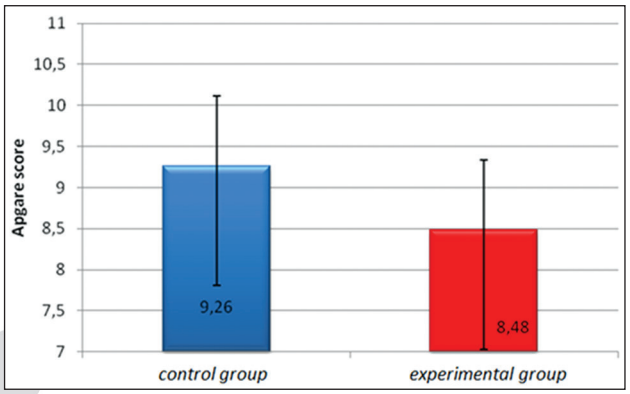
The average birth weight of infants in a controlled group was 3459 ± 435g while for the women of the experimental group they amount to 3248 ± 550g. Statistically, there is a significant difference in the average weight of newborns with control and test group, p = 0.003. Subjects in the control group gave birth to children of higher body weight compared to those of the study group. The reason for this difference is that the patients in the study group gave birth before term so the differences in gestational age also causes the different weight of newborns.

The average body length of newborns in a control group was 51.22 ± 1.89 cm while of those in the experimental group was 49.57 ± 3.89cm. There was statistically a significant difference in the average length of newborns in control and test

groups, p <0.005. Subjects in the control group gave birth to children of increased body length in relation to the respondent group.



Graph 2. Body length of infants of subjects in control and experimental group



Graph 3. Apgar scores of newborns in control and experimental group

The average value of the Apgar score of newborns in the control group was 9.26 ± 0.86, while the avera-

Table 1. Average TIBC values in relation to the trimester of pregnancy in control and experimental groups ***(µmol/L)***

Trimester	Group	Number of subjects	Average score	Std. Deviation	Std. Error
I	Control group	100	65.83 µmol/L	6.39137	.63914
	Experimental group	100	45.30µmol/L	4.34730	.43473
II	Control group	100	73.16 µmol/L	5.00852	.50085
	Experimental group	100	51.74 µmol/L	4.90026	.49003
III	Control group	100	78.65 µmol/L	4.77234	.47723
	Experimental group	100	78.02 µmol/L	4.47209	.44721

Table 2. Average values of UIBC in reference to trimester of pregnancy in control and experimental group ***(µmol/L)***

Trimester	Group	Number of subjects	Average score	Std. Deviation	Std. Error
I	Control group	100	43.19 µmol/L	8.09002	.80900
	Experimental group	100	20.39 µmol/L	3.60386	.36039
II	Control group	100	51.88 µmol/L	5.23253	.52325
	Experimental group	100	21.14 µmol/L	2.66674	.26667
III	Control group	100	56.79 µmol/L	3.62175	.36217
	Experimental group	100	51.72 µmol/L	9.01299	.90130

Table 3. Average values of hemoglobin (mmol / l) compared to the trimester of pregnancy in control and experimental group - (ANOVA test)

Trimester	group	N	average value (mmol/l)	Std. Deviation	Std Error	95% interval		Minimum	Maximum
						Lower limit	Upper limit		
I	Control	100	8.66	0.52	0.05	8.55	8.76	7.51	9.99
	Experimental	100	7.40	0.37	0.04	7.33	7.48	6.70	9.00
	Total	**200**	8.03	0.77	0.05	7.92	8.14	6.70	9.99
II	Control	100	7.23	0.33	0.03	7.16	7.29	6.21	8.13
	Experimental	100	6.74	0.37	0.04	6.67	6.82	6.21	7.51
	Total	**200**	6.99	0.42	0.03	6.93	7.04	6.21	8.13
III	Control	100	7.90	0.62	0.06	7.77	8.02	6.39	9.25
	Experimental	100	6.54	0.31	0.03	6.47	6.60	5.77	7.51
	Total	**200**	7.22	0.84	0.06	7.10	7.33	5.77	9.25

ge value of the experimental group was 8:46 ± 1:45. Independent t-test showed a statistically significant difference in Apgar scores between the control and experimental group, t (200) = 4.63, p <0.005. Although both groups' newborns had favorable Apgar score, which was the first minute after birth within the normal range, the control group had a higher Apgar score compared to that of the experimental group.

Independent t-test showed a statistically significant difference in average values of TIBC betwe-

Table 4. Correlation of dependent variables (Er, Hb, Htc) on trimestralnom level in the control and experimental group

Groups	Controlled variable – first trimestar			Hbg (I)	Htc (I)
Control group	Er (I)	Hb (I)	Correlation	1.000	.012
			Sig. (2-tailed)	.	.903
			Df	0	97
		Htc (I)	Coleration	.012	1.000
			Sig. (2-tailed)	.903	.
			Df	97	0
Experimental group	Er (I)	Hb (I)	Correlation	1.000	.104
			Sig. (2-tailed)	.	.308
			Df	0	97
		Htc (I)	Correlation	.104	1.000
			Sig. (2-tailed)	.308	.
			Df	97	0

Grupe	Controlled variable – second trimestar			Hb (II)	Htc (II)
Control group	Er (II)	Hb (II)	Correlation	1.000	-.128
			Sig. (2-tailed)	.	.208
			Df	0	97
		Htc (II)	Correlation	-.128	1.000
			Sig. (2-tailed)	.208	.
			Df	97	0
Experimental group	Er (II)	Hb (II)	Correlation	1.000	-.054
			Sig. (2-tailed)	.	.594
			Df	0	97
		Htc (II)	Correlation	-.054	1.000
			Sig. (2-tailed)	.594	.
			Df	97	0

Grupe	Controlled variable – third trimestar			Hb (III)	Htc (III)
Control group	Er(III)	Hb (III)	Correlation	1.000	-.008
			Sig. (2-tailed)	.	.938
			Df	0	97
		Htc (III)	Correlation	-.008	1.000
			Sig. (2-tailed)	.938	.
			Df	97	0
Experimental group	Er (III)	Hb (III)	Correlation	1.000	-.152
			Sig. (2-tailed)	.	.134
			Df	0	97
		Htc (III)	Correlation	-.152	1.000
			Sig. (2-tailed)	.134	.
			Df	97	0

en the control and experimental group at the first and second trimester level, p <0.005, while for the third trimester there is no statistically significant difference p = 0389.

Independent t-test showed that there is a statistically significant difference in average values of UIBC between control and experimental group, p <0.005

Analysing the average value of hemoglobin in experimental and control group, at the trimester level of pregnancy, and the application of statistical test ANOVA, we came to data which show that there is statistically a significant difference in mean values of hemoglobin, p <0.005.

Hemoglobin levels in the control group decreased linearly but in the third trimester remained in physiological values of 7.22 mmol / L, while the experimental group also had a linear decline of the average hemoglobin levels in the first and second trimester. In the third trimester value increased but all values were lower than the lower limit of the normal range of hemoglobin.

• There is no correlation between the average number of red blood cells and the average values of Htc and Hb during all three trimesters of pregnancy of the control group, p = 0903, p = 0.208, p = 0.938

• There is no correlation between the average number of red blood cells and the average values of Htc and Hb during all three trimesters of pregnancy of the experimental group, p = 0.308, p = 0.594, p = 0.134

Discussion

Iron deficiency is the leading cause of iron deficiency anemia, which affects more than half a billion people worldwide. Two key stages of iron deficiency are the depletion of reserves of iron without anemia and iron depletion of reserves with anemia. In the U.S., as in Europe, iron deficiency is most common in women during the pregnancy and as a result of bleeding. (11)

Extremely low hemoglobin levels are directly associated with lower Apgar score and a higher risk of asphyxia, which may even lead to fetal death. (12) Preterm birth is defined by WHO as a birth before the age of 37 weeks of pregnancy, and low body weight at birth is the one below 2,500 grams. (13) Preterm birth is the major cause of perinatal mortality in the industrialized countries whose incidence is not declining in the last 10 years. (14) Low birth weight is a major cause of morbidity and mortality in the United States, and in children with IUGR there is a great incidence of hypertension in adulthood. Also, children in the first year of life have a higher risk of psychomotor disturbances. (15)

Body weight, body length, and Apgar scores of newborns of mothers who belonged to the experimental group had statistically significantly lower values compared to infants of mothers without iron deficiency anemia.

In his study, Msolla showed that the average weight of newborns whose mothers had a hemoglobin concentration is below 7.4g/dl was 2500g (2 160 ± 228g). Pregnant women who had a hemoglobin concentration greater than 9.5g/dl, gave birth to the children of average weight about 3000g (3142 ± 329g). (16)

Low values of serum iron with increased value of transferrin and its unsaturated part (UIBC) are absolute diagnostic values in diagnosing of iron deficiency anemia, as well as medical finding of anulocita in the peripheral blood smear. Morphological analysis of peripheral blood smear with medical finding of anulocita and findings of reduced iron values with increased UIBC and TIBC and the reduced value of ferritin, the diagnosis of iron deficiency anemia is established. Other laboratory analysis certainly help solve diagnostic problems with other types of anemia. (17)

Analyzing the average value of hemoglobin in experimental and control group at the trimester level of pregnancy, and the application of statistical test ANOVA, we came to the conclusion that there is a statistically significant difference in average values of hemoglobin, p <0.005.

In his study, which was carried out in India (2008), Sharma followed 3698 pregnant women, and came to the data that 80% of the women were anemic. Average hemoglobin values ranged, at 20% of subjects up to 5-8g/dL (2.77 to 4.43 mmol / L). These patients were treated with intra- muscular application of iron. (18)

Conclusions

Iron deficiency anemia is a pathological condition often accompanying pregnancy, and pregnant women who have not started timely treatment of this type of anemia. As a result of inadequate treatment many complications related to the fetus, the weight and length, Apgar score occur. In addition, pregnant women with untreated iron deficiency anemia are considered to be of high-risk pregnancy.

References

1. *Greer F. Wintrobe Clinical Hematology-12th Edition, Lippincot Williams & Wilkins, Wolters Kluwer; 2009; 779.*
2. *Vrhovec B. Interna Medicina Treće dopunjeno izdanje, Naklada Ljevak; 2003; 136.*
3. *Marshall AL,William JW. Williams hematology 7 edit, 2006; 449*
4. *International Journal of Hematology Increased incidence of iron deficiency anemia secondary to inadequate iron intake in institutionalized, young patients with cerebral palsy Springer Japan. 2008; 88(5): 495-497*
5. *National Institutes of Health. "Dietary Supplement Fact Sheet: Iron". United States of America, Department of Health and Human Services. http://ods.od.ni Braunwald E. et al.Harrisonova načela interne medicine. 2 /. - 15 izd. New York: Lange Medical Books : McGraw-Hill, 2006; 294-333*
6. *Pernilla N, et. al. Health Education to Prevent Anemia Among Women of Reproductive Age in Southern India, Medical Sociology; Women; Health Care for Women International, 2006; 27(2): 131 - 144*
7. *Lindsay HA: Anaemia and iron deficiency: effects on pregnancy outcome, American Journal of Clinical Nutrition, 2000; 492-501.*
8. *Hodin RA, Matthews BJ. Smal Intestine Essential Practice of Surgery Springer New York May, 2006; 251-268*
9. *h.gov/factsheets / iron.asp. Retrieved 2009-03-30.*
10. *World Health Organization. National strategies for overcoming micronutrient malnutrition. Document A 45/3, 1992.*
11. *Auerbach M, Goodnough LT, Picard D, Maniatis A. The role of intravenous iron in anemia management and transfusion avoidance. Transfusion 2008; 48: 988.*
12. *Martin Y. Bioreactors for tissue mass culture: Design, characterization, and recent advances. 2005; 26(35): 7481-7503*
13. *World Health Organization. Adverse birth outcomes in United Republic of Tanzania - impact and prevention of maternal risk factors. Bull World Health Organ vol 85 (1), 2007*
14. *Van den Broek NR, Ntonya C, Mhango E, White SA. Diagnosing anaemia in pregnancy in rural clinics: assessing the potential of the Haemoglobin Colour Scale. Bull World Health Organ 1999; 77: 15–21*
15. *Gillespie TW. Anemia in cancer: therapeutic implications and interventions. Cancer Nurs . 2003; 26(2): 119-28.*
16. *Msolla MJ, Kinabo JL. Prevalence of anaemia in pregnant women during the last trimester. Int J Food Sci Nutr. 1997; 48(4): 265-70.*
17. *Rockville US. Preventive Services Task Force. Screening for Anemia—Including Iron Supplementation for Children and Pregnant Women:2006*
18. *Sharma A, Patnaik R, Garg S, Prema Ramachandran. Detection & management of anaemia in pregnancy in an urban primary health care institution. Nutrition Foundation of India, New Delhi, India. 2009.*

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Determinant factors of differential diagnosis in cases with tuberculous meningitis and neurobrucellosis

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Abstract

Background: Similar clinical, laboratory and CSF findings may be seen among cases with Tuberculous meningitis (TBM) and Neurobrucellosis (NB) during referral to hospital. Appropriate treatment should be initiated prior to confirmation of diagnosis based on results of CSF examination and culture.

Methods: In this trial, 15 TBM and 15 NB cases were evaluated, who were treated and followed-up with related diagnoses in Department of Infectious Diseases and Clinical Microbiology of Gaziantep University Medical Faculty during January-2007 and December-2010.

Results and Conclusions: Patients referring with headache and complaints related to involvement of cranial nerves, exhibiting findings of motor loss in lower extremities and involvement of cranial nerves in physical examination, with laboratory findings of elevated ESR and CRP, positive tuberculin skin test, increase in cell count, protein, LDH and ADA in CSF and decrease in glucose values, together with basal involvement, hydrocephalus and tuberculoma in Magnetic Resonance Imaging (MRI) should be considered as TBM, while cases referring with lumbar pain, with laboratory findings of lymphocyte dominance in whole blood cell count and positive standard tube agglutination test in blood, bone marrow and CSF in addition to increased signals in periventricular white matter should be regarded as NB.

Key words: Brucellosis, Meningitis, Neurobrucellosis, Tuberculosis.

Introduction

Tuberculous meningitis (TBM) is a common disease observed in developed and developing countries, presenting as the most severe clinical form of tuberculosis. In general, agent pathogen is Mycobacterium tuberculosis. Examination of cerebrospinal fluid (CSF) is essential in diagnosis of TBM. Diagnosis is confirmed by observation of acid-resistant bacteria (ARB) in CSF by direct Ehrlich Ziehl - Neelson (EZN) staining method and/or growth of tuberculosis bacteria in CSF culture (1).

Neurobrucellosis (NB) is defined as involvement of central nervous system in brucellosis cases; it's encountered in less than 5% of cases and usually presents as acute or chronic meningitis. Essential findings in diagnosis of NB are consumption of unpasteurized milk and dairy products, residing in a rural area and/or contact with animals in medical history, presence of clinical findings compatible with meningitis or meningoencephalitis as well as typical CSF findings, positivity of blood, bone marrow or CSF cultures or positive serological tests for neurobrucellosis (2).

TBM and NB cases may refer with similar clinical, laboratory and CSF findings. Appropriate treatment should be initiated before obtaining results of CSF examination and culture for a confirmed diagnosis. In this trial, we evaluated demographic characteristics, clinical signs and symptoms, CSF findings and radiological findings of patients with TBM and NB and we investigated the factors which may be indicative in terms of differential diagnosis and appropriate treatment approaches.

Material and method

A total of 15 NB and 15 TBM cases, diagnosed with isolation of the agent pathogen, treated and followed up in Department of Infectious Diseases and Clinical Microbiology of Gaziantep University Medical Faculty during January-2007 and December-2010 were evaluated.

Gaziantep University Medical Faculty Hospital is located in Southeast Anatolian region of Turkey,

providing outpatient and inpatient health services with an inpatient bed capacity of 930. Department of Infectious Disease and Clinical Microbiology is a regional reference department, with an inpatient bed capacity of 12. The trial was initiated upon approval of local ethical committee.

Diagnosis of tuberculous meningitis was confirmed by determination of ARB in CSF by direct EZN staining method and/or growth of M. tuberculosis in CSF, in addition to assessment of clinical features and accompanying CSF findings. As per these assessments, the following was regarded as diagnostic criteria; clinical findings suggestive of tuberculous meningitis, presence of other tuberculosis foci in addition to subacute meningitis findings, impaired consciousness, neurological deficits, cell count > 10 cells/mm^3, high protein levels and low glucose levels in CSF and response to antituberculous treatment (1).

Diagnosis of neurobrucellosis was based on the following findings; presence of clinical findings in compliance with meningitis or meningoencephalitis, cell count>10 cells/mm^3, high protein levels and low glucose levels in CSF and presence of positive culture or serological tests for neurobrucellosis in blood, bone marrow or CSF (2).

Demographic characteristics (distribution of age and gender), complaints, physical examination, laboratory and imaging findings of patients with a confirmed diagnosis were collected from patient files in hospital archive and recorded in an Excell file. Data were analyzed with Microsoft Excell 2007 and SPSS (version 13.0, SPSS INC., Chicago, IIIinois, USA) programs. Values were given as nominal values and percentages. Comparison of data was performed by Student-t test. P values <0.05 were regarded as significant.

Results

A total of 30 patients, 15 TBM and 15 NB cases were evaluated. Among TBM and NB cases, mean duration of complaints at referral were 3.3±2.6 and 2.32±1.93 months respectively.

While number of female and male patients among cases with a diagnosis of tuberculous meningitis was approximately the same, ratio of female/male patients was determined as 4/1 among patients with neurobrucellosis and the difference was statistically significant (p‹0.05). In 13 (86.6%) of 15 NB patients, consumption of milk and dairy products, residing in a rural area and/or contact with animals were found in medical history.

Referral symptoms evaluated as significant were headache and complaints related to involvement of cranial nerves in TBM patients and lumbar

Table 1. Clinical findings of cases

	Neurobrucellosis n=15	**TBC meningitis n=15**	**p**
Age,years (mean±SD)	33.9±12.2	40.87±18.5	NS
Gender (M/F)	3/12	8/7	p<0.05
Symptom n (%)			
Fever	9 (60)	10 (66.6)	NS
Malaise	10 (66.6)	12 (80)	NS
Headache	9 (60)	13 (86.6)	p<0.05
Nausea-vomiting	8 (53.3)	8 (53.3)	NS
Perspiration	7 (46.6)	5 (33.3)	NS
Lumbar pain	7 (46.6)	0	P<0.05
Involvement of cranial nerves	2 (13.3)	10 (66.6)	p<0.05
Duration of symptoms, months (mean±SD)	2.32±1.93	3.3±2.6	NS
Physical examination n (%)			
Fever	37.6±0.8	38.5±0.53	NS
Neck rigidity	10 (66.6)	13 (86.6)	NS
Kernig sign	3 (20.0)	5 (33.3)	NS
Brudzinski sign	8 (53.3)	10 (66.6)	NS
Motor loss in lower extremities	2 (13.3)	7 (46.6)	p<0.05
Findings related to involvement of cranial nerves	2(13.3)	5 (33.3)	p<0.05

pain in NB cases (p‹0.05). Physical examination of patients with a diagnosis of TBM revealed significant findings of motor loss in lower extremities and complaints related to involvement of cranial nerves (p‹0.05). Clinical features of patients are shown in table 1.

Statistically significant laboratory findings in cases with TBM were as follows; elevated erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) values, positive tuberculin skin test, increase in cell count, protein, lactate dehydrogenase (LDH) and adenosine deaminase (ADA) and decrease in glucose in CSF; corresponding significant findings in NB cases were as follows; lymphocyte dominance in whole blood count, positive standard tube agglutination test in blood, bone marrow or CSF (p<0.05). Laboratory findings of cases are summarized in table 2.

Cranial Magnetic Resonance Imaging (MRI) findings of 15 TBM patients revealed basal involvement in 46.6%, hydrocephalus in 33.3% and diffuse tuberculoma in 20%. In cranial MRI of 15 NB cases, no pathology was detected in 80% while increased signals were determined in periventricular white matter in 20%. MRI findings of cases are summarized in table 3.

Discussion

The most severe clinical form of tuberculosis is central nervous system tuberculosis and is fatal, unless provision of appropriate treatment. Diagnosis of tuberculous meningitis is confirmed by determination of ARB in CSF by direct EZN staining and/or growth of M. tuberculosis in CSF (1). However, sensitivity of detecting ARB with EZN staining is 2-87% and the corresponding rate for growth in CSF culture is between 25-75%, with results obtained in several weeks (3,4). Since delay in diagnosis and treatment increases neurological complications and mortality, early diagnosis and treatment in tuberculous meningitis is of utmost importance.

Neurobrucellosis, which is described as direct invasion of central nervous system during the course of brucellosis, is seen in 5-10% of the cases (5). Neurological complications may develop

Table 2. Laboratory findings of cases

	NB patients	TBM patients	p
Blood leucocytes (μL)	7573.6±2802.5	11031.43±5778.50	NS
ESR (mm/h)	11.9±7.2	48,33±14,96	p< 0.05
CRP (mg/l)	4.9±2.9	44,35±32,79	p< 0.05
PMN (%)	57.2±10.9	70,47±14,51	NS
Lymphocytes (%)	30.1±10.8	19,52±14,62	p< 0.05
Positive STA in blood, n (%)	15 (100)	0	p< 0.05
Positive STA in CSF, n (%)	11 (73.3)	0	p< 0.05
CSF (cells/ mm^3)	186.7±119.9	370.43±469.80	p< 0.05
CSF protein (mg/dl)	122±81	216,85±123.72	p< 0.05
CSF LDH (U/L)	38.4±16.2	118.29±61.03	p< 0.05
CSF glucose (mg/dL)	107.2±29.4	28.46±17.54	p< 0.05
CSF ADA (U/L)	6.2±1.7	24.14±17.03	p< 0.05
PPD (mm)	7.17±8.03	16.18±5.72	p< 0.05

ESR, erythrocyte sedimentation rate; CRP, C reactive protein; CSF, cerebrospinal fluid; STA, standard tube agglutination test; PPD, Purified Protein Derivative; ADA, adenosine deaminase

Table 3. MRI findings of cases

	Findings	n (%)
TBC cases (n=15)	Basal involvement Hydrocephalus Tuberculoma	7 (46.6) 5 (33.3) 3 (20)
Neurobrucellosis cases(n=15)	Normal Periventricular lesion	12 (80) 3 (20)

at any stage of the disease. Essential criteria in diagnosis of NB are medical history, presence of clinical findings compatible with meningitis or meningoencephalitis as well as typical CSF changes, positive culture or positive serological test results in blood, bone marrow or CSF. Elevated protein and decreased glucose values are more prominent in TBM cases, as compared to NB patients; in addition, CSF findings in NB cases are more similar to CSF findings in aseptic meningitis (6).

Since clinical, laboratory and CSF findings at referral may be similar in cases with TBM and NB, appropriate treatment should be initiated while awaiting CSF test results for differential and confirmed diagnosis. Therefore, demographic features, CSF findings and radiological imaging test results of cases diagnosed with TBM and NB at referral stage should be evaluated and indicative factors should be determined regarding differential diagnosis and appropriate treatment approaches prior to confirmation of diagnosis.

Although TBM may be seen in almost every age group, it's more common in the first 5 years during childhood (20%) and over 50 years of age in adult age group (60%) (6). Mean age of our patients was determined as 40.87±18.5 years. Mean age of NB patients in the current trial was 33.9±12.2 years. Age was not specified as a differentiating characteristic but nevertheless, prevalence of TBM is observed to be increased in advanced ages.

In literature, no such trial was detected, evaluating the comparison of findings of TBM and NB cases. In the trial conducted by Gul et al., the most common referral symptoms in NB were determined as headache and fever while the most common physical examination findings were findings related to meningeal irritation, impaired consciousness and organomegaly (7). In a trial performed by Christensen et al., the most common referral symptoms among patients with TBM were fever, headache and weight loss; the most common findings of physical examination were determined as findings of meningeal irritation, confusion and paralysis of cranial nerves (8). In the current trial, headache, fever and malaise were observed as common referral symptoms in both groups; complaints related to involvement of cranial nerves in TBM cases and lumbar pain in NB patients were detected as additional primary symptoms. Duration of symptoms was 3.3±2.6 months in patients with TBM and 2.32±1.93 months in NB cases; duration of symptoms prior to referral in TBM cases was longer than NB cases. Findings of physical examination were fever and positive meningeal irritation findings in both patient groups. In cases with a diagnosis of TBM, motor loss in lower extremities and findings related to involvement of cranial nerves were observed as well. During referral to hospital, headache and complaints related to involvement of cranial nerves were specified as determinant factors for TBM patients while corresponding determinant factors for NB patients was specified as lumbar pain.

In the current trial, cell count in CSF among TBM cases was higher than NB cases and total protein values of CSF did not show a prominent increase in NB cases, in contrast to TBM cases. The most relevant low values in TBM cases were CSF glucose values. CSF findings suggestive of TBM were regarded cell count> 10/mm^3, high protein levels and low glucose levels. Similar CSF findings were observed in cases suggestive of NB. However, increase in cell count, protein and LDH and decrease in glucose in CSF among TBM cases were more prominent, as compared to NB patients. CSF smear revealed prominent mononuclear cell (lymphocytic pleocytosis) dominance in both diseases.

In diagnosis of TBM, measurement of adenosine deaminase (ADA) enzyme in CSF is regarded as supportive since rapid access to results and sensitivity was indicated to be superior to PCR in several publications (1,9,10). Moghtaderi et al. determined sensitivity of the test as 81% and specificity as 86%, regarding ADA limit values in CSF as 10.5 IU/L (11). On the other hand, Rana et al. recognized ADA limit value in CSF as 10 IU/L and calculated sensitivity and specificity rates of 66.6% and 90%, respectively (10). In the current trial, ADA activity in CSF was measured in all cases with TBM; mean value was determined as 24.14± 17.03 U/L and supported the diagnosis. On the other hand, ADA values in CSF in NB patients were not as significant as TBM cases and mean values were determined as 6.2±1.7 U/L.

Purified Protein Derivative (PPD) test results in the two patient groups revealed a mean value of 16.18±5.72 mm in TBM patient group and 7.17±8.03 mm in NB patient group. High PPD values in NB group were associated with routine administration of Bacillus Calmette-Guerin vaccine

in our country. High cell count, prominently high protein and ADA levels and low glucose values in CSF and high PPD values were observed to support clinical diagnosis in TBM cases.

CSF gram staining, culture and test for Brucella-specific antibodies are valuable in differential diagnosis of TBM and NB. In diagnosis of NB, CSF Gram stain is generally negative and culture is found to be positive in less than ¼ of the patients. Observation of ARB in EZN staining of CSF is substantial in diagnosis of TBM. However, sensitivity of ARB positivity in CSF is 2-87% while rate of growth in CSF culture is between 25-75% (3,4). Diagnosis of NB is confirmed by positivity of specific antibodies in CSF or blood, in addition to CSF findings. On the other hand, Brucella standard tube agglutination test in blood and CSF is negative in TBM patients. In our trial, positive Brucella-specific antibody was determined in CSF and blood in all of the 15 patients with NB, in addition to CSF findings compliant with NB. In both patient groups, agent pathogen was grown in CSF cultures obtained prior to treatment.

In tuberculous meningitis, lesion is mostly located in basal meninges of brain and no involvement is seen in convex regions in general. Cranial nerves and large vessels in basal regions and choroid plexus in ventricules are covered with exudate. In brain tissue under these regions covered with exudate, inflammatory changes like edema, perivascular infiltration and microglial reaction are observed. During late stages of the disease, hydrocephalus develops due to arachnoid adhesions (1,9,12,13). Hydrocephalus is the most common complication of tuberculous meningitis. Computerized brain tomography (CBT) and MRI methods are auxiliary diagnostic methods used to determine tuberculoma, hydrocephalus, basillary arachnoiditis and cerebral infarction (1,14,15). According to MRI results, basal involvement was determined in 7 of 15 of our patients followed up with a diagnosis of TBM, while hydrocephalus was seen in 5 cases and diffuse tuberculoma was found in 3 patients (Figure 1). In 2 patients with hydrocephalus, external shunt was inserted for therapeutic purposes; 1 patient with tuberculomas was operated and pathological diagnosis of operation material was determined as caseified granulomatous inflammation.

MRI and CBT imaging methods are also valuable in diagnosis of NB and MRI is regarded as more sensitive in terms of diagnosis, as compared to CBT. MRI of patients with NB may reveal 4 types of radiological findings, namely normal appearance, alterations in white matter of brain parenchyma, vascular changes and radiological findings related to inflammation (16). These radiological findings should be assessed with clinical findings of patients. In the current trial, diffuse hyper-intense lesions were detected in periventricular white matter in 3 patients and this appearance was shown to regress with treatment, as indicated by control MRIs (Figure 2). MRI findings of remaining 12 patients were reported as normal with no determined pathology.

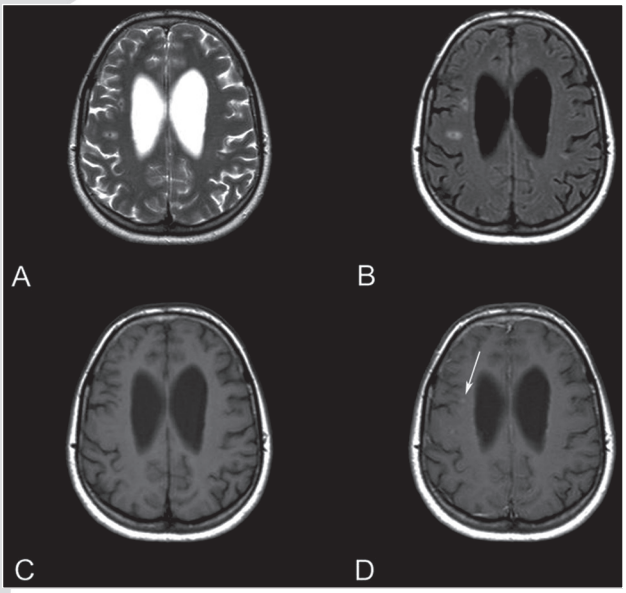

Figure 1. Hyperintense signal changes in accordance with edema at right corona radiata level in T2 weighted (A) and FLAIR (B) appearances in a patient with TBM; millimetric lesions in T1 weighted appearances (C), converting to opaque granuloma (D) following injection of hypointense intravenous contrast material (white arrow).

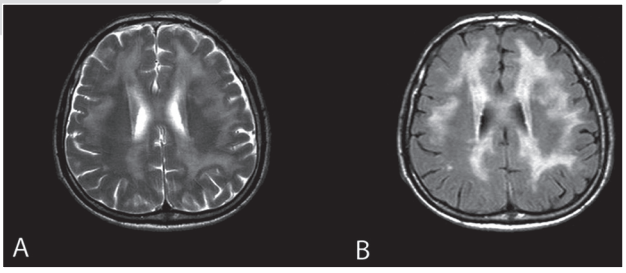

Figure 2. Diffuse increase in signals in bilateral peripheral subcortical white matter and localizations in accordance with arcuate fasciculus in T2 weighted (A) and FLAIR (B) axial MRI appearances in a patient with NB.

MRI results are significant in terms of diagnosis of TBM. However, MRI is considered insignificant in terms of NB diagnosis and seems to be performed only for differential diagnosis for TBM.

Prognostic factors in TBM diseases are age of the patient, duration of symptoms and neurological deficits (6). In the current trial, complaints related to involvement of cranial nerves were found in 10 of 15 TBM patients (66.6%), motor loss was observed in lower extremities in physical examination of 7 patients (46.6%) and signs related to involvement of cranial nerves were found in physical examination of 5 patients (33.3%). Neurological deficits are rarely seen in NB cases, as compared to patients with TBM. Complaints related to involvement of cranial nerves and motor loss in lower extremities was detected in 2 of our 15 patients with NB (13.3%).

TBM and NB are diseases requiring long term treatment. TBM is a disease with high mortality, requiring quadruple antituberculous treatment for at least 12 months; in NB cases where mortality is low (0.5%), preferred approaches are double or triple antimicrobial treatment for 2-15 months (mean: 5 months), with treatment pursued until clinical and CSF findings are normalized (6). In all of the 15 patients in the current trial who were followed-up with a diagnosis of NB, full recovery was provided with appropriate antimicrobial treatment with normalization of CSF findings; however, full recovery was observed in 11 of 15 TBM cases, while death associated with the disease was seen in 4 patients. Mortality rate in TBM disease is high while the rate is extremely low in NB disease. In the current trial, all patients with a diagnosis of NB recovered without any sequellae.

Conclusion

Although clinical, laboratory and CSF findings of TBM and NB cases at referral stage are similar, a diagnosis of TBM should be considered in cases with the following until the diagnosis is confirmed by CSF examination and culture; headache, complaints due to involvement of cranial nerves, motor loss in lower extremities and signs of involvement of cranial nerves in physical examination, laboratory findings as high ESR and CRP, positive tuberculin skin test, increase in cell count, protein, LDH and ADA and decrease in glucose in CSF and basal involvement, hydrocephalus and tuberculoma in cranial MRI; accordingly, NB should be considered in cases with the following findings prior to confirmation of disease by CSF examination and culture; lumbar pain as referral symptom, laboratory findings as lymphocyte dominance in whole blood count, positive standard tube agglutination test in blood, bone marrow and CSF and increased signals in periventricular white matter.

References

1. *Haas DW, Prez R MD. Mycobacterium tuberculosis. In: Mandell GL, Bennett JE, Dolin R (eds). Principles and Practice of Infectious Diseases. New York: Churchill Livingstone, 1995; 2213-43.*
2. *Sanchez-Sousa A, Torre C, Campello MG, et al. Serological diagnosis of neurobrucellosis. J Clin Pathol. 1990; 43: 79-81.*
3. *Leonard JM, Des Prez RM. Tuberculous meningitis. Infect Dis Clin North Am. 1990; 4: 769-87.*
4. *Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM. Tuberculous meningitis: a 30-year review. Clin Infect Dis. 1993; 17: 987-94.*
5. *Shakir RA. Brucellosis. In: Shakir RA, Neuman PK, Poser CM, eds. Tropical Neurology. Cambridge: WB Saunders; 1996: 168-79.*
6. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases Sixth Edition. 2005; 1135-6.*
7. *Gul HC, Erdem H, Bek S Overview of neurobrucellosis: a pooled analysis of 187 cases International Journal of Infectious Diseases 2009; 13, 339-43.*
8. *Christensen ASH, Andersen AB, Thomsen V, H Andersen P, S Johansen I. Tuberculous meningitis in Denmark: a review of 50 cases BMC Infectious Diseases 2011; 11: 47.*
9. *Pottage JC, Harris AA. Chronic meningitis. In: Gorbach SL, Bartlett JG, Blacklow NR (eds). Infectious Diseases. Philadelphia: WB Saunders Company, 1998; 1415-24.*
10. *Rana SV, Chavko F, Lal V, Arora SK, Parbhakar S, Sharma SK, Singh K. To compare CSF adenosine deaminase levels and CSF-PCR for tuberculous meningitis. Clin Neurol Neurosurg. 2010; 112: 424-30.*

11. *Moghtaderi A, Niazi A, Alavi-Naini R, Yaghoobi S, Narouie B. Comparative analysis of cerebrospinal fluid adenosine deaminase in tuberculous and non-tuberculous meningitis. Clin Neurol Neuros. 2010; 112: 459-62.*

12. *Molavi A, LeFrock JL. Tuberculosus meningitis. Med Clin North Am 1985; 69: 315-31.*

13. *Byrd T, Zinser P. Tuberculosis Meningitis. Curr Treat Options Neurol. 2001; 3 : 427-32.*

14. *Holdiness MR. Management of tuberculous meningitis. Drugs 1990; 39: 224-33.*

15. *Andronikou S, Smith B, Hatherhill M, Douis H, Wilmshurst J. Definitive neuroradiological diagnostic features of tuberculous meningitis in children. Pediatr Radiol 2004; 34: 876-85.*

16. *Al-Sous MW, Bohlega S, Al-Kawi MZ, Alwatban J, Mclean DR. Neurobrucellosis: Clinical and Neuroimaging Correlation AJNR Am J Neuroradiol 2004; 25: 395-401.*

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The effects of Human Papilloma Virus infection on the survival time in patients with head and neck squamous carcinoma

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Abstract

Human papillomavirus (HPV) is recognized to play an important role in the pathogenesis of a subset of head and neck squamous cell carcinomas (HNSCCs), especially with oropharyngeal carcinoma. We conducted this retrospective study to investigate the association between HPV status and survival outcomes in Chinese patients with HNSCCs as well as the relationship between HPV status and other clinical characteristics in these patients**.** Seventy-eight HNSCC patients were enrolled. The HPV16/18DNA positive rate was 62.8% by PCR. The HPV16 DNA and 18 DNA positive rate was 47.4% and 1.28% by ISH. The survival time was 44 months in HPV16/18 positive group and 25 months in negative group. Low differentiated patients, the survival time was 44 months in HPV positive subgroup patients, and 15 months in negative subgroup. COX regression analysis revealed that the major risk factors for poor survival outcome were Ki67 expression, lymph node metastasis, not the status of HPV16/18 infection.

Key words: Human Papillomavirus, Head and neck squamous carcinoma, Survival time.

Introduction

About 645,000 new cases of HNSCC were diagnosed globally per year. In recent years, the annual incidence of HNSCC was about 15.2/100,000 in Chinese population and accounted for 4.45% of total malignancies. It have been known that the development of HNSCC has an intimate relation with smoking and drinking (smoking and alcohol use are the major risk factors for development of HNSCC, however, more and more emerging evidence showed that HPV infection is an important oncogenic factor for HNSCC (1-6). In 2001, a meta-analysis conducted by Miller and Johnstone, including 4680 HNSCC specimens from 94 studies, showed that HPV-16 and HPV-18 can be detected in 99% of HPV positive oral squamous cell carcinomas. Other high risk type of HPV was less than 1% (7).

HPV is an 8kb circular DNA virus. It has several species specificity and has a high tendency for adhesion to basal epithelial and mucosa cells of human (8). The infection of HPV relies on the basal epitheliums which are in the proceeding of proliferation and differentiation. The virus, after passing through the superficial layers of stratified squamous epithelium, stays in the basal layers of squamous epithelial cells. The carcinogenic effects are involved in the expression of two carcinogenic genes, E6 and E7, and then the malignant transformation in the infected cells. E6 protein promotes p53 degradation and induces activation of the telomerase. E7 protein might help the infected cells to escape the cell cycle checkpoint, leading to erroneously DNA duplication by the way of enhancing pRb degradation and increasing the expression of p16 (9, 10).

There are great differences in clinical characteristics of HNSCC patients with or without HPV infection. The average age of HNSCC patients associated with HPV infection was 5 years younger than non HPV infected patients. Furthermore, most studies showed that the HPV positive patients had better prognoses than the negative if the tumors were in the same staging. The risk of death in the HPV positive patients decreased 60-80% than the HPV negative. A prospective study, conducted by

Carole Fakhry and his colleagues, including 96 patients with oropharyngeal or laryngeal cancer at the grade of □ or □, found a better response to chemotherapy and radiation in the HPV positive patients than the negative patients. After an average follow-up of 39.1 months, compared to the negative group, the risks of disease free progression and death reduced 72% and 79% respectively in the HPV positive group. Therefore, these studies suggested that HPV infection is a valuable prognostic and predictive indicator of better therapeutic response and survival in HNSCC (11).

HPV infection is not the only oncogenic factor in HNSCC. However, considering its high value in predicting better therapeutic response and survival time, detection of HPV infection in tumor tissue to guide the therapeutic choices seems of great clinical significance. In this study consisted of 78 HNSCC patients confirmed by histopathology. The data of clinical characteristics, HPV infection status and other pathogenic factors were collected. The relationship between these factors and the effects of these factors on survival time were further investigated.

Materials and Methods

Patients

Patients, who were newly diagnosed with head and neck cancer from January 1, 2004 to September, 2009, underwent surgery and confirmed by pathologists at Peking Union Medical College Hospital, were retrieved and identified from the hospital registry and pathology records. These include the tumors originated in the lips, oral cavities, oropharynx and hypopharynx. The clinical stages were classified according to the international cancer alliance criteria (UICC, 6th edition). The information of patients' demographic distribution, smoking, alcohol, treatment, and survival was collected from their medical records. Six patients were lost to follow-up (7.7%) after operation. The other patients had a median follow-up for 21.5 months (4-72 months).

Ethics

This study is a retrospective study, no new samples were collected in this study and the patients were not accepted any new treatment. The patients lived in difference province of China, so the patients (if they alive) or relative of patients (if they death) were follow-up by phone and the verbal informed consent was collected by phone too. The ethics committees of Peking Union Medical College Hospital approved this consent procedure. The retrospective study was approved by Ethics committees of Peking Union Medical College Hospital.

DNA extraction from the paraffin embedded tumor tissues

First, a diagnosis of squamous cell carcinoma was confirmed by a pathologist after reading the slides. Then, tissue sections, scratched from paraffin embedded tumor tissues, were put into a 1.5 ml centrifugal tube. After 1.2 ml of xylene was added, the centrifugal tube was vortex shaken. The tube was centrifuged at a speed of 12000rpm for 5min. After abandoning supernatant, 1.2 ml anhydrous alcohol was added. After shaking and blending, the tube was centrifuged at a speed of 12000rpm for 2min. After abandoning supernatant, the tube stayed open at 37 ℃ for residual ethanol vaporization. Then, FFPE tissue DNA extraction kit (Gene Co, Shanghai, China) was applied to extract the DNA, according to the instruction book. The Ultrospec 2100 pro ultraviolet spectrophotometer was applied to measure the DNA concentration, which was adjusted to 10ng/μl.

Real-time quantitative PCR to detect the HPV16/18 infection

HPV 16/18 subtypes nucleic acid amplification fluorescence detection kit (Da'an Gene Co, Guangzhou, China) was used for quantitative PCR (real time PCR, RT-PCR). The extracted DNA 2μl and standard substance 10^7,10^6,10^5,10^4 provided by the kit was put into each PCR tube according to the instruction book. The PCR reaction conditions was denaturation at 93 ℃ for 2min, renaturation at 93 ℃ for 45s, elongation at 55 ℃ for 60s, for 10 cycles, then denaturation at 93 ℃ for 30s, elongation at 55 ℃ for 45s, for 30 cycles. An IQ5 real-time quantitative PCR instrument and its software were used for calculating the copy numbers of HPV DNA in each sample.

In situ hybridization(ISH) to detect the HPV16 and HPV18 infection

Formalin-fixed, paraffin-embedded tumor specimens were used for detecting HPV16 or HPV18 DNA by in situ hybridization, using catalyzed-sig-

nal-amplification method and biotinylated probes (TBD science, Tianjin, China). Briefly, tissue sections were subjected to deparaffinization, heat induced target retrieval, and digestion with proteinase K (TBD science, Tianjin, China). Tumor specimens were then hybridized to a biotinylated type-specific HPV16 probe (TBD science, code FISH001), HPV18 probe (TBD science, code FISH003) or a negative control probe (Dako, code FISH002). An HPV16-positive and HPV18-positive tumor specimen (squamous cell carcinoma of cervix) was used as a positive control. Slides were scored as positive for HPV16 or 18 if a punctate signal specific to tumor cell nuclei was present. The criteria of classification: the percentage of positive cells less than 20% was defined as negative. Otherwise, the percentage of positive cells at 20-100% was defined as positive.

Immunohistochemistry assay to evaluate the expression of Ki67, p53

The expression status of p53 and Ki67 were evaluated by immunohistochemistry staining. Briefly, after 5-um sections were deparaffinized, antigen retrieval was performed by use of heat-induced epitoperetrieval with 10 mM citrate buffer. Sections were incubated with a monoclonal antibody against Ki67, p53 (Santacruz) at 1 : 500 dilution. The Ki67, p53 antibody was detected using the avidin-biotin-peroxidase technique (DakoLSAB Kit, Dako). The expression of the, p53, and Ki67 were determined by a pathologist. The positive rate of Ki67 was defined by the percentage of Ki67 positive cells. The classification of "-, +, ++, +++, ++++" was defined by the percentage of p53 positive cells at the level of <10%, 10 -25 %, 26-50%, 51%-75% and 76-100%, respectively.

Statistical analysis

SPSS software (version 13.0) was used for statistical analysis. Measurable data were expressed by x±SD. Kappa consistency test was applied to evaluate the consistency of two methods for detection of HPV infection. Spearman relationship test and χ^2 test were used for evaluating the relationship between HPV16/18 DNA positive status and the expression of Ki67, p53 and the other various clinical characteristics. Kaplan-Meier method was used for survival analysis. The differences between groups were compared by Log-rank test. A multiple factors Cox regression model was used for survival time analysis. $P<0.05$ indicates the difference is of significance.

Results

The demographic characteristics of the patients

Seventy-eight new HNSCC patients were diagnosed at Peking Union Medical College Hospital by operation and pathology from January 2004 to September 2009. The characteristics of patients were listed in Table 1.

Table 1. Patient Characteristics

Characteristics	Percentage of Patients-no. (%)
Age-year	
Median	59.1±13.6
Range	34-91
Sex-no. (%)	
Male	52(66.7%)
Female	26(33.3%)
Smoke-no. (%)	
Smoke	41(52.6%)
Non-smoke	37(47.4%)
Alcohol-no. (%)	
Alcohol	52(66.7%)
Non-alcohol	26(33.3%)
Region-no. (%)	
Lip	6(7.7%)
Oral cavity	48(61.5%)
Oropharynx	10(12.8%)
Hypopharynx	14(17.9%)
Differentiation grade-no. (%)	
G1	26(33.3%)
G2	18(23.1%)
G3	34(43.6%)
Nodal stage -no. (%)	
N0	54 (69.2%)
N1	8 (10.3%)
N2	15 (19.2%)
N3	1 (1.3%)
Total tumor stage-no. (%)	
0	2(2.6%)
I	8(10.3%)
II	25(32.1%)
III	16(20.5%)
IV	27(34.6%)
Radiotherapy-no. (%)	
Yes	36(46.2%)
No	42(53.8%)
Chemotherapy-no. (%)	
Yes	6(7.7%)
No	72(92.3%)

Incidence of HPV16/18 infection detected by real time PCR and ISH in tumor tissues

HPV16/18 DNA was detected in 62.8% of the patients by real time PCR (RT-PCR) The incidence of HPV16/18 DNA positive rate was the highest in oropharyngeal squamous cell carcinoma, reaching 70%. The HPV16 DNA positive rate was 47.4% by ISH (Figure 1). Only one patient (1.28%) was HPV18 DNA positive by ISH, who was HPV16 DNA positive as well. The difference between the tumor regions were of no significance (P>0.05, Table 2). Both RT-PCR and ISH had a high consistency in detecting HPV 16/18 infection (*Kappa* = 0.595, P = 0.000).

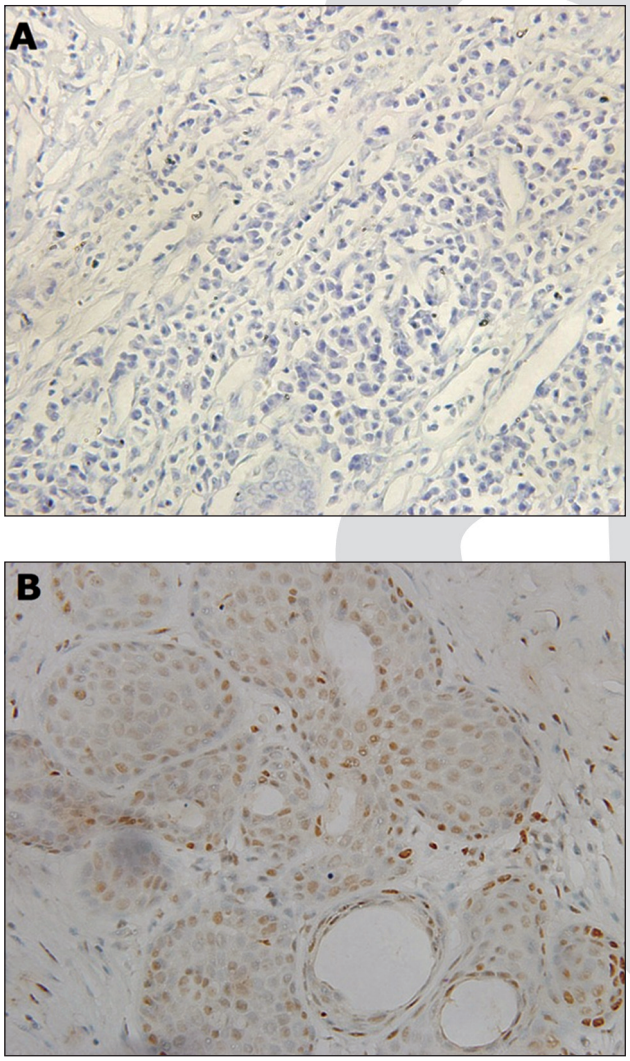


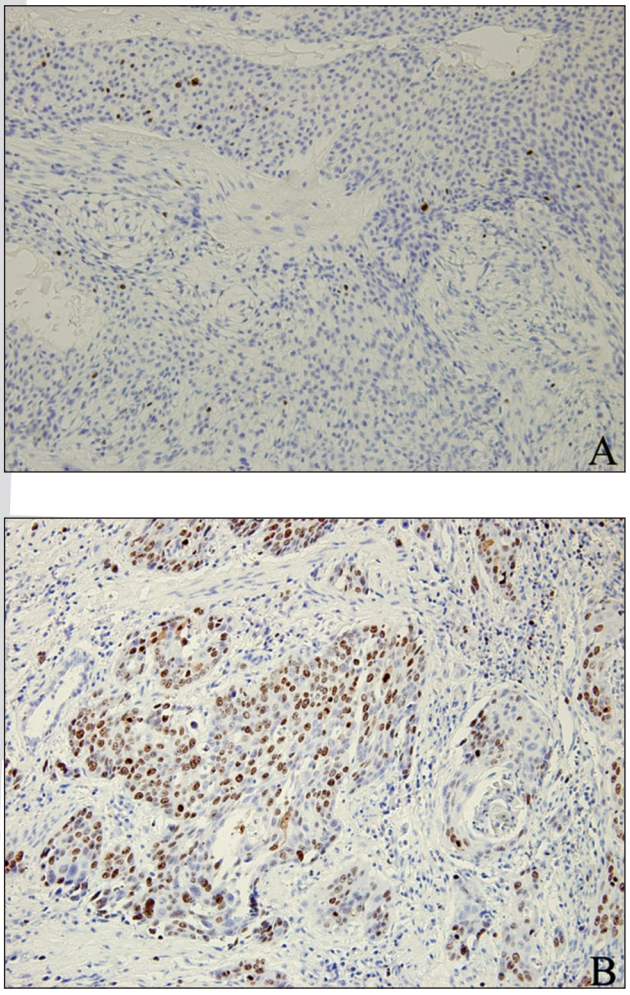
Figure 1. Results of HPV16 DNA ISH in HNSCC tumor tissues: A and B represented negative results and positive results

Table 2. The positive rate of HPV16/18 DNA in different regions by RT-PCR and ISH

Regions	Case (no.)	Positive rate (%)	
		RT-PCR	ISH
Lip	6	33.3%	33.3%
Oral cavity	48	66.7%	43.8%
Oropharynx	10	70.0%	60.0%
Hypopharynx	14	57.1%	57.1%
Total	78	62.8%	47.4%

The expression of Ki67 and p53 protein in HNSCC tumor tissues

Ki67 was expressed in each tumor tissues with the positive rate of 2~70% (Figures 2A, 2B). Forty-two of 78 patients (53.8%) were p53 negative, 16 (20.5%) were p53(+), 15 (19.2%) were p53(++), 5 (6.4%) were p53(+++) (Figures 2C, 2D).



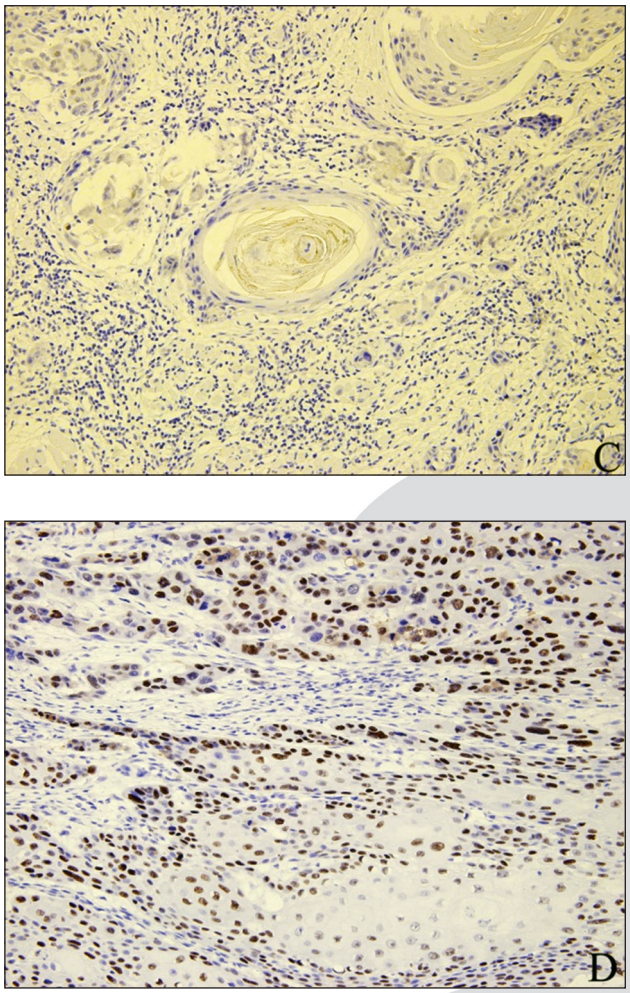


Figure 2. Expression of Ki67, p53 in tumor tissues (microscopic amplification 10×10). A and B represented for the expression of Ki67 at the level of 3% and 50% in different tumor tissues. C and D represented for the expression of p53 (-) and p53 (+++)

The relations between HPV16/18 infection and other clinical characteristics

There were no significant association between HPV16/18 infection and sex, age, smoking, drinking, clinical tumor stages, tumor locations, tumor differentiation and lymph nodes metastasis (all $P>0.05$, Table 3). The HPV16/18 infection had an approximately association with the expression of Ki67 ($P=0.069$), but had no association with the expression of p53 ($P=0.147$) . Stratified analysis revealed that, the patients whose tumor located in oropharynx and hypopharynx, the expression of p53 had a negative association with HPV16/18 infection, indicating less p53 expressed in HPV 16/18 infected tumor tissues. In patients of poorly differentiated tumors (G3), the expression of p53 ($P=0.002$) were decreased in HPV 16/18 infected tumor tissues.

*Table 3. The relationship between HPV16/18 status and other factors (*p<0.05)*

Factors	HPV infected condition			
	RT PCR		ISH	
	κ value	p value	κ value	p value
Sex	0.439	*0.508*	1.260	*0.262*
Smoke	1.673	*0.196*	2.453	*0.117*
Alcohol	1.345	*0.246*	0.026	*0.873*
Co-cancer	0.000	*0.984*	1.888	*0.333*
Region	2.863	*0.413*	1.916	*0.590*
Differentiation grade	13.292	*0.001**	16.129	*0.000**
Stage	1.515	*0.824*	2.875	*0.576*
Lymph node metastasis	2.899	*0.409*	1.544	*0.672*
P53	5.366	*0.147*	6.903	*0.075*
Ki67	Z=-1.816	*0.069*	Z=-0.609	*0.542*

Multiple factor Cox regression analysis

The main risk factors for the survival time of HNSCC patients were the expression of Ki67 ($P=0.012$) and the lymph nodes metastasis ($P=0.007$). HPV infection status, age, smoking, drinking, clinical tumor stages, tumor differentiation, radiotherapy and chemotherapy had no significant influence on the survival time (Table 4).

*Table 4. Possible risk factors which might influence the prognosis in HNSCC (*p<0.05)*

Covariant	All patients with data imputed (N=72)	
	Relative Risk (95%CI)	*p* value
HPV status RT PCR	1.012(0.974-1.051)	*0.547*
Age	1.034(0.224-4.776)	*0.966*
Sex	1.012(0.974-1.051)	*0.547*
Smoke	1.204(0.283-3.615)	*0.800*
Alcohol	0.777(0.287-5.057)	*0.680*
Region	0.836(0.437-1.697)	*0.587*
Differentiation grade	1.244(0.724-2.137)	*0.430*
Stage	1.192(0.691-2.056)	*0.527*
Lymph node metastasis	2.341(1.255-4.365)	*0.007**
Ki67	1.049(1.011-1.089)	*0.012**
p53	1.031(0.613-1.733)	*0.909*
Radiotherapy	0.836(0.328-2.219)	*0.768*
Chemotherapy	2.797(0.650-12.043)	*0.167*

Survival time analysis

Among 78 HNSCC patients, 6 of them were lost follow-up after their operations. The rest had a median follow-up of 21.5 months. The endpoint of the follow-up is their death or till September 2010, which was the last time of follow-up. The HPV16/18DNA positive group had a longer median survival time than the negative group (44 vs. 25 months, *P*=0.508, Figure 3).

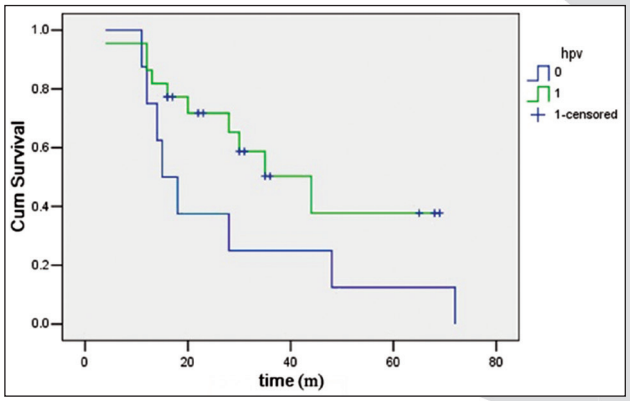


Figure 3. The relation of the survival time and HPV16/18 infection

The patients were divided into the survival or the death group, and the HPV infection rate were 73.33% and 48.48% in the living and death group (*P*=0.033), respectively. The one-year survival rate in HPV16/18 DNA negative and positive groups was 92.9 and 85.9% (*P*=0.337). The two-year survival rate in HPV16/18 DNA negative and positive groups was 57.7% and 70.8% (*P*=0.33). The three-year survival rate in the HPV16/18 DNA negative and positive group was 41.2% and 51.1% (*P*=0.412).

Other survival time analysis was conducted in different subgroups according to the histologic grades, regions, clinical tumor stages, smoking and alcohol use (Table 5). In all subgroups, the median survival time in the HPV positive group was longer than the negative group, although they were no statistically significant. In patients at G3 group (whose tumors were in low differentiation), the median survival time in HPV positive and negative subgroups were 44 and 15 months (*P*=0.098, Figure 4), respectively.

*Table 5. The median survival time in different subgroups of HNSCC according to their HPV status (*p<0.05)*

	HPV Negative Median Survival Time(month) (95%CI)	**HPV Positive Median Survival Time(month) (95%CI)**	***P* value**
Differentiation grade			
G1			
G2	18(12.1-23.9)	27(22.4-31.6)	0.129
G3	15(25.1-65.9)	44(25.1-65.9)	0.098
Region			
Lip+Oral cavity	28(20.4-35.6)	44(19.2-68.8)	0.593
Oropharynx+Hypopharynx	25(4.6-45.5)	28(15.3-40.7)	0.696
Stage			
0+I+II	28(0.0-59.7)		0.553
III+IV	18(8.0-28.0)	30(25.0-35.0)	0.770
Age			
<50	25(23.9-26.1)	35(26.4-53.6)	0.601
≥50	28(19.8-36.2)	44(15.5-72.5)	0.595
Smoke			
No	48(6.5-89.5)		0.395
Yes	25(23.7-26.3)	30 (25.4-34.7)	0.567
Alcohol			
No	28(0.0-58.0)		0.567
Yes	25(19.6-30.5)	28(24.0-31.8)	0.525

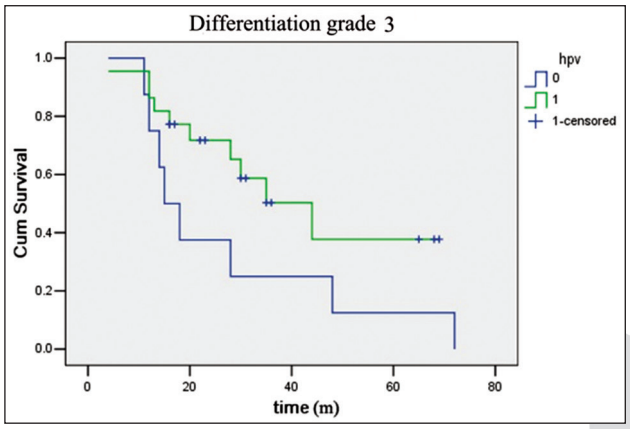


Figure 4. In poorly differentiated (G3) HNSCC patients, the accumulated survival rate was higher in HPV positive subgroup than negative subgroup

The effects of different therapeutic modalities on survival time of HNSCC patients were also evaluated. 65.5% (19/29) of HPV negative and 34.7% (17/49) of HPV positive patients received local site radiotherapy (P= 0.008). At the same time, 17.2 % (5/29) of HPV negative and 2.0% (1/49) of HPV positive patients got systemic chemotherapy (P = 0.015).

Discussion

In situ hybridization (ISH) to detect HPV DNA was considered to be the gold standard for the diagnosis of HPV infection (12, 13). In this study, we test the HPV16/18 infections status in HNSCC tumor tissues by both the RT-PCR and ISH. It revealed that 62.8% of the HNSCC tumor tissues were HPV16/18 DNA positive by RT-PCR, and 47.4% was HPV16 DNA positive by ISH (including one case had HPV18 DNA probe positive simultaneously). These two assays have a high consistency in detection of HPV 16/18 DNA (Kappa = 0.595, P = 0.000) in tumor tissues. RT-PCR is highly sensitive and can be conducted in many clinical laboratories. ISH has a high specificity, but it is time-consuming, labor intense, relatively low sensitivity, and high cost. Our results suggested that it is reasonable and convenient for clinical practice to detect HPV16/18 infection by RT-PCR instead of ISH.

The study also found that only one patient (1.28%) was HPV18DNA positive by ISH, who also had HPV16DNA positive simultaneously. This is accordance with the previous literature reports showing that HPV16 was the most frequent subtype (≥95%) associated with HNSCC (5, 7). The incidence of HPV infection varied in different tumor regions. The highest incidence of HPV infection was in the region of oropharynx (70%) and the lowest was in the lips (33.33%). This trend was also consistent with reports from other domestic and international studies (1, 2, 5, 6, 14).

HPV infection is also associated with the tumor histologic grade. Poorer differentiated tumors have higher HPV detectable rate. The incidence of HPV infection was 18.4%, 28.6% and 53.0% in G1 (well-differentiated), G2 (moderately differentiated) and G3 (poorly differentiated), respectively, by RT-PCR. The HPV16 infection had positively correlated with the tumor pathological grading (P = 0.001), this part should be in the result section). Similar results were also been observed by Poetsch M (15).

The survival time analysis revealed that the HPV positive group had a longer survival time than the negative, 44 vs. 25 months (P=0.508) by RT-PCR, and 30 vs. 28months (P=0.631) by ISH. No significant statistical difference was observed between the positive and negative groups, partially due to the following reasons: (1) the regions of tumors. There were only 10 cases of HNSCC in oropharynx. Recent researches suggested that only in the patients with HNSCC in oropharynx, HPV positive group had a longer disease free survival time than negative (16). (2) The follow-up time was too short. (3) As a retrospective designed research, we did not evaluate the effects of PS scores on the survival time. There were no consensus therapeutic regimes for the HNSCC patients in any HPV status, which might influence the results. (4) Our study had a small sample size. Even though we had these limitations, the results still indicated a trend of longer survival in HPV positive HNSCC patients.

The subgroup survival time analysis showed the similar results. In patients with poorly differentiated tumors, the median survival in HPV positive group is much longer than HPV negative group (44 vs. 15 months, P=0.098), which is consistent with the results from the other clinical studies (15). It suggested that, in the HNSCC patients with poorly differentiated tumor, HPV infection was a protective factor, which might be explained by the intact expression of p53 (15).

It seemed that there is a different tumorigenesis in molecular mechanism for HNSCC between HPV infection and other oncogenic factors (such as smoking), which rationalize the individualized therapeutic approaches. In the HNSCC patients with the definitive evidence of HPV infection, targeting the virus may be a new experimental option. HPV therapeutic vaccines, which might eliminate HPV infection in residual tumor lesions, may block the progression of disease. HPV vaccine has already been applied clinically to prevent the cervical carcinoma.

Reference

1. *Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev, 2005; 14: 467-475.*

2. *Sugiyama M, Bhawal UK, Kawamura M, et al. Human papillomavirus-16 in oral squamous cell carcinoma: clinical correlates and 5-year survival. Br J Oral Maxilofac Surg, 2007; 45: 116-122.*

3. *Jo S, Juhasz A, Zhang K, et al. Human papillomavirus infection as a prognostic factor in oropharyngeal squamous cell carcinomas treated in a prospective phase II clinical trial. Anticancer Res, 2009; 29: 1467-1474.*

4. *Chuang AY, Chuang TC, Chang S, et al. Presence of HPV DNA in convalescent salivary rinses is an adverse prognostic marker in head and neck squamous cell carcinoma. Oral Onco l, 2008; 44: 915-919.*

5. *Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst; 2008; 100: 407-420.*

6. *De Petrini M, Rittà M, Schena M, et al. Head and neck squamous cell carcinoma: role of the human papillomavirus in tumour progression. New Microbiol, 2006; 29: 25-33.*

7. *Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2001; 91: 622-635.*

8. *Doorbar J. The papilloma virus life cycle. J Clin Virol, 2005; 32(Suppl 1): S7–S15.*

9. *Hubbert NL, Sedman SA, Schiller JT. Human papillomavirus type 16 E6 increases the degradation rate of TP53 in human keratinocytes. J Virol, 1992; 66: 6237-6241.*

10. *McKaig RG, Baric RS, Olshan AF. Human papillomavirus and head and neck cancer: epidemiology and molecular biology. Head Neck, 1998; 20: 250-265.*

11. *Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst, 2008; 100: 261-269.*

12. *Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer, 2007; 121: 2465-2472.*

13. *Shi W, Kato H, Perez-Ordonez B, et al. Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. J Clin Onco l, 2009; 27: 6213–6221.*

14. *Zhao QL, Li HY. The association between the expression of HPV E7, Ki67 and micro-vessels in tumor tissues from adult pharyngeal papilloma. Shandong University Academic Journal, 2007; 45: 1030-1033.*

15. *Poetsch M, Lorenz G, Bankau A, Kleist B. Basaloid in contrast to nonbasaloid head and neck squamous cell carcinomas display aberrations especially in cell cycle control genes. Head Neck, 2003; 25: 904-910.*

16. *Allen CT, Lewis JS, El-Mofty SK, Haughey BH, Nussenbaum B. Human papillomavirus and oropharynx cancer: biology, detection and clinical implications. Laryngoscope, 2010; 120: 1756-1772.*

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The efficiency of L-ornithine L-aspartate in hepatic injury due to acetaminophen poisoning in rats

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Abstract

Aim: It is aimed to observe the effect of L-ornithine L-aspartate (LOLA) use in addition to antidotal therapy following single dose acetaminophen (APAP) toxicity in rats.

Patients-Method: In this study, 48 randomly selected Sprague-Dawney male rats weighing 200-250 grams were used. The rats were divided into 6 groups so that each group contained 8 rats; *Group 1*: control group, *Group 2*: acetaminophen group, *Group 3*: L-ornithine L-aspartate group, *Group 4*: acetaminophen + N-acetyl cysteine (NAC) group, *Group 5*: acetaminophen + L-ornithine L-aspartate group, *Group 6*: acetaminophen + N-acetyl cysteine + L-ornithine L-aspartate group. APAP 1gr/kg was administered as a single dose via oral route with the help of a gastric tube after dilution with distilled water. One hour after APAP administration, the prespecified groups were given NAC 1.5 gr/kg as a single intraperitoneal dose, and LOLA 200 mg/kg as a single dose via oral route with the help of a gastric tube after dilution with distilled water. All rats were observed 24 hours after the therapy.

Results: Our study showed an increased TOS level, and a decreased TAS level together with a resultant markedly increased OSI level in APAP group. Our study showed an increased TOS level, and a decreased TAS level together with a resultant markedly increased OSI level in APAP group. We observed that, when compared with APAP group, APAP+NAC group showed a decreased TOS level, and an increased TAS level together with a resultant markedly decreased OSI level. In our study, LOLA group and the control group had similar AST, ALT, serum TOS, and serum TAS levels. In addition, LOLA group and APAP+LOLA group had significant differences with respect to biochemical parameters. APAP+LOLA group had almost similar biochemical parameters to APAP group, which suggests that LOLA was not effective biochemically in acetaminophen toxicity-induced liver injury.

Discussion: We concluded that LOLA had no effects similar or superior to NAC in terms of antioxidant and hepatoprotective properties when used alone or in combination with NAC in treatment of acetaminophen-induced liver injury.

Key words: Acetaminophen toxicity, liver injury, N-acetyl cysteine, L-ornithine L-aspartate.

Introduction

Known as paracetamol, acetaminophen (N-acetyl-p-amino-phenol, APAP), is a drug with analgesic and antipyretic properties that is safely used in therapeutic doses. However, its overdose may lead to serious liver injury in both humans and experimental animal models. APAP poisoning develops either acutely with overdose (usually within 1 hour when taken as a single dose) or gradually with long term supratherapeutic ingestions (1). N-acetyl-p-benzoquinomine (NAPQI) is created by cytochrome p450 (CYP 450) enzymes which are abundant in liver and it is a very sensitive metabolite. It is responsible from the APAP toxicity (2). In therapeutic doses of APAP, this metabolite is effectively detoxified by glutathione (GSH) that is increased in the renally-cleared cysteine and mercapturic conjugates (1). In exposure to high dose APAP, more NAPQI is formed, which depletes hepatic GSH depots. The remaining NAPQI is covalently bound to cellular macromolecules and leads to cellular death (3). APAP-induced hepatotoxicity is typically defined as excessive elevation of aspartate aminotransferase (AST) and alanine transaminase (ALT) activities. These enzymes rise above 1000 IU/L. Excessive increases in AST and ALT are generally related to

histopathological changes of centrilobular necrosis (1,4). N acetyl cysteine is an effective antidote used in APAP overdoses. Antidotal therapy with NAC restores amount of hepatocellular GSH and decreases APAP hepatotoxicity (5). In literature, there are some studies comparing the protective effect of NAC with that of other antioxidants in experimental rat models (6).

L-ornithine L-aspartate is a stable salt of natural amino acid ornithine and aspartic acid. It has been both experimentally and clinically proved effective in the treatment of hepatic encephalopathy (HE) and is widely used. In addition, LOLA shows a hepatoprotective effect by stabilizing peroxidant/antioxidant balance of liver cells thanks to its antioxidative effects (7).

The experimental animal model of liver toxicity induced by APAP in rats is characterized by clinical observations, and biochemical and histopathological definitions. Liver injury that develops in rats following APAP application resembles human liver injury both in biological and morphological terms (8). Although there are plenty of studies showing the efficiency of LOLA in HE, data on its direct effect in acute poisonings toxic to liver (9) is insufficient. In this study, we aimed to observe the effect of LOLA use in addition to antidotal therapy following single dose APAP toxicity in rats.

Method

Animals and the Experimental Protocol

In this study, 48 randomly selected Sprague-Dawney male rats weighing 200-250 grams were used from among those cared at the Dicle University Health Sciences and Research Center (Diyarbakir, Turkey). The rats were housed in wooden cages of size 14×9×8 cm. Before the experiment, all rats were fed standard rat chow and water ad libitum and were kept in an air-conditioned room at 21 ˚C, with 12h: 12h light: dark cycle and were handled humanely. Animals were fasted for before experiment. Dicle University Local Ethical Committee for Animal Research (Diyarbakir, Turkey) approved the study.

The rats were divided into 6 groups so that each group contained 8 rats.

Group 1: control group,
Group 2: acetaminophen group,
Group 3: L-ornithine L-aspartate group,
Group 4: acetaminophen + N-acetyl cysteine group,
Group 5: acetaminophen + L-ornithine L-aspartate group,
Group 6: acetaminophen + N-acetyl cysteine + L-ornithine L-aspartate group.

APAP 1gr/kg was administered as a single dose via oral route with the help of a gastric tube after dilution with distilled water. The dose level was set at the maximum tolerated dose level, based on clear signs of toxicity with little or no lethality (10). One hour after APAP administration, the prespecified groups were given NAC (11) 1.5 gr/kg as a single intraperitoneal dose, and LOLA (12) 200 mg/kg as a single dose via oral route with the help of a gastric tube after dilution with distilled water. All rats were given anesthetized using ketamine hydrochloride (50 mg/kg intramuscularly) 24 hours after the therapy. All rats were prostrated in supine position for surgical procedure. Laparotomy with midline incision was applied to each rat. Subsequently, liver tissue samples and blood samples were taken from all rats. All rats were sacrificed with exsanguination method at the end of procedure.

Biochemical analysis and oxidant-antioxidant parameters

Maximum-amount blood samples that were taken via intracardiac route were centrifuged and the serum samples were stored at -70 ˚C until the time of biochemical tests. Part of the liver tissue was homogenized and the supernatant of the resultant homogenate was properly transferred to eppendorf tubes with plastic cover. These transferred homogenates were stored at -70 ˚C until the time of study. Serum AST, ALT were determined with the Abbott Architect c16000 Autoanalyzer and expressed as U/L.

Biochemical Steps and Analyses

Blood samples were centrifuged at 3000 rpm for 10 min to obtain plasma. Plasma samples were used for total antioxidant capacity (TAC). Liver tissues for the estimation of tissue oxidant and antioxidant levels were prepared at 4 °C. Tissues were weighed and cut into small pieces. Tissues were homogenized in 10 volumes of ice-cold phosfate buffer solution (PBS) (50 mM/L, pH 7.0) using a homogenizer (Ultra-Turrax T8 dispersing

homogenizator, Staufen, Germany). The homogenate was centrifuged at 15,000 rpm for 10 min at 4 °C to obtain supernatant. Supernatant samples were used for the determined of total oxidant status (TOS) and TAC.

Measurement of the Total Antioxidant Status (TAS)

TAC of supernatant fractions was determined using a novel automated measurement method developed by Erel (13). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample against the potent-free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values, lower than 3%. The results are expressed as nmol Trolox Equiv./mg protein.

Measurement of Total Oxidant Status (TOS)

TOS of supernatant fractions was determined using a novel automated measurement method, developed by Erel (14*)*. Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 Equiv./mg protein.

Determination of Oxidative Stress Index (OSI)

Percent ratio of TOS level to TAC level was accepted as OSI. OSI value was calculated according to the following formula (15*)*; OSI (Arbitrary Unit) = TOS (nmol H_2O_2 Equiv./mg protein) / TAC (nmol Trolox Equiv./mg protein). The results are expressed as Arbitrary Unit.

Histopathologic Evaluation of Liver Injury

The liver samples were fixed in %10 formalin solution for 48 hours. After routin histological tissue preparation , embedded in parafin, sectioned to a thickness of 4-5 µm and the sections were stained with hematoxylin-eosin (H&E). For histopathological evaluation, all samples were examined under light microscopy by a pathologist blinded to the study group: liver injury of hepatic lobul, including cell death, ballooning and inflamation around the central veins were scored for each rat according to the following criteria: (0); normal, (I); involvement less than 20% of the hepetic lobul, (II); involvement of 20-70% of the hepatic lobul, (III); more than 70% of the hepatic lobul (16).

Statistical Analysis

Whole measured information was uploaded and assessed with SPSS 11.0 All the groups showed normal distribution, so parametric statistical methods were used to analyze the data. A one-way analysis of variance test was performed, and post hoc multiple comparisons were done with the Bonferroni method. The chi-square (Fisher's exact) test was used to categorize the data analysis. The Spearman's rho test was used for the correlation analysis. Results were presented as means ±SD. A $p<0.05$ was considered to be statistically significant.

Results

Biochemical Parameters

AST, ALT, liver tissue and serum TOS, TAS, and OSI levels are shown on Table 1. While TOS, AST, and ALT levels were markedly high in Group 2, TAS level was the lowest.

Comparison of group 1 vs. group 2, there was statistically difference in liver tissue OSI ($p=0.013$), there were statistically significant difference in the other parameters ($p<0.0001$). Comparison of group 1 vs. group 3, there was statistically difference in liver tissue TOS($p=0.004$), there were no statistically difference in other parameters ($p>0.05$).

Comparison of group 2 vs. group 4, there were no statistically significant difference in some parameters ($p>0.05$) except AST, serum OSI, liver tissue TOS, liver tissue TAS ($p<0.0001$). Compa-

Table 1. Results of Biochemical Analyses

	Groups						
	Parameters	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
	AST(IU/L)	116.98±16.35	257.35±38.82	148.35±27.36	165.02±42.44	229.33±29.43	167.30±12.73
	ALT(IU/L)	48.90±8.00	80.73±10.80	54.20±7.62	71.37±12.59	80.03±7.31	64.12±3.46
Liver	TOS(μmol/L)	120.46±7.99	62.64±10.52	142.56±16.92	93.36±5.06	65.53±11.56	102.64±10.65
	TAS(mmol/L)	3.64±0.47	1.29±0.21	3.21±0.64	2.55±0.23	1.79±0.57	3.26±0.76
	OSI(%)	3.34±0.35	4.69±0.91	4.10±0.96	3.69±0.42	3.61±0.81	3.29±0.80
Serum	TOS(μmol/L)	14.06±1.75	25.93±3.46	15.65±2.28	23.88±5.63	24.22±3.42	19.77±1.72
	TAS(mmol/L)	0.48±0.09	0.18±0.03	0.44±0.14	0.34±0.14	0.25±0.05	0.43±0.15
	OSI(%)	3.02±0.83	14.79±4.31	3.85±1.14	7.72±2.90	10.22±3.50	4.98±1.51

AST: aspartate aminotransferase; ALT: alanine aminotransferase;
TOS: Total oxidant status; TAS: Total antioxidant status; OSI: oxidative stress index

Table 2. Results of histopathological examination

Parameter	**Grade**	**Groups**					
		Group 1	**Group 2[a]**	**Group 3[b]**	**Group 4**	**Group 5**	**Group 6[c]**
Centrilobular cellular death	(0)	8	0	8	1	0	3
	(I)	0	1	0	5	3	4
	(II)	0	2	0	2	4	1
	(III)	0	5	0	0	1	0
Ballooning	(0)	8	5	5	8	8	5
	(I)	0	3	3	0	0	3
	(II)	0	0	0	0	0	0
	(III)	0	0	0	0	0	0
Inflammation	(0)	8	0	8	0	2	4
	(I)	0	2	0	5	1	3
	(II)	0	2	0	3	3	0
	(III)	0	4	0	0	2	1

[a]*; Separate comparisons of Group 2 with Group 1 and Group 4 revealed significant differences (p<0.026) with respect of cellular death(III) injury, no significant difference was present between Group 2 and Group 5 (p>0.05).*
[b]*; Separate comparisons of Group 3 with Group 1 and Group 5 did not reveal significant differences with respect of any parameter (p>0.05).*
[c]*; Separate comparisons of Group 6 with Group 4 and Group 5 did not reveal significant differences with respect of any parameter (p>0.05).*

rison of group 2 vs. group 5, there was statistically difference in serum OSI (p=0.023), there were no statistically significant difference in the other parameters (p>0.05).

Comparison of group 3 vs. group 5, there were statistically significant difference in some parameters (p<0.0001) except liver tissue OSI (p>0.05). Comparison of group 4 vs. group 6, there were no statistically significant difference in all the parameters (p>0.05). Comparison of group 5 vs. group 6, there were statistically significant difference in liver tissue TOS and TAS (p<0.0001), there were statistically difference in AST, ALT, serum TAS, serum OSI (respectively p=0.002, p=0.012, p=0.038, p=0.005), there were no statistically significant difference in the other parameters (p>0.05).

Histopathological view sections of the groups are presented in Figure 1 and results of histopathological examination are shown in Table 2.

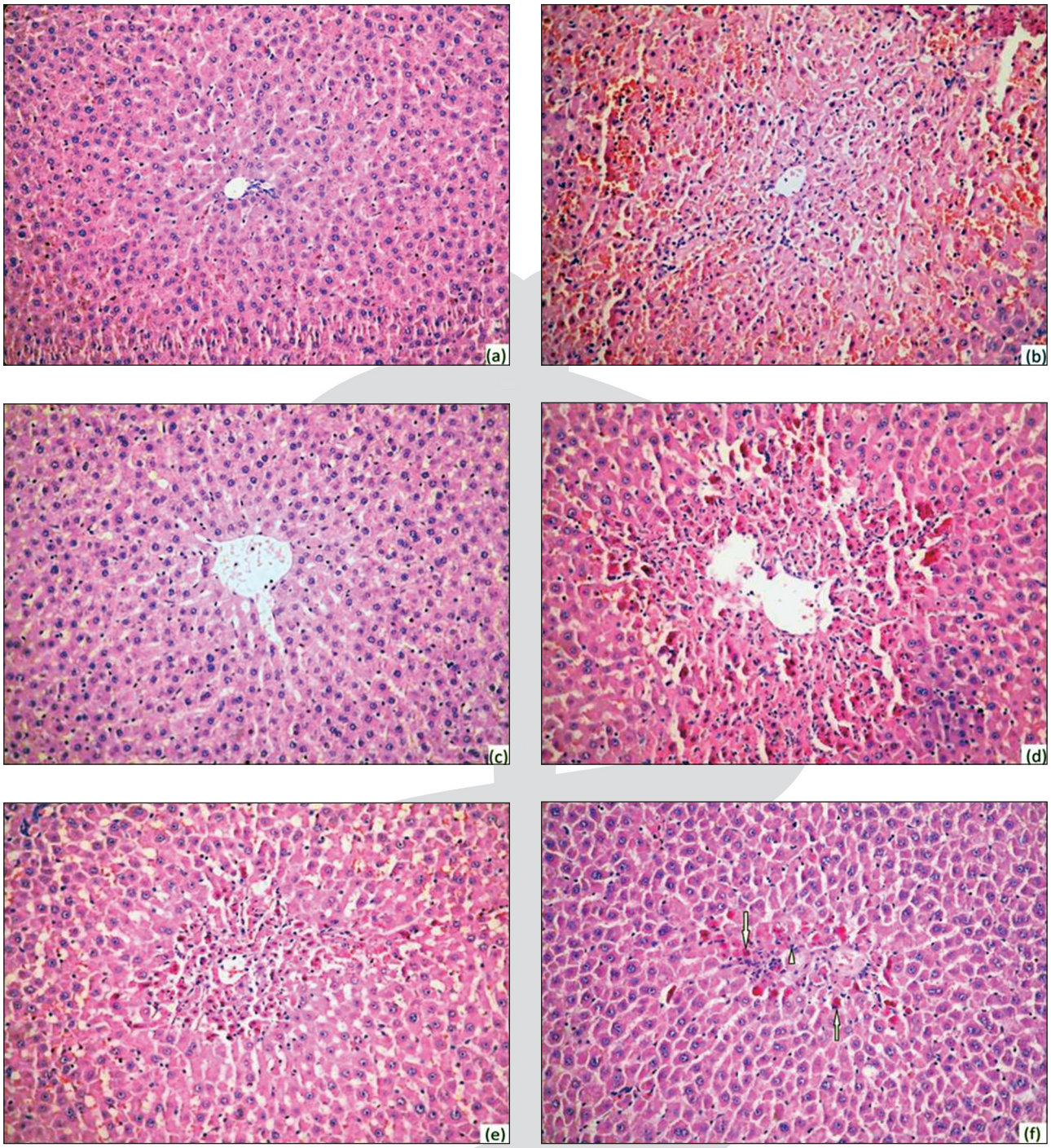

Figure 1. (a) Control group, Hepatic lobule (H&E, x200); **(b) APAP group**, Massive hepatic lobul injury. Centrilobular cell death (+++) and inflammation (+++) (H&E, x200); **(c) LOLA group**, Protected hepatic lobule (H&E, x200), **(d) APAP + NAC group**, Moderate centrilobular cell death (++) and inflammation (++). (H&E, x200); **(e) APAP + LOLA group**, Mild centrilobular cell death (+) and inflammation (+). (H&E, x200); **(f) APAP + LOLA + NAC group**, Prominent improvement in the hepatic lobul injury. Minimal centrilobular cell death (+) and inflammation (+). Arrows demonstrate apoptotic body. Arrow head indicate inflamatuar cell (lemphocyte) (H&E, x200).

Discussion

Acetaminophen is a widely-used analgesic and antipyretic agent. It may lead to hepatic necrosis and even death in both humans experimental animal models when taken in excessive doses (17,18). Acetaminophen is safe at therapeutic doses; however, it may cause intoxication in adults at a single dose of 140 mg/kg or at long term doses above 7.5 gr/day. Overdose ingestions are typically part of suicidal attempts in adults, while accidental ingestions predominate in children (19-21). In humans, the first case of APAP overdose leading to fatal hepatotoxicity was reported in 1966 (1). Since then, APAP poisoning has been the most common cause of acute liver failure in developed regions/countries such as United Kingdom, Canada, Australia, and Scandinavia (22-25).

Reactive oxygen and nitrogen products have an important role in acetaminophen-induced hepatotoxicity (26). NAPQI which is formed by breakdown of acetaminophen by hepatic CYP 450 enzymes is responsible for this toxicity. NAPQI is bound to macromolecules in liver and cause liver injury first, and then hepatic necrosis. AST and ALT are released from the injured hepatocytes; therefore, the classical laboratory finding of hepatotoxicity is increase in ALT and AST (20). In our study, we observed a marked increase in AST and ALT levels in PCT group. In addition, clinical factors such as elevated INR (International normalized ratio) and plasma creatinine, metabolic acidosis, and encephalopathy are used to detect APAP-induced hepatotoxicity (1). AST and ALT are weak prognostic factors for liver injury or severity of acute liver failure although an increase of their activity is a marker of hepatocellular injury (27). Many studies have shown acetaminophen-induced oxidative injury consisting of tissue lipid peroxidation, enzymatic activation, and antioxidatory defense systems of hepatocytes, an increase of oxidant parameters following oxidative injury that results from acetaminophen intoxication, and a decrease in antioxidant parameters (28, 29). Our study showed an increased TOS level, and a decreased TAS level together with a resultant markedly increased OSI level in PCT group.

N-acetyl cysteine, a precursor of cysteine, is the standard therapeutic agent used to prevent or reduce acetaminophen-induced liver injury (30). In addition, owing to its known antioxidant effects, NAC is also used in various liver injuries (31). In the present study we observed that, when compared with PCT group, PCT+NAC group showed a decreased TOS level, and an increased TAS level together with a resultant markedly decreased OSI level.

Known as an agent used in treatment of hepatic encephalopathy, LOLA also exerts a hepatoprotective effect by stabilizing the peroxidant/antioxidant balance in liver cells by virtue of its antioxidant properties (7). Although studies showing the efficiency of LOLA in HE are abundant, data with respect to its direct effect on hepatotoxic acute poisonings (9) is insufficient. In our study, LOLA group and the control group had similar AST, ALT, serum TOS, and serum TAS levels. In addition, LOLA group and PCT+LOLA group had significant differences with respect to biochemical parameters. PCT+LOLA group had almost similar biochemical parameters to PCT group, which suggests that LOLA was not effective biochemically in acetaminophen toxicity-induced liver injury.

Acetaminophen causes a liver injury characterized by hemorrhagic centrilobular necrosis in both humans and experimental animal models (28, 29). Our study demonstrated a more prominent histopathological centrilobular cellular death in PCT and PCT+LOLA groups. However, no significant histopathological differences existed when PCT+NAC+LOLA group was individually compared with PCT+NAC and PCT+LOLA groups, which suggests that LOLA was not histopathologically effective in liver injury associated with acetaminophen toxicity.

We concluded that LOLA had no effects similar or superior to NAC in terms of antioxidant and hepatoprotective properties when used alone or in combination with NAC in treatment of acetaminophen-induced liver injury. We believe that the limitations of our study are related with lack of administration of maintenance doses of NAC and LOLA during the 24-hour follow-up. We think that this study will shed light on future experimental animal models of acetaminophen-induced liver injury.

References

1. *Kalsi S, Wood DM, Waring WS, Dargan PI. Does cytochrome P450 liver isoenzyme induction increase the risk of liver toxicity after paracetamol overdose? Open Access Emergency Medicine 2011; 3: 69-76.*

2. *Laine JE, Auriola S, Pasanen M, Juvonen RO. Acetaminophen bioactivation by human cytochrome P450 enzymes and animal microsomes. Xenobiotica 2009; 39: 11-21.*

3. *Gul H, Uysal B, Cakir E, Yaman H, Macit E, et al. The protective effects of ozone therapy in a rat model of acetaminophen-induced liver injury. Environmental Toxicology and Pharmacology 2012; 34: 81-86.*

4. *Bruss M, Homann J, Molderings GJ. Dysferlinopathy as an extrahepatic cause for the elevation of serum transaminases. Med. Klin. 2004; 99: 326-329.*

5. *Grypioti AD, Mykoinatis M, Demopoulos CA, Kostopanagiotou G. Recombinant platelet-activating factor-acetylhydrolase attenuates paracetamol-induced liver oxidative stress, injury, and regeneration. Dig Dis Sci 2007; 52: 192-199.*

6. *Acharya M, Lau-Cam C. Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. Journal of Biomedical Science 2010; 17(Suppl 1): 35-45.*

7. *Najmi AK, Pillai KK, Pal SN, Akhtar M, Aqil M, Sharma M. Effect of l-ornithine l-aspartate against thioacetamide-induced hepatic damage in rats. Indian J Pharmacol 2010; 42(6): 384-387.*

8. *Prescott LF. New perspectives on paracetamol. Drugs 2003; 63: 51-56.*

9. *Jiang Q, Jiang XH, Zheng MH, Chen YP. l-ornitine l-aspartate in the management of hepatic encephalopathy: A meta analysis. J Gastroenterol Hepatol 2009; 24: 9-14.*

10. *Jin SM, Kil HR, Park K, Noh C. Gene expression in rat hearts following oral administration of a single hepatotoxic dose of acetaminophen. Yonsei Med J 2012; 53(1): 172-180.*

11. *Wang T, Qiao S, Lei S, Liu Y, et al. N-acetylcysteine and allopurinol synergistically enhance cardiac adinopectin content and reduce myocardial reperfusion injury in diabetic rats. PLoS ONE 6(8): e23967. doi: 10.1371/journal.pone.0023967.*

12. *Maliankot M, Jayalekshmi H, Chakrabarti A, Vasudevan DM. Effects of exogenous l-ornitine l-aspartate on ethanol induced testicular injury in wistar rats. Indian Journal of Clinical Biochemistry 2009; 24(1): 94-97.*

13. *Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004; 37: 112–119.*

14. *Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38: 1103–1111.*

15. *Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. BMC Infect Dis. 2005; 5: 95*

16. *Naiko-Ito A, Asamoto M, Naiki T, Ogawa K, Takahashi S et al. Gap junction dysfunction reduces acetaminophen hepatotoxicity with impact on apoptotic signaling and connexin 43 protein induction in rat. Toxicologic Pathology 2010; 38: 280-286.*

17. *Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical Reviews in Toxicology 2001; 31(1): 55-138.*

18. *Maddrey WC. Drug-induced hepatotoxicity. Journal of Clinical Gastroenterology 2005; 39(Suppl 2): 83-89.*

19. *Barlett D. Acetaminophen toxicity. Journal of Emergency Nursing 2004; 30: 281-283.*

20. *Hung OL, Nelson LS. Acetaminophen. In: Tintinalli JE, Kelen GD and Stapczynski JS(eds). Emergency Medicine: A Comprehensive Study Guide. New York, NY: McGraw-Hill 2004, pp: 1088-1094.*

21. *Mladenovic D, Radosavljevic T, Ninkovic M, Vucevic D, Jesic-Vukicevic R and Todorovic V. Liver antioxidant capacity in the early phase of acute paracetamol-induced liver injury in mice. Food and Chemical Toxicology 2009; 47: 866-870.*

22. *Brandsaeter B, Hockerstedt K, Friman S, et al. Fulminant hepatic failure: outcome after listing for highly urgent liver transplantation-12 years experience in the nordic countries. Liver Transpl 2002; 8(11): 1055-1062.*

23. *Tessier G, Villeneuve E, Villeneuve JP. Etiology and outcome of acute liver failure: experience from a liver transplantation centre in Montreal. Can J Gastroenterol 2002; 16(10): 672-676.*

24. *Larson AM, Polson J, Fontana RJ, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005; 42(6): 1364-1372.*

25. *Bernal W, Cross TJ, Auzinger G, et al. Outcome after wait-listing for emergency liver transplantation in acute liver failure: a single centre experience. J Hepatol 2009,50(2): 306-313.*

26. *James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepayotoxicity. Drug Metabolism and Disposition 2003; 31: 1499-1506.*

27. *Huang L, Heinloth AN, Zeng ZB, Paules RS, Bushel PR. Genes related to apoptosis predict necrosis of the liver as a phenotype observed in rats exposed to a compendium of hepatotoxicants. BMC Genomics 2008; 9: 288.*

28. *Isik B, Bayrak R, Akcay A, Sogut S. Erdosteine against acetaminophen induced renal toxicity. Molecular and Cellular Biochemistry 2006; 287: 185-191.*

29. *Kuvandik G, Duru M, Nacar A, et al. Effects of erdosteine on acetaminophen-induced hepatotoxicity in rats. Toxicologic Pathology 2008; 36: 714-719.*

30. *Yağmurca M, Bas O, Mollaoglu H, et al. Protective effects of erdosteine on doxorubicin induced hepatotoxicity in rats. Archives of Medical Research 2007; 38: 380-385.*

31. *Baniasadi S, Eftekhari P, Tabarsi P, et al. Protective effect of N-acetylcysteine on antituberculosis drug induced hepatotoxicity. Europan Journal of Gastroenterology and Hepatology 2010; 22(10): 1235-1238.*

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Relationship between depressive personality and DNA damage in Mexican community-dwelling elderly adults

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Abstract

Objectives: Several studies have suggested that depressive personality might be a risk factor for several diseases and it may also affect the course of medical diseases, linked to DNA damage. We assessed the relationship between depressive personality and DNA damage in elderly people.

Method: A case-control study was carried out in a sample of 80 Mexican older subjects: i) 25 with depressive personality (mean age 67.0 ± 5.8 years old), and ii) 55 without depressive personality (mean age 67.5 ± 5.4 years old). We measured DNA damage by the single cell gel electrophoresis technique (comet assay) in peripheral blood lymphocytes. The depressive personality was determined using the Minnesota Multiphasic Personality Inventory 2 (MMPI-2) adapted to the Spanish language.

Results: We observed that DNA damage was markedly increased in elderly with depressive personality than control group (tail length 39.04 ± 4.2 vs. 27.08 ± 1.4 mm, $p< 0.05$). We observed a positive correlation between number of cells with damage and T-score of MMPI-2 related to depressive personality ($r = 0.450$, $p < 0.05$). Using logistic regression analysis to control pro-oxidant variables (gender, smoking, alcohol intake, overweight, age, and sedentarism) we found that the subjects with depressive personality had four-fold more risk for DNA damage than control group with an odds ratio (OR) of 5.28 (95%CI: 1.63 – 17.14, $p< 0.01$).

Conclusions: Our findings suggest that depressive personality can be a relevant risk factor for oxidative DNA damage in elderly people.

Key words: Depressive personality, DNA damage, Mexican elderly.

Introduction

Depression has been linked to chronic diseases and is the leading global cause of life-years lived with disability and permanent psychological stress, and it ranks fourth for disability-adjusted life-years worldwide [1, 2].

On the other hand, the depressive personality conceptualized as a chronic disorder characterized by depressive symptoms such as low mood, low self-esteem, poor concentration, sleep disturbances, fatigue and feelings of hopelessness [3]. This disorder is very frequent in the older people [4, 5], however, a high percentage them are undertreated, being a risk factor for major depression and chronic diseases [6, 7].

Chronic activation of stress response mediators, although adaptive in the short term, can result in chronic "wear and tear" to tissues or organ compartments; McEwen (2003) [8] has modeled mood and anxiety disorders as chronic stresses with chronic biological adaptations that result in long-term biological damage.

Moreover, many studies have demonstrated the role of psychosocial and behavioral risk factors in the etiology and pathogenesis of some disorders, principally cardiovascular events; these behaviors across the life form the personality [9]. In depressed mood, the subjects have a tendency to experience negative emotions, and therefore more somatic symptoms, and have an attention bias towards adverse stimuli [10].

At the same time, several studies have showed that "normal" aging is accompanied by abnormalities in neuroendocrine responses to psychosocial or physical stress [11]. In addition; there is evidence

of derangement of oxidant and antioxidant defense systems in depression. As well, oxidative stress (OS) causes deleterious effects in brain that may include lipid and protein peroxidation, and DNA oxidation, that may eventually lead to cell death [12].

It has been demonstrated a causal relation between acute stressors and DNA-damage in animals and a significant correlation between psychological factors (e.g., depression, coping) and DNA-damage in humans [13]**.** On the other hand, the depression might be an etiologic factor for several diseases and it may also affect the course of medical diseases, linked to DNA damage [14, 15].

It is for this reason that it is supposed that depressive personality keep a dysregulated activation of the generalized stress response during his/her life [16]; thus, the aim of this study was to assess the relationship between depressive personality and DNA damage in elderly people.

Materials and Methods

Subjects and study design

A case-control study was carried out in a convenience sample of 80 Mexican older subjects: I) 25 with depressive personality (mean age 67.0 ± 5.8 years old), and II) 55 without depressive personality (mean age 67.5 ± 5.4 years old). The subjects were community-dwelling Mestizo Mexican elderly living in Mexico City for 10 years or more. Informative brochures were distributed in the community specifying the objectives of the study and admission criteria.

All subjects were physically and mentally functional, without antioxidant supplement intake, and healthy at study entry (arterial hypertension, diabetes mellitus or cancer were ruled out) and well-nourished [Mini Nutritional Assessment score >23.5, caloric intake was between 2,000–2500 kcal/d, and the alimentation had the nutrients requirements (protein, fat, carbohydrate, vitamins and minerals) between Recommended Dietary Allowance (RDA) measured by 24-h dietary recalls, and serum albumin >35 g/L] [17], as assessed by medical history and physical examination. Also, we applied a structured pro-oxidants questionnaire that included: age, gender, smoking, alcohol intake, and no physical activity.

The subjects agreed to participate in the study after giving their informed consent. The Ethics Committee of the Universidad Nacional Autónoma de México (UNAM) Zaragoza Campus approved the research protocol for this study.

Anthropometric measurements

After clinical history and physical examination were conducted, we performed the following anthropometric measurements: weight was measured while the subject was wearing underwear and a clinical smock and in a fasted state (after evacuation). A Torino® scale (Tecno Lógica, Mexicana, México, TLM®) was used, calibrated before each weight measurement. Height was obtained with an aluminum cursor stadiometer graduated in millimeters. The subject was barefoot, back, and head in contact with the stadiometer in Frankfurt horizontal plane. BMI was calculated by dividing weight (in kilograms) by squared height (in meters); it was considered as overweight when BMI $\geq$ 27 [18].

Assessment of depressive personality

The depressive personality was determined using the Minnesota Multiphasic Personality Inventory 2 (MMPI-2) adapted to the Spanish language The MMPI-2 includes three validity scales and ten clinic scales, in where scale 2 is depression. Each subject of the sample was submitted to MMPI-2 self-descriptive with 566 items. It was considered as depressive personality when the test showed predominant scales in groups 1, 21 and 33, and the depressive intensity in agree with T-score in clinic scale [19].

Blood sampling and biochemical analysis

Blood samples were collected after a 12-h fasting period by venopuncture and placed in vacutainer/siliconized test tubes containing a separating gel and no additives, and heparin as anticoagulant agent (Becton-Dickinson, Mexico City, Mexico). Blood samples containing heparin were analyzed using a hemoglobin test protocol (including hemoglobin, hematocrit, and leukocyte counts) in a Celly 70 autoanalyser (Chronolab, Mexico City, Mexico). Serum thus obtained from samples was tested for glucose, blood urea nitrogen (BUN), creatinine, urate, cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), and albumin concentrations using a chemical chemistry autoanalyser (Shimadzu, Columbia, MD, USA). Glucose le-

vels were measured with glucose oxidase method; BUN levels were measured with Berthelot urease method; creatinine levels by Jaffe method without deproteinization, and urate levels by uricase colorimetric method. Albumin levels were measured by bromocresol green technique; cholesterol was analyzed using CHOD-PAP technique; triglycerides by GPO-Trinder technique; whereas HDL-c were assessed employing CHOD-PAP technique after precipitation of low and very-low lipoproteins using a phosphotungstic acid/magnesium chloride solution. All reagents employed in biochemical tests were obtained from Randox Laboratories, Ltd. (Crumlin, Co, Antrim, UK). We include a high and normal control serum as quality control (Randox Laboratories, Ltd). The intra- and inter- assay variation coefficients were less 5% in all the determinations. These tests were used as screening measurements for diagnosis of clinically healthy subjects, and cut-off points for reference values for Mexican elderly persons were determined at the Gerontologic Clinical Research Laboratory of the Universidad Nacional Autónoma de México (UNAM) Zaragoza Campus in Mexico City [20].

Alkaline unicellular electrophoresis

Alkaline unicellular electrophoresis assay in peripheral blood lymphocytes was performed as described by Tice et al. (1992) [21]. A small volume (10 mL) of cells was mixed with 75 mL of 0.5% low-melting agarose maintained at 37°C, and 75 mL of this mixture was pipetted onto a slide with 180 mL normal agarose and immediately covered with a coverglass, to make a microgel on the slide. Slides were placed in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed and 75 mL agarose was layered as previously. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris-base, and 1% Na-sarcosinate; pH 10). After lysis at 4°C for 1 h, slides were placed on the horizontal electrophoresis unit. The DNA was allowed to unwind for 20 min in electrophoresis running buffer solution (300 mM NaOH and 1 mM Na2EDTA; pH 13). Electrophoresis was conducted for 20 min at 25 V and 300 mA.

All technical steps were conducted using very dim indirect light. After electrophoresis, the slides were gently removed and the alkaline was pH-neutralized with 0.4 M Tris (pH 7.5). Ethidium bromide (75 mL of a 20 mg/mL solution) was added to each slide and a coverglass was placed on the gel. The slides were stored in a humidified box at 4°C to prevent drying of the agarose. Individual cells were visualized at 20× magnification on a Nikon Optiphoto-2 microscope (Nikon, Japan) with fluorescence attachments (excitation filter, 515–560 nm; barrier filter, 590 nm) and the extent of migration (i.e. image length, of both the head and tail of the comet) was measured with a scaled ocular. To evaluate DNA migration, 100 cells were scored for each person. Another criterion for evaluation was the assignment of cells into five categories corresponding to the following amounts of DNA in the tail: no DNA damage, ≤ 5%; low-level DNA damage, 5–20%; medium-level DNA damage, 20–40%; high-level DNA damage, 40–95%, and total DNA damage, ≥ 95%. Migration was the difference between length and diameter.

The cut-off of high magnitude value was considered to be the median of the number of cells with DNA damage. A high grade of DNA damage was established as when amounts of DNA migration in electrophoresis were ≥ 40% in relation to nuclear diameter [22,23].

Statistical analysis

Data were processed by use of standard statistical software SPSS V. 15.0 (SPSS Inc. Chicago, IL, USA). The descriptive statistics were mean ± standard error (SE), and they were compared using Student's- t test, and frequencies and percentage compared with c^2. Also it was calculated lineal regression and the odds ratio (OR) with a 95% confidence interval (CI) using logistic regression analysis to simultaneously control the pro-oxidant risk factors. It was considered risk factor when OR >1 and range of CI not includes the value 1.0. In all the tests, a p-value <0.05 was considered significant.

Results

Biochemical characteristics

Biochemical-hematologic parameters and body mass index (BMI) of elderly study participants are presented in Table 1. It was observed that elderly adults in both groups have similar values in all parameters (p >0.05).

Table 1. Biochemical and hematologic parameters and body mass Index by group

Parameter	Depressive personality (n = 25)	Control group (n = 55)
Glucose (mg/dL)	107 ± 6.9	117 ± 7.4
BUN (mg/dL)	31 ± 1.6	32 ± 1.2
Urate (mg/dL)	5.3 ± 0.3	4.8 ± 0.2
Creatinine (mg/dL)	0.90 ± 0.04	0.83 ± 0.03
Cholesterol (mg/dL)	219 ± 11.8	235 ± 6.0
Triglycerides (mg/dL)	178 ± 18.0	170 ± 9.7
HDL-c (mg/dL)	48 ± 2.4	52 ± 2.4
Albumin (mg/dL)	4.4 ± 0.08	4.3 ± 0.07
Hemoglobin (g/dL)		
Female	13.8 ± 0.3	14.2 ± 0.2
Male	15.4 ± 0.6	14.6 ± 0.4
Hematocrit (%)		
Female	44 ± 1.0	45 ± 0.5
Male	47 ± 1.6	46 ± 1.0
Total leukocytes/mm^3	6133 ± 303	6472 ± 204
BMI (kg/m^2)	28.57 ± 0.89	28.31 ± 0.48

Data show means ± standard error. BUN: blood ureic nitrogen, HDL-c: high density lipoprotein cholesterol, BMI: body mass index.

DNA damage and depressive personality

We found 32 elderly (40%) with DNA damage of the 80 participants, however in the analysis by group the DNA damage, this was markedly increased in elderly with depressive personality than control group: sixteen (50%) elderly with depressive personality had DNA damage against nine (19%) control group ($p< 0.01$). On the other hand, the DNA damage intensity was significantly higher in the subjects with depressive personality (tail length 39.04 ± 4.2 vs. 27.08 ± 1.4 mm, p<0.05) (Figure 1).

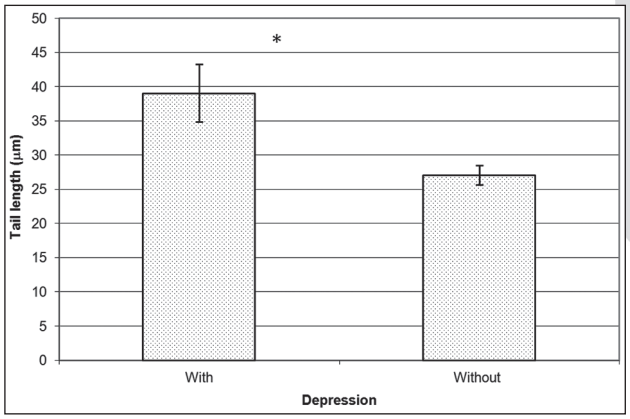

Figure 1. DNA migration in lymphocytes of elderly with and without depressive personality
**Student's t test, p< .05.*

It was determined the relationship between DNA oxidative damage and depressive personality; therefore, we carried out a simple linear regression between the number of cells with oxidative DNA damage and T-score of MMPI-2, and observed a positive correlation with depressive personality scale ($r = 0.450$, $p <0.05$) (Figure 2). There was not association in subjects of the control group.

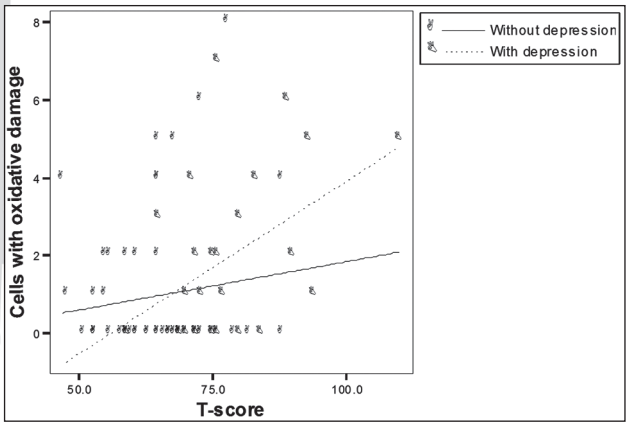

Figure 2. Lineal regression of cells with DNA damage and T-score by study group.

Elderly with dysthymia. r = 0.450, $r^2 = 0.202$, p= .024; elderly without depressive personality r = 0.122, $r^2 = 0.015$, p = .375.

Pro-oxidant risk factors

Regard pro-oxidant risk factors, we found a higher percentage of depressive personality in men than in women. Other risk factors not show difference (Table 2).

Depressive personality as risk factor for DNA damage

Using logistic regression analysis to control pro-oxidant variables like (gender, smoking, alcohol intake, obesity, age, and no physical activity) we found that subjects with depressive personality can have four-hold more risk for DNA damage than control group with an odds ratio (OR) of 5.28 (95%CI: 1.63 – 17.14, $p<0.01$) (Table 3).

Discussion

During aging process the accumulative oxidative stress causes deleterious oxidative damage in the brain. The effects of stressors on the aging process and age-related diseases are complex, involving the nervous, endocrine and immune systems [11]. Fiske et al. (2009) suggest that the onset and maintenance of depression in late life can be understood as an interaction between certain vulnerabilities, including genetic factors, cognitive diathesis and age-associated neurobiological changes, and the types of stressful events that occur with greater frequency in late life than earlier in the lifespan [24].

Table 2. Age, gender, life styles and body mass index by study group

Variable	Depressive personality (n = 25)	Control group (n = 55)
Age		
60 – 69 years	18 (35%)	33 (65%)
≥ 70 years	7 (24%)	22 (76%)
Gender		
Female	12 (22%)	42 (78%)
Male	13 (50%)*	13 (50%)
Smoke		
Positive	2 (18%)	9 (82%)
Negative	23 (34%)	46 (66%)
Alcohol intake		
Positive(≥ 2 cups/day)	8 (23%)	27 (77%)
Negative	17 (38%)	28 (62%)
Weight		
Overweight (BMI≥27 kg/m^2)	15 (30%)	35 (70%)
Normal weight	10 (33%)	20 (66%)
Sedentarism		
Positive	18 (27%)	48 (63%)
Negative	7 (50%)	7 (50%)

c^2 test, $p< .05$.

Table 3. Risk factors to DNA damage in Mexican elderly

Pro-oxidant risk factor	OR	95% CI	p value*
Depressive personality	5.28	1.63 – 17.14	.006
Overweight (≥ 27 kg/m^2)	2.56	0.78 – 8.45	.121
Age (≥ 70 years)	2.15	0.72 – 6.41	.169
Alcohol intake (≥ 2 cup/day)	1.15	0.33 – 3.71	.872
Gender (male)	0.84	0.22 – 3.20	.841
Sedentarism	0.36	0.09 – 1.50	.157
Smoking	0.29	0.05 – 1.73	.174

Logistic regression analysis, $R^2 = 0.283$, $p = .010$.

Stressful stimuli induce physiological and behavioral reactions which are necessary to reduce the harmful effects of the challenge on the organism. However, uncontrollable and repetitive stress experiences may lead to inadequate or insufficient bodily responses, which ultimately can contribute to the expression and/or exacerbation of diverse pathologies, including depression [25].

A paucity of studies has investigated personality in the context of health status and disease, probably because of the inconsistencies in findings in research on the Type A Behavior Pattern (TABP) [26]. However, personality factors may explain individual differences in health outcomes above and beyond demographic and clinical risk factors [27]. The older people with depressive personality have being many years with dysthymia, producing permanent high OS and consequent oxidative damage to biomolecules, including DNA. In this study we found that the elderly with depressive personality have more oxidative DNA damage compared with control group elders, and we observed a positive correlation between the number of cells with oxidative damage and T-score of the MMPI-2 in old adults with depressive personality, ie, greater intensity in the clinical scale of depressive personality, greater number of damaged cells. In this sense, it demonstrates how a stress-induced anabolic/catabolic imbalance—characterized in part by high cortisol, glucose, and insulin, and low androgens and growth hormones—may lead to OS and systemic inflammation, which in turn impair cell aging processes [28]; specifically, it has also been documented a causal relation between acute stressors and DNA damage [13].

As well, we observed that depressive personality is the major risk factor to DNA damage, controlled by others pro-oxidant factors. With regard to this, some researches had showed a positive relationship between depressive symptoms and OS measured by antioxidant status, lipoperoxides and 8-hydroxy-2-deoxyguanosine (8-OHdG) [29-31]. Furthermore, Andreazza et al. (2007) found that the frequency of DNA damage using the single cell gel electrophoresis comet assay, correlated with the severity of symptoms of depression and mania in bipolar disorder [32]. It is important to note that although we found a higher proportion of men with depressive personality, gender was not a risk factor for DNA damage, probably by the small sample size.

Recently, Type D personality has been described as the tendency to experience a high joint occurrence of negative affectivity and social inhibition. Patients with a Type D personality tend to experience increased levels of anxiety, irritation, and depressed mood across situations and time, while not sharing these emotions with others because of fear of rejection or disapprove [27]; and it was related with chronic heart failure (CHF), principally. Type D personality is a vulnerability factor for general psychological distress that affects mental and physical health status and is associated with disease-promoting mechanisms and work-related problems in apparently healthy individuals [33]. As for this, it was described that CHF patients with Type D personality are characterized by an increased OS burden, apparent in the decreased antioxidant levels and an increased OS ratio [34].

In addition, previous studies reported that psychological stress would alter susceptibility to illnesses such as cancer [14, 15], and depressed individuals become vulnerable to comorbid medical illness [32], and recently in animal models, Costantini et al. (2008) suggests that different animal personalities may be accompanied by differences in oxidative status, which may predict differences in longevity [35]. Therefore we think that is important to identify older people with depressive personality to indicate a preventive or therapeutic alternative to avoid or defer multiple comorbid conditions.

Conclusions

Our findings suggest that depressive personality keeping during the life is a relevant risk factor for oxidative DNA damage in elderly people, and consequently higher risk for major depression and chronic diseases.

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References

1. *Dent OF, Waite LM, Bennett HP, Casey BJ, Grayson DA, Cullen JS, Creasey H, Broe GA. A longitudinal study of chronic disease and depressive symptoms in a community sample of older people. Aging Ment Health. 1999; 3: 351-357.*

2. *Insel TR, Charney DS. Research on major depression. JAMA. 2003; 289: 3167-8.*

3. *Barzega G, Maina G, Venturello S, Bogetto F. (2001). Dysthymic disorder: clinical characteristics in relation to age at onset. J Affec Disord. 2001; 66: 39-46.*

4. *Devanand DP, Nobler MS, Singer T, Kiersky JE, Turret N, Roose SP, Sackeim HA. Is dysthymia a different disorder in the elderly?. Am Jl Psychiatry. 1994; 151: 1592-1599.*

5. *Comijs HC, van Marwijk HW, van der Mast RC, Naarding P, Oude Voshaar RC, Beekman AT, Boshuisen M, Dekker J, Kok R, de Waal MW, Penninx BW, Stek ML, Smit JH. The Netherlands study of depression in older persons (NESDO); a prospective cohort study. BMC Res Notes. 2011; 5: 4:524. doi: 10.1186/1756-0500-4-524.*

6. *Shelton RC, Davidson J, Yonkers KA, Koran L, Thase ME, Pearlstein T, Halbreich U. The undertreatment of dysthymia. J Clin Psychiatry. 1997; 58: 59-65.*

7. *Hybels CF, Pieper CF, Blazer DG, Steffens DC. The course of depressive symptoms in older adults with comorbid major depression and dysthymia. Am J Geriatr Psychiatry. 2008; 16: 300-309.*

8. *McEwen BS. Mood disorders and allostatic load. Biol Psychiatry. 2003; 54: 200-7.*

9. *Ursano RJ, Epstein RS, Lazar SG. Behavioral responses to illness: personality and personality disorders. In: Wise MG, Rundell JR, eds. The American psychiatric publishing yextbook of consultation-Liaison psychiatry. Psychiatry in the medically Ill, 2nd ed. Washington DC: American Psychiatric Publishing; 2002. p. 107–25.*

10. *Watson D, Pennebaker JW. Health complaints, stress, and distress: exploring the central role of negative affectivity. Psychol Rev. 1989; 96: 234–54.*

11. *Pedersen WA, Wan R, Mattson MP. Impact of aging on stress-responsive neuroendocrine systems. Mech Ageing Dev. 2001; 122: 963-83.*

12. *Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408: 239-47.*

13. *Gidron Y, Russ K, Tissarchondou H, Warner J. The relation between psychological factors and DNA-damage: a critical review. Biol Psychol. 2006; 72: 291-304.*

14. *Irie M, Asami S, Ikeda M, Kasai H. Depressive state relates to female oxidative DNA damage via neutrophil activation. Biochem Biophys Res Común. 2003; 311: 1014-8.*

15. *Irie M, Miyata M, Kasai H. Depression and possible cancer risk due to oxidative DNA damage. J Psychiat Res. 2005; 39: 553-60.*

16. *Sanchez-Rodriguez, M., Arronte-Rosales, A., & Mendoza-Nuñez V.M. (2008). Depression as a risk factor for oxidative DNA damage in elderly people. Free Radical Biology and Medicine, 45: S100.*

17. *Vellas B, Guigoz Y, Baumgartner M, Garry PJ, Lauque S, Albarede JL. Relationship between nutritional markers and the Mini-Nutritional assessment in 155 older persons. J Am Geriatr Soc. 2000; 48: 1300-9.*

18. *Reuben DB, Greendale GA, Harrison GG. Nutrition screening in older persons. J Am Geriatr Soc. 1995; 43: 415-25.*

19. *Lucio-Gómez-Maqueo E, Pérez y Farías JM, Ampudia A. Un estudio de confiabilidad test-retest del MMPI-2 en un grupo de estudiantes mexicanos. Rev Mex Psicol. 1997; 1: 55-62.*

20. *Sánchez-Rodríguez MA, Mendoza-Núñez VM, García-Sánchez A, González-González B, Rodríguez-Torres E, González-Obregón A. Valores de referencia para una población de ancianos y adultos de la ciudad de México. Parámetros bioquímicos y hematológicos. Acta Bioquim Clin Latinoam. 1998; 32: 812-21.*

21. *Tice RR, Straus GHS, Peters WP. High-dose combination alkylating agents with autologous bone-marrow support in patients with breast cancer: preliminary assessment of DNA damage in individual peripheral blood lymphocytes using the single cell electrophoresis assay. Mutat Res. 1992; 271: 101-13.*

22. *Anderson D, Yu TW, Phillips BJ, Schmerzer P. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the comet assay. Mutat Res. 1994; 307: 261-71.*

23. *Mendoza-Núñez VM, Sánchez-Rodríguez M, Retana-Ugalde R, Altamirano-Lozano M, Vargas-Guadarrama LA. Age and Gender as Risk Factors for DNA damage in Lymphocytes of elderly. Mech Ageing Dev 2001; 122:835-847.*

24. *Fiske A, Wetherell JL, Gatz M. Depression in older adults. Annu Rev Clin Psychol. 2009; 5: 363-89.*

25. *Isovich E, Mijnster MJ, Flûgge G, Fuchs E. Chronic psychosocial stress reduces the density of dopamine transporters. Eur J Neurosci. 2000; 12: 1071-8.*

26. *Pedersen SS, Denollet J, Ong AT, Serruys PW, Erdman RA, van Domburg RT. Impaired health status in type D patients following PCI in the drug-eluting stent era. Int J Cardiol. 2007; 114: 358-65.*

27. *Denollet J. DS14: Standard assessment of negative affectivity, social inhibition, and type D personality. Psychosom Med. 2005; 67: 89-97.*

28. *Eppel ES. Psychological and metabolic stress: A recipe for accelerated cellular aging? Hormones. 2009; 8: 7-22.*

29. *Lesgards JF, Durand P, Lassarre M, Stocker P, Lesgards G, Lanteaume A, et al. Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. Environ Health Perspect. 2002; 110: 479-87.*

30. *Tsuboi H, Shimoi K, Kinae N, Oguni I, Hori R, Kobayashi F. Depressive symptoms are independently correlated with lipid peroxidation in a female population. Comparison with vitamins and carotenoids. J Psychosom Res. 2004; 56: 53-8.*

31. *Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2-deoxyguanosine in clinical pepression. Psychosom Med. 2006; 68:1-7.*

32. *Andreazza AC, Frey BN, Erdtmann B, Salvador M, Rombaldi F, Santin A, et al. DNA damage in bipolar disorder. Psychiatry Res. 2007; 153: 27-32.*

33. *Mols F, Denollet J. Type D personality in the general population: a systematic review of health status, mechanisms of disease, and work-related problems. Health Qualt Life Outcomes. 2010; 8: 9. Available in: http://www.hqlo.com/content/8/1/9.*

34. *Kupper N, Gidron Y, Winter J, Denollet J. Association between type D personality, depression, and oxidative stress in patients with chronic heart failure. Psychosom Med. 2009; 71: 973-80.*

35. *Costantini D, Carere C, Caramaschi D, Koolhaas JM. Aggressive and nonaggressive personalities differ in oxidative status in selected lines of mice (Mus musculus). Biol Lett. 2008; 4: 119-22.*

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Relationship of endothelial dysfunction and inflammation with renal function in preeclampsia

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Abstract

Purpose: Preeclampsia is an important cause of pregnancy related renal pathologies and manifests itself as endothelial dysfunction and increased inflammation. However, little is known regarding the contribution of endothelial dysfunction and increased inflammation to renal dysfunction in preeclampsia. The aim of this study is to analyze the relationship of endothelial dysfunction and inflammatory markers with glomerular filtration rate (GFR) in preeclamptic patients.

Methods: Eighty-eight consecutive pregnant women with preeclampsia followed in our clinic and 40 healthy pregnant women were enrolled in the study. Urine was collected from all subjects and creatinine clearance was calculated. The relation of endothelial dysfunction markers [vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), plasminogen activator inhibitor-1 (PAI-1)] and inflammatory markers [tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), high sensitive CRP (hs-CRP)] to creatinine clearance was investigated.

Results: There was no significant difference in age, gestational week and BMI between the patient group and the control group. While GFR was significantly lower in the patient group than the control group, blood creatinine levels were higher among preeclamptic patients ($p<0.001$). GFR was negatively correlated with hs-CRP, VCAM-1, ICAM-1, TNF-α, blood pressure and blood creatinine levels and positively correlated with BMI. A significant relationship was found between GFR and the markers hs-CRP and VCAM-1.

Conclusion: GFR is decreased in preeclampsia. Markers of endothelial dysfunction and inflammation and blood pressure are negatively correlated with the decrease in GFR. The endothelial dysfunction marker VCAM-1 and the inflammatory marker hs-CRP may be independent determinants of the decrease in GFR due to preeclampsia.

Key words: Preeclampsia, renal function, glomerular filtration rate, VCAM-1, hs-CRP.

Introduction

Preeclampsia, which is an important cause of maternal morbidity and mortality today, is characterized by the manifestation of hypertension and proteinuria after the 20th gestational week and occurs in 2-8% of all pregnancies [1]. The most well known risk factors of preeclampsia are chronic hypertension, diabetes, renal disease, insulin resistance, nulliparity, family history and molar pregnancy. Uterine ischemia in the early stages of placental development as a result of incomplete remodeling of uterine spiral arteries caused by the insufficient invasion of extravillous cytotrophoblasts to uterine spiral arteries, probably due to a change in angiogenic factors, is implicated in the pathogenesis of preeclampsia [2]. Endothelial dysfunction, which is an important factor for the systemic effects of preeclampsia, is caused by Soluble Fms-like tyrosine kinase 1 (sFlt1), endoglin, TNF-α, IL-6 and thromboxane that are released from the ischemic placenta [3]. Soluble Fms-like tyrosine kinase has an antiangiogenic effect and plays a key role in the pathogenesis of preeclampsia by blocking the interaction between vascular endothelial growth factor (VEGF) and its specific receptor via binding VEGF. VEGF is secreted from glomerular podocytes in high concentration and is important for glomerular filtration, and the function of glomerular podocytes and endothelial cells. VEGF deficiency causes glomerular endothelial dysfunction, loss of fenestrations, endotheliosis and proteinuria. Glomerular endotheliosis is the characterized renal

manifestation of preeclampsia. Swelling of glomerular endothelial cells and hypertrophy is seen by electron microscopy, and the occlusion of capillary lumens causes the glomeruli to appear 'bloodless' [4]. Preeclampsia is one of the most important causes of renal function loss in pregnancy. Although GFR increases by approximately 50% in a normal pregnancy, it is reported that GFR is reduced by about 30-40% in pregnant women with preeclampsia compared to healthy pregnant women at similar gestational age [5].

Endothelial dysfunction is known to cause systemic complications such as hypertension, cerebral edema and liver dysfunction in preeclamptic patients. It has been shown a certain number of studies that proinflammatory cytokines are increased in preeclampsia [6,7,8]. But the effect of endothelial dysfunction and inflammation on systemic complications in preeclampsia is yet unknown. Gadonski *et al.* have shown that IL-6, a proinflammatory cytokine, is increased in RUPP (reduction in uterine perfusion pressure in pregnant) rats and on the other hand the infusion of IL-6, comparable to levels observed in RUPP rats, raises blood pressure and reduces renal plasma flow (RPF) and GFR [9].

There are no previous studies of pregnant women with preeclampsia which focus on the effect of endothelial dysfunction and inflammation in determining GFR. The aim of this study was to evaluate the relation of endothelial dysfunction (VCAM-1, ICAM-1, PAI-1) and inflammatory markers (TNF- α, IL-6, hs-CRP) to preeclampsia and GFR in pregnant women.

Materials and Methods

Patient

This prospective case-controlled study was conducted in the Department of Obstetrics and Gynecology of Dicle University Medical Hospital. Eighty-eight consecutive preeclamptic patients followed in our clinic and 40 healthy pregnant women were enrolled in the study. The study protocol was approved by the Ethics Committee of Dicle University. All subjects provided informed consent. Patients with placenta previa, abruptio placenta, polyhydramnios, multiple pregnancy, major fetal anomaly, history of hypertension, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) renal disorder, diabetes mellitus, chronic systemic disease, and maternal or fetal infection were excluded from the study. Preeclampsia was defined as systolic blood pressure higher than 140mmHg or diastolic blood pressure higher than 90mmHg in more than 2 separate readings taken at least six hours apart together with proteinuria (spot urine >1+ or > 300 mg/24 h, in the absence of urinary tract infection) after 20 weeks of pregnancy in a pregnant woman who previously had normal blood pressure. A systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥110 mmHg or 5gr/24 h proteinuria was defined as severe preeclampsia.

Sampling

Blood samples were drawn from the antecubital vein after an overnight fasting period. Blood samples were collected in tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged at 3000 rpm for 10 minutes and the supernatant was stored at -80 °C until analysis.

TNF-α, IL-6 and PAI-1 levels were measured using a chemiluminescent enzyme-immunometric assay (IMMULITE Automated immunoassay system; Immulite DPC, Los Angeles, CA, USA). VCAM-1 and ICAM-1 measurements were performed by using a commercially available enzyme-linked immunosorbent assay kit (Biosource, California, USA). An enzyme-linked immunosorbent assay (ELISA) was used for high sensitivity C-reactive protein (hs-CRP) levels (Diasource Immunoassays S.A., Belgium).

Complete blood count was measured using the Abbott Cell Dyn 3700 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL, USA). Serum biochemical parameters were assayed using an autoanalyzer Roche/Hitachi Moduler P 800 (Roche/Hitachi; Mannheim, Germany), and urine analysis was performed using the IQ 200 urine analyzer (Iris Diagnostics, Chatsworth, CA, USA).

Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the data were distributed normally. One-way ANOVA was applied on parametrically grouped variables that had a normal distribution. Kruskal-Wallis was applied to nonparamaetric groups of variables that were not

normally distributed. Mann-Whitney U test was performed on statistically significant groups to search for dual significance after the Kruskal-Wallis was performed and afterwards Bonferroni correction was applied. $p \leq 0.017$ was accepted as the threshold of statistical significance. Pearson's analysis was applied to parametric variables and Spearman's rho correlation analysis was applied to nonparametric variables for correlation analysis. Linear regression analysis was performed in order to investigate independent variables that effect GFR.

Results

Eighty-eight pregnant women with preeclampsia and 40 healthy pregnant women (control group) were enrolled in the study. The preeclampsia group consisted of 58 patients with mild preeclampsia and 30 patients with severe preeclapsia. There was no significant difference between the three groups regarding age, parity, gestational age and BMI (Table 1). When the biochemical parameters, endothelial markers and inflammation markers of the three groups were compared, there was a significant difference between GFR, PAI, TNF-α, IL-6, VCAM-1, ICAM-1, serum BUN, serum creatinine and proteinuria/day values (Table 2).

In the correlation analysis between GFR, endothelial dysfunction, and cytokines there was a significant negative correlation between GFR and hs-CRP, VCAM-1, ICAM-1, TNF- α, systolic blood pressure, diastolic blood pressure and serum creatinine levels, while GFR and BMI were positively correlated (Table 3). hs-CRP and VCAM-1 were found to be correlated with GFR in the linear regression analysis (Table 4)

Table 1. Clinical characteristics of groups

	Control group	Mild Preeclampsia group	Severe Preeclampsia group	*p*
Age	27.33±4.16	27.41±4.53	25.50±4.97	0.129
Parity	1.20±0.62	1.00±0.59	0.73±0.28	0.259
Gestational age	32.63±3.79	32.37±1.89	31.60±3.42	0.518
BMI	30.40±3.90	30.66±3.79	29.12±3.34	0.159
Systolic blood pressure (mmHg)	108.66±7.76	145.86±6.76	161.00±18.07	<0.001
Diastolic blood pressure (mmHg)	68.50±4.18	91.03±5.52	102.00±11.56	<0.001

BMI: Body mass index.

Table 2. Laboratory and biomarker parameters of groups

	Control group	Mild Preeclampsia group	Severe Preeclampsia group	*p*
GFR	160.66±27.49	137.75±24.01	129.68±33.20	<0.001[a], <0.001[b], 0.244[c]
PAI-1	4547.13±2103.66	5305.97±1902.07	5787.10±1469.27	0.103[a], 0.011[b], 0.193[c]
TNF-α	4283.10±3186.12	5871.84±1720.02	7445.43±1275.67	0.015[a], <0.001[b], <0.001[c]
IL-6	13.48±10.88	19.03±11.03	44.21±76.72	0.006[a], 0.038[b], 0.084[c],
hs CRP	0.16±0.03	0.13±0.04	0.58±2.39	0.158
VCAM-1	968.53±418.44	2144.00±799.82	2677.53±1038.75	<0.001[a], <0.001[b], 0.078[c]
ICAM-1	600.57±349.86	1069.93±391.73	1922.20±514.28	<0.001[a], <0.001[b], <0.001[c]
Fasting Glucose	72.03±9.30	71.03±8.97	67.57±6.01	0.154
Serum BUN level	16.36±4.82	22.27±7.19	28.20±12.76	<0.001[a], <0.001[b], 0.032[c]
Serum creatinine level	0.54±0.05	0.63±0.10	0.70±0.25	<0.001[a], <0.001[b], 0.819[c]
Proteinuri/day	0.30±0.13	1.31±0.88	4.60±3.88	<0.001[a], <0.001[b], <0.001[c]

a: between control and mild preeclampsia,
b: between control and severe preeclampsia,
c:between mild and severe preeclampsia,
GFR: glomerular filtration rate, VCAM-1: vascular cell adhesion molecule 1, ICAM-1: intercellular adhesion molecule 1, PAI-1: plasminogen activator inhibitor-1, TNF- α: tumor necrosis factor- α, IL-6: interleukin-6, hs-CRP:high sensitive CRP

Table 3. Correlation analysis for GFR and clinical and biomarker parameters

Parameters	*r*	*p*
TNF-α	**-0.220**	**0.017**
PAI-1	-0.154	0.097
IL-6	-0.141	0.128
hs-CRP	**-0,228**	**0.013**
VCAM-1	**-0,285**	**0.002**
ICAM-1	**-0,338**	**<0.001**
Systolic blood pressure (mmHg)	**-0,229**	**0.013**
Diastolic blood pressure (mmHg)	**-0,269**	**0.003**
Gestational age (week)	0.034	0.712
Fasting Glucose	0.026	0.782
Serum BUN level	**-0,600**	**<0.001**
Serum creatinine level	**-0,893**	**<0.001**
Proteinuri/day	**-0,365**	**<0.001**
BMI	**0,359**	**<0.001**

GFR: glomerular filtration rate, VCAM-1: vascular cell adhesion molecule 1, ICAM-1: intercellular adhesion molecule 1, PAI-1: plasminogen activator inhibitor-1, TNF- α: tumor necrosis factor- α, IL-6: interleukin-6, hs-CRP:high sensitive CRP

Table 4. Lineer regression analysis for glomerular filtration rate

Parameters	B	Std. Error	Beta	*t*	*P*
TNF-α	0.001	0.001	-0.031	-0.466	0.643
PAI-1	0.001	0.001	0.039	0.623	0.535
IL-6	0.014	0.059	0.015	0.243	0.809
hs-CRP	0.031	0.012	0.240	2.665	**0.009**
VCAM-1	0.000	0.000	0.275	2.230	**0.028**
ICAM-1	-0.006	0.007	-0.103	-0.824	0.412
Systolic blood pressure (mmHg)	0.212	0.235	0.130	0.902	0.370
Diastolic blood pressure (mmHg)	-0.397	0.360	-0.156	-1.103	0.273

VCAM-1: vascular cell adhesion molecule 1, ICAM-1: intercellular adhesion molecule 1, PAI-1: plasminogen activator inhibitor-1, TNF- α: tumor necrosis factor- α, IL-6: interleukin-6, hs-CRP:high sensitive CRP,

Discussion

The main findings of this single-center study are: 1) GFR levels were lower and serum creatinine levels were higher among patients with preeclampsia compared to the control group, 2) PAI-1, TNF-α, IL-6, VCAM-1 and ICAM-1 levels were higher in the patient group compared to the control group, 3) while GFR and TNF-α, hs-CRP, VCAM-1, ICAM-1, systolic blood pressure, diastolic blood pressure had significant negative correlation, a significant positive correlation was found for GFR and body mass, and 4) hs-CRP and VCAM-1 were independent variables related to GFR.

Preeclampsia is one of the most important causes of maternal and perinatal mortality [10]. Although the exact reason is still unknown, endothelial dysfunction is frequently mentioned in recent publications [11,12]. Improper placental development and placental ischemia may trigger oxidative stress, leading to the release of anti-angiogenic factors [13,14]. Some researchers even point out that inflammation also exists in normal pregnancy, however preeclampsia is a result of excessive inflammation [15,16]. Reduced placental growth factor (PIGH), tissue growth factor (TGF-ß) and VEGF levels together with excessive inflammation have peen proposed as pathophysiological mechanisms in recent years [17]. The process that leads from normal pregnancy to preeclampsia can be divided into two processes: vascular damage and excessive systemic inflammation. Vascular damage occurs in the time before the 20th week of pregnancy [18]. Markers like sFlt-1 and soluble endoglin that inhibit VEGF/TGF-ß contribute to endothelial dysfunction and are increased in both the amniotic fluid and

placenta in this term, and remain elevated until the end of pregnancy [19,20]. These markers also reduce the synthesis of nitric oxide [21]. The second process is systemic excessive inflammation, which begins with the 20th week of pregnancy when preeclampsia becomes manifest. Decreases in angiogenic factors continue in this period. This decrease triggers endothelial cell dysfunction and causes the release of various inflammatory markers and cytokines. Briefly, increasing inflammation accompanied by a decrease in angiogenesis leads to elevated blood pressure, renal injury and proteinuria that are clinical manifestations of preeclampsia [18].

Facca *et al.* have emphasized that the metabolic stress triggered by preeclampsia may cause endothelial injury and thus pave the way for future cardiovascular and chronic renal diseases [22]. How the mechanisms mentioned above effect renal injury in preeclampsia is not completely understood. VEGF is a crucial factor for glomerulus and podocyte functions and is the most studied angiogenic factor in the process of glomerulosclerosis caused by preeclampsia. A decrease in VEGF levels contributes to the development of glomerulosclerosis, as in preeclampsia, and is manifested as proteinuria [23,24]. It is shown in a study that the administration of exogenous VEGF causes a regression in arterial hypertension and proteinuria [25]. However, the data on the relationship between markers of endothelial dysfunction and renal function in patients with preeclampsia is limited. Wang *et al.* have shown that blood pressure and proteinuria regress in preclamptic rats and outcomes of pregnancy are better after administration of anti-ICAM [26]. In our study, ICAM-1 and VCAM-1, markers of endothelial injury, were correlated with decreased GFR in patients with preeclampsia. VCAM-1 was determined to be an independent variable that affects GFR. Szarka *et al.* have reported that VCAM-1 is related to kidney and liver functions [27]. Glomerular endothelial cells have fenestrations and are an important part of the glomerular filtration barrier. When endothelial signaling is suppressed and endothelial injury becomes manifest, the size and density of the fenestrations are reduced and the endothelium becomes thicker and swollen, forming 'endotheliosis' [28]. Adhesion molecules such as VCAM-1 and ICAM-1 enhance the adhesion of inflammatory cells like leukocytes, lymphocytes and thrombocytes to the glomerular endothelium and contribute to glomerular injury. However, histological studies are needed to address the role of adhesion molecules in glomerular endotheliosis.

The relation between inflammation and renal functions, the other mechanism in the pathogenesis of preeclampsia, has not been extensively investigated in comparison to the relation between the reduction of angiogenic factors such as VEGF and renal functions. Gadonski *et al*, have shown that blood pressure rises and renal perfusion flow decreases in rats infused with IL-6 [9]. But exactly how IL-6 causes this effect is not precisely known. Proinflammatory cytokines may be causing the sympathetic tonus and plasma renin levels to increase as well as triggering the course of events leading to endothelial dysfunction. There is a study reporting that heme oxygenase (HO), an antioxidant and anti-inflammatory enzyme, is reduced in preeclampsia. This observation suggests the possibility of using statins for their anti-inflammatory properties and their effect in increasing HO activity for the treatment of preeclampsia [29]. In this study, we attempted to partially address a lack of data in literature regarding the relationship between inflammation and renal function in patients with preeclampsia by evaluating the effects of inflammatory markers on GFR. As stated in our findings, markers like TNF-α, IL-6 and hs-CRP that play a key role in inflammation are negatively correlated with GFR. In particular, hs-CRP, widely used in everyday practice, is an independent variable that affects GFR, an observation that may lead to the use of this marker in patients with preeclampsia for predicting and evaluating renal dysfunction.

Limitations of the study

This is a one-center study with a limited number of patients. We evaluated only a small portion of the large number of proinflammatory cytokines and endothelial dysfunction markers. Although independent risk factors that effect GFR were evaluated by linear regression, it is possible that various residual and unknown factors may exist. We do not have any data to indicate how the levels of the mentioned markers may change during the course of preeclampsia. Moreover, the relation of inflam-

matory and endothelial dysfunction markers and the changes in renal functions were not evaluated because we were unable to follow the patients for long periods of time.

Conclusion

This study, conducted in a small population of patients with preeclampsia, has shown that there is a relation between inflammation and impaired renal function. The correlation of GFR with an affordable biochemical parameter such as hs-CRP may contribute vastly to monitoring and evaluating renal functions in our daily practice in preeclamptic patients and may light the way for new studies. Although the relationship between inflammation and preeclampsia is well known, there is insufficient evidence regarding the effect of inflammation on renal injury in preeclamptic patients. We hope this study leads the way to many studies with larger numbers of patients that measure all inflammatory and endothelial dysfunction markers and we may very well be discussing the use of anti-inflammatory drugs in cases of preeclampsia in the coming years.

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References

1. *Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Seminars in Perinatology 2012; 36(1): 56–59*

2. *Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe preeclamptic pregnancies. Br J Obstet Gynaecol 1994; 101(8): 669–674*

3. *Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 2011; 123(24): 2856–2869*

4. *Kincaid-Smith P. The renal lesion of preeclampsia revisited. J Kidney Dis 1991; 17(2): 144–148*

5. *Karumanchi SA, Maynard SE, Stillman IE, Epstein FH, Sukhatme VP. Preeclampsia: A renal perspective. Kidney Int 2005; 67(6): 2101-2113*

6. *Moffett A, Hiby SE. How does the maternal immune system contribute to the development of pre-eclampsia? Placenta 2007; 28(Suppl. A): 51–56*

7. *Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JN Jr, et al. Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. Am J Obstet Gynecol 1999; 181(4): 915–920*

8. *Xie C, Yao MZ, Liu JB, Xiong LK. A meta-analysis of tumor necrosis factor-alpha, interleukin-6, and interleukin-10 in preeclampsia. Cytokine 2011; 56(3): 550–559*

9. *Gadonski G, LaMarca BB, Sullivan E, Bennett W, Chandler D, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of interleukin 6. Hypertension 2006; 48(4): 711–716.*

10. *World Health Organization. Make Every Mother and Child Count: the World Health Report 2005. Available at http://www.who.int/whr/2005/en/ (accessed on Novamber 1, 2012)*

11. *Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol 1998; 179(5): 1359-1375*

12. *Ahmed A. New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications. Thromb Res 2011; 127(Suppl 3): 72-75*

13. *Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta 2009; 30(6): 473-482*

14. *Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol 2010; 63(6): 534-543*

15. *Willis C, Morris JM, Danis V, Gallery ED. Cytokine production by peripheral blood monocytes during the normal human ovulatory menstrual cycle. Hum Reprod 2003; 18(6): 1173-1178*

16. *Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999; 180(2 Pt 1): 499-506*

17. *Ahmed A. Heparin-binding angiogenic growth factors in pregnancy. Trophoblast Res 1997; 10: 215–258.*

18. *Ramma W, Ahmed A. Is inflammation the cause of pre-eclampsia? Biochem Soc Trans 2011; 39(6): 1619-1627*

19. *Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004 12; 350(7): 672-683*

20. *Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006; 12(6): 642-649.*

21. *Jerkic M, Rivas-Elena JV, Prieto M, Carrón R, Sanz-Rodríguez F, Pérez-Barriocanal F, et al. Endoglin regulates nitric oxide-dependent vasodilatation. FASEB J 2004; 18(3): 609-611*

22. *Facca TA, Kirsztajn GM, Sass N. Preeclampsia (marker of chronic kidney disease): from genesis to future risks. J Bras Nefrol 2012; 34(1): 87-93*

23. *Stillman IE, Karumanchi SA. The glomerular injury of preeclampsia. J Am Soc Nephrol 2007; 18(8): 2281-2284.*

24. *Hara M, Yanagihara T, Kihara I, Higashi K, Fujimoto K, Kajita T. Apical cell membranes are shed into urine from injured podocytes: a novel phenomenon of podocyte injury. J Am Soc Nephrol 2005; 16(2): 408-416*

25. *Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, et al. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. J Biol Chem 2003; 278(15): 12605-12608*

26. *Wang Z, Zou H, Yu Y, Song Y. Monoclonal antibody to intercellular adhesion molecule-1 as a novel therapy for preeclampsia: preliminary results from a rat model. J Matern Fetal Neonatal Med 2012; 25(6): 855-859*

27. *Szarka A, Rigo J, Lazar L, Beko G, Molvarec A. BMC Immunology 2010, 11: 59*

28. *Satchell SC, Braet F. Glomerular endothelial cell fenestrations: An integral component of the glomerular filtration barrier. Am J Physiol Renal Physiol 2009; 296(5): 947-956*

29. *Zenclussen AC, Lim E, Knoeller S, Knackstedt M, Hertwig K, Hagen E, et al. Heme oxygenases in pregnancy II: HO-2 is downregulated in human pathologic pregnancies. Am J Reprod Immunol 2003; 50(1): 66-76.*

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Biocompatibility of wrist joint fusion and functions in the patients with traumatic carpal arthritis after titanium plate implantation

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Abstract

Objective: To evaluate the biocompatibility of wrist joint fusion and its motional functions in the patients with traumatic carpal arthritis after steel plate implantation.

Methods: 31 patients with traumatic carpal arthritis who were treated by the placement of internal fixation material with steel plates for wrist joint fusion in Department of Orthopedics in our hospital from March 2011 to December 2012 were selected in this study, of which 24 patients were followed up for more than 9 months, including 13 males and 11 females with the mean age of 45 years old (31~60 years old).

Results: There were 13 cases of slight dorsal extension dysfunction in metacarpophalangeal joints, which mainly occurred in thumb, index and middle fingers without confined motions. 8 patients had slight initiative dorsal extension dysfunction in thumb interphalangeal joints, and 2 patients complained limited forearm rotation when distal radioulnar joints or ulnar wrist joints ached. In the 24 patients, 15 felt no pain completely, 6 felt pain in wrist joints after heavy physical labor which could be tolerable, and 3 suffered from pain in daily life or forearm rotation which affected the work. The grip strengths of 16 patients reached higher than 80% of that of the other side, those of 6 patients reached 60% to 80%, and those of 2 patients were lower than 60%.

Conclusion: Pain can be significantly alleviated after the fusion of wrist joints with steel plates placed, but there might be loss of some functions in wrist joints after the surgery. There is good biocompatibility between steel materials and human body without triggering special adverse reactions.

Key words: Wrist joint fusion, internal fixation material, steel plate, bone implant, biocompatibility.

Introduction

Wrist joints consist of 3 rows of joints, namely the joints between radius and proximal row of carpus, those between proximal and distal rows of carpus, and those between distal row of carpus and metacarpal base. Carpal arthritis refers to various joint inflammations involving wrist, including rheumatic, rheumatoid and traumatic arthritis, osteoarthritis and septic arthritis, etc. Carpal arthritis can be divided into acute and chronic types according to the symptoms and courses of disease. Acute carpal arthritis has an acute onset, with the major signs of heat, redness, swelling and pain in wrist joints, dysfunction and systemic symptoms such as fever. Chronic carpal arthritis is mainly manifested as joint swelling, pain, deformity and varying degrees of dysfunction [1-3]. In the early stage, arthritis only injures the synovium of joint, while articular cartilage and bone quality may be changed or even destroyed in later phase. The damages of wrist joints are often accompanied by significant trauma, wrist weakness and wrist activity problems. There is no obvious swelling or pain in minor injuries, in which pain can only be felt upon sharp wrist motion. If the wrist is badly sprained, patients may suffer from swelling, severe pain, and cannot move wrist joints during motion [4-6].

The fusion of wrist joints, which is considered to be optimal in treating traumatic carpal arthritis, aims to relieve the pain of wrist joints via diverse fusion methods and fixed materials. In recent years, the materials of internal fixation with steel plates have been increasingly used in the fusion of

wrist joints, the advantages of which include desirable steel plate compression and rigid fixation of wrist joints, thereby moving adjacent joints in an early stage and preventing joint stiffness [7].

Materials and methods

Objects

31 patients with traumatic carpal arthritis who were treated with placement of internal fixation material with steel plates for wrist joint fusion in the Department of Orthopedics of our hospital from Mar. 2011 to Dec. 2012 were selected in this study, of which 24 patients were followed up, including 13 males and 11 females, with a mean age of 45 years old (31~60 years old). All patients were right-handed. There were 15 cases of C3 distal radius fracture (AO type), 9 cases of arthritis caused by old scaphoid fracture, including 2 cases of ulnar fracture. 2 patients had undergone open reduction of fracture, and ulnar open reduction and internal fixation respectively before the surgery.

Methods

The surgery was mainly completed by performers who had been engaged in orthopedic professional work for 10 years or above as experienced doctors whose qualification met the requirements for post technical standards. After brachial plexus or general anesthesia, pneumatic tourniquet was used, S-shaped incision was made on dorsal wrist which was cut open layer-by-layer to reveal extensor tendon retinaculum, the third extensor tendon sheath was cut open sharply, the fourth extensor tendon sheath was dissociated along the surface of wrist joint capsule via the third extensor tendon sheath to stretch the extensor pollicis longus muscle tendon to the radialis side and the finger extensor tendon to the ulnar side; dorsal interosseous nerve was searched from the base of the fourth extensor tendon sheath, a nerve segment of 1.5 cm long was resected, the radius and ulna interosseous membrane was made a longitudinal incision of 2.0 to 3.0 cm to find palmar interosseous nerve which was then resected by 1.5 cm [8].

The dorsal carpal joint capsule was made a longitudinal incision for the sharp dissection of the periosteum on the dorsal surface of the third metacarpal bone, carpus and radius; the Lister node was chiseled away by osteotome, and bone rongeur was used to cut the cartilage facet of adjacent surface of radius, scaphoid bone, semilunar bone, capitate bone as well as small and large multangulums. The lilium spongy bone was implanted into the gap between the radiocarpal bone and median wrist; the straight titanium plate (Synthe, U.S.A.) was bent by 15º; the third metacarpal bone, head bone and radius were fixed with steel plates, and the gap between the steel plates and the wrist was filled with lilium spongy bone. During the surgery, C-arm X-ray system was used to observe the position of fusion of wrist joints and the positions of plate screws. It was carefully observed whether the ulna was too long and whether it would affect the pronation and supination of wrist joints; the head of ulna was resected if necessary. The wound was washed, the steel plates were covered with the surrounding soft tissues as far as possible, and the joint capsule and the fourth extensor tendon sheath were sutured with 5/0 absorbable sutures. A drainage catheter was placed in the surface of wound, and then skin was sutured. The forearm was fixed with outer plaster support for two weeks [9].

Anteroposterior and lateral X-ray examination was performed during follow-up visit to observe the healing of wrist joints and the positions of plate screws.

The pain degree of wrist joints was evaluated by visual analog scale, 0 of pain value for no pain and 10 for intolerable severe pain. The grip strength of both hands was measured using Jamar wrist developer, 3 times for either side for averaging [10].

Results

Basic information of participants

All subjects felt wrist pain, limited rotation, decreased grip strength, who received fusion of wrist joints. The materials for internal fixation were straight titanium plates made by Synthe (USA). The positions of internal fixation materials placed were the third metacarpal bone, head bone and radius, one plate for each position. The other basic conditions are shown in Table 1.

Overall evaluation results

24 subjects were subjected to anteroposterior and lateral X-ray examination during follow-up

Table 1. Basic information of participants

No.	Gender	Age	Disease cause	Fracture position feature	Injury side	Time from injury to surgery (d)
1	Male	34	Contusion	Distal radius fracture	Right	3
2	Male	36	Tumble injury	Old scaphoid fracture	Right	2
3	Male	49	Tumble injury	Distal radius fracture	Right	3
4	Female	38	Tumble injury	Distal radius fracture	Left	3
5	Female	56	Contusion	Distal radius fracture	Left	2
6	Male	31	Chop wound	Old scaphoid fracture	Right	2
7	Female	44	Tumble injury	Old scaphoid fracture	Right	1
8	Male	37	Contusion	Distal radius fracture	Left	1
9	Male	42	Chop wound	Old scaphoid fracture	Left	3
10	Female	41	Contusion	Distal radius fracture	Left	2
11	Male	39	Contusion	Distal radius fracture	Left	3
12	Male	51	Tumble injury	Distal radius fracture	Right	2
13	Female	55	Tumble injury	Old scaphoid fracture	Right	2
14	Female	50	Tumble injury	Old scaphoid fracture	Left	1
15	Male	47	Contusion	Distal radius fracture	Left	2
16	Female	34	Tumble injury	Old scaphoid fracture	Right	2
17	Male	60	Tumble injury	Distal radius fracture	Left	1
18	Male	39	Tumble injury	Distal radius fracture	Right	2
19	Female	58	Contusion	Distal radius fracture	Left	2
20	Male	48	Contusion	Distal radius fracture	Right	2
21	Female	44	Contusion	Old scaphoid fracture	Right	3
22	Female	42	Tumble injury	Old scaphoid fracture	Right	1
23	Male	56	Contusion	Distal radius fracture	Right	2
24	Female	37	Tumble injury	Distal radius fracture	Left	2

visit, the results of which showed good fusion condition, no occurrence of plates and screws fracture, corrosion, loosening or twisting, nor inflammation, rejection or other side effects (Table 2).

Follow-up results

24 patients were followed up for 9 to 24 months, 16 months on average. 1) Results of subjective function evaluation: There were a patient complaining wound discomfort, scar formation, affecting appearance during the follow-up visit, 8 patients with fully normal function, 14 cases of restricted wrist joints in certain motions and 2 cases of dysfunction. 2) Joint range of motion: There were 13 cases of slight dorsal extension dysfunction in metacarpophalangeal joints, which mainly occurred in thumb, index and middle fingers, without confined motion in metacarpophalangeal joints; 8 patients had slight initiative dorsal extension dysfunction in thumb interphalangeal joints, and 2 patients complained limited forearm rotation when distal radioulnar joint or ulnar wrist joints ached. 3) Degree of pain: The value of preoperative pain after forced weight bearing was 4.9 (3～8). During the follow-up, all patients complained that the degree of pain of wrist joints was significantly alleviated compared with that before, with the postoperative pain value of 1.0 (0~2) and pain value after forced weight bearing was 1.9 (0~3). In the 24 patients, 15 felt no pain completely, 6 felt pain in wrist joints after heavy physical labor which could be tolerable and 3 suffered from pain in daily life or forearm rotation, which affected the work. 4) Grip strength: The grip strength was 14 (4~26) kg before the surgery, 33 (24～39) kg during the follow-up visit, and 36 kg on the normal side. The grip strength of 16 patients reached HIGHER than 80% of that of the other side; 6 patients 60% to 80% and 2 patients below 60%. 5) Overall function evaluation after fusion of wrist joints: The Buck-Gramcko/Lohmannn score was 9.4. 6) Subjective functional evaluation after fu-

Table 2. Overall evaluation results

No	Surgery time (min)	Intraoperative bleeding (ml)	Follow-up time (month)	Function evaluation			Buck-Gramcko/ Lohmannn score	DASH investigation scale
				Joint movement	Pain degree	Grip strength (kg)		
1	80	60	11	Slightly limited joint movement	0	39	10	29
2	70	50	9	Tolerable	0	24	9	31
3	110	50	10	Slightly limited joint movement	0	36	12	36
4	100	60	13	Tolerable	1	37	8	31
5	60	30	16	Tolerable	0	32	6	33
6	70	50	14	Slightly limited joint movement	0	28	8	35
7	50	40	16	Tolerable	0	29	9	36
8	90	50	12	Tolerable	0	28	7	36
9	110	60	17	Tolerable	1	31	10	30
10	80	80	24	Tolerable	2	35	11	29
11	70	70	18	Slightly limited joint movement	0	38	10	37
12	70	60	17	Slightly limited joint movement	0	37	8	36
13	80	50	9	Tolerable	0	33	9	29
14	90	40	12	Tolerable	0	34	13	28
15	80	60	13	Tolerable	0	35	12	34
16	100	30	15	Slightly limited joint movement	1	36	8	36
17	70	40	10	Tolerable	0	30	9	33
18	80	50	9	Slightly limited joint movement	0	28	10	35
19	60	60	16	Tolerable	0	24	9	27
20	120	40	17	Tolerable	2	26	11	28
21	90	30	14	Tolerable	0	28	12	35
22	110	50	20	Slightly limited joint movement	0	31	14	31
23	80	60	21	Slightly limited joint movement	1	28	9	28
24	70	70	9	Tolerable	0	34	10	29

sion of wrist joints: The upper limb function was assessed using the DASH questionnaire, the average value of which was 34, indicating that the surgery had a relatively great impact on affected limb function, which was mainly manifested in all restricted daily motions in the need for flexible use of wrist joints, and the work of much physical strength was obviously affected. 3 patients complained wrist pain, and 1 patient numb and needle-like pain in wrist which affected sleep.

Adverse reactions

After the surgery, there were 2 cases of hemorrhage in wound surface which were healed by first intention after taking out part of the sutures and drainage; 2 cases of wound skin irritation which were cured after the use of antibiotics; 1 case of skin edge necrosis which was cured by dressing change; and 3 cases of hemorrhage after cutting cancellous bone from ilium which was healed by taking out part of the sutures and drainage.

Discussion

The follow-up results of internal fixation with steel plates in fusion of wrist joints had distinct differences. Cavaliere et al. reported that a total of 50 patients got 82 cases of complications in 73 patients who received AO/AISF plate fixation in wrist joints [11]. Gaulke et al. reported that the incidence rate of postoperative complication was 29%, which included wound hemorrhage, wound infection, irritation of steel plates on extensor tendon and tendon rupture, deep branch of ulnar nerve injury and carpal tunnel syndrome [12]. Through follow-up, it was found in this study that early postoperative complications were mainly wound hemorrhage and infection which were cured after corresponding treatment, and in the late stage, steel plates caused extensor tendon adhesions, affecting the initiative dorsal extension activities of metacarpophalangeal joints. Some scholars have reported that after fusion of wrist joints, the incidence rate of carpal tunnel syndrome is up to 25%, the reason of which is that iliac bone implanted in wrist joints oppresses palmar joint capsule, causing neurothlipsis, but no similar results were found in the follow-up visit in this study [13-15].

The follow-up found that the motion of metacarpophalangeal joints and thumb interphalangeal joints were slightly affected in some patients after fusion of wrist joints. There were 13 patients with initiative dorsal extension dysfunction in metacarpophalangeal joints after the surgery which was mainly manifested in the metacarpophalangeal joints on the radial side obviously constrained, but the metacarpophalangeal joints of ring and little fingers hardly limited. It was mainly because the steel plates used were relatively wide, and were difficult to be completely covered with soft tissues, which caused the steel plates had a direct contact with the radial side of extensor tendon, leading to adhesions. The constrained dorsal extension function of thumb interphalangeal joint was associated with the resection of the Lister node and loss of fulcrum in extensor pollicis longus muscle tendon [16-18].

The follow-up visit found that nearly 70% of the patients could not feel pain after fusion of wrist joints, but 30% of the patients appeared wrist joints pain which was mostly in the ulnar side. Rodgers and Toma also found that there were still varying degrees of pain in a small part of the patients after fusion of wrist joints. By analysis, the reasons might lie in the following aspects: before surgery, the patients might not only suffer from radial wrist and mediocarpal joint injuries, but also be accompanied by distal radioulnar joint and TFCC injuries which were not treated during fusion of wrist joints; multiple articular cartilage surfaces were cut during the surgery, and compression fixation with steel plates might lead to too long ulna relative to radius, resulting in postoperative ulnar impaction syndrome, so as to cause ulnar pain of wrist joints and forearm rotation dysfunction [19,20]. Therefore, we believe that wrist should be carefully examined before surgery, especially for patients with ulnar pain in wrist joints, to prevent the missed diagnosis of distal radioulnar joint or TFCC injuries. In addition, C-arm X-ray system is used during the fusion for fluoroscopy, when if ulna is observed relatively too long, the ulnar head might be resected to prevent pain caused by postoperative ulnar impaction syndrome.

The Buck-Gramcko/Lohmmannn score after fusion of wrist joints was 9.4, indicating that hand function is less affected after the fusion of wrist joints. However, by further in-depth evaluation of the influence of fusion of wrist joints on upper limb function using the DASH questionnaire for patient self evaluation, the score was 34, which shows that fusion of wrist joints has a great impact on daily life, and the loss of activity is mainly manifested in flexible use of wrist joints and heavy physical labor. After fusion of wrist joints, the heavy physical labor of some patients were affected, which might be related with the reason that wrist strength can not be fully recovered and there are still some patients suffering from postoperative wrist joint pain. Therefore, patients should be explained clearly in preoperative communication before fusion of wrist joints, and put emphasis on early functional exercises after this surgery.

References

1. *Thabet AM, Kowtharapu DN, et al. Wrist fusion in patients with severe quadriplegic cerebral palsy. Musculoskelet Surg, 2012; 96(3): 199-204.*
2. *Lehner B, Jung M, et al. Total wrist fusion with vascularized fibula graft after tumor resection of the distal radius. Oper Orthop Traumatol, 2012; 24(3): 186-95.*
3. *Richards AA, Afifi AM, et al. Four-corner fusion and scaphoid excision using headless compression screws for SLAC and SNAC wrist deformities. Tech Hand Up Extrem Surg, 2012; 15(2): 99-103.*
4. *Iagnocco A, Perella C, et al. Magnetic resonance and ultrasonography real-time fusion imaging of the hand and wrist in osteoarthritis and rheumatoid arthritis. Rheumatology (Oxford), 50(8): 1409-13.*
5. *Berkhout MJ, Shaw MN, et al. The effect of radioscapholunate fusion on wrist movement and the subsequent effects of distal scaphoidectomy and triquetrectomy. J Hand Surg Eur Vol, 2010; 35(9): 740-5.*
6. *Gupta RK, Chauhan DS, et al. Non-union scaphoid: Four-corner fusion of the wrist. Indian J Orthop, 2010; 44(2): 208-11.*
7. *Kalb K, Prommersberger KJ, et al. Total wrist fusion using the AO wrist fusion plate. Oper Orthop Traumatol, 2009; 21(4): 498-509.*
8. *Terzis JK, Barmpitsioti A, et al. Wrist fusion in posttraumatic brachial plexus palsy. Plast Reconstr Surg, 2009; 124(6): 2027-39.*
9. *Bedford B, Yang SS, et al. High fusion rates with circular plate fixation for four-corner arthrodesis of the wrist. Clin Orthop Relat Res, 2009; 468(1): 163-8.*
10. *Gaulke R, O'Loughlin PF, et al. Radiolunate fusion in the rheumatoid wrist via three point fixation with a mini-titanium-T-plate and oblique screw. Technol Health Care, 2009; 17(4): 345-51.*
11. *Cavaliere CM, Oppenheimer AJ, et al. Reconstructing the rheumatoid wrist: a utility analysis comparing total wrist fusion and total wrist arthroplasty from the perspectives of rheumatologists and hand surgeons. Hand (N Y), 2010; 5(1): 9-18.*
12. *Gaulke R, Suppelna G, et al. Radiolunate fusion in the rheumatoid wrist with Shapiro staples: clinical and radiological results of 22 cases. J Hand Surg Eur Vol, 2010; 35(4): 289-95.*
13. *Gong HS, Jeon SH, et al. Ulnar-sided wrist pain after four-corner fusion in a previously-asymptomatic ulnar positive wrist: a case report. Hand Surg, 2010; 14(1): 49-51.*
14. *Masuko T, Iwasaki N, et al. Radiolunate fusion with distraction using corticocancellous bone graft for minimizing decrease of wrist motion in rheumatoid wrists. Hand Surg, 2011; 14(1): 15-21.*
15. *Givissis PK, Antonarakos P, et al. Management of posttraumatic arthritis of the wrist with radiolunate fusion enhanced with a sliding autograft: a case report and description of a novel technique. Tech Hand Up Extrem Surg, 2010; 13(2): 90-3.*
16. *Muhldorfer M, Hohendorff B, et al. Medium-term results after radioscapholunate fusion for post-traumatic osteoarthritis of the wrist. Handchir Mikrochir Plast Chir, 2009; 41(3): 148-55.*
17. *Ho PC. Arthroscopic partial wrist fusion. Tech Hand Up Extrem Surg, 2010; 12(4): 242-65.*
18. *Bialocerkowski AE. Activity limitations and compensatory mechanism use following limited wrist fusion. Arthritis Rheum, 2011; 59(10): 1504-11.*
19. *Rodgers JA, Holt G, et al. Scaphoid excision and limited wrist fusion: a comparison of K-wire and circular plate fixation. Hand (N Y), 2011; 3(3): 276-81.*
20. *Toma CD, Machacek P, et al. Fusion of the wrist in rheumatoid arthritis: a clinical and functional evaluation of two surgical techniques. J Bone Joint Surg Br, 2012; 89(12): 1620-6.*

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The evaluation of sexual dysfunctions in men with thyroid disorders

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Abstract

Introduction: The aim of this study was to evalaute the sexual dysfunctions in males both before and after the treatment of thyroid disorders.

Material and Methods: Between January 2009 and February 2011 a total of 46 men with hypothyroidism and hyperthyroidism were evaluated prospectively. Patients were divided into three groups as fallows; Group 1 (n=35) control group, group 2 (n=25) hypothyroid patients group and group 3 (n=21) hyperthyroid patients group. The erectile status of the patients were evaluated with International Index of Erectile Function (IIEF) form. Additionally psychologic assesment of the patients were evaluated.

Results: The mean age of the patients in group 1, group 2 and group 3 was 54.71±5.76 years, 54.32±8.78 years and 54.57±14.37 years, respectively (P=0.988). Before the thyroid disease treatments the mean IIEF scores were 23.74±4.22, 20.56±10.40 and 22.10±8.66 in group 1, group 2 and group 3, respectively. After the establishment of euthyroidism the mean IIEF scores improved significantly only in group 2 (p=0.022). At the initial evaluation, the premature ejaculation rates were 22.9% (n=8), 44% (n=11) and 61.9% (n=13) in group 1, 2 an 3, respectively. Premature ejaculation rates were significantly higher in group 3 in comparison to group 1 (p=0.013). After the normalization of thyroid functions these ratios detected as 40% and 38.1% in group 2 and in group 3, respectively. In group 3, the improvement rate of premature ejaculation was higher compared to group 2. Mean BDI score improved both in group 2 and group 3. Beck Anxiety Inventory score was significantly decreased in group 3 in posttreatment period compared to pretreatment period (p=0.001).

Conclusions: According to these results we can say that hyperthroidism and hypothyroidism may cause sexual dysfunction in men. In men presenting with erectile dysfunction and ejaculatory disorders thyroid dysfunctions should be considered in the etiology of male sexual dysfunction.

Key Words: Erectile dysfunction, premature ejaculation, hyperthyroidism, hypothyroidism.

Introduction

Penile erection involves a complex interaction between the central nervous system and local factors. To achieve an erection the penile vascular smooth muscle integrity in the corpus cavernosum is very important. During tumescence, by parasympathetic stimulus the release of nitric oxide stimulates smooth muscle relaxation in the corpus cavernosum (1). The resulting dilation of the cavernosal arterioles and sinuses results in increased blood flow and a subsequent rise in intracavernosal pressure. This initial rise in intracavernosal pressure activates a veno-occlusive mechanism to limit the outflow of blood and further increases the pressure inside the cavernosal bodies. The erectile response ensues as the force of the elevated pressure expands the outer tunica albuginea of the penis, resulting in the increased penile length and diameter characteristic of erection (2). Normally, this erectile process results in ejaculation. In this erection mechanism, several signal molecules and neurotransmitters may play a role (1,2). For normal penile erection the integration of normal psychological, neurological, and normal vascular processes are required. Several factors can disrupt the normal physiologic mechanisms involved in penile erection. Thus, in any problem occurs in the integrity of this system, erectile problems develop. Erectile dysfunction (ED) is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance (3). The etiology of ED is multifactorial and is associated with several recognized risk factors, including hypertension, heart disease, aging, obesity, diabe-

tes mellitus, pelvic surgery, hyperlipidemia, smoking, psychiatric diseases, and medications (4). Some of the hormones such as adrenocorticotropic hormone, oxytocin, prolactin, androgens, especially testosterone and thyroid hormones, have also been implicated in the modulation of erectile function (5). There are two principal thyroid hormones which are produced and secreted by the thyroid, **thyroxine** (commonly known as **T4**) and **triiodothyronine** (**T3**). The synthesis of thyroid hormones is controlled by hypothalamic thyrotropin-releasing hormone (TRH) and pituitary thyroid-sitimulating hormone (TSH). Thyroid hormones play a critical role in normal human physiology with effects on almost all tissues (6). Most of these effects are mediated via mechanisms that stimulate resting metabolic rate, increase ATP expenditure, and modulate adrenergic receptor number and thus responsiveness to catecholamines (7). The association between ED and thyroid hormones have been reported in a limited number of studies (8,9). Similar to ED premature ejaculation is also considered as one of the most common sexual complaints in men. As in the mechanism of erectile process, hormones directly or indirectly regulate ejaculation mechanism (10). A few animal and clinical studies have investigated the anatomic and physiologic interactions between ejaculation disorders and thyroid hormones (10).

Although in a few studies authors have been addressed the relationship between thyroid disorders and sexual dysfunction, this relationship between ED and ejaculatory problems and thyroid hormones have not been studied extensively. The aim of this study was to prospectively evaluate the prevalence of sexual dysfunctions in male patients with hyperthyroidism and hypothyroidism and to compare the sexual dysfunctions of the patients before and after the treatment of these conditions.

Material and methods

Between January 2009 and February 2011 a total of 46 men with thyroid disorders who admitted to endocrinology outpatient department and referred to urology department were evalauted prospectively. The local ethical committee approval (Ethical approval number; 09-GEKTIP-021) was obtained and all of the men gave written informed consent at the beginning of the study as well. Patients were evaluated with physical examination, plain abdominal graphy, urinalysis, hormon profile including testosterone, prolactine, follicle stimulating hormone (FSH), **luteinizing hormone** (LH), triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), routine hematologic and biochemical analysis and urinary system ultrasonography if needed. If patients had symptoms of prostate inflammation/infection, the Meares-Stamey test was performed. Patients were divided into three groups as fallows; Group 1 (n=35) control, group 2 (n=25) hypothyroid patients group and group 3 (n=21) hyperthyroid patients group. The International Index of Erectile Function (IIEF) questionnaire was administered to all patients to detect of the degree of the ED. A score higher than 26 was considered as normal, between 11 and 25 was diagnostic of mild to moderately ED, and 10 or less was to indicate severe ED. In the diagnosis of premature ejaculation, stopwatch measurements was used by the patient or his partner. Psychiatric status of the patients was evaluated by the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) scales. All parameters of initial evaluation were repeated at 24 to 36 weeks after the achievement of euthyroidism. Exclusion criteria were as follows; neurologic conditions such as spinal cord injury and multiple sclerosis, serum creatinine >200 mol/l, age less than 18 years, maximum urinary flow <15 ml/sec, active urinary infection, analgesic/narcotic dependency and chronic usage of drugs such as antidepressants, antihistaminics and anxiolytic agents.

Initially all hyperthyroid patients were given to an iodine deficient diet concomitant with an antithyroid drug (150 to 300 mg propylthiouracil/day). After 2 months of medical treatment patients were enrolled to receive maintenance medical treatment. The high initial starting dose was gradually reduced when the patient became euthyroid to maintenance dose of 50-200 mg propylthiouracil. Treatment of hypothyroidism was initiated with 25-100 μg T4 daily and the patients were reviewed at 2-3 weeks and if all is well the dose of T4 was then increased.

Thyroid function and male reproductive hormone axis were investigated using electrochemiluminescence immunoassay (ECLIA) commercial kits. The normal reference ranges for thyroid hor-

mones were as follows: TSH, 0.005-100.0 μIU/mL; T_4, 4.5–12.5 μg/dl; free T_4 (fT_4), 0.023- 7.77 ng/dL (0.300- 100.0 pmol/L); T_3, 0.8–2.2 ng/ml; fT_3, 2,0- 4.4 pg/mL (3.1-6.8 pmol/L). The normal reference ranges for reproductive hormones were as follows: LH, 0.100-200 mIU/mL; PRL, 1.00-10000 μIU/mL (0.0470-470 ng/mL); total testosterone (tT), 0.025-15.0 ng/mL (0.087-52.0 nmol/L); free testosterone (fT), 12–40 pg/ml (40–140 pmol/liter); estradiol (E_2), 18.4 -1578 pmol/L (5.00-4300 pg/mL); SHBG, 10–55 nmol/liter.

Statistical Analysis

Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables were normal. Accordingly, it was seen that some continuous variables not displayed a normal distribution. Two independent sample t test or Mann Whitney U test was used to compare the urologic and psychometric parameters between groups. Wilcoxon rank sum test was used to compare the urologic and psychometric parameters between pre and post-treatment terms separately for groups. The continuous variables were presented as the mean ±standard deviation for normally distributed variables and median and interquartile range (IQR, Q1 to Q3) for non-normally distributed variables. Chi-Square test was used to compare the categorical variables between groups. Mc-Nemar test was used to compare the categorical variables between pre and post-treatment terms separately for groups. Categorical variables were presented as a count and percentage. A p-value <0.05 was considered significant. Analyses were performed using commercial software (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY)

Results

The mean age of the patients in group 1, group 2 and group 3 was 54.71±5.76 years, 54.32±8.78 years and 54.57±14.37 years, respectively (P=0.988). Mean serum TSH levels at the initial evaluation was 19.26 IU/ml (median 0.06, range 0.001 to 0.33), 33.68 IU/ml and 0.054 IU/ml in group 1, group 2 and group 3, respectively. After the treatment TSH levels were detected as 3.37 IU/ml and 2.55 IU/ml in group 2 and group 3, respectively. At the initial evaluation, the premature ejaculation rates were 22.9% (n=8), 44% (n=11) and 61.9% (n=13) in group 1, group 2 and group 3, respectively. Premature ejaculation rates were significantly higher in group 3 compared to group 1 (p=0.013). After the treatment, the premature ejaculations ratios were detected as 40% and 38.1% in group 2 and group 3, respectively (p>0.05). In group 3, the improvement rate of premature ejaculation was higher in comparison to group 1. Before the antithyroid hormone replacement theraphy the mean IIEF scores were 23.74±4.22, 20.56±10.40 and 22.10±8.66 in group 1, 2 and 3, respectively. Although IIEF scores were lower in group 2 and in group 3 compared to group 1, there were no statistically significant difference between the groups. After the normalization of thyroid function with treatment the IIEF scores were 23.96±8.78 and 21.71±8.37 in group 2 and group 3, respectively. The IIEF scores improved significantly only in group 2, after the euthyroidsm was achived (p=0.022). The mean testosterone levels were detected as 5.03±1.64, 4.69±1.84 ng/mL and 5.25±1.65 ng/mL in group 1, 2 and 3, respectively. The clinical and demographic characteristics of the patients are shown in table 1 and table 2. All hormone prophiles were within normal ranges. The mean FSH, LH, prolactine, total testesterone and estradiol levels were not statistically different in pretreatment and post treatment periods between the groups. In pretreatment period the BDI scores were detected as 1.43±4.36, 4.40±6.16 and 3.90±7.52 in group 1, in group 2 and in group 3, respectively (p=0.013). After the treatment of thyroid disorders the mean BDI scores were 4.40±6.16 and 3.90±7.52 in group 2 and in group 3 (p=0.270, p=0.067), respectively. In pretreatment period, the BAI scores were significantly higher in group 2 and group 3, compared to group 1 (p<0.05). Beck Anxiety Inventory scores were significantly decreased in group 3 in posttreatment period compared to pretreatment period (p=0.001).

Discussion

Male sexual dysfunctions can be divided into two main categories as ED and ejaculatory disorders. Erectile dysfunction which causes a substantial negative impact on intimate relationships, quality of life, and self-esteem is a common sexual problem in men with a reported prevalence of 52% in men aged 40 to 70 years in the United States

Table 1. The comparison of the clinical and demographic characteristics of the groups.

		Control (n=35)	Hypothyroidy (n=25)	Hyperthyroidy (n=21)	[1]p
Age (year)		54.71±5.76	54.32±8.78	54.57±14.37	0.988
IIEF	Pre-Treatment	23.74±4.22	20.56±10.40	22.10±8.66	0.295
	Post-Treatment	-	23.96±8.78	21.71±8.37	0.383
	[2]p	-	**0.022**	0.944	
Beck Anxiety Total Score	Pre-Treatment	0.69±2.00	4.24±5.95	9.52±7.84	**<0.001**[a]
	Post-Treatment	-	3.69±5.34	2.86±5.07	0.597
	[2]p	-	0.403	**<0.001**	
Beck Depression Total Score	Pre-Treatment	1.43±4.36	5.48±7.77	6.33±8.04	**0.013**[b]
	Post-Treatment	-	4.40±6.16	3.90±7.52	0.807
	[2]p	-	0.270	0.067	
Pre-Ejaculation	Pre-Treatment	8 (22.9%)	11 (44.0%)	13 (61.9%)	**0.013**[b]
	Post-Treatment	-	10 (40.0%)	8 (38.1%)	1.000
	[2]p	-	1.000	0.125	
FSH		6.57±3.12	5.70±2.76	6.89±7.44	0.641
LH		6.10±2.39	5.31±1.62	6.15±3.73	0.445
Prolaktin		9.46±4.26	9.17±4.08	7.60±2.71	0.206
Total Testesteron		5.03±1.64	4.69±1.84	5.25±1.65	0.524
Estradiol		27.45±6.44	28.81±6.36	29.83±6.24	0.384

Data were presented as mean±standart deviation and n (%).
[1]: Statistical results of the comparison between Hypothyroidy and Hyperthyroidy groups.
[2]: : Statistical results of the comparison comparison between pre and post treatments.
[a]: There was statistically significant differences between control and Hypothyroidy and between control and Hyperthyroidy groups.
[b]: There was statistically significant differences between control and Hyperthyroidy groups.

Table 2. The distrubitions of erectile dysfunctions' degree in groups.

Erectile Dysfunction	Severe (n, %)	Moderate (n, %)	Mild (n, %)	Normal (n, %)
Hypothyroidy (n=25)	6 (24)	3 (12)	6 (24)	10 (40)
Hyperthyroidy (n=21)	3 (14.28)	2 (9.52)	9 (42.85)	7 (33.33)
Total (n=46)	9 (19.56)	5 (10.86)	15 (32.60)	17 (36.95)
Control Group	2 (4.34)	2 (4.34)	24 (52.17)	18 (39.13)

(11) and 49% in men aged 50 to 80 years in Europe (12). In the etiology of ED several factors have been reported such as; vascular, neuronal, psychologic, drugs, metabolic, and endocrine diseases. The endocrine and metabolic diseases including diabetes mellitus, obesity, metabolic syndrome, osteoporosis, osteopenia, mild-moderate hypovitaminosis D, dyslipidemia, hyperthyroidism and hypothyroidism are among the most common contemporary human afflictions in the world (13). The endocrine system effects almost all human body systems and studies have reported evidence of hormonal abnormalities in about 25-35% of impotent men (14). According to the literature review it is understood that, not only the most frequent causes of endocrine sexual dysfunction, such as hypogonadism and hyperprolactinemia, but other extragonadal endocrinopathies including hypercortisolism and hypocortisolism, steroidal secreting tumors, hyperthyroidism and hypothyroidism may have a great or lesser effect on male sexual functions (15). Thyroid dysfunction is prevalent among endocrine disorders (14). In the United States, hypothyroidism is present in 4.6% of the population and hyperthyroidism is present in 1.3% of the population (13). Thyroid hormones regulate many cellular processes in almost all body organs of human including the growth, the basal metabolic rate, the respiratory rate, the rate at which calories are burned, maintenance of the skin, maturation and turnover rates of bone, heat production, fertility, digestion, myocardial con-

tractility and functional differentiation of central nervous system (16). In this context, in numerous studies, it has been reported that thyroid diseases may play a role in the development of atherosclerotic cardiovascular diseases, hypertension, depression and dementia, arrhythmia, heart failure, stroke and male infertility (16-18). Although the effects of both hyperthyroidism and hypothyroidism on several body systems have been well established, the relationship between the thyroid disorders and male sexual functions have not been studied extensively. For this reason, the impact of these disorders on male sexual functions remains controversial. In a few studies the effects of hyperthyroidism and hypothyroidism on sexual functions were evaluated in males both before and after thyroid hormone treatment. In a small case series authors showed that nine of 14 men who presented with hypothyroidism had sexual dysfunction (19). In another clinical study, patients with thyroid diseases and obese subjects were compared with control group (20). In this study, ED was more frequent in obese subjects (42%) and in patients affected by thyroid diseases (59%) than in controls (30%). Both below and above the age of 50 years, ED score was worse in thyroid patients than control subjects. To determine the effect of hyperthyroidism and hypothyroidism on male sexual functions in a total of seventy-one men (27 hyperthyroid and 44 hypothyroid) and a similar number of controls were evaluated by Krassas et al in a study (21). Authors found that approximately 80% of their patients with thyroid dysfunction had ED, compared with 34% of controls. Additionally, ED was severe in 21 of these patients (37.5%). After 8–9 months of euthyroidism, 30% of their patients had ED, which was similar to controls. According to these results, authors recommended that specific treatment for ED should be postponed in such patients for at least 6 months after achieving euthyroidism. In present study the IIEF scores were lower in group 2 and group 3 compared to group 1. In addition, severe ED rates was higher in patients with hypothyroidism and hyperthyroidism compared to controls.

In previous studies, it has been shown that ED may improve after the normalization of the thyroid disorder. In a multicenter prospective study, included 48 adult men, 34 with hyperthyroidism and 14 with hypothyroidism, the prevalence of sexual dysfunctions and their resolution after normalization of thyroid hormone levels were evaluated (19). In this study a statistically significant improvement was detected in IIEF scores after euthyroidism was achieved. In present study, IIEF scores significantly improved in hypothyroid patients. Similarly in another study, after restoration of euthyroidism, the prevalence of ED decreased significantly to levels comparable with those of controls (21).

To explain the relationship between ED and thyroid diseases several theories have been proposed. The presence of thyroxine hormone receptors in the corpus cavernosum and smooth muscle cells in the penis have been demonstrated by Carosa et al (22). This result supported the possibility of the relationship between thyroid disorders and male sexual dysfunction. In an experimental study it has been shown that thyroid hormones may affect the release of nitric oxide from nitrergic nerves and endothelium in hypothyroidism induced rabbit model (23). In this study, reduction of relaxation response in hypothyroid rabbits corpus cavernosum has also been detected. In addition, thyroid hormone receptors have been described in the testis (24). Studies have shown that thyroid hormone is an important factor in maturation of Leydig cells. Wortsman *et al (25).* investigated eight hypothyroid male patients aged 37–77 year. All patients had evidence of hypogonadism, five were hypergonadotropic, and three were hypogonadotropic. Similarly, Kumar *et al (26)* studied reproductive and endocrine function in eight males with primary hypothyroidism during the hypothyroid phase and again after achieving euthyroidism with T_4. The authors detected high mean Gn levels, low serum testosterone, low SHBG, and subnormal testosterone responses to hCG at the end of the study. These authors concluded that gonadal function abnormalities were common in men with primary hypothyroidism. However these changes were usually seen hypothyroidism persisting relatively longer periods. Indeed, induction of hypothyroidism in mature male rat had little effect on histopathology of the testes, spermatogenesis, or serum testosterone concentrations. It would therefore appear that hypothyroidism affects the immature, but not the mature, testis (27). Also, most studies indicated that hypothyroid men with hypogonadism have normal LH and FSH levels, suggesting

that the primary defect is not in Leydig cells and presumably results from a defect at the hypothalamus and/or pituitary level. Blunted Gn responses to GnRH support this notion, and it would therefore seem that primary hypothyroidism impairs the ability of the pituitary gland to respond to GnRH (28). In adult-onset hyperthyroidism, gonadotropins are often within the normal range, whereas SHBG is invariably elevated (19, 29). In male hyperthyroidism, the increase in SHBG leads to a rise in circulating tT levels (19). However, the fT is generally not affected. In a study, all reproductive hormones were within or very close to the reference range, despite that consistent fluctuations were observed with the normalization of thyroid hormones (19). Similarly, in our study all reproductive hormones were within normal ranges. Hypothyroidism may be associated with high prolactine levels. The higher prolactine levels observed in hypothyroid subjects may have affected the central machinery of sexual drive as previously shown (30). In contrast, males with primary hypothyroidism seldom exhibit elevated serum prolactine concentrations, except those with long-standing and severe hypothyroidism.

The psychological **status of the patient is also very important factor in the etiology of the ED.** In hyperthyroid patients, ED might have been precipitated by an increased adrenergic tone that, in predisposed subjects, leads to an insufficient corpora cavernosa relaxation and venocclusive mechanism. **As reported in numerous previous studies t**hyroid hormones have a dramatic effect on human behavior and relations. It is well known that hypothyroidism provokes somnolence, lethargy, and depression, whereas hyperthyroidism is associated with nervousness, emotional lability, and hyperkinesia (31). In this context, most of the studies which evaluate the association of thyroid disorders and male psychologic status revealed that there is a strong connection between thyroid hormones and depression, anxiety and panic disorder (32). Probably, due to this condition, patients with thyroid hormone disorders may experience a variety of sexual symptoms.

Although ED has become the most well-known aspect of male sexual dysfunction, recently the most prevalent male sexual disorders are ejaculatory dysfunctions. Ejaculation is a complex process as erection, in which both peripheral and central signals as well as both sympathetic and parasympathetic signals, are integrated into the ejaculation center of the spinal cord through input from the thoracolumbar sympathetic, sacral parasympathetic, and somatic spinal pathways. The spectrum of ejaculatory dysfunction extends from premature ejaculation to delayed ejaculation and anejaculation (33). The prevalance rates of ejaculatory disorders changes between 4% and 58.43% (34-36). Premature ejaculation is one of the most common male sexual problem in all countries with the prevalence of 20–30% (35). Although previously it was thought that this condition was primarily psychologically or interpersonally based, recently it has been shown that many neurotransmitters are involved in the control of ejaculation including dopamine, norepinephrine, serotonin, acetylcholine, oxytocin, gamma-aminobutyric acid (GABA), 5-hydroxy tryptamine, and nitric oxide (37). The most significant of these seem to be the central serotonergic and dopaminergic neurons. In addition, in the etiology of the ejaculatory disorders several clinical factors such as prostatitis, diabetes mellitus, penile hypersensitivity, hyperexcitable ejaculatory reflex, hyperarousability, genetic predisposition, erectile difficulties, selective serotonin reuptake inhibitos, chronic pelvic pain syndrome and thyroid disorders have been reported (38). Although the effect of hyperthyroidism on ejaculatory physiology in rats using para-chloroamphetamine-induced ejaculation model have been reported, the mechanism of action was not understood completely (39). Association between hyperthyroidism and premature ejaculation was evaluated in a limited number of clinical studies. Waldinger et al. (10) did not find any relationship between PE and thyroid dysfunction in a cohort of 620 men with PE. In contrast, in a study hyperthyroidism was associated with PE in a population of men with sexual dysfunctions collected in an endocrine department (40). In a consecutive series of 755 men presenting with sexual dysfunction showed that 28% of men with hyperthyroidism had PE (41). In a recent study a total of 43 patients with hyperthyroidism and no history of hyperthyroidism treatment were evalauted to determine the prevalence of premature ejaculation and observed IELT alterations before and after hyperthyroidism treatment. Premature ejaculation was observed in 31 of the 43 patients (72.1%). Authors detected a statistically significant improve-

ment in IELT after the achievement of euthyroidism (40). The main sexual complaint found in a multicenter, prospective study of hyperthyroid patients was PE (50%), whereas in hypothyroid subjects, it was delayed ejaculation (19). Although the first was considered the most frequent sexual complaint, affecting around 20% of the normal population, the latter was an infrequent symptom relatively (<5% of general population) (42). Both ejaculatory dysfunctions reverted upon achievement of euthyroidism in the absence of any other treatment for the sexual symptoms. After thyroid hormone normalization in hyperthyroid subjects, PE prevalence decreased from 50 to 15%, whereas delayed ejaculation was improved in half of the treated hypothyroid men. In our study, after thyroid hormone normalization in hyperthyroid subjects, PE prevalence decreased from 61.9% to 38.1%.

Although the relationship between thyroid hormones and ejaculatory disorders has not been completely understood, a few mechanisms have been hypothesized. These mechanisms include the sympathetic nervous system, the serotoninergic pathway, and the endocrine/paracrine system (19). Most of the manifestations of thyrotoxicosis and sympathetic nervous system activation overlap. This may suggest a similar action of both systems on ejaculation. In studies it has been reported that thyroid hormones augment sensitivity to β-adrenergic agonists (43). This situation leads to an increased sympathetic activity with normal circulating catecholamine levels. In hyperthyroid patients, the increased adrenergic tone may act on anxiety and irritability directly. The opposite may occur in hypothyroid patients. However, plasma catecholamines and their urinary metabolites are usually normal in hyperthyroidism (43). In present study the anxiety scores were significantly higher in group 3 compared to controls. In posttreatment period only in group 3 the BAI scores significantly decreased. Similarly, in a study in patients with hyperthyroidism, who had PE, anxiety scores were determined to be higher (40). Due to widespread distribution of thyroid hormone nuclear receptors within the brain, it can be hypothesized that thyroid hormones specifically may alter the central serotoninergic pathway (44), leading to diminished ejaculation control. This relationship have been reported in animals with experimentally induced hypothyroid states (45). According to another thought, the effects of thyroid hormone on the ejaculatory mechanism may be exerted through estrogen metabolism. Hyperthyroidism increases SHBG, which binds androgens with higher affinity than estrogens, leading to a relative hyperestrogenism. It has been demonstrated in hypogonadic rabbits that estrogens, but not androgens, fully restore oxytocin-induced epididymal contractility, up-regulating oxytocin receptor gene and protein expression, and that deprivation of endogenous estrogens induces oxytocin hyporesponsiveness (46). Because oxytocin is involved in the ejaculatory mechanism, both centrally and peripherally, this may account for the correlation between hyperthyroidism and PE.

It is well known that erection mechanism is a complex process and several factors may cause erectile dysfunction to affect negatively on this system. Among these factors thyroid disorders should always be considered in the etiology of the male sexual dysfunctions.

References

1. *Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med. 2010; 7: 445-75.*

2. *Eardley I, Donatucci C, Corbin J, El-Meliegy A, Hatzimouratidis K, McVary K, Munarriz R, Lee SW. Pharmacotherapy for erectile dysfunction. J Sex Med. 2010; 7: 524-40.*

3. *Lue TF, Giuliano F, Montorsi F. Summary of the recommendations on sexual dysfunctions in men. J Sex Med. 2004; 1: 6–23.*

4. *Hatzimouratidis K, Amar E, Eardley I, Giuliano F, Hatzichristou D, Montorsi F. European Association of Urology. Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. Eur Urol. 2010; 57: 804-14.*

5. *Morales A, Buvat J, Gooren LJ, Guay AT, Kaufman JM, Tan HM. Endocrine aspects of sexual dysfunction in men. J Sex Med. 2004; 1: 69-81.*

6. *Flynn RW, Bonellie SR, Jung RT, MacDonald TM, Morris AD, Leese GP. Serum thyroid-stimulating hormone concentration and morbidity from cardiovascular disease and fractures in patients on long-term thyroxine therapy. J Clin Endocrinol Metab. 2010; 95: 186-93*

7. *Park SB, Choi HC, Joo NS. The relation of thyroid function to components of the metabolic syndrome in Korean men and women. J Korean Med Sci. 2011; 26: 540-5.*

8. *Carani C, Isidori AM, Granata A, Carosa E, Maggi M, Lenzi A. Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. J Clin Endocrinol Metab. 2005; 90: 6472–9.*

9. *Buvat J, Maggi M, Gooren L, Guay AT, Kaufman J, Morgentaler A. Endocrine aspects of male sexual dysfunctions. J Sex Med. 2010; 7: 1627-56.*

10. *Waldinger MD, Zwinderman AH, Olivier B, and Schweitzer DH. Thyroid-stimulating hormone assessments in a Dutch cohort of 620 men with lifelong premature ejaculation without erectile dysfunction. J Sex Med. 2005; 2: 865–870.*

11. *Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: Results of the Massachusetts male aging study. J Urol 1994; 151: 54–61.*

12. *Rosen R, Altwein J, Boyle P. Lower urinary tract symptoms and male sexual dysfunction: The multinational survey of the aging male (MSAM–7). Eur Urol. 2003; 44: 637–49.*

13. *Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Clinical review: Prevalence and incidence of endocrine and metabolic disorders in the United States: a comprehensive review. J Clin Endocrinol Metab. 2009; 94: 1853-78.*

14. *Kilicarslan H, Bagcivan I, Yildirim MK, Sarac B, Kaya T. Effect of hypothyroidism on the NO/cGMP pathway of corpus cavernosum in rabbits. J Sex Med. 2006; 3: 830-7.*

15. *Lombardo F, Gandini L, Jannini EA, Sgrò P, Gilio B, Toselli L. Diagnosing erectile dysfunction: instruments for endocrine diagnosis. Int J Androl. 2005; 28 Suppl. 2: 53-5.*

16. *Brix K, Führer D, Biebermann H. Molecules important for thyroid hormone synthesis and action - known facts and future perspectives. Thyroid Res. 2011; 3; 4 Suppl 1: S9.*

17. *Franco M, Chávez E, Pérez-Méndez O. Pleiotropic effects of thyroid hormones: learning from hypothyroidism. J Thyroid Res. 2011; 2011: 321030.*

18. *Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010; 31: 702-55.*

19. *Carani C, Isidori AM, Granata A, Carosa E, Maggi M, Lenzi A. Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. J Clin Endocrinol Metab. 2005; 90: 6472-9.*

20. *Veronelli A, Masu A, Ranieri R, Rognoni C, Laneri M, Pontiroli AE. Prevalence of erectile dysfunction in thyroid disorders: comparison with control subjects and with obese and diabetic patients. Int J Impot Res. 2006; 18: 111-4.*

21. *Krassas GE, Tziomalos K, Papadopoulou F, Pontikides N, Perros P. Erectile dysfunction in patients with hyper- and hypothyroidism: how common and should we treat ? Clin Endocrinol Metab. 2008 93: 1815-9.*

22. *Carosa E, Di Sante S, Rossi S, Castri A, D'Adamo F, Gravina GL. Ontogenetic profile of the expression of thyroid hormone receptors in rat and human corpora cavernosa of the penis. J Sex Med. 2010; 7: 1381-90.*

23. *Yildirim MK, Bagcivan I, Sarac B, Kilicarslan H, Yildirim S, Kaya T. Effect of hypothyroidism on the purinergic responses of corpus cavernosal smooth muscle in rabbits. Int Urol Nephrol. 2008; 40: 691-9.*

24. *Wagner MS, Wajner SM, Maia AL. The role of thyroid hormone in testicular development and function. J Endocrinol. 2008; 199: 351-65.*

25. *Wortsman J, Rosner W, Dufau ML. Abnormal testicular function in men with primary hypothyroidism. Am J Med. 1987; 82: 207–212*

26. *Jaya Kumar B, Khurana ML, Ammini AC, Karmarkar MG, Ahuja MM. Reproductive endocrine functions in men with primary hypothyroidism: effect of thyroxine replacement. Horm Res. 1990; 34: 215–218*

27. *Oncu M, Kavakli D, Gokcimen A, Gulle K, Orhan H, Karaoz E. Investigation on the histopathological effects of thyroidectomy on the seminiferous tubules of immature and adult rats. Urologia Internationalis. 2004; 73: 59–64.*

28. *Velázquez EM, Bellabarba Arata G. Effects of thyroid status on pituitary gonadotropin and testicular reserve in men. Arch Androl. 1997; 38: 85–92*

29. *Sarne DH, Refetoff S, Rosenfield RL, Farriaux JP. Sex hormone-binding globulin in the diagnosis of peripheral tissue resistance to thyroid hormone: the value of changes after short term triiodothyronine administration. J Clin Endocrinol Metab. 1988; 66: 740-6.*

30. *El-Sakka AI, Hassoba HM, Sayed HM, Tayeb KA. Pattern of endocrinal changes in patients with sexual dysfunction. J Sex Med. 2005; 2: 551-8.*

31. *Denicoff KD, Joffe RT, Lakshmanan MC, Robbins J, Rubinow DR. Neuropsychiatric manifestations of altered thyroid state. Am J Psychiatry. 1990; 147: 94-9.*

32. *Panicker V, Evans J, Bjøro T, Asvold BO, Dayan CM, Bjerkeset O. A paradoxical difference in relationship between anxiety, depression and thyroid function in subjects on and not on T4: findings from the HUNT study. Clin Endocrinol (Oxf). 2009; 71: 574-80.*

33. *Rowland D, McMahon CG, Abdo C, Chen J, Jannini E, Waldinger MD. Disorders of orgasm and ejaculation in men. J Sex Med. 2010; 7: 1668-86.*

34. *Laumann EO, Nicolosi A, Glasser DB, Paik A, Gingell C, Moreira E. GSSAB Investigators' Group. Sexual problems among women and men aged 40-80 y: prevalence and correlates identified in the Global Study of Sexual Attitudes and Behaviors. Int J Impot Res. 2005; 17: 39-57.*

35. *McCarty E, Dinsmore W. Dapoxetine: an evidence-based review of its effectiveness in treatment of premature ejaculation. Core Evid. 2012; 7: 1-14.*

36. *Vakalopoulos I, Dimitriadis G, Varnava C, Herodotou Y, Gkotsos G, Radopoulos D. Prevalence of ejaculatory disorders in urban men: results of a random-sample survey. Andrologia. 2011; 43: 327-33.*

37. *Giuliano F, Hellstrom WJ. The pharmacological treatment of premature ejaculation. BJU Int. 2008; 102: 668-75*

38. *Jannini EA, Maggi M, Lenzi A. Evaluation of premature ejaculation. J Sex Med. 2011; 8 Suppl 4: 328-34.*

39. *Yonezawa A, Yoshizumi M, Ebiko M, Iwanaga T, Kimura Y, Sakurada S. Evidence for an involvement of peripheral serotonin in p-chloroamphetamine-induced ejaculation of rats. Pharmacol Biochem Behav. 2005; 82: 744-50.*

40. *Cihan A, Demir O, Demir T, Aslan G, Comlekci A, Esen A. The relationship between premature ejaculation and hyperthyroidism. J Urol. 2009; 181: 1273-80.*

41. *Corona G, Petron L, Mannucci E. Psycho-biological correlates of rapid ejaculation in patients attending an andrologic unit for sexualdysfunctions. Eur Urol. 2004; 46: 615–622.*

42. *Jannini EA, Simonelli C, Lenzi A. Disorders of ejaculation. J Endocrinol Invest. 2002; 25: 1006–1019.*

43. *Bilezikian JP, Loeb JN. The influence of hyperthyroidism and hypothyroidism on α- and β-adrenergic receptor systems and adrenergic responsiveness. Endocr Rev. 1983; 4: 378–388.*

44. *Sandrini M, Vitale G, Vergoni AV, Ottani A, Bertolini A. Effect of acute and chronic treatment with triiodothyronine on serotonin levels and serotonergic receptor subtypes in the rat brain. Life Sci. 1996; 58: 1551–1559.*

45. *Kulikov AV, Jeanningro R. The effects of hypothyroidism on 5-HT1A and 5-HT2A receptors and the serotonin transporter protein in the rat brain. Neurosci Behav Physiol. 2001; 31: 445–449.*

46. *Filippi S, Morelli A, Vignozzi L, Vannelli GB, Marini M, Ferruzzi P. Oxytocin mediates the estrogen-dependent contractile activity of endothelin-1 in human and rabbit epididymis. Endocrinology. 2005; 146: 3506-17.*

47. *Oxytocin mediates the estrogen-dependent contractile activity of endothelin-1 in human and rabbit epididymis. Endocrinology. 146: 3506–3517*

48. *Cahangirov A, Cihan A, Murat N, Demir O, Aslan G, Gidener S, Esen AA. Investigation of the neural target level of hyperthyroidism in premature ejaculation in a rat model of pharmacologically induced ejaculation. J Sex Med. 2011; 8: 90-6.*

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Myopia, axis oculi and bone age in youth: Determination and relationships

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Abstract

Aims: To determine the relationship among degree of myopia, corneal curvature, axis oculi, body height, body weight and bone age in students aged 6~14 years.

Methods: All subjects underwent pupil enlargement for refractive examination and optometric examination to measure cycloplegia, corneal curvature and axial length. Body height, weight, and visual acuity were measured, and bone age was determined by X-imaging of the carpal bone of both hands.

Results: We found that axis oculi length, body height, body weight and difference in bone age were positively correlated with age, while equivalent spherical degree was negatively correlated with age. Body height, body weight, age, bone age and difference of bone age were negatively correlated with equivalent spherical degree and positive correlated with axis oculi length. Difference in axis aculi length showed weak positive correlations with difference in bone age, especially in myopia students aged 9 to <12 years, in females aged 6 to <9 years and in males aged 9 to <12 years.

Conclusions: Myopia in adolescents was related to changes in total body growth and development.

Key words: Myopia, corneal curvature, axial oculi, height, weight, bone age.

Introduction

Myopia is defined as the focus of parallel light rays from a distant source in front of the retina [1]. The mechanism by which individuals develop myopia has not been fully elucidated, although genetic factors, growth and development factors, and extrinsic factors have been suggested [2]. Myopia in adolescence arises during the process of body growth and development. It remains unclear, however, whether the degree of myopia is related to the level of growth and development in an individual.

Myopia thought to be due primarily to elongation **of the axis oculi, especially in** children and adolescents [17-24]. Although axis oculi length has been positively correlated with body height[5-11], a relationship between body height and condition of refraction has not been observed in Australian children. [12] Moreover, degree of myopia was not correlated with the size of the orbit. [13] Refraction has been found to correlate with body height, but not body weight [14], although others [15] have reported a positive correlation between body weight and condition of refraction in Singapore Chinese children aged 7-9 years. A study in English children found that body weight increased more rapidly in those with developing than stable myopia.[16] Taken together, these findings indicate unclear relationships among axial oculi length, degree of myopia and growth of total body.

Increased body height is the most outstanding manifestation of total body growth and development, with body height mainly determined by skeletal growth. Genes important in skeletal growth are also expressed in the cornea and retina and influence the development of experimental myopia[27,28]. Myopia therefore may be related with skeletal growth [29]. Earlier growth and development in adolescence has been associated with a greater increase in axis oculi length and the development of juvenile myopia[30]. Children with myopia grow and develop more rapidly and mature early, as shown by the greater incidence of myopia in girls with early menarche [31].

Bone age differs from chronological age[32] . In children of the same chronological age, those with greater bone age show faster increases in physique growth, body weight and body height than children with lower bone age[4]. Skeletal age increases with bone growth, especially body height, during adolescence [33-34]. We have therefore assessed the association between the development of myopia and markers of growth and development in Chine-

se children aged 6-14 years. Parameters measured included axis oculi length, chronological age, bone age, differences in axis oculi length and bone age. We hypothesized that individuals who grew and developed earlier and more rapidly would show greater elongation of the axis oculi and earlier and more rapid development of myopia.

Methods

Subjects

A cross-sectional, descriptive, and observational study was designed, in which individuals were selected by gender- and age-stratified sampling from July to December 2009 at the Center for Disease Control in the BaoShan area of ShangHai. We selected 1620 students (3240 eyes), aged 6-14 years attending 3 middle and elementary schools were chosen. Of these, 630 students had lower vision levels and were further examined at the Ophthalmology Department of the Baoshan Branch Institute in Shanghai Shuguang Hospital to identify the cause of low vision and conditions of refraction. Non-myopic individuals were excluded, including 9 with amblyopia or farsightedness students. The remaining 621 students included 283 boys (45.6%) and 338 girls (54.4%). The research protocol of this study was approved by the Ethics Committee of the Shanghai Shuguang Hospital, and the parents of each subject provided written informed consent for in hospital examinations.

Characteristics of the study subjects

The 621 students included 283 boys (45.6%) and 338 girls (54.4%). Age distribution was skewed slightly. The youngest was 5.90 years old and the oldest was 14.88. The minimum and maximum differences in bone age were -2.61 and 3.83 years, respectively.

Examinations

In school measurements included body height, body weight, vision and computer optometry. Vision examinations were performed at a distance of 5 m in a broad and bright indoor environment using the international standard logarithmic visual acuity chart.

In hospital, students were examined by a high-grade optometrist using a Nikon RK-8000 computer eye refractometer. Each examination included optometry and measurements of corneal curvature, axis oculi length, and bone age. Medical optometry examinations were performed by placing 5% tropicamide eyedrops in both eyes for 5 minutes, followed by a rest for 20 minutes in a darkroom and performing girdle-shaped band light retinoscopy. Degree of corneal curvature was measured to the nearest 0.1 mm using a Nikon RK-8000 computer optometer. Bone age was measured by X-imaging of the carpus of both hands, as described [4].

Statistical analysis

All statistical analyses were performed using the Excel SPSS17.0 software package. Relationships between parameters were assessed using Spearman coefficient correlation tests.

Results

Relationship among equivalent sphere mirror, axis oculi length and corneal curvature

Equivalent sphere mirror has been defined as the sum of half the sphere mirror degree and half the cylindrical lens degree. K_1 and K_2 have been defined as the corneal curvature upon minimus and maximum radialis, respectively, of corneal refractive power .We found that the correlation coefficient of the equivalent sphere mirror was 0.93 in both the left and right eyes, that the correlation coefficient of the axis oculi length was 0.91 in both the left and right eyes, and that the correlation coefficients of K1 and K2 in the left and right eyes were 0.91 and 0.92, respectively, indicating that the parameters in the two eyes of subjects were highly correlated. Therefore, in analyzing the relationship among refraction parameters, were report only the results of right eyes.

Equivalent sphere mirror degree, axis oculi length and corneal curvature are non-normal bivariable data. Using the Spearsman coefficient correlation test, we found that equivalent sphere mirror degree was strongly negatively correlated with axis oculi length (r_s=-0.606, P <0.05) (Figure 1.), but was not correlated with corneal curvature (K_1: r_s =-0.068, P>0.05; K_2: r_s =-0.075, P >0.05). (Figure 2.)

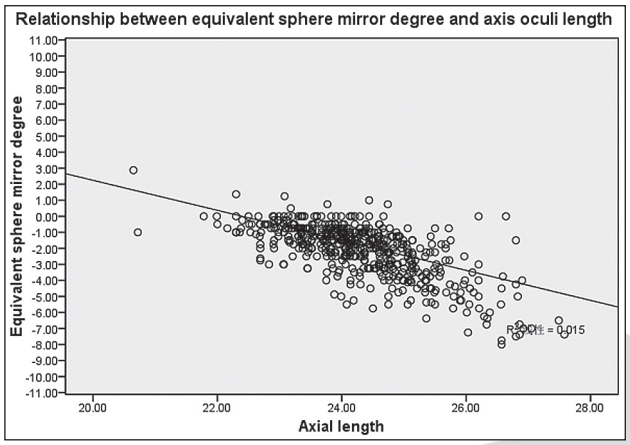
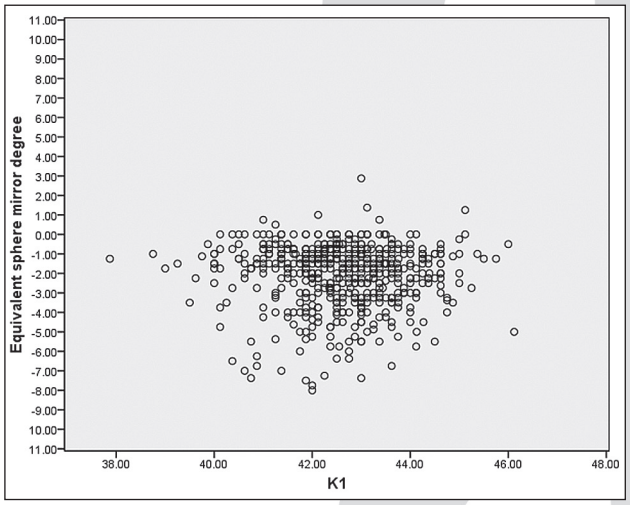


Figure 1. Equivalent sphere mirror degree was strongly negatively correlated with axisoculi length (r_s=-0.606, P <0.05) .



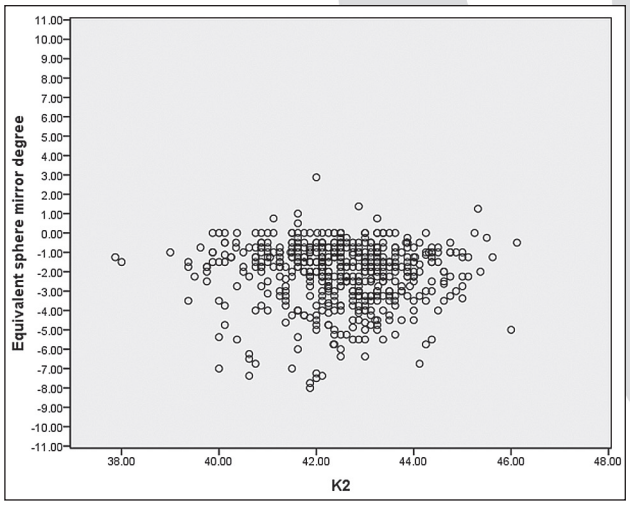


Figure 2. Equivalent sphere mirror degree was not correlated with corneal curvature (K1: r_s =-0.068, P>0.05; K2: r_s =-0.075, P >0.05).

Age distribution of equivalent sphere mirror degree, axis oculi length, corneal curvature, body height and weight, difference of axis oculi and difference of bone age

Census databases obtained from students in 11 schools in the Baoshan area aged 6-14 years showed that the mean normal axis oculi lengths in emmetropic 22.15±0.11 mm in those aged 6 to <9 years, 22.86±0.07 mm in those aged 9 to >12 years, and 23.98±0.15 mm in those aged 12-14 years.

Although corneal curvature was unrelated to age (r_s =-0.067, *P*=0.179), body height (r_s =0.854, *P*<0.05) and body weight (r_s =0.689, *P*<0.05) showed strong positive correlations with age. Difference in bone age had a weak positive relationship to age (r_s =0.148, *P*<0.05).

Equivalent sphere mirror degree was negatively correlated with age (r_s =-0.293, *P*<0.05), indicating that equivalent sphere mirror degree gradually decreased and tended toward myopia with age. (Figure 3.)

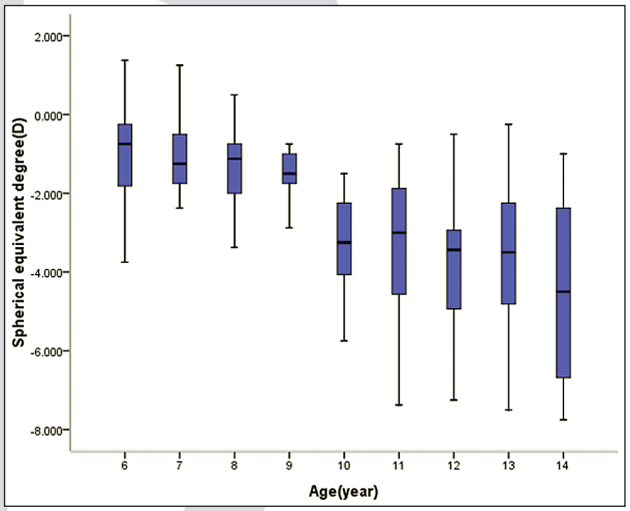


Figure 3. Equivalent sphere mirror degree was negatively correlated with age (r_s =-0.293, P<0.05).

Axial length in groups of subjects aged 6 to <9, 9 to <12, and 12-14 years . Axial length was positively correlated with age (r_s =0.324, *P*<0.05). There were significant differences between each pair of groups (**P*<0.05). (Figure 4.)

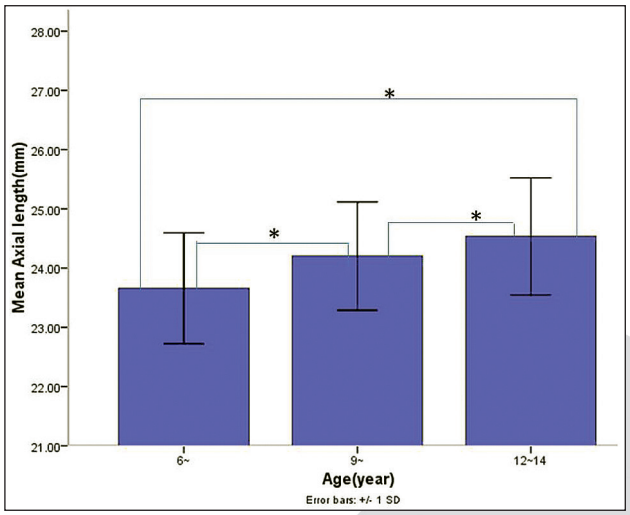


*Figure 4. Axial length in groups of subjects aged 6 to <9, 9 to <12, and 12-14 years. There were significant differences between each pair of groups (*P<0.05).*

Axis difference in groups of subjects aged 6 to <9, 9 to <12, and 12-14 years. Axis difference was negatively correlated with age (r_s =-0.285, P<0.05). There were significant differences between each pair of groups (*P<0.05). (Figure 5.)

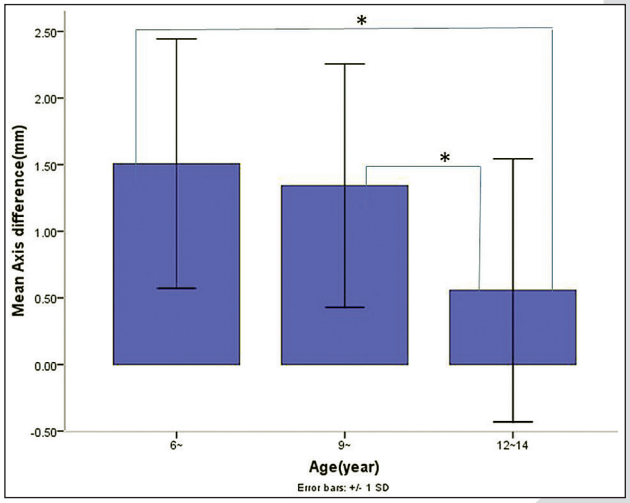


*Figure 5. Axis difference in groups of subjects aged 6 to <9, 9 to <12, and 12-14 years. Axis difference was negatively correlated with age (r_s =-0.285, P<0.05). There were significant differences between each pair of groups (*P<0.05).*

Comparison of the age and bone age distributions of axial length.Age distribution of axial length showed that axial elongation was rapid and volatile in subjects aged 6-11 years, while leveling off in those aged 11-14 years. Bone age distribution of axial length showed that axial elongation was stable in subjects of bone age 6-12 years, becoming rapid and volatile in those of bone age over 12 years. As shown in Figure 6a and Figure 6b.

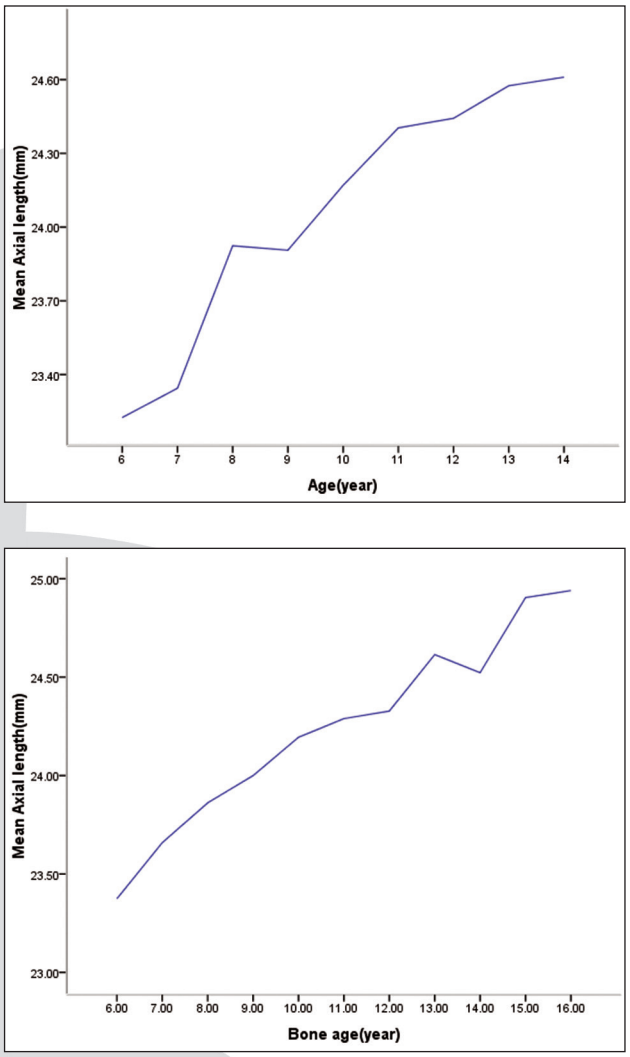


Figure 6. Comparison of the age (a) and bone age (b) distributions of axial length. (a) Age distribution of axial length showed that axial elongation was rapid and volatile in subjects aged 6-11 years, while leveling off in those aged 11-14 years. (b) Bone age distribution of axial length showed that axial elongation was stable in subjects of bone age 6-12 years, becoming rapid and volatile in those of bone age over 12 years.

Relationship between biomeasurement parameters of eyeballs and body development

We found that difference in bone age were more positive, differences in axis oculi were larger, bone ages were more advanced, and axis oculi lengths were greater than emmetropia (Figure 7).

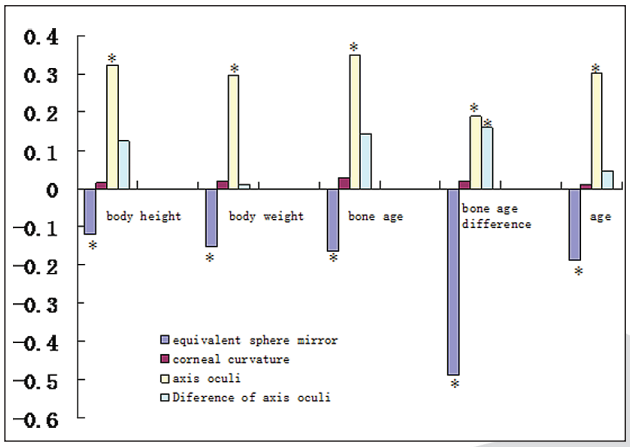


*Figure 7. Body height, body weight, age, bone age, and difference in bone age were weakly negatively correlated with equivalent sphere mirror degree (*P<0.05), weakly positively correlated with axis oculi length (*P<0.05) and showed no relationship with corneal curvature. Body height, body weight, age, and bone age showed no correlations with difference of axis oculi. Difference in bone age had a weak positive relationship with difference of axis oculi (*P<0.05).*

Body height, body weight, age, bone age, and difference in bone age were weakly negatively correlated with equivalent sphere mirror degree (**P*<0.05), weakly positively correlated with axis oculi length (**P*<0.05) and showed no relationship with corneal curvature .Body height, body weight, age, and bone age showed no correlations with difference of axis oculi. (Figure 7)

Before delamination analysis, difference in bone age had a weak positive relationship with difference of axis oculi (*P<0.05) as shown in Figure5. Analyses by age and gender are shown in Figure 8.

Correlations between difference in bone age and difference in axis oculi in boys and girls aged 6 to <9, 9 to <12, and 12-14 years. In students aged 9 to <12 years old, the difference in axis oculi length showed a weak positive correlation with difference in bone age (* *P*<0.05). Difference in axis oculi was positively correlated with difference in bone ages in girls (**P*<0.05), but not in boys, aged 6 to <9 years; and these two parameters were positively correlated in boys (**P*<0.05), but not in girls, aged 9 to <12 years. (Figure 8)

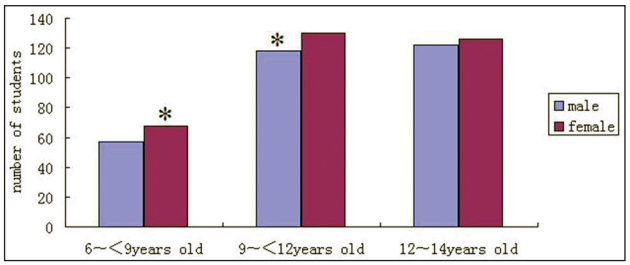


Figure 8. Correlations between difference in bone age and difference in axis oculi in boys and girls aged 6 to <9, 9 to <12, and 12-14 years. In students aged 9 to < 12 years old, the difference in axis oculi length showed a weak positive correlation with difference in bone age (P<0.05). Difference in axis oculi was positively correlated with difference in bone ages in girls (*P<0.05), but not in boys, aged 6 to < 9 years; and these two parameters were positively correlated in boys (*P<0.05), but not in girls, aged 9 to <12 years.*

Discussion

We have shown here that equivalent sphere mirror degree had a strong negative relationship with axis oculi length in myopic schoolchildren aged 6-14 years. As age increased, axis oculi length gradually increased while equivalent sphere mirror degree gradually decreased, tending toward myopia. These findings suggest that, with increased age, the elongated degree of axis oculi length became gradually aggravated, resulting in an increased difference in axis oculi. The ages at which the largest difference in axis oculi occurred corresponded to the peak age for development of myopia. In contrast, we found that the difference in axis oculi length was negatively related to age distribution, suggesting that the axial elongation was correlated with development. The increase in axis oculi length per year did not differ in subjects who were myopic and emmetropic, although the yearly increase in eyeball transverse diameter was lower in myopia than in emmetropia [25]. Axis oculi length increased more rapidly before age 10 years, increasing more slowly thereafter. The growth in axis oculi and body height cease at around the same age[26]. These findings suggest the need for additional measurements of eyeball growth, including eyeball trans diameter and volume and orbit volume. Due to intergender differences in development, further delamination analysis is essential in relation to age and gender.

As expected, body height and weight and differences in bone age increased with age in students aged 6-14 years. Difference in axis oculi length showed a weak positive relationship with difference in bone age. These findings indicate that individuals with more advanced bone age had longer axis oculi length than individuals with emmetropia, with their condition of refraction tending toward myopia. These findings suggest that the development of myopia during adolescence was related to the timing of body growth and development.

Peak height velocity (PHV) of skeleton development in Chinese children occurs at bone ages of 11.56±1.33 years in boys and 11.0±1.36 years in girls, and at chronological ages of 12.46±0.75 years and 10.58±1.15 years, respectively [35]. The age of development is about 11-13 years in boys and 9-11 years in girls, showing that girls mature about 2 years before boys.

To investigate the relationship between of myopia and growth and development by gender and age, we performed delamination analysis.We found that the difference in axis oculi was weakly positively correlated with difference of bone age in myopic students aged 9~ <12 years, the peak age of development. This correlation was significant in girls aged 6~ <9 years and in boys aged 9~ <12 years.

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References

1. *D Ning H, Ren Y, Zhu F. Lv. myopia subject; The People's Medical Publishing House; 2009: 3.*
2. *J Ge, H.ZJ Wang, YZ Qing. ZH Sun. JQ Chen. K Yao et al, Ophthalmology; The People's Medical Publishing House; 2002; 224.*
3. *CY Ji. Hygiene of children and adolescents,fifth edition.BeiJing: The People's Medical Publishing House, 2004; 92.*
4. *YY Ye, Chinese children skeletal age Score methods. BeiJing: The People's Medical Publishing House, 2005.*
5. *Wu HM, Gupta A, Newland HS, Selva D, Aung T, Casson RJ. Association between stature, ocular biometry and refraction in an adult population in rural Myanmar: the Meiktila eye study. Clin Experiment Ophthalmol. 2007; 35(9): 834-839.*
6. *Wong TY, Foster PJ, Johnson GJ, Klein BE, Seah SK. The relationship between ocular dimensions and refraction with adult stature: the Tanjong Pagar Survey. Invest Ophthalmol Vis Sci. 2001; 42(6): 1237-1242.*
7. *Lee KE, Klein BE, Klein R, Quandt Z, Wong TY. Association of age, stature, and education with ocular dimensions in an older white population. Arch Ophthalmol. 2009; 127 (1): 88-93.*
8. *Pereira GC, Allemann N. Ocular biometry, refractive error and correlation with height, age, gender and years of formal education. Arq Bras Oftalmol. 2007; 70(3): 487-493.*
9. *Selović A, Juresa V, Ivankovic D, Malcic D, Selović Bobonj G. Relationship between axial length of the emmetropic eye and the age, body height,and body weight of schoolchildren. Am J Hum Biol. 2005; 17(2): 173-177.*
10. *Eysteinsson T, Jonasson F, Arnarsson A, Sasaki H, Sasaki K. Relationships between ocular dimensions and adult stature among participants in the Reykjavik Eye Study. Acta Ophthalmol Scand .2005; 83(6): 734-738.*
11. *Saw SM, Chua WH, Hong CY, Wu HM, Chia KS, Stone RA, et al. Height and its relationship to refraction and biometry parameters in Singapore Chinese children. Invest Ophthalmol Vis Sci. 2002; 43(5): 1408-1413.*
12. *Ojaimi E, Morgan IG, Robaei D, Rose KA, Smith W, Rochtchina E, et al. Effect of stature and other anthropometric parameters on eye size and refraction in a population-based study of Australian children. Invest Ophthalmol Vis Sci. 2005; 46(12): 4424-4429.*

13. *Chau A, Fung K, Pak K, Yap M. Is eye size related to orbit size in human subjects? Ophthalmic Physiol Opt. 2004; 24(1): 35-40.*

14. *De C, Wang J, Zhang XB, Kong WY, Huang MG. Influence of body height and body weight upon refraction condition of eyeball and biological parameter. International ophthalmology journal, 2011; 11(11): 1902-1906.*

15. *Saw SM, Carkeet A, Chia KS, Stone RA, Tan DT. Component dependent risk factors for ocular parameters in Singapore Chinese children. Ophthalmology. 2002; 109(11): 2065-2071.*

16. *Gardiner PA. Physical growth and the progress of myopia. Lancet. 1955; 266: 952-953.*

17. *Larsen JS. The sagittal growth of the eye. IV. Ultrasonic measurement of the axial length of the eye from birth to puberty. Acta Ophthalmol (Copenh). 1971; 49(6): 873-886.*

18. *Zadnik K. The Glenn A. Fry Award Lecture (1995). Myopia development in childhood. Optom Vis Sci. 1997; 74 (8): 603-608.*

19. *Zadnik K, Mutti DO, Mitchell GL, Jones LA, Burr D, Moeschberger ML. Normal eye growth in emmetropic schoolchildren. Optom Vis Sci. 2004; 81(11): 819-828.*

20. *Fledelius HC. Ophthalmic changes from age of 10 to 18 years. A longitudinal study of sequels to low birth weight. IV. Ultrasound oculometry of vitreous and axial length. Acta Ophthalmol (Copenh). 1982; 60(3): 403-411.*

21. *Qi ZX, Wu XM, Chen JX; Study of relationship between adolescence myopia diopter and cornea refractive power. Int Ophthalmol. J between Chin Am. 2002; 9(3): 44-45.*

22. *Lam CS, Edwards M, Millodot M, Goh WS. A 2-year longitudinal study of myopia progression and optical component changes among Hong Kong schoolchildren. Optom Vis Sci. 1999; 76(6): 370-380.*

23. *Saw SM, Chua WH, Gazzard G, Koh D, Tan DT, Stone RA.. Eye growth changes in myopic children in Singapore. Br J Ophthalmol. 2005; 89(11): 1489-1494.*

24. *Zadnik K, Mutti DO, Friedman NE, Qualley PA, Jones LA, Qui P, et al. Ocular predictors of the onset of juvenile myopia. Invest Ophthalmol Vis Sci. 1999; 40(9): 1936-1943.*

25. *Song HT, Kim YJ, Lee SJ, Moon YS. Relations between age, weight, refractive error and eye shape by computerized tomography in children. Korean J Ophthalmol. 2007; 21(3): 163–168.*

26. *Goss DA, Cox VD, Herrin-Lawson GA, Nielsen ED, Dolton WA. Refractive error, axial length, and height as a function of age in young myopes. Optom Vis Sci. 1990; 67(5): 332-338.*

27. *Wang Q, Zhao G, Xing S, Zhang LN, Yang X. Role of bone morphogenetic proteins in form-deprivation myopia sclera. Mol Vis. 2011; 17: 647-657.*

28. *Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth Factors. 2004; 22(4): 233-241.*

29. *Pohlandt F. Hypothesis: myopia of prematurity is caused by postnatal bone mineral deficiency. Eur J Pediatr. 1994; 153(4): 234-236*

30. *Zhang YX, Wang SR. Chinese school doctor.2005; 19(5): 448-451*

31. *Ye GJ. Modern hygiene of children and adolescents BeiJing: The People's Medical Publishing House, 1999; 446.*

32. *Medical subject headings ocular fixation character obedien table (MeSHAAL); Chinese Academy of Medical Science; Institute of Social Science Medical Information; BeiJing: The People's Medical Publishing House, 1984.*

33. *Gilsanz V, Ratib O. Hand Bone Age. A Digital Atlas of Skeletal Maturity. Springer-Verlag Berlin Heidelberg. Germany. 2005; 7.*

34. *Hagg U, Taranger J. Skeletal stages of the hand and wrist as indicators of the pubertal growth spurt. Acta Odontol Scand. 1980; 38 (3): 187-200.*

35. *Pan FP, Zhang GD. Tracing observation upon relationship between skeletal age and adolescence development. China Preventive Medicine Journals. 1985; 2: 79-81.*

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The significance of serum and urinary neopterin levels and Behçet's Disease current activity form in determining activity of Behçet's Disease

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Abstract

Background and design: A consistent laboratory marker, which indicates activity of Behçet's disease (BD) is still lacking. The clinical significance of *neopterin measurement* is well known in various diseases, high levels suggest the activation of cellular immunity and allow monitoring the disease activity and the response to the treatments. Recently, BD Current Activity Form (BDCAF) is used for establishing the activity of BD.The purpose of this study is to investigate the serum and urinary neopterin levels and BDCAF scores in BD patients.

Materials and Methods: Forty-eight patients with BD and 24 healthy controls were enrolled in the study. The patients were also divided in the active and inactive groups based on their clinical presentation. The serum and urine neopterin levels were measured and BDCAF scores were recorded for all patients.

Results: Increased levels of both serum and urinary neopterin were detected in patients with BD ($p<0.05$). No significantly different levels of neopterin were obtained from the active and inactive patients ($p>0.05$). However, BDCAF scores were significantly higher in the active patients ($p<0.05$).

Conclusion: The serum and urinary neopterin levels were higher in BD patients but they cannot be used as a reliable laboratory marker for determining the activity of BD since the neopterin levels do not increase in the active stage. However, higher BDCAF scores may identify the active stage of BD.

Key words: Behçet's disease, Serum neopterin, Urinary neopterin, Activity of Behçet's disease, Behçet's Disease Current Activity Form.

Introduction

Behçet's disease (BD), described for the first time in 1937 by a Turkish dermatologist Hulusi Behçet, as a triad of symptom complex; oral aphthae, genital ulcers and hypopyon uveitis [1]. The etiopathogenesis of the disease remains to be elucidated [2-5]. There is no widely accepted clinical or laboratory finding that represents the activity of the BD which allows following up the clinical course of the disease and treatment strategies [6-8].

Neopterin (NPT) and its derivates are synthesized from guanosine triphosphate. Dihydro NPT triphosphate (NH_2TP), the first product of NPT pathway, is essential for the synthesis of catecholamines and serotonin in neuroendocrine tissues and lymphocytes. NH_2TP is broke down to tetra-hydrobiopterin (BH4) that actually participates in the enzymatic reactions of those hormones as an electron donor. The lack of enzymes for synthesis of BH_4 from NH_2TP in monocytes and macrophages leads to hydrolysis of BH_4 to dihydro neopterin namely NPT [8-13]. NPT *is secreted* by activated monocytes/macrophages upon the stimulation with interferon-gamma (INF-γ). It has been reported that lesser amounts of NPT also released from umbilical vein and T-lymphocytes but not from B-lymphocytes, granulocytes, natural killer cells or tumoral cells. Interleukin-2 stimulates T-cells, which will finally promote the secretion of NPT via INF-γ. So, increased levels of NPT are associated with cell-mediated immunity. Studies demonstrated high levels of NPT in many infectious, malignant and inflammatory diseases. Recently, NPT is accepted as a reliable biomarker for various cell-mediated diseases [10-16].

The Behçet's Disease Current Activity Form (BDCAF) was developed as an outcome of the

studies of the International Scientific Committee on Behçet's Disease. The content of the BDCAF was based on previous work [17] that compared two schemes which were available to assess the disease activity: the Iranian Behçet's Disease Dynamic Measure (IBDDAM) [18] and an European scheme initially developed in the UK. Recently, BDCAF has been proposed as a useful instrument to generate an efficient index of the disease activity [7,19]

In the current study, our aim was to compare the serum and urine NPT levels between the patients with BD and healthy individuals considering that NPT may also be an activity indicator in BD. We also intended to collate the results, with the BDCAF scores in the active and inactive phases of disease.

Methods

Patients who were presented to the dermatology clinic with the diagnosis of BD based on the International Study Group criteria and as well as age- and sex-matched healthy control subjects were enrolled in the study. Subjects in control group did not have oral aphtosis, BD or any systemic diseases. The patients without any systemic diseases except BD were participated to the study. Medical history of the patient including; duration of the disease, past symptoms of the disease, medication use and family history were inquired. After a general physical examination, eye and skin examinations were also performed. Assessment of the previous studies encouraged us to maintain the disease activity based on modified criteria of which Erturan et al. used in their study [20-23]**.** In dermatologic examination, patients who have oral ulcer and additionally, at least one of the clinical findings such as genital ulcer or the other skin lesions were considered in the active period [22]. Patients who had one of the systemic findings, with or without oral ulcer, such as arthritis, neurological findings, active uveitis or involvement of the great vessels were assumed in the active period [22]. However, Erturan et al. [23] accepted that even one and only mucocutaneous manifestation was sufficient to maintain the active period. We did not consider the patients with only one oral ulcer as in the active phase. In clinical practice, most of patients have an aphtous ulcer in repetition of attacks and remissions of the BD. Also, most of patients have unremitting aphtous ulcers without any other symptoms. A dermatologist performed BDCAF to all patients. We used an adaptation of BDCAF to Turkish language described by Hamuryudan et al. [24] Of the routine laboratory tests, the complete blood count, biochemical parameters, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured in the patient and the control groups. Patients underwent the pathergy tests by the same physician. The study protocol was approved by the Medical School Ethics Committee. Fasting blood was drawn from all participants to determine the NPT levels. To determine the urine NPT levels, 20 milliliters of urine was taken simultaneously with the blood sample. Some of the urine was transmitted to biochemistry laboratory in order to evaluate spot creatinine levels. We preferred the RIA technique in determining the NPT levels which enables accurate and convenient screening for NPT [25,26].

The comparison of serum and urine NPT levels between the patient and the control groups, was performed by independent-samples t-test. Analysis of variance (Anova) was performed for comparing the serum and urinary level of the active and inactive patients and the control group. Also independent-samples t-test was used for the comparison of serum and urine NPT levels of patients who were treated with colchicine in the active group and inactive groups. Kruskal-Wallis H test was used to compare the serum and urine NPT levels of the patients treated with colchicine, systemic corticosteroid or cyclosporine and untreated patients. Since these three groups were not normally distributed and homogeneous we preferred to select nonparametric tests in comparison of the groups.

Unpaired T test was used for determining the association between the pathergy positivity and the NPT levels. Regression analysis was used to evaluate the correlation of independent samples; a p value less than 0.05 was considered as statistically significant.

Results

The study group consisted of 27 (56,25 %) female and 21 (43,75 %) male patients. There were 10 (41,66 %) males and 14 (58,33 %) females in the control group. There were no statistically

differences between the patient and the control groups based on the gender (p>0.05). The mean ages of the patient group and the healthy controls were 35.20 ± 10.43 and 37.9 ± 11.73, respectively. There was no significant difference in the mean ages between the patient and the control groups (p>0.05). Mean duration of the disease was 4.8 ± 4.28 years. Pathergy positivity was observed in 18 patients (37.5 %). Seven of the patients (14.5%) had family history of BD.

Based on the clinical manifestations, 24 (50%) of the patients were in the active and 24 (50%) in the inactive phase of the disease. There were 10 (41,66 %) males and 14(58,33 %) females in the active group, and 11 (45,83 %) males and 13 (54,17 %) females in the inactive group. The mean age was 33.4 ± 8.21 in the active group and 37.0 ± 10.25 in the inactive group. The mean duration of the diseases was 4.16 ± 3.24 in the active group and 5.6 ± 4.88 in the inactive group. There were no significant differences between the active and inactive groups based on the gender, mean ages and the duration of the diseases (p>0.05).

All of the patients had 100 percent recurrent oral ulcers. Of these patients 27 (56,2 %) were in the active and 21 (43,80 %) were in the inactive group. The clinical symptoms were as follows in the groups: Genital ulcers: 75%, 10 (27,77 %) in active 26 (72,33 %) in inactive group; ocular involvement 20.8%, 4 (36,66 %) in active, 7 (63,63 %) in inactive group; erythema nodosum 64.5%, 9 (29 %) in active, 22 (70,96 %) in inactive group; papulopustuler lesions 93.7%, 13 (28,8 %) in active, 32 (71,11 %) in inactive group; arthritis 72.9%, 6 (17,14 %) in active, 29 (82,85 %) in inactive group; artralgia 72.9%, 6 (17,14 %) in active, 29 (82,85 %) in inactive group; recurrent superficial tromboflebitis 18.7%, 3 (33,33 %) in active, 6 (66,67 %) in inactive group; neurologic involvement 6.2%, 2 (66,67 %) in active, 1 (33,33 %) in inactive group. The main artery involvement was seen in 2 (4.1%) of the patients. One patient had *pulmonary artery aneurysm and* another one had a history of *vena cava superior syndrome.* None of the patients had a history of gastrointestinal involvement. The patients' clinical presentation was summarized in Table 1 (Table 1).

Table 1. Active and inactive clinical presentations in patient group

	Active	Inactive	Total (n=48)	95 %CI*	Range
	n (%)	n (%)	n (%)		%
Recurrent oral aphthous ulcers	27(56,2)	21(43,7)	48(100)	±0	100-100
Genital ulcers	10(4,8)	26(54,1)	36(75)	±12.25	62,75-87.25
Eye involvement	4(8,3)	7(14,5)	11(22,8)	±11.87	10.93-34.67
Erythema nodosum	9(18,7)	22(45,8)	31(64,5)	±13.54	50.96-78.94
Papulopustular lesion	13(27)	32(45,8)	45(93,7)	±6.87	86.83-100.5
Arthritis	6(12,5)	29(60,4)	35(72,9)	±12.57	60.33-85.47
Arthralgia	6(12,5)	29(60,4)	35(72,9)	±12.57	60.33-85.47
Recurrent superficial thrombophlebitis	3(6,2)	6(12,5)	9(18,7)	±11.03	7.67-29.73
Neurological involvement	2(4,1)	1(2,0)	3(6,2)	±6.82	-0.62-13.02
Large vessel involvement	1(2,0)	1(2,0)	2(4,1)	±5.61	-1.51-9.71

**95% Confidence Interval*

Table 2. Medications of patients in Active and Inactive groups

Medications	Active group, n=24	Inactive group, n=24
	n (%)	n (%)
Colchicine	10(41,66)	20(83,3)
Colchicine + Azathioprine	1(4,16)	-
Colchicine + Salasopyrin	1(4,16)	1(4,16)
Colchicine+ Systemic corticosteroids	2(8,3)	-
No systemic treatment	6(25)	1(4,16)
Systemic corticosteroids	4(16,6)	1(4,16)
Cyclosporine	-	1(4,16)

The assessment of the last month's treatment was performed. The medications that patients were using were as follows: 30 (10 in active, 20 in inactive group) colchicine, 1 (in active group) azathioprine along with colchicine, 2 (1 in active, 1 in inactive group) salazopyrin along with colchicine, 2 (in active group) systemic corticosteroid along with colchicine. Thus, 14 patients in the active group and 21 patients in the inactive group were taking colchicine either alone or along with another systemic agent. Of the patients 5 (4 in the active, 1 the in inactive group) were using only systemic corticosteroid, and 1 (in inactive group) was using only cyclosporine. Total of 7 patients (6 in active and 1 in inactive group) were not using any systemic treatment. The treatment status of the patients' was shown in the Table 2 (Table 2).

Renal and liver function tests and as well as the complete blood counts were within the normal limits in all groups. The CRP levels were 13,3 ± 29,71 mg/l in active and 3,6 ± 5,6 mg/l in inactive group. The ESR were 27,2± 17,2 mm/h in active and 13,5±12,9 mm/h in inactive group. BDCAF scores were 17,9±5,4 and 10,7±6,1 in active and inactive groups respectively. There were statistically significant differences between the patients in active and inactive groups regarding the mean values of CRP, ESR and BDCAF scores ($p<0.05$, $p<0.05$ and $p<0.001$ respectively). There was no correlation between CRP, ESR and BDCAF scores with the positivity of the pathergy test in the patient group ($p>0.05$).

The serum and urine NPT levels were 8.53 ± 6.75 nmol/L and 422.2 ± 672.73 in the patients, and 5.53 ± 3.46 nmol/L and 203.3 ± 147.74 lmol/mol creatinine in the controls, respectively. The serum and urine NPT levels of patients were higher than controls and the difference was statistically significant (t= 2,287, p=0.025) The urine NPT levels of patients were higher than controls and the difference was statistically significant (t= 2,158, p=0.035) (Table 3). There was a positive correlation between the serum NPT and urine NPT levels in the patient and the control groups (r ¼ 0.42, P ¼ 0.000 and r ¼ 0.38, P ¼ 0.008, respectively). The serum and the urine NPT levels were 9.085±1.13 nmol/L and 479.2±20.46 lmol/mol creatinine in active patients, and 7.5±0.94 nmol/L and 365.2± 38.83 lmol/mol creatinine in inactive patients, respectively. There was no statistically significant difference between

Table 3. Serum and urinary neopterin levels

	stastical method	**serum neopterin (nmol/l)**	**urinary neopterin (lmol/mol)**
Patient group, n=48		8.53 ± 6.75	422.2 ± 672.73
Control group, n=24		5.53 ± 3.46	203.3 ± 147.74
	Independent-samples t-test	t=2,287 p=0,025	t=2,158 p=0,035
Active group, n=24		9.085±1.13	479.2±20.46
Inactive group, n=24		7.5±0.94	365.2± 38.83
Control group, n=24		5.53 ± 3.46	203.3 ± 147.74
	Anova	f=1,830, p=0,168	f=1,474 p=0,235
Colchicine alone or with another systemic agent, vn=35		8,63 ± 7,2	431.16 ± 724.13
Systemic agent except colchicine (cyclosporine, azathioprine, systemic steroid), n=6		8,55 ± 6,73	442.78 ±731.85
No systemic agent, n=7		6,93 ± 5,94	291.95 ± 345.13
	Kruskal-Wallis H test	χ^2 = 1,631 p=0,443	χ^2 = 0,370 p=0,831
Only colchicine in the active group, n=14		12,07 ± 9,97	506.95 ± 753.32
Only colchicine in the inactive group, n=21		6,37 ± 3,16	381.95 ± 718.31
	Independent-samples t-test	t=2,44 p=0,02	t=0,495 p=0,624

the serum NPT levels in active and inactive patients and control group (f= 1,830, p=0.168). There was no statistically significant difference between the urine NPT levels in active and inactive patients and control group (f=1,474 p=0.235) (Table 3).

The serum and the urine NPT levels of the patients' were as follows: 8,63 ± 7,2nmol/L and 431.16 ± 724.13 lmol/mol respectively using colchicine alone or with another systemic agent, 8,55 ± 6,73nmol/L and 442.78 ± 731.85 lmol/mol using systemic agent (cyclosporine, azathioprine, systemic steroid) except colchicine, 6,93 ± 5,94 nmol/L and 291.95 ± 345.13 lmol/mol not using any systemic agent. There were no statistically significant differences between the groups (χ^2=1,631, p=0.443 and χ^2=0,370, p=0.831 respectively) (Table 3). The serum and urine NPT levels of the patients' who were treated only with colchicine in the active and the inactive groups were 12,07 ± 9,97± 2.52 nmol/L and 506.95 ± 753.32 lmol/mol and 6,37 ± 3,16± 0.8 nmol/L and 381.95 ± 718.31 lmol/mol (Table 3). The serum NPT levels of the patients in the active group who were taking colchicine were higher than those of the inactive group and the difference was statistically significant (t=2,44, p=0.02). The urine NPT levels of the patients' who were treated with colchicine in the active group were higher than those of the inactive group but the difference was not statistically significant (t=0,495, p=0.624)(Table 3). Comparison of the mean values of the serum and urine NPT levels between the patients with or without positive pathergy test showed no significant difference (t=0,804, p=0.651 and t=0,486, p=0,273).

Discussion

It is known that increased T- and B-cell response against several antigenic stimuli acts in the etiopatogenesis of BD. So, it is argued that cytokines or other products of these pathways may reflect the disease activity[18]. In numerous studies, Th1 and Th2 cytokines, alone or together, were reported to play role in the disease pathogenesis [27,31]. IFN-γ, as a cytokine of Th pathway, induces monocytes/macrophages for secretion of NPT. And also, IFN-γ is the most efficient inducer of NPT. IFN-γ has a pivotal role in the pathogenesis of BD. The number of γδ-T-cell subtype, which produces IFN-γ, was increased initially without any association to the disease activity in BD [32-34].

Detection of elevated levels of NPT in active periods of many diseases can be thought as the cell-mediated immunity entirely causes the relapse, or distinct factors that lead to relapse can secondarily affect the cell-mediated immunity. NPT measurements are thought to be helpful in BD to determine the disease activity and have been performed in body fluids including; cerebrospinal fluid (CSF), serum and urine [15,21]. Furukawa et al. [35] reported high levels of NPT in CSF of BD patients with neurological involvements. They

Table 4. Comparion of studies investigating neopterin levels in Behçet Disease

	Body fluid used for detection of neopterin	Significant deference between patients and controls	Significant deference between active and inactive patients	Correlation of neopterin with BDCAF+
Furukawa et al.[35]	Cerobrospinal fluid	Higher in patients	Correlated to the clinical severity	-
Samsonov et al.[36]	serum	Higher in patients	-	-
Keser et al.[21]	serum	Higher in patients	No difference	-
Kose et al.[38]	serum	Higher in patients	Higher in active patients	-
Altindağ et al.[41]	urine	Higher in patients	Higher in active patients	-
Coskun et al.[39]	serum	Higher in patients	Higher in active patients	-
Kökcam et al.[37]	serum	Higher in patients	-	-
Lee et al.[40]	serum	Higher in patients	Correlated to the clinical severity	-
Erturan[23]	serum&urine	Higher in patients	No difference	-
Current study	serum&urine	Higher in patients	No difference	No difference

+*BDCAF: Behçet's Disease Current Activity Form*

concluded that the levels of NPT were associated to the clinical severity. Some studies showed significantly high levels of NPT in the serum of patients with BD [36,37]. Additionally, a few study mentioned high levels of NPT in the active phase of the disease [38-41]. Conversely, Keser et al. [21] and Erturan et al. [23] found increased levels of NPT in BD compared to healthy controls but the difference was not statistically significant between the active and the inactive phase of the disease. Similar results were observed in the present study. The results of these studies are summarized in Table 4 (Table 4). In conclusion, the increased levels of NPT in patients with BD as in all previous studies, points out chronic cellular immunity activation with remissions and relapses. The cell-mediated immunity is not one and the only cause for relapsing, in other words, several factors leading to exacerbation do not impact cell-mediated immunity in BD. Different mechanisms other than the cell-mediated immunity could be responsible for exacerbations. On the other hand, similar levels of serum and urinary NPT in the active and inactive periods may be a result of permanent cell-mediated immune activation [3,9].

Most of the patients (85,4%) participated to our study were administered at least one of the medications such as colchicine, systemic corticosteroids, *azathioprine or cyclosporine*. The patients' systemic agent use has not been interrupted in the current study, which could affect the clinical status. However, colchicine, and systemic immunosuppressive agents like; *azathioprine and cyclosporine have been reported to decrease* NPT *levels [41] Altindağ et al. [41] also, did not terminate colchicum use during their study. Erturan et al. [23] indicated that the patients did not have any medications for two months in their study. We could not find any detailed information about the medication use in the study of Köse et al. [38] and Samsonow et al. [36]*.

In our study there were no significant differences in the NPT levels between the patients who were on systemic medications and the ones who were not on any medications ($p>0.05$). Even though this finding might cause the thought of systemic therapies did not affect the NPT levels, the patients' clinical presentation has significant importance. From this point of view, only the NPT levels of the patients' who were treated with colchicine in the active and inactive groups were compared. As a result, the serum NPT levels of the patients' in the active group were found higher than those of the inactive group ($p<0.05$). Urine NPT levels were also higher in the active patients' only using colchicine, but the difference was not statistically significant. These findings are not sufficient to assess the effect of the therapeutic agent or the clinical status on the NPT levels. So, a study conducted with newly diagnosed patients without any treatment might be more reliable. In addition, a longitudinal study will be more beneficial to determine the value of the NPT level in measuring the disease activity.

To the best of our knowledge, this is the second study in evaluating both urine and serum NPT levels for BD [23]. Keser et al. [21] reported that measuring serum NPT levels is not reliable to determine the disease activity and suggested to measure urine NPT levels instead. In our study there was a correlation between the serum and urine NPT levels in BD. Investigators claimed that urinary NPT measurement could be more meaningful because the urine NPT levels are at least 500–1000 times higher than serum NPT [42]. Altindağ et al. [41] reported higher urinary levels in the active patients compared to inactive ones, but they did not study the serum NPT levels. In many studies regarding different diseases, investigators proposed to evaluate serum and urinary NPT levels together.

Similar to some studies in the literature, we did not find any correlation between NPT, CRP, ESR and pathergy positivity [21-23]. However, converse results have also been reported [36,38]. Certainly, CRP, ESR and pathergy positivity are not accepted as activity criteria. On the other hand, this is the first study, evaluating both the BDCAF scores and the NPT levels in BD. We found significantly higher BDCAF scores in the active patients. However, there was no correlation with BDCAF scores and the NPT levels both in the serum and urine. Even though BDCAF is the most commonly used activity index to assess the activity of disease, it comprises some insufficiencies [17-19]. BDCAF scores reflects clinical status of the last month. Also, it does not exactly represent all the systemic involvements of BD. It was thought to be more reliable in the patients with mucocutaneous and ocular involvements [24].

The serum NPT level is a sensitive marker that indicates endogenous IFN-γ secretion [43]. Detecting serum IFN-γ levels with NPT could be more valid. In the present study, we found higher levels of serum and urinary NPT level in BD compared to the healthy controls. But there were no statistically differences between the active and inactive patients.

Follow-up data of the same patients in active and remission phases would be more informative. Also, serum NPT levels were found to be associated with the disease activity in SLE and systemic vasculitis [44]. As mentioned in the study of Erturan et al. [23] the patients with active SLE or systemic vasculitis could be included as a positive control group in the study.

Conclusion

In conclusion, the current study suggests that BD is a T cell–mediated inflammatory disease. However, surely, this suggestion does not clarify the etiopathogenesis of the disease. Even though some contrary reports exist, we support the suggestion that the serum and urinary NPT measurements cannot be used as a reliable laboratory marker in determining the activity of BD. Despite having some disadvantages, BDCAF scores may identify the active stage of BD.

References

1. *Behçet H. Über rezidiverende aphtöse, durch ein Virus verursachte Geschwure am Mund, am Auge und an den Genitalien. Dermatol Wochenschr 1937; 105: 1152.*
2. *Evereklioglu C, Er H, Turkoz Y, et al. Serum levels of TNF-a , sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behçet's disease. Mediators of Inflammation 2002; 11: 87–93.*
3. *Kapsimali VD, Kanakis MA, Vaiopoulos GA, et al. Etiopathogenesis of Behçet's disease with emphasis on the role of immunological aberrations. Clin Rheumatol 2010; 29: 1211–1216.*
4. *Al-Mutawa SA, Hegab SM. Behcet's disease. Clin Exp Med 2004; 4: 103-31.*
5. *Krause I, Weinberger A. Behçet's disease. Curr Opin Rheumatol 2008; 20: 82-7.*
6. *Azizlerli G, Kose AA, Sarica R, et al. Prevalence of Behçet's disease in Istanbul, Turkey. Int J Dermatol 2003; 42: 803–806.*
7. *Tursen U. Activation Markers in Behcet Disease. Turkderm 2009; 43: 74-86.*
8. *Reibnegger G, Bollbach R, Fuchs D, et al. A simple index relating clinical activity in Crohn's disease with T cell activation: hematocrit, frequncy of liquid stools and urinary NPT as parameters. Immunobiology 1986; 73: 1–11.*
9. *Suzuki KM, Suzuki N. Behcet's disease. Clin Exp Med 2004; 3: 10–20.*
10. *Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflamm Res 2003; 52: 313–321.*
11. *Davatchi F. New and innovative therapies for Behçet's disease. APLAR J Rheumatol 2004; 7: 141–145.*
12. *Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. J Clin Pharm Ther 2001; 26: 319–329.*
13. *Frick J, Aulitzky W, Fuchs D, et al. The value of urinary neopterin as an immunological parameter in patients with malignant tumor of the genitourinary tract. Urol Int 1985; 40: 155–159.*
14. *Daito K, Suou T, Kawasaki H. Clinical significance of serum and urinary neopterin levels in patients with various liver diseases. Am J Gastroenterol 1992; 87: 471–476.*
15. *Reibnegger G, Auhuber I, Fuchs D, et al. Urinary neopterin levels in acute viral hepatitis. Hepatology 1998; 8: 771–774.*
16. *Kaleli I,Demir M, Cevahir N, et al. Serum neopterin levels in patients with replicative and non-replicative HBV carriers. BMC Infect Dis 2006; 31: 157.*
17. *Bhakta BB, Brennan P, James TE, et al. Behçet's disease: evaluation of a new instrument to measure clinical activity. Rheumatology 1999; 38: 728-33.*
18. *Shahram F, Khabbazi A, Nadji A, et al. Comparison of existing disease activity indices in the follow-up of patients with Behçet's disease. Mod Rheumatol 2009; 19: 536-41.*
19. *Neves FS, Moraes JC, Kowalski SC, et al. Cross-cultural adaptation of the Behçet's Disease Current Activity Form (BDCAF) to Brazilian Portuguese language. Clin Rheumatol 2007; 26: 1263-7.*

20. *Ghate JV, Jorizzo JL. Behçet's disease and complex aphthosis. J Am Acad Dermatology 1999; 40: 1-18.*

21. *Keser G, Oksel F, Aksu K, et al. Serum neopterin levels in Behçet's syndrome. Clin Rheumatol 2000; 19: 326–329.*

22. *Evereklioglu C, Turkoz Y, Er H, et al. Increased nitric oxide production in patients with Behçet's disease: is it a new activity marker? J Am Acad Dermatol 2002; 46: 50–54.*

23. *Erturan I, Basak PY, Ozturk O, et al. Is there any relationship between serum and urine neopterin and serum interferon-c levels in the activity of Behcet's disease? JEADV 2009; 23: 1414–1418.*

24. *Hamuryudan V, Fresko I, Direskeneli H,et al. Evaluation of the Turkish translation of a disease activity form for Behçet's syndrome. Rheumatology 1999; 38: 734-6.*

25. *Barak M, Merzbach D, Gruener N. Neopterin measured in serum and tissue culture supernates by a competitive enzyme-linked immunosorbent assay. Clin Chem 1989; 35: 1467–1471.*

26. *Wachter H, Fuchs D, Hausen A, et al. Neopterin as a marker of cellular immunity: immunologic basis and clinical application. Adv Clin Che 1989; 27: 81–141.*

27. *Koarada S, Haruta Y, Tada Y, et al. Increased entry of CD4+ T cells into the Th1 cytokine effector pathway during T-cell division following stimulation in Behcet's disease. Rheumatology 2004; 43: 843–851.*

28. *Sugi-Ikai N, Nakazawa M, Nakamura S, et al. Increased frequencies of interleukin-2 and interferon-gamma-producing T cells in patients with active Behcet's disease. Invest Ophthalmol Vis Sci 1998; 39: 996–1004.*

29. *Aridogan BC, YildirimM, Baysal V, et al. Serum levels of IL-4, IL-10, IL-12, IL-13 and IFN-gamma in Behcet's disease. J Dermatol 2003; 30: 602–607.*

30. *Raziuddin S, Al-Dalaan A, Bahabri S, et al. Divergent cytokine production profile in Behcet's disease. Altered Th1/Th2 cell cytokine pattern. J Rheumatol 1998; 25: 329–333.*

31. *Hamzaoui K, Hamzaoui A, Guemira F, et al. Cytokine profile in Behcet's disease patients. Relationship with disease activity. Scand J Rheumatol 2002; 31: 205–210.*

32. *Psarra K, Kapsimali V, Vaiopoulos G, et al. Immunophenotype and Th1/ Th2 cytokines in patients with Adamantiades-Behcet's disease. Adv Exp Med Biol 2003; 528: 249–253.*

33. *Sharquie KE, Al-Hayani RK, Najim RA. Dilsen's pathergy test in Behcet's disease: positive correlation with clinical manifestations. Adv Exp Med Biol 2003; 528: 331–332.*

34. *Koarada S, Haruta Y, Tada Y, et al. Increased entry of CD4+ T cells into the Th1 cytokine effector pathway during T-cell division following stimulation in Behcet's disease. Rheumatology 2004; 43: 843–851.*

35. *Furukawa Y, Nisshi K, Kondo T, et al. Significanca of CSF total neopterin and bipterin in inflammatory neurological disseases. J Neeurol Sci 1992; 111: 65-72.*

36. *Samsonov M, Prokaeva TB, Madanet V, et al. Serum neopterin in Behçet's disease. Klin Med 1995; 73: 53–55.*

37. *Kokcam I, Naziroglu M. Effects of vitamin E supplementation on blood antioxidants levels in patients with Behcet's disease. Clin Biochem 2002; 35: 633–639.*

38. *Kose O, Arca E, Akgul O, et al. The levels of serum neopterin in Behçet's disease-Objectivemarker of disease activity. J Dermatol Sci 2006; 42: 128–130.*

39. *Coskun B, Saral Y, Godekmerdan A, et al. Activation markers in Behçet's disease. Skinmed 2005; 4: 282–286.*

40. *Yoon J, Lee SH, Bang D, et al. Elevated serum levels of neopterin in patients with Behcet's disease. Ann Dermatol 1993; 5: 74–78.*

41. *Altindağ ZZ, Werner-Felmayer G, Şahin G, et al. Colchicine derivates inhibit neopterin production in human peripheral blood mononuclear cells. Clin Exp Immunol 1997; 107; 574-577.*

42. *Palmer HE, Giovannoni G, Standford MR, et al. Urinary neopterin in idiopathic retinal vasculitis. Br J Opthalmol 2001; 85: 30–33.*

43. *Niederwieser A, Joller P, Seger R, et al. Neopterin in AIDS, other immunodeficiencies, and bacterial and viral infections. Klin Wochenschr 1986; 64: 333–337.*

44. *Samsonov MY, Tilz GP, EgorovaO, et al. Serum soluble markers of immune activation and disease activity in systemic lupus erythematosus. Lupus 1995; 4: 29–32.*

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Impact of obesity on health related quality of life among children and adolescents in Slovenia

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Abstract

Background: The prevalence of obesity among children and adolescents in Slovenia has increased substantially over the last decade as a consequence of unhealthy contemporary lifestyle. The aim of our study was to examine the relationship between health related quality of life (HRQOL) and body weight in a group of obese children/adolescents compared with normal weight controls.

Methods: The study included 69 obese children/adolescents between 3 and 18 years, as well as 64 children/adolescents with normal weight. The questionnaire was developed to assess the main aspects of HRQOL: general health, physical activity, social status, psychological and specific aspects (including eating habits). Logistic regression analysis was performed to estimate the risks associated with child obesity.

Results: Results showed that parental educational level is an important risk factor for the development of obesity in children/adolescents. Children and adolescents who were obese had significantly less physical strength and were less physically active. Obese children and adolescents were breastfed for a shorter period of time and solid foods were introduced earlier than in the group of children with normal weight.

Conclusion: Obese children/adolescents had lower scores on a number of HRQOL domains. However, they don't feel excluded from society and are predominantly satisfied with their HRQOL. They did not feel depressed, nor sad.

Key words: Adolescents, children, health-related quality of life, obesity, parental education.

Introduction

The global prevalence of overweight and obesity among children and adolescents has reached alarming proportions over the last two decades, as a consequence of the unhealthy contemporary lifestyle (1). It is characterized by numerous changes in the social, economic and physical environment related to the nutrition transition, which is usually associated with an increase in the consumption of energy-dense foods, sugars and sweetened drinks, a decrease in physical activity and a more sedentary lifestyle (2). Slovenia is following this trend (3). Juvenile obesity is rapidly becoming one of the most important health concerns, due to its direct correlation with overweight in adulthood and due to possible adverse health implications such as development of type 2 diabetes mellitus, hypertension, obstructive sleep apnea, asthma, dyslipidemia, osteoporosis, and overall decreased quality of life (4, 5). It has an immediate consequence on a child's physical appearance, but it can also lead to additional psychosocial consequences, such as low self-esteem, social alienation, lack of self-confidence, discrimination, and more commonly in girls, depression (2, 6, 7). In addition, peers have a critical role for psycho-social development in children. Children being constantly teased by their peers and having problems with making friends, not being socially accepted are likely to become depressed. This consequently leads to social alienation and overweight or obesity due to a lack of physical activity (8, 9). The prevalence of juvenile obesity has been shown to be more common among children originating from families with lower social status and in children of parents with a lower educational level (10, 11). Family environment is one of the important factors that influence the food choices and eating behaviour of children. Parents determine the food behaviour of their children through their influence on the availability of food, meal structure, their own body weight, socioeconomic status, food preferences and physical activity (12). Moreover,

socioeconomic status has an influence on the type of food consumed. It has been shown that diets in families with low socioeconomic status are mainly composed of meat, fatty foods and, sugars, with fewer vegetable products and fruits (10). Furthermore, obesity today is unreported, as parents seem to be unaware of the problem until severe complications related to obesity appear. They often describe their children as ″vigorous″ rather than overweight, with a healthy diet and good appetite. The term health related quality of life (HRQOL)is based on the well known and broad definition of health as given by the World Health Organization (WHO), which defines health as a condition of complete physical, mental and social well-being and not merely as the absence of disease or disability (13). The evaluation of HRQOLis a method used to assess global psychosocial functioning (14). In clinical studies regarding chronic health problems, including juvenile obesity, HRQOL is an important indicator of outcome complementary to clinical and laboratory markers (15). It is a multidimensional form with several core aspects, including general health and physical, psychological and social functioning. HRQOL reveals the individual's subjective evaluation of his/her own well-being and functioning (14). It has already been shown that severely obese children and adolescents have poorer HRQOL across all domains of functioning (physical, psychological, social and educational) when compared to children and adolescents with normal weight, and at the same time have a similar HRQOL as those diagnosed with cancer (6, 16).

To our knowledge, the impacts and consequences of juvenile obesity on the everyday life of children and adolescents in Slovenia have not been characterized yet. For this reason, we analyzed both, the impact and the consequences of obesity on children' and adolescent's daily activities, physical activity, social interactions, and emotional well-being.

The aim of the present study was to compare the self-reported HRQOL of obese children and adolescents with normal-weight controls using an HRQOL questionnaire developed for the study. Furthermore, we wanted to determine any correlation between participant characteristics, BMI percentile status and HRQOL to give information about potential risk factors within obese children and adolescents for decreased HRQOL.

Patients and methods

The correlations of HRQOL and child/adolescent body weight were analysed at the Pediatric Clinic of the University Medical Center Maribor, Slovenia. Maribor is the second largest city in Slovenia and the largest city of the traditional region of Lower Styria, with population of 350 000 inhabitants which incline to this center.

The case- control study was carried out on subjects who were newly referred for the evaluation of obesity associated conditions to the Pediatric Clinic of the University Medical Center Maribor during December 2007 through February 2011. Obese children who came to different specialist outpatient clinics, as well as those obese children who came to a general outpatient clinic at the Pediatric clinic were included in the study. The control group consisted of children and adolescents who showed a BMI within the normal range and who came to the Pediatric Clinic for their check-ups following their hospitalization due to a particular health condition such as pneumonia, urinary tract infection, otitis media, diarrhoea, etc. During their visit, parent(s) and child/adolescent received information regarding the study. After obtaining the written consent, participants were handed in the questionnaire.. The study included 69 preschool and school children and adolescents (31 girls, 38 boys) between 3 and 18 years of age (mean 13 ± 3.3 years), with BMI above 97th percentile for age and sex. The youngest obese child included in the study was 3 years old.

The control group consisted of 64 preschool and school children and adolescents (41 girls, 23 boys), with a BMI within the normal range. A total of 133 children and adolescents completed the questionnaire developed for youths. However, four obese participants did not reach inclusion criteria and were consequently excluded form the study. The reasons for exclusion were, hypertension (1 adolescent), chronic kidney disease (1 adlescent), and Down syndrome (2 children). In addition, three obese adolescents, and 10 children and adolescents from the control group refused to participate.

As the study focused on HRQOL of obese children/adolescents, overweight participants with BMI between 85th and 95th percentile for sex and age were excluded. Children's and adolescent's anthropometric measurements, body height (cm)

and body weight (kg) were collected by medical personnel using standardized procedures.

The Questionnaire

No pediatric international HRQOL questionnaire has been validated in Slovenia, at the time of conducting the study. Hence a 91 item questionnaire was designed to obtain information on five main aspects of HRQOL in children and adolescents: general health, physical activity, social status, psychological aspects and other specific aspects (including eating habits).

Parents were asked to fill in the questionnaire in the case, when children were younger than 14, while adolescents were asked to fill it in themselves. The parents of all participants (both patients and control group) also completed the part of the questionnaire regarding their social background, information about medical problems in the family and, parental education level, as well as the specific part of the questionnaire encompassing breastfeeding and the child's eating habits. Questionnaire was formulated in such a way that both parents and their children were able to fill in the details together. For that reason, answers from parents and children could not be analyzed separately. All questions were answered and questionnaires completed in the presence of the interviewer at the Pediatric Clinic. Parental education level was divided into three categories: low (primary school, ≤ 10 years of education), middle (secondary school, 10-12 years of education) and high (university, > 12 years of education). The socioeconomic parameter combines family income, parental education, profession of parents, place of residence (own flat/house), and way of spending holidays, and was also divided into categories low, middle and high. Each question had multiple possible answers rated from the lowest to the highest score. Physical activity, including type and duration, was also assessed with the questionnaire, and was classified into two mutually exclusive groups: actively training for sports in a club (0, occasionally, 1, 2, 3 or more times/week for at least 1 h), and recreationally training for sports (0, occasionally, 1, 2, 3 or more times/week for at least 1 h).

Watching television or playing computer games was classified into the following categories: 0-1, 1-2, 3 or more hours/day. The psychological aspect of HRQOL included the sociability of subjects, satisfaction with their appearance and quality of life, functioning in society, school success etc. The specific part of the questionnaire analyzed the eating habits of the study population based on diet records. BMI was calculated as the weight in kilograms divided by the square of height in meters and adjusted for age and sex. Obesity was defined according to standard international definitions, using age and sex adjusted BMI cut-off percentiles (15). BMI percentiles of the study population were categorized into two mutually exclusive groups: (1) obese (BMI > 97^{th} percentile) and (2) normal weight (BMI 5^{th} - 85^{th} percentile). Reference data of BMI for age and sex from the 2000 Centers for Disease Control and Prevention were used (16).

The study was approved by the National medical ethics committee.

Statistical methods

The background information was presented through percentage distributions. Chi-square test was used to calculate statistically significant differences of background information. Logistic regression analysis was performed to calculate the risks associated with child obesity. The significance, odds ratios and confidence intervals were calculated for univariate analysis. Statistical analysis was performed with the SPSS 15.0 statistical package for Windows. All statistical values were considered significant at the P level of 0.05.

Binary logistic regression was calculated to obtain (a) variables independently connected to risk of obesity, and (b) a model for predicting obesity occurrences according to these independent variables. All measured variables were dichotomous and had values entered "1" for "yes" and "2" for "no". Table 4 presents univariate statistics of all measured variables potentially related to risk of obesity. Eight variables reached statistical significance.

After multivariate regression analysis two out of eight statistically significant variables entering the model using Wald statistics were composed in the prediction model. Both remaining variables (breastfeeding ≤ 6 months and daily number of meals < 5) were also primarily independently connected to risk of children obesity (Table 5).

Results

Differences between obese children and adolescents and the control group

A total of 69 patients and 64 controls completed the questionnaires and were included in the analysis. Demographic and weight characteristics of the study population are shown in Table 1. Using BMI percentiles for age and sex, all of the 69 patients involved in the study were considered obese, with body mass index (BMI) above the 97th percentile. None of the patients involved in the study was in the overweight group with BMI between the 85th and 97th percentile, nor in the group with BMI less than the 85th percentile. The control group consisted of normal-weight participants with BMI less than the 85th percentile. A significant difference ($\chi^2 = 0.036$, $p < 0.05$) was found between the weight category and gender of participants, males being classified as obese in higher proportion than females (62.3 % obese males vs 43.1 % obese females). However, because males and females did not differ significantly by age, paternal or maternal education, location of residence and socioeconomic status, both genders were combined for statistical analysis and processed as a unique group. Considerable differences in HRQOL were found between the group of obese children and adolescents and the control group, as shown by differences in several domains of the questionnaire. In addition to lower BMI percentiles of children and adolescents, a higher level of paternal education was found in the control group. In the study population, 14.9 % of fathers and 17.4 % of mothers had less than 10 years of education, 76.1 % of fathers and 69.6 % of mothers had 10 to 12 years, and 9.0 % of fathers and 13.0 % mothers with more than 12 years of education. Fathers' education level ($\chi^2 = 0.018$, $p < 0.05$) had a greater influence on the obesity of children and adolescents than mothers' ($\chi^2 = 0.125$, $p > 0.05$) education level (Table 1). Furthermore, obese children and adolescents were less engaged in sports training ($\chi^2 = 0.022$, $p < 0.05$) (Table 1). Physical strength in the study population of obese children and adolescents was distinctly lower than in the control group ($\chi^2 = 0.010$, $p < 0.05$). Results also indicate a weak association between low self-esteem and obesity.

Table 1. Descriptive characteristics of study population

	Normal weight No. (%)	Obese No. (%)	*P*- value
Age groups (y)			0.167
3 - 13	30 (46.9)	41 (59.4)	
≥ 14	34 (53.1)	28 (40.6)	
Sex			**0.036**
Male	23 (35.9)	38 (55.1)	
Female	41 (64.1)	31 (44.9)	
Maternal education (y)			0.125
≤ 10	8 (12.7)	12 (17.4)	
10 - 12	38 (60.3)	48 (69.6)	
> 12	17 (27.0)	9 (13.0)	
Paternal education (y)			**0.018**
≤ 10	5 (8.1)	10 (14.9)	
10 - 12	40 (64.5)	51 (76.1)	
> 12	17 (27.4)	6 (9.0)	
Mother's employment			0.370
Unemployed	9 (14.1)	14 (20.3)	
Part-time	3 (4.7)	6 (8.7)	
Full-time	52 (81.2)	49 (71)	
Father's employment			0.422
Unemployed	5 (7.9)	9 (13.4)	
Part-time	2 (3.2)	4 (6.0)	
Full-time	56 (88.9)	54 (80.6)	

Number of brothers/sisters			0.858
None	12 (18.8)	13 (18.8)	
One	36 (56.2)	39 (56.5)	
Two	10 (15.6)	13 (18.8)	
Three or more	6 (9.4)	4 (5.8)	
Location of residence			1,00
Urban	22 (35.5)	25 (36.8)	
Rural	40 (64.5)	43 (63.2)	
Parent's opinion regarding child's appearance			**< 0.001**
Appropriate	47 (73.4)	4 (5.8)	
Oversized	13 (20.3)	54 (78.3)	
Markedly oversized	0 (0.0)	11 (15.9)	
Lean	4 (6.3)	0 (0.0)	
Physical strength			**0.010**
Normal	45 (71.4)	40 (58.0)	
Poor	8 (12.7)	23 (33.3)	
Very poor	1 (1.6)	3 (4.3)	
Above average	9 (14.3)	3 (4.3)	
Actively training sports			**0.022**
Yes	36 (52.2)	20 (31.7)	
No	33 (47.8)	43 (68.3)	
Recreationally training for sports			0.165
Yes	36 (58.1)	31 (45.6)	
No	26 (41.9)	37 (54.4)	
Socioeconomic status			0.849
Very good	14 (22.2)	13 (18.8)	
Satisfactory	40 (63.5)	47 (68.1)	
Low	9 (14.3)	9 (13.0)	
School success			0.235
Very successful	18 (56.3)	24 (36.4)	
Average	5 (15.6)	11 (16.7)	
Sufficient	7 (21.8)	27 (40.8)	
Poor	2 (6.3)	4 (6.1)	
Social functioning			0.412
Very social	31 (49.2)	41 (59.4)	
Average	30 (47.6)	25 (36.2)	
Antisocial	2 (3.2)	3 (4.3)	
Satisfaction with QOL			0.325
Very satisfied	25 (41.0)	21 (30.9)	
Mostly satisfied	33 (54.1)	40 (58.8)	
Mostly dissatisfied	3 (4.9)	7 (10.3)	

Clinical characteristics of the study population of obese children and adolescents

Results showed that 72.4 % of obese children and adolescents from the study population had at least one family member who was overweight or obese (Table 2). Furthermore, the most common medical problems in these families were elevated blood pressure (63.7 %), type 2 diabetes mellitus (44.9 %), elevated lipids (36.2 %) and cardiovascular problems (30.4 %) (Table 2).

Of obese children and adolescents, 32.8 % reported having had problems with their weight less than five years, 49.2 % five years or more, and 14.8 % having had weight problems since birth - but surpri-

Table 2. Medical problems in families of obese children and adolescents

Medical problem	No. (%) of obese children/ adolescents with a particular medical problem in the family	No. (%) of normal weight children/ adolescents with a particular medical problem in the family
Overweight or obesity in family	50 (72.4)	10 (15.6)
Elevated blood pressure	44 (63.7)	35 (54.7)
Type 2 diabetes mellitus	31 (44.9)	6 (9.4)
Elevated lipids	25 (36.2)	15 (23,4)
Cardiovascular problems	21 (30.4)	25 (39.1)
Thyroid	14 (20.3)	10 (15.6)
Neurological problems	3 (4.4)	2 (3.1)
Atherosclerosis	2 (2.9)	2 (3.1)

Table 3. Specific aspect of HRQOL of study population (including eating habits)

	Normal weight No. (%)	Obese No. (%)	*P*- value
Type of diet			0.108
Normal	61 (95.2)	68 (100)	
Vegetarian	3 (4.8)	0 (0)	
Number of daily meals			**0.005**
Two to three	15 (23.8)	34 (50)	
Five	41 (63.5)	31 (45.6)	
More than five	8 (12.7)	3 (4.4)	
Eats breakfast			**0.003**
Yes	56 (86.7)	43 (63.2)	
No	8 (13.3)	25 (36.8)	
Frequent eating out			0.365
Yes	4 (6.3)	8 (12.3)	
No	60 (93.7)	57 (87.7)	
Duration of breast feeding			**0.003**
Not at all	6 (9.8)	10 (15.4)	
3 months	20 (31.1)	36 (55.4)	
4 months or more	38 (59.0)	19 (29.2)	
Introduction of solid foods			**0.049**
Before 4^{th} month	0 (0)	3 (4.6)	
Between 4^{th} and 6^{th} month	38 (58.6)	46 (70.8)	
After 6^{th} month	26 (41.4)	16 (24.6)	
Family meal			**0.050**
Every day	38 (59.7)	26 (40.6)	
Occassionally	26 (40.3)	38 (59.4)	
Time spent in front of computer (daily)			
> 1h	50 (78.1)	49 (74.2)	**0.022**
> 2h	11 (17.2)	5 (7.6)	
3 h and more	3 (4.7)	12 (18.2)	
Time spent in front of television (daily)			**0.017**
> 1h	41 (64.1)	28 (42.4)	
> 2h	21 (32.8)	29 (43.9)	
3 h and more	2 (3.1)	9 (13.6)	

singly, only 18 % of them had ever been part of an overweight prevention program. Furthermore, 34.4 % of our study population had other medical problems besides weight (i.e. type 2 diabetes mellitus, cardiovascular problems, elevated lipids, asthma or hypertension). Obese children and adolescents visit their doctor more than once a year (63.9 %); 8.2 % of them visit their doctor on a monthly basis.

Very interesting is the parent's opinion regarding the dietary habits of their children: 8.2 % of parents think their children are eating too little, despite the fact they are seriously overweight, 44.3 % think their children eat adequately, and 45.9 % think their children eat too much. On the other hand, 80.3 % of the parents of obese children and adolescents are aware their children are overweight, and only 1.6 % think their children have a normal weight. Breakfast should be a part of a healthful diet and lifestyle in children and adolescents, but only 63.2 % of obese children and adolescents from the present study consumed this meal on a daily basis (Table 3). Three of the participants from the control group of children and adolescents were vegetarians, while no participants from the obese group of children and adolescents were vegetarian, all of them had a normal diet and consumed at least one warm meal per day (Table 4). In both children and adolescents, physical inactivity was the strongest predictor of increased BMI. Physical inactivity, as measured by watching television, playing computer games or lower physical strength was associated with greater adiposity in children and adolescents. An almost equal number of obese children and adolescents (74.2 %) and normal-weight participants (78.1 %) spent 1 h daily in front of the computer. On the other hand, an obvious difference between these two groups was in a longer time spent in front of computer (3 h and more), where almost one fifth of obese children and adolescents spent daily 3 h in front of the computer (18.2 %) unlike the control group (4.7 %). A similar situation occurred with television watching. While more children and adolescents of normal weight (64.1 %) watched television for shorter periods of time (up to 1 h daily) in comparison to obese children and adolescents (42.4 %), the inverse happened in the case of longer periods of time spent in front of the television (3 h or more) with only 3.1 % of normal-weight children and adolescents watching that much television, and 13.6 % of obese children and adolescents. In logistic regression, watching television for more than 3 h/day was a factor that was strongly associated with obesity in children/adolescents (OR 4.9, 95% CI 1.0 - 23.6), as well as playing computer games for more than 3 h/day (OR 4.5, 95% CI 1.2 - 16.9) (Table 4). Overweight children are no longer a rare phenomenon in modern society. It is interesting that nowadays obesity does not affect the wellbeing of children and adolescents and does not represent a barrier to their normal social functioning. This is obviously due to the changes in society during the last few decades and the increasing prevalence of obesity among adults and children/adolescents. As a consequence these children are no longer a subject of teasing nor excluded from society. In addition, they feel as good as their normal weight peers in both school and home environment and are mostly satisfied with their quality of life (Table 1, figure 1A and 1B).

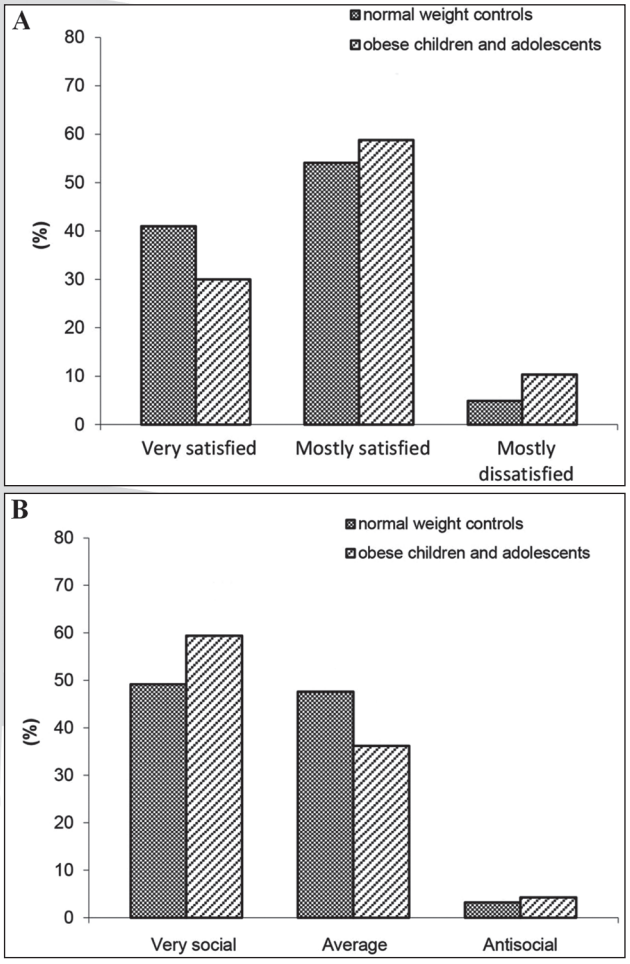

Figure 1. Satisfaction with the quality of life of obese children/adolescent when compared to normal-weight controls (A) and sociability of obese children/adolescent when compared to normal-weight controls (B).

Table 4. Univariate statistics of measured variables potentially related to risk of obesity

Child group Variable	Normal (n=64)	Obese (n=68)	Chi-square	OR	95% CI	*P*-value
Without breakfast (%)	13.3	36.8	8.5	3.8	1.5-9.2	0.004
Breastfeeding ≤ 6 months (%)	41.0	70.8	11.0	3.5	1.7-7.3	0.001
Mixed nutrition ≤ 6 months (%)	58.6	75.4	3.9	2.2	1.0-4.7	0.049
Infrequent family meal (%)	40.3	59.4	4.5	2.2	1.1-4.4	0.034
Computer ≥ 3 hours daily (%)	4.7	18.2	5.0	4.5	1.2-16.9	0.025
TV ≥ 3 hours daily (%)	3.1	13.6	3.9	4.9	1.0-23.6	0.048
Daily number of meals < 5 (%)	23.8	50.0	9.2	3.2	1.5-6.8	0.002
Without daily hot meal (%)	1.6	1.5	0.0	0.9	0.1-15.1	0.956
Vegetarian nutrition (%)*	4.8	0.0	/	/	/	0.108
Visiting fast food restaurants on a weekly basis (%)	31.6	20.6	1.8	0.6	0.2-1.3	0.174
Without fish meal on a weekly basis (%)	28.6	46.2	4.2	2.1	1.0-4.5	0.041
Without daily fruits (%)	28.6	34.8	0.6	1.3	0.6-2.8	0.445
Without daily vegetables (%)	43.5	41.5	0.5	0.9	0.5-1.9	0.819
Without daily meat (%)	63.5	54.8	1.0	0.7	0.3-1.4	0.326
Frequent meals outside home (%)	6.3	12.3	1.3	2.1	0.6-7.3	0.256

** odd ratio could not be calculated since no child from the obese group had vegetarian nutrition*

Table 5. Variables independently related to risk of obesity

Variable	Chi-square	OR	95 % CI	*P*-value
Breastfeeding ≤ 6 months	10.4	3.9	1.7-8.9	0.001
Daily number of meals < 5	9.9	4.1	1.7-9.9	0.002

The calculated prediction model had a specificity of 70.5 % and sensitivity of 75.2 %. The Hosmer-Lemeshow test was not significant, indicating little deviation from a perfect fit (chi-square 0.988, degrees of freedom 2, P = 0.610).

Discussion

In order to determine the association between HRQOL and obesity in children and adolescents, 69 obese children and adolescents were examined and compared to 64 normal-weight controls using a questionnaire which consisted of 91 questions. Our results confirmed the hypothesis, that obese adolescents experience lower HRQOL when compared to normal-weight controls, particularly regarding physical health and to a lesser extent social functioning. As reported elsewhere, obesity tends to aggregate within families (19, 20). For both genders, having an overweight or obese family member considerably increased the likelihood for juvenile obesity, probably as a consequence of unhealthy lifestyles. Our results showed that 72.4 % of obese children and adolescents had a family member with weight problems. Contrary to former studies (7, 10, 16), we found that in Slovenia, socioeconomic status did not have an important impact on childhood obesity. Children and adolescents from all three socioeconomic classes appeared to be at equal risk for being overweight. Our results also showed that parental education level is an important risk factor for the development of juvenile obesity and could be important in preventing obesity during childhood. Obese children and adolescents in our study had fathers with a lower education level ($\chi^2 = 0.018$, $p < 0.05$) when compared to normal-weight controls.

Numerous studies have documented a rapid increase in the prevalence of childhood obesity over the last two decades (1, 3, 7). During these decades of rising obesity prevalence, physical activity among children and adolescents has declined; meanwhile, time spent in sedentary activities such as watching television and playing computer games has increased (21). Lack of physical activity (particularly playing on the computer and watching television) might be one of the main factors determining the increase of juvenile obesity. Obese

children and adolescents from the present study spent significantly more time in front of computer ($\chi^2 = 0.022$, $p < 0.05$) and television ($\chi^2 = 0.017$, $p < 0.05$) than normal-weight children and adolescents. Watching television or playing computer games was frequently accompanied by consuming excessive amounts of food, including fruit (37.7 %), chocolate (23.2 %), potato chips (21.7 %), etc. Television watching is thought to promote weight gain by displacing physical activity and increasing energy intake, as well as exposing children and adolescents to advertisements for food products which in themselves are fattening (22, 23).

We have also found an inverse association between the duration of breastfeeding and obesity. Obese children and adolescents from our study were breastfed for a shorter period of time when compared to the control group ($\chi^2 = 0.003$, $p < 0.05$). Our results concord with the findings of the Ebbeling et al. (23), who showed that bottlefed children were at higher risk for obesity later in childhood than those who were breastfed. Three recent studies (24, 25, 26) performed on large population samples have had a follow-up at least until preschool age. These studies agree in defining breastfeeding as a protective factor against childhood obesity. A possible explanation for this is control over the feeding rate (baby versus parent) in contrast to feeding in childhood and later during adolescence, when parents provide the eating environment for their children. There are also differences in macronutrient intake between breast-fed and bottle-fed children, suggesting that a higher protein intake early in life might increase the risk of later obesity in bottlefed children (27). Furthermore, children fed with formula double their birth weight earlier than the breast-fed ones, and the weight increase is relatively faster than the height increase (12). We have also found that solid foods were introduced earlier in the group of obese children and adolescents ($\chi^2 = 0.049$, $p < 0.05$). The early introduction of solid foods determines the lack of the protective effects of human milk and is directly associated with an increase in the percentage of body fat and weight by the age of seven (28).

The dietary causes of obesity are complex and poorly understood. While individual nutrients and foods have been implicated in obesity, few attempts have been made to identify eating patterns in this regard (28). For this reason, besides breastfeeding, we have also analyzed eating patterns of obese children and adolescents (including fast food restaurant consumption), beverage consumption, portion sizes, meal patterns, school meal consumption, and dietary quality. Usually, the main nutritional mistakes in children and adolescents are: excessive energy intake, low intake of vegetables, fruits and fiber, a high intake of simple sugars, skipping breakfast, high intake of snacks and junk food, and frequent fast-food meals (12). When analyzing the eating habits of our study population, we found that the majority of obese children and adolescents had a healthy diet; 62.1 % eat fruits each day, 53.8 % eat vegetables six time a week, and 33.8 % eat fish two to three times a month. Our results showed that children and adolescents who reported eating breakfast every day were less likely to be obese ($\chi^2 = 0.003$, $p < 0.05$). Even though breakfast has been labelled the most important meal of the day, 36.8 % of obese children and adolescents as well as 13.3 % of children and adolescents from the control group skipped this meal. Evidence suggests that children who consume breakfast regularly tend to have better nutritional profiles than their breakfast-skipping peers. The latter had a higher daily percentage of energy from fat and lower intake of protein, vitamins, and minerals (21, 30). In addition, children and adolescents who skipped breakfast consumed snacks more often, and had higher plasma total cholesterol levels (21).

In addition, the results show that children and adolescents with a consistent meal pattern (i.e. five meals a day) are leaner than those with an inconsistent meal pattern. Obese children and adolescents from our study consumed fewer meals (two to three meals, including one warm meal) a day (50 %) than children and adolescents of normal weight, the majority of whom consumed five (63.5 %) or more meals a day ($\chi^2 = 0.005$, $p < 0.05$). This suggests that skipping meals may play an important role in the development of obesity in children and adolescents. Furthermore, 59.7 % of children and adolescents with normal weight had a family meal every day, in comparison to obese children and adolescents (59.4 %), who had family meals only occasionally ($\chi^2 = 0.050$, $p < 0.05$). The latter indicates that the change in the traditional pattern of the family eating together might also contribute to the development of childhood obesity, as parental pre-

sence at the meal influences child's or adolescent's food choice. Therefore, it seems that the general problem is the amount of the food consumed (i.e. portion size, meal patterns and meal frequency) and not the quality of food chosen. Our results also indicate that in the management of juvenile obesity, physical activity and eating patterns within families should be targeted. In this way, early intervention could have important long-term benefits in decreasing the prevalence of obesity.

On the other hand, despite being obese, children and adolescents from the present study did not feel depressed, nor sad. The majority of them were very successful in school (38.8 %) and surprisingly, they did not feel excluded from society (82.4 %). Their most common problems included: headache (37.7 %), tiredness (34.8 %) and nervousness (27.5 %). However, despite the problems they had, they were happy and mostly satisfied with their appearance, probably because they perceived social support from their classmates and friends and were not teased by their peers. These results concord with two recent studies (14, 16), who showed that obese youth who perceived social support from their parents and friends are less depressed and may have better HRQOL.

Limitations of the study

As no validated questionnaire has been available prior to the study, the benchmarking with other countries is not possible.

Conclusion

Previous studies have reported that obesity among children and adolescents is associated with problems in the domains of general health and physical, emotional and social functioning (4, 31). In contrast to the other recent studies (4, 6, 32) we have only found an association between obesity and decreased quality of life in the domain of physical functioning and to a lesser extent, social functioning. Our results also showed that parental education level is an important risk factor for the development of juvenile obesity and could be important in preventing obesity during childhood. Futhermore, scores for the emotional and educational domains of obese children and adolescents were similar to the normal-weight controls. Accordingly, obese children and adolescents were not stigmatized and appeared to be content with the quality of their life. In order to generalize data on the national level, further research is needed in other pediatric departments.

Contributors

D Mičetić Turk was responsible for drafting the protocol of the study.

M Šikić Pogačar, E Turk, J Dolinšek and N Marčun Varda were responsible for the conduct of the study.

D Mičetić Turk, M Šikić Pogačar and E Turk were responsible for data interpretation.

M Šikić Pogačar was responsible for the first draft of the Materials and Methods.

D Mičetić Turk assumed the main responsibility for the writting of the final manuscript.

All authors agreed upon the final version.

D Mičetić Turk is the coordinator of the whole study.

References

1. *Erickson SJ, Robinson TN, Haydel KF, Killen JD. Are overweight children unhappy? Body mass index, depressive symptoms, and overweight concerns in elementary school children. Arch Pediatr Adolesc Med 2000; 154: 931-5.*

2. *Doak CM, Visscher TLS, Renders CM, Seidell JC. The prevention of overweight and obesity and adolescents: a review of interventions programmes. Obes rev 2006; 7: 111–36.*

3. *Avbelj M., Saje-Hribar N., Seher-Zupančič M., Brcar P., Kotnik P., Iršič A., et al. Prevalenca čezmerne prehranjenosti in debelosti med pet let starimi otroki in 15 oziroma 16 let starimi mladostnicami in mladostniki v Sloveniji. Za Skupino za raziskavo telesne teže pri otrocih in mladostnikih. ZdrV 2005; 74: 753–9.*

4. *Friedlander SL, Larkin EK, Rosen CL, Palermo TM, Redline S. Decreased quality of life associated with obesity in school-aged children. Arch Pediatr Adolesc Med 2003; 157: 1206-11.*

5. *Knöpfli BH, Radtke T, Lehmann M, Schätzle B, Eisenblätter J, Gachnang A, et al. Effects of a multidisciplinary inpatient intervention on body composition, aerobic fitness, and quality of life in severely obese girls and boys. J Adolesc Health 2008; 42: 119–27.*

6. *Schwimmer JB, Burwinkle TM, Varni JW. Health-related quality of life of severely obese children and adolescents. JAMA 2003; 289 (14): 1813-19.*

7. *Swallen KC, Reither EN, Haas SA, Meier AM. Overweight, obesity, and health-related quality of life among adolescents: The national longitudinal study of adolescent health. Pediatrics 2005; 115: 340-47.*

8. *Pratt KJ, Lamson AL, Swanson MS, Lazorick S, Collier DN. The importance of assessing for depression with HRQOL in treatment seeking obese youth and their caregivers. Qual Life Res 2011; 103-106.*

9. *Rubin KH, Coplan RJ, Bowker JC. Social withdrawal in childhood. Annu Rev Psychol 2009; 60: 141-71.*

10. *Danielzik S, Czerwinski-Mast M, Langnäse K, Dilba B, Müller MJ. Parental overweight, socioeconomic status and high birth weight are the major determinants of overweight and obesity in 5–7-year-old children: baseline data of the Kiel Obesity Prevention Study (KOPS). Int J Obes 2004; 28: 1494–502.*

11. *Bralic I, Vrdoljak J, Kovacic V. Associations between parental and child overweight and obesity. Coll Antropol 2005; 29 (2): 481–6.*

12. *Tabacchi G, Giammanco S, La Guardia M, Giammanco M. A review of the literature and a new classification of the early determinants of childhood obesity: from pregnancy to the first years of life. Nutr Res 2007; 27: 587–604.*

13. *World Health Organization. World Health Organization Constitution. In: Basic Documents, Geneva: World Health Organization, 1948.*

14. *Zeller MH, Modi AC. Predictors of health-related quality of life in obese youth. Obes 2006; (14) 1: 122-30.*

15. *de Beer M, Hofsteenge GH, Koot HM, Hirasing RA, Delamarre van de Waall RJBJ. Health-related-quality-of-life in obese adolescents is decreased and inversely related to BMI. Acta Pædiatr 2007; 96: 710–14.*

16. *Williams J, Wake M, Hesketh K, Maher E, Waters E. Health-related quality of life of overweight and obese children. JAMA 2005; 293 (1): 70- 6.*

17. *Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000; 320: 1240–43.*

18. *Centers for Disease Control and Prevention (http: // www.cdc.gov/).*

19. *Burke V, Beilin LJ, Dunbar D. Family lifestyle and parental body mass index as predictors of body mass index in Australian children: a longitudinal study. Int J Obes Relat Metab Disord 2001; 25: 147-57.*

20. *Fuentes RM, Notkola IL, Shemeikka S, Tuomilehto J, Nissinen A. Tracking of body mass index during childhood: a 15-year prospective population-based family study in eastern Finland. Int J Obes Relat Metab Disord 2003; 27: 716-21.*

21. *McDonald CM, Baylin A, Arsenault JE, Mora-Plazas M, Villamor E. Overweight is more prevalent than stunting and is associated with socioeconomic status, maternal obesity, and a snacking dietary pattern in school children from Bogota, Colombia. J Nutr 2009; 139(2): 370-6.*

22. *Berkey CS, Rockett HRH, Gillman MW, Field AE, Colditz GA. Longitudinal study of skipping breakfast and weight change in adolescents. Int J Obes 2003; 27: 1258–66.*

23. *Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. Lancet 2002; 360: 473–82.*

24. *Lobstein T, Baur L, Uauy R. Obesity in children and young people: a crisis in public health. Obes rev 2004; 5 (1): 4-85.*

25. *von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, et al. Breast-feeding and obesity: cross sectional study. BMJ 1999; 319: 147-50.*

26. *Gillman MW, Rifas-Shiman SL, Camargo CA, Berkey CS, Frazier AL, Rockett HRH, et al. Risk of overweight among adolescents who were breastfed as infants. JAMA 2001; 285: 2461-7.*

27. *Hediger ML, Overpeck MD, Kuczmarski RJ, Ruan WJ. Association between infant breast-feeding and overweight in young children. JAMA 2001; 285: 2453-60.*

28. *Arenz S, Rückerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity—a systematic review. Int J Obes 2004; 28: 1247–56.*

29. *Wilson AC, Forsyth JS, Greene SA, Irvine L, Hau C, Howie PW. Relation of infant diet to childhood health: seven year follow up of cohort of children in Dundee infant feeding study. BMJ 1998; 316: 21-5.*

30. *Nicklas TA, Baranowski T, Cullen KW, Berenson G. Eating patterns, dietary quality and obesity. J Am Col Nutr 2001; 20 (6): 599–608.*

31. *Rampersaud GC, Pereira MA, Girard BL, Adams J, Metzl JD. Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. J Am Diet Assoc 2005; 105 (5): 743-60.*

32. *Koletzko B, Toschke AM. Meal patterns and frequencies; do they affect body weight in children and adolescents? Crit Rev Food Sci Nutr 2010; 50: 100-105.*

33. *Moreno LA, Rodriguez G, Fleta J, Bueno-Lozano M., Bueno G. Trends of dietary habits in adolescents. Crit Rev Food Sci Nutr 2010; 50: 106-112.*

34. *Szajewska H., Ruszcynski M. Systematic review demonstrating that breakfast consumption influences body weight outcomes in children and adolescents in Europe. Crit Rev Food Sci Nutr 2010; 50: 113-119.*

35. *Varni JW, Limbers CA, Burwinkle TM. Impaired health-related quality of life in children and adolescents with chronic conditions: a comparative analysis of 10 disease clusters and 33 disease categories/ severities utilizing the PedsQL™ 4.0 Generic Core Scales. Health and Quality of Life Outcomes 2007; 5: 43.*

36. *Larsson U, Karlsson J, Sullivan M. Impact of overweight and obesity on health-related quality of life—a Swedish population study. Int J Obes 2002; 26: 417–24.*

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Heat-conductive properties of restorative lining materials

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Abstract

Aim: The aim of this study was to examine the thermal conductivity of five different cavity lining materials *ex vivo*.

Materials and Methods: Zinc oxide eugenol cement, zinc phosphate cement, two types of calcium hydroxide liner and glass ionomer cement were examined. These materials were prepared in accordance with the manufacturers' instructions and applied to standard moulds. Three samples of each material were prepared. Measurements were taken using a Heat Conduction Unit (P.A. Hilton Ltd. Stockbridge, Hants, UK). The thermal conductivity coefficient was calculated for each sample using the Fourier equation. Coefficients were analyzed statistically by the Kruskal–Wallis test.

Results: Significant differences were found for thermal conductivity between some materials ($P < 0.05$). The conductivity coefficient of zinc oxide eugenol cement was found to be higher than those of the other materials ($P < 0.05$). The conductivity coefficient of zinc phosphate cement was found to be lower than that of zinc oxide eugenol cement but higher than those of calcium hydroxide and glass ionomer cements ($P < 0.05$). No statistically significant differences were found between the calcium hydroxide and glass ionomer cements ($P > 0.05$).

Conclusions: The results showed that restorative lining materials functioned as thermal insulators and had different heat-conductive properties that depended on their composition.

Key words: Heat conduction, heat insulation, lining materials.

Introduction

For many years, the possible damaging effect of increases in temperature during restorative treatment on the pulp tissue has been a matter of concern in dentistry (1). Such temperature rises might present a biological problem, because a previous histological study in animals has demonstrated that healthy pulp failed to recover from an intrapulpal temperature rise of 5.55°C in 15% of situations. When the intrapulpal temperature increased by 11.1 and 16.65°C, 60% and 100% of the pulp, respectively, lost vitality (2). Although dentin has poor heat conduction and thus protects the pulp from harmful thermal agitation (3), in deep cavities, the remaining dentin layer is inadequate to provide effective heat insulation.

If a temperature difference arises inside a solid material, heat is transferred from areas of higher temperature to those of lower temperature by the process of heat conduction. Heat conduction can be explained by Fourier's law of heat conduction: $Q = -k A\Delta T/\Delta x$. This equation is used in particular to estimate the thermal conductivity coefficient of solids and fluids (4-6). The units of heat that are transferred in time (Q) are directly proportional to the cross-section of the material used in the experiment (A), and the heat difference between the two points (ΔT), but indirectly proportional to the thickness of the material in which the direction of the heat produced is transferred (Δx). k is the thermal conductivity coefficient, which is one of the most important properties of the material.

Heat conduction occurs in teeth during both daily life and dental procedures. The thermal environment of teeth during daily life varies widely between 0 and 70°C (7). The thermal insulating effect of restorative lining materials is clinically important in minimising the discomfort that patients might experience during consumption of hot/cold food or drinks, especially with metallic restoration. Approximately 20–30% of patients suffer from postoperative sensitivity, when a range of different liner and base materials are used for fillings (8). The heat generated during dental procedures (e.g. polishing or photocuring) can cause

damage to the pulp (9,10). Traditional teaching advocates the use of lining materials to protect and insulate the pulp from extreme thermal stimuli during and after restorative procedures (11-13) but the thermal insulatory effect of lining materials varies significantly depending on the composition (14). The aim of the research reported herein was to examine the heat conduction properties of lining materials of different compositions.

Materials and Methods

The thermal conductivity of the following materials was tested: zinc oxide eugenol cement (Cavex; Haarlem, Netherlands); zinc phosphate cement (Adhesor; Spofa-Dental, Jicin, Czech Republic); two type of calcium hydroxide liners (Calcimol & Calcimol LC; VOCO, Cuxhaven, Germany) and glass ionomer cement (Ionoseal; VOCO). Teflon moulds were made with an inner diameter of 25 mm and thickness of 1.5 mm for the preparation of sample discs. Test materials were prepared in accordance with the manufacturer's instructions and then used to fill the moulds. After setting, sample discs were removed from the moulds and their thickness was measured with the help of an electronic compass. Moulds of polyurethane, which is an insulating material, were prepared with the same dimensions as the original moulds and the test samples were embedded in the polyurethane (Figure 1). Three samples were prepared for each material.

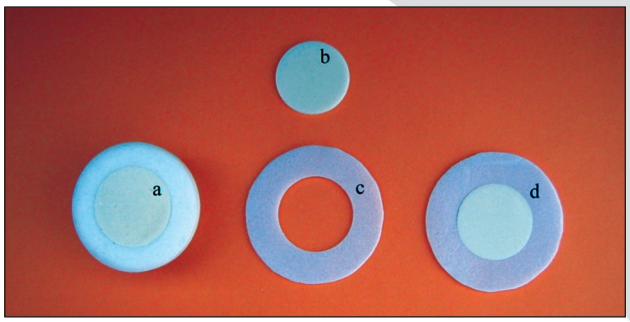

Figure 1. (a) Set sample disc in Teflon mould. (b) Sample disc which was removed from Teflon mould. (c) Polyurethane mould. (d) Test sample which was embedded in the polyurethane mould

Heat conduction experiment

The linear heat conduction module of the Heat Conduction Unit (P.A. Hilton Ltd. Stockbridge, Hants, UK) was used to determine the heat conductivity of the sample (Figure 2). To enable higher surface contact between the sample and the part of the apparatus, both sides of the sample were coated with a heat-conducting paste. The sample to be tested was placed in the sample slot of the linear module of the conduction apparatus, between the heating and cooling compartments. The pieces of the apparatus were then locked in a suitable form. For every sample that was tested, the heat entrance point of the module was heated with 10 W energy, and the heat pit section was cooled with water. Thus, the apparatus was heated on one side of the test sample and cooled on the other side until the desired stability was reached. Even though the time required for the system to attain stability varied among samples, the average time needed was 40–60 min. When the experimental system reached stability, the heat values was recorded from the thermostat temperature sensors that were situated on both sides of the test sample using a digital heat reader.

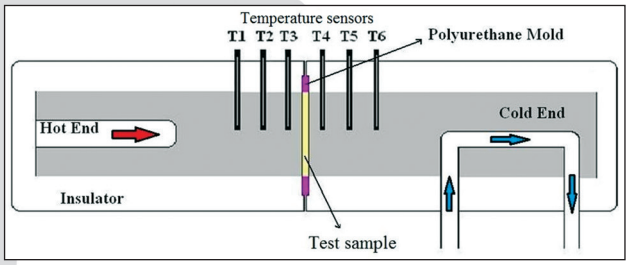

Figure 2. Diagrammatic representation of the apparatus used to measure heat conduction

Three temperature sensors were placed in both the heated and cooled sections; the sensors closest to the sample were at a distance of 5 mm from the sample, and there was a distance of 10 mm between each sensor. The recorded temperature values were subjected to regression curve analysis, and the heat of the heated surface (Ta) and cooled surface (Tb) of the test samples were determined using Microsoft Excel 2007 (Table 1).

Table 1. The temperatures (°C) of the heated surface (Ta) and cooled surface (Tb) of the test samples

Material	Ta	Tb
	Mean ± SD	Mean ± SD
Cavex	23.74 ± 0.59	17.11 ± 0.39
Adhesor	25.13 ± 0.25	17.28 ± 0.10
Calcimol	31.40 ± 0.45	16.38 ± 0.14
Calcimol LC	31.00 ± 0.52	16.28 ± 0.24
Ionoseal	31.70 ± 0.86	16.57 ± 0.73

In this way, the temperature at eight points was recorded for every sample. Then, using the Fourier equation, the value of *k* was calculated for each sample, as W/mK, with Excel.

Statistics

Coefficients were compared by performing the Kruskal–Wallis test (SPSS 10.0; SPSS, Chicago, IL, USA) and differences were considered to be statistically significant at $P < 0.05$.

Results

Significant differences with respect to thermal conductivity were found between some materials ($P < 0.05$). The thermal conductivity coefficient of zinc oxide eugenol cement was found to be higher than that of the other materials ($P < 0.05$). The thermal conductivity coefficient of zinc phosphate cement was found to be lower than that of zinc oxide eugenol cement and higher than those of calcium hydroxide and glass ionomer cement ($P < 0.05$). No significant differences were found between calcium hydroxide and glass ionomer cement ($P > 0.05$) (Table 2). The average temperature values and heat conduction graphics for each measuring point for all the materials are shown in Figure 3.

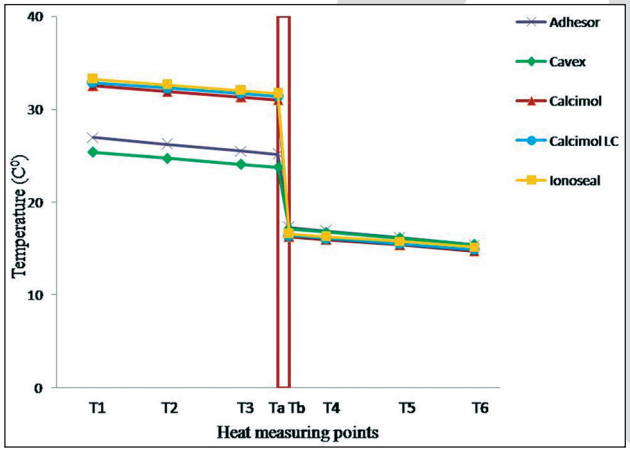

Figure 3. Average temperature values and heat conduction schema from each measuring point for all the specimens

Table 2. The thermal conductivity coefficients ($Wm^{-1}K^{-1}$) of the tested materials

Material	Mean value ± SD
Cavex	$4.6198^{a} \pm 0.1415$
Adhesor	$3.8960^{b} \pm 0.0871$
Calcimol	$2.0802^{c} \pm 0.0991$
Calcimol LC	$2.0367^{c} \pm 0.0751$
Ionoseal	$2.0213^{c} \pm 0.0182$

Superscript letters a, b, and c represent statistically significant differences ($P < 0.05$)

Discussion

When a temperature difference occurs inside a solid material, heat is transferred from regions of higher temperature to those of lower temperature by conduction. The various methods to measure thermal conductivity fall into two categories: steady state and non-steady state. In steady-state methods, the specimen is subjected to a temperature profile that does not vary over time; after equilibrium has been reached, thermal conductivity is determined directly by measuring both the rate of heat flow per unit area and the temperature gradient (15). The thermal conductivity of dental tissues and materials has been examined by many researchers using different methods (11,16-18).

In the present study, Fourier's law of heat conduction could be applied easily. The heat conduction properties of the test samples were examined by placing the samples in a test apparatus between heated and cooled sections that were equipped with temperature sensors. In this system, heat is transferred from the heated to the cooled end by means of conduction through the test material. If the tested material has high thermal conductivity, the heat produced in the heated section can be transferred easily to the cold end. Thus, the system can reach a steady state without having more increase of heat in the heated section. If the thermal conductivity of the tested material is poor, less heat is transferred to the cold end and the temperature at the hot end increases. Once the rate at which heat is generated is equal to the rate at which it is removed, steady state conditions exist, temperatures will become fairly constant, and readings can be taken.

In the test procedure used, heat conducting paste is applied to both surfaces of the test samples. The function of this paste is to convey the heat efficient-

ly from the heated section to the test sample. The paste does not affect the thermal conductivity of the test material. When heat conducting paste is not used, there might not be full contact between the heated section and the test samples. Hence, the heat produced cannot be transferred fully to the samples.

Zinc oxide eugenol as been the classic dental liner/base material for many generations of dentists, who were encouraged to use thick linings to ensure good thermal insulation. *In vitro* and *in vivo* studies by Tibbets *et al,* showed that zinc oxide eugenol had the lowest thermal conductivity among all materials tested (19). In contrast, a study performed using a steady-state method reported that zinc oxide eugenol was the most heat conductive material (11). The present study revealed that the thermal conductivity coefficient of zinc oxide eugenol was higher than that of the other materials tested ($P < 0.05$). In addition, we found that the conductivity coefficient of zinc phosphate cement was lower than that of zinc oxide eugenol but higher than that of the calcium hydroxide and glass ionomer cements ($P < 0.05$). The thermal conductivity of these materials seemed to depend on the composition and inorganic filler content of the main components. According to the thermal conductivity data, zinc oxide had a higher thermal conductivity coefficient (~54 $Wm^{-1}K^{-1}$) than calcium phosphate (~1.3 $Wm^{-1}K^{-1}$) (14). The study was conducted *in vitro*, and thus, the results could be misleading clinically. Clinically, even though zinc oxide eugenol conducts more heat than other materials, this might not be noticed by the patient, because of the obtundent effect of zinc oxide eugenol (due to eugenol) on the pulp (13).

The present study revealed that the calcium hydroxide and glass ionomer cements had the lowest thermal conductivity and there was no significant difference between these two materials. Both calcium hydroxide and glass ionomer cements have become lining materials of choice: calcium hydroxide because of its easy handling, alkaline pH, and excellent pulpal response; and glass ionomer because of its ability to adhere to tooth structures, its potential to be etched and bonded reliably, and its apparent ability to reduce postoperative sensitivity when used as a liner under restorative materials. In addition, the results of our study demonstrated that these materials should be preferred because of their effectiveness in thermal insulation. Liners are placed as thin coating on the surface of a cavity preparation (20). The thickness of the specimens analyzed in the present study differed from that used clinically. However, even though a difference in the thickness of the material alters the amount of heat conducted, it cannot change the thermal conductivity coefficient of the material.

Dentin is a poor thermal conductor (18,21) and a small difference in thickness may have a large effect on the conduction of heat (22). Dentin may be removed partially or totally during vital pulp therapy or insufficient dentin may remain in deep cavity preparations for it to provide effective thermal insulation. Using a liner that has low thermal conductivity will protect the pulp against increases in temperature that are produced during treatment procedures (i.e. polishing), as well as during intraoral rises in temperature that occur during daily life and function.

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References

1. *Hannig M, Bott B. In-vitro pulp chamber temperature rise during composite resin polymerization with various light-curing sources. Dent Mater 1999; 15: 275-81.*
2. *Zach L, Cohen G. Pulp Response to Externally Applied Heat. Oral Surg Oral Med Oral Pathol 1965; 19: 515-30.*
3. *Lin M, Xu F, Lu TJ, Bai BF. A review of heat transfer in human tooth--experimental characterization and mathematical modeling. Dent Mater 2010; 26: 501-13.*
4. *Saatçi B, Maraşlı N, Gündüz M. Thermal conductivities of solid and liquid phases in Pb-Cd and Sn-Zn binary eutectic alloys. Thermochimica Acta 2007; 454: 128-234.*
5. *Meydaneri F, Saatçi B, Özdemir M. Thermal conductivities of solid and liquid phases for pure Al, pure Sn and their binary alloys. Fluid Phase Equilibria 2010; 298: 97-105.*

6. *Garrido PL, Hurtado PI, Nadrowski B. Simple one-dimensional model of heat conduction which obeys fourier's law. Phys Rev Lett 2001; 86: 5486-9.*

7. *Barclay CW, Spence D, Laird WR. Intra-oral temperatures during function. J Oral Rehabil 2005; 32: 886-94.*

8. *Weiner R. Teaching the use of liners, bases, and cements: a 10-year follow-up survey of North American Dental Schools. Dent Today 2006; 25: 74, 76, 78-79.*

9. *Jones CS, Billington RW, Pearson GJ. The effects of lubrication on the temperature rise and surface finish of glass-ionomer cements. J Dent 2006; 34: 602-7.*

10. *Jones CS, Billington RW, Pearson GJ. The effects of lubrication on the temperature rise and surface finish of amalgam and composite resin. J Dent 2007; 35: 36-42.*

11. *Little PA, Wood DJ, Bubb NL, Maskill SA, Mair LH, Youngson CC. Thermal conductivity through various restorative lining materials. J Dent 2005; 33: 585-91.*

12. *Grajower R, Kaufman E, Rajstein J. Temperature in the pulp chamber during polishing of amalgam restorations. J Dent Res 1974; 53: 1189-95.*

13. *Tyas MJ. Pulp protection under restorations--do you need a liner? Aust Endod J 1998; 24: 104-8.*

14. *Saitoh M, Masutani S, Kojima T, Saigoh M, Hirose H, Nishiyama M. Thermal properties of dental materials--cavity liner and pulp capping agent. Dent Mater J 2004; 23: 399-405.*

15. *Akbulut S, Ocak Y, Keşlioğlu K, Maraşlı N. Thermal conductivities of solid and liquid phases for neopentylglycol, aminomethylpropanediol and their binary alloy. J Phys Chem Solids 2009; 70: 72-8.*

16. *Panas AJ, Zmuda S, Terpilowski J, Preiskorn M. Investigation of the thermal diffusity of human tooth hard tissue. Int J Thermophys 2003; 24: 837-47.*

17. *Civjan S, Barone JJ, Reinke PE, Selting WJ. Thermal properties of nonmetallic restorative materials. J Dent Res 1972; 51: 1030-7.*

18. *Figueiredo de Magalhaes M, Neto Ferreira RA, Grossi PA, de Andrade RM. Measurement of thermophysical properties of human dentin: effect of open porosity. J Dent 2008; 36: 588-94.*

19. *Tibbetts VR, Schnell RJ, Swartz ML, Phillips RW. Thermal diffusion through amalgam and cement base: comparison of in vitro and in vivo measurements. J Dent Res 1976; 55: 441-51.*

20. *Weiner R. Liners, bases, and cements in clinical dentistry. A review and update. Dent Today 2003; 22: 88-93.*

21. *Sweatman TL, Baumgartner JC, Sakaguchi RL. Radicular temperatures associated with thermoplasticized gutta-percha. J Endod 2001; 27: 512-5.*

22. *Dominici JT, Clark S, Scheetz J, Eleazer PD. Analysis of heat generation using ultrasonic vibration for post removal. J Endod 2005; 31: 301-3.*

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Effect of various enamel etching on shear bond strength in orthodontics

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Abstract

Objective: Laser ablation has been proposed as an alternative method to acid etching; however, previous studies have obtained contrasting results. This study compared the tensile strength of bracket-tooth bonds obtained after preparation of the enamel by conventional acid-etching, by Er: YAG laser etching and combined treatments.

Materials and Methods: Sixty nine premolars were randomly divided to three groups. The teeth received the following treatments: Group I 37% phosphoric acid, Group II Er: YAG laser ablation and Group III Er: YAG laser ablation + 37% phosphoric acid gel .ConTec LC adhesive was used in all groups for bracket bonding. Samples were stored in 0.9% sodium-chloride solution at 4°C until experiment. The shear debonding test was performed at a crosshead speed of 5 mm/min. Failed brackets were examined by a stereomicroscope at 10x magnification to determine the bond failure interface using a modified adhesive remnant index (ARI). SBS values were compared using One-way analysis of variance (ANOVA).

Results: Mean SBS values (MPa) and standard deviations for the groups were: Group I 16.26±7.25; Group II 13.10 ±10.18 and Group III 18.55±5.25. No significant differences were observed in the SBS of all the groups evaluated.

Conclusion: All the methods of enamel conditioning tested in this study are potentially adequate for orthodontic bonding according to the SBS values.

Key words: Er: YAG laser, orthodontic brackets, bonding.

Introduction

While Buonocore [1] in 1955 demonstrated a new concept of acid etching in order to improve bonding between tooth surfaces and dental resins, Newman was the first who described direct application of orthodontic brackets [2]. The procedure consists of etching the enamel using acid than adhesive and bracket application. Finding the adequate technique for bonding brackets that is fast enough and takes care of the enamel surface is still a great challenge in clinical orthodontics [3-8]. Among the various proposed methods, the application of 37% phosphoric acid is the most widely accepted technique [9].

The advantage of etching with phosphoric acid is the high level of bracket bond strength achieved. On the other hand, the loss of mineral crystals, essentially the acid-protecting barrier, is inevitable. Because of this mineral loss, the acid-etched region may be vulnerable to successive acid attacks in the oral environment.

In spite of the fact that the acid-etching technique is a useful method, there is a need to simplify the technique to decrease the complexity and to improve the bonding procedure in an effort to maintain clinically useful bond strengths while minimizing the amount of enamel loss.

Modern dentistry has focused on preventive methods and conservative techniques to apply less-invasive procedures to tooth structure [10, 11]. One of the new technologies that has been applied in almost all dentistry fields is the laser. Many different types of lasers exist and they produce different results in hard tissue [12-14]. Laser etching is painless and does not involve vibration or heat.

Maiman [15] first reported the application of laser in medical treatment and after him Goldman et al. [16] explored the application of laser energy to hard dental tissues. Among the various laser types used in dentistry, the Er: YAG laser the most often recommended, because its wavelength (2.94 μm) corresponds to the main absorption band of water (about 3.0 μm) and it is also well absorbed by the –OH groups in hydroxyapatite [17,18].

The aim of this study was to compare the shear bond strength (SBS), enamel roughness and adhesive remnant index (ARI) of orthodontic brackets bonded to enamel that has been conditioned with 37% phosphoric acid, Er: YAG laser irradiation, or a combination of these.

Material and methods

Sixty nine premolars extracted for orthodontic reasons from patients aged 10-18 years were used in this study. Criteria for the teeth were: intact tooth surface which was not exposed to chemical agences (hydrogen peroxide) and with no cracks originated from pressure during extraction. The teeth were cleaned and stored in 0.9% sodium-chloride solution at 4˚C until the experiment. Prior to the experiment, the buccal enamel surfaces of the teeth were polished with a rubber cup and fluoride-free polishing paste rinsed with a water spray and dried with compressed air. The teeth were then randomly divided into three groups. The remaining three teeth didn't undergo the shear test but were prepared for scanning electron microscope (MIRA3 TeScan FEG-SEM) evaluation after different surface treatments.

Group I consisted of teeth that were treated with 37% phosphoric acid gel (Kerr Corporation) + ConTec Prime (Dentaurum GmbH & Co. KG, Ispringen, Germany); in Group II, teeth were treated with Er: YAG laser + ConTec Prime (Dentaurum GmbH & Co. KG, Ispringen, Germany) and in Group III teeth were treated with Er: YAG laser and 37% phosphoric acid gel (Kerr Corporation) + ConTec Prime (Dentaurum GmbH & Co. KG, Ispringen, Germany). Stainless steel orthodontic brackets for premolars (Discovery, Dentaurum, Roth 022) with the average bracket base area of 14.7 mm^2 were used. The brackets were bonded to the buccal surfaces with ConTec LC adhesive (Dentaurum GmbH & Co. KG, Ispringen, Germany) according to the manufacturer's instructions. For the acid-etching technique, we applied 37% phosphoric acid gel to the bonding area for 15 seconds. In Group II enamel surfaces were irradiated with Er: Yag laser (AT Fidelis, Fotona, Ljubljana, Slovenia) of a 2940 nm wave length, at 300 mJ/pulse, 10 Hz, 10 seconds. The surface was irradiated in a light contact mode using a 300 μm optical fiber with a contra-angle hand piece under water spray.

The enamel surface ached by acid or/and laser was covered with a thin layer of ConTec Prime using a brush, gently air-dried and cured with a halogen light source for 20 seconds. ConTec LC adhesive paste was applied to the base of the bracket pad and the bracket was pressed firmly onto the tooth. Excess adhesive was removed from the periphery of the bracket base with a sponge. The adhesive was cured for 20 seconds from the mesial and distal sites.

After the bonding procedure all samples were stored in demineralized water at 37°C for 24 h. Before testing, the teeth were vertically placed into a custom-made mold made of acrylic resin, to ensure that the direction of force in relation to the bracket was the same for every specimen. Samples were tested for the force at bond failure with an Universal Testing Machine (Instron 1332, Mass), retrofited by FastTrack 8800 Compact Digital Control Electronics, with the crosshead speed of 5 mm/min at 500N load cell, at the room temperature. Each tooth was oriented so that its facial surface was parallel to the direction of force during the Shear bond Strength (SBS) testing. The SBS values were calculated in megapascals (MPa) by dividing the force measured in Newton (N) by the area of the bracket base (14.7 mm^2).

Statistical analysis

Descriptive statistics, including the mean, standard deviation, minimum and maximum values were calculated for each group (Table 1.). Comparisons of the shear bond strengths for the different etching types were performed with the ANOVA test. According to One-way ANOVA test no statistically significant difference was found between the groups evaluated for the SBS test ($p = 0.085$). The frequency distribution of ARI scores for the three groups tested is shown in Chart 1. All statistical evaluations were made with a software program (SPSS for Windows, 15.0, Chicago, Ill)

Results

Descriptive statistics for the comparison of shear bond strengths and ARI scores for the three groups are given in Table 1 and Chart 1. The combined Er: Yag fallowed by acid-etching group yielded the highest mean shear bond strength (18.55 ± 5.27MPa). The mean shear bond strengths for the acid and laser group were 16.26 ±7.25 MPa and 13.10±10.18 MPa.

Table 1. The mean values of bond strength between the bracket and tooth enamel differently treated

	Group I	Group II	Group III
N	22	22	22
Mean	16.26	13.10	18.55
SD	7.25	10.18	5.27
Median	15.58	10.54	18.84
Minimum	6.05	4.01	8.71
Maximum	29.73	34.76	26.39
ANOVA	F=2.565	df=2	p=0.085

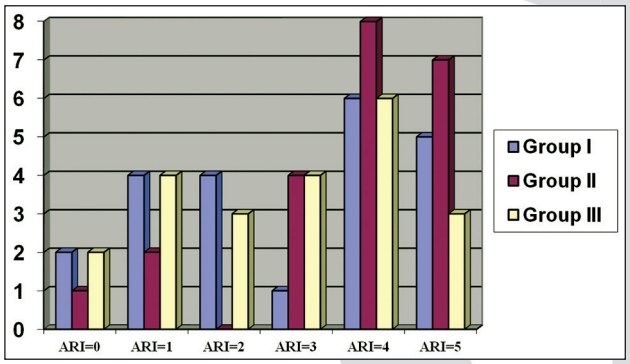


Chart 1. Distribution of groups according to ARI score

SEM examination

Figure 1 shows the enamel surface after phosphoric acid etching treatment. This morphology revealed type III etched pattern with mixed prism centers and prism periphery etching [19]. Dissolution of hydroxyapatite by phosphoric acid produced tags and rough surface that afforded the mechanical lock for resin. After 300 mJ/pulse, 10 Hz and 10 seconds of Er: YAG laser irradiation (Figure 2 and figure 3), rough irradiated surface with micro-cracks were found. Less regular, inhomogeneous ablated patterns, comparable with those of acid treatment, were also observed. After Er: YAG laser (300 mJ/pulse, 10 Hz, 10 seconds) followed by acid etching (Figure 4 and figure 5), the enamel surface was similar to that of the acid group and revealed type I (preferential prism center etching) etching pattern, having a scaly appearance and circular depressions with a relatively flat-surface structure.

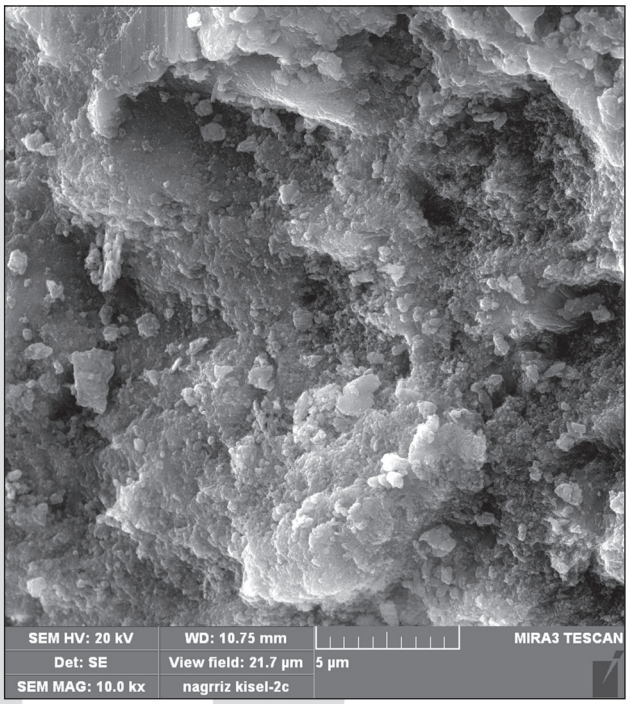
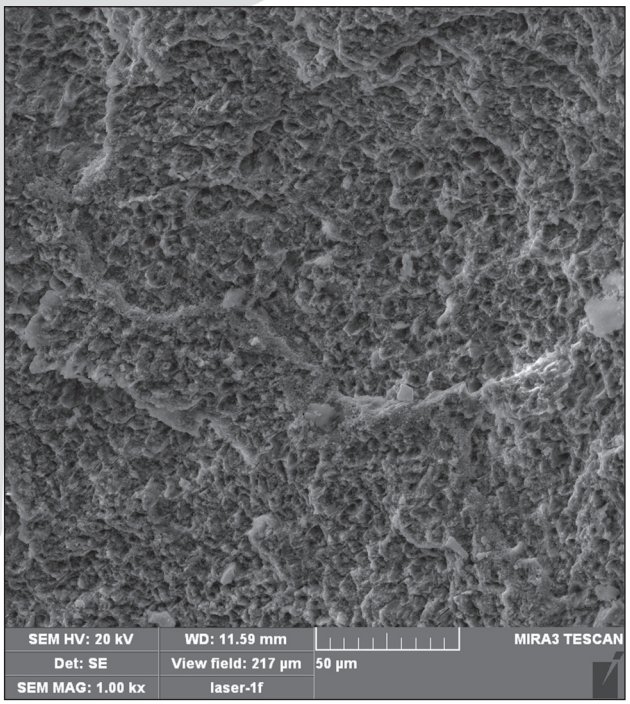


Figure 1. SEM of treated enamel with 37% phosphoric acid



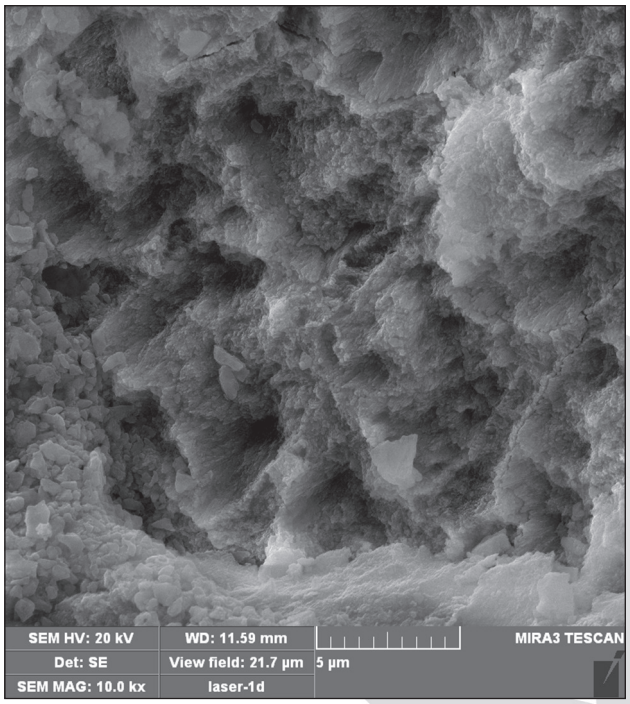


Figure 2. and Figure 3. SEM of irradiated enamel with ER: YAG laser

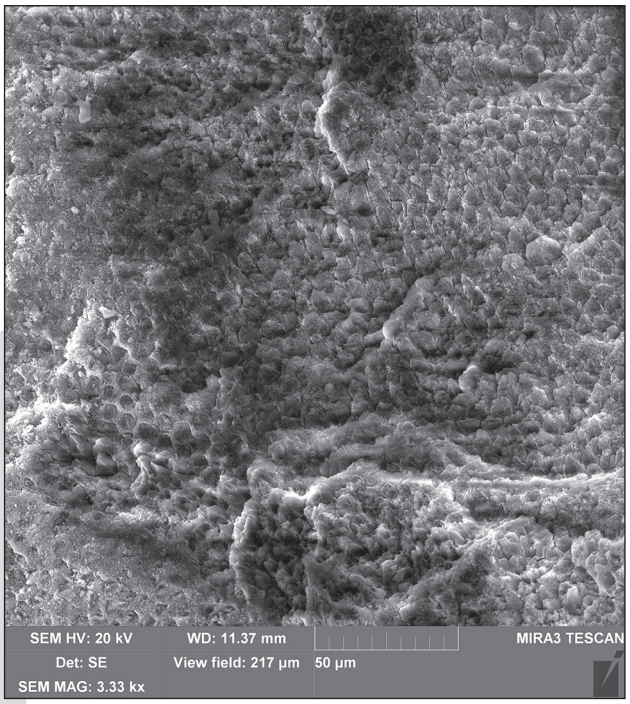
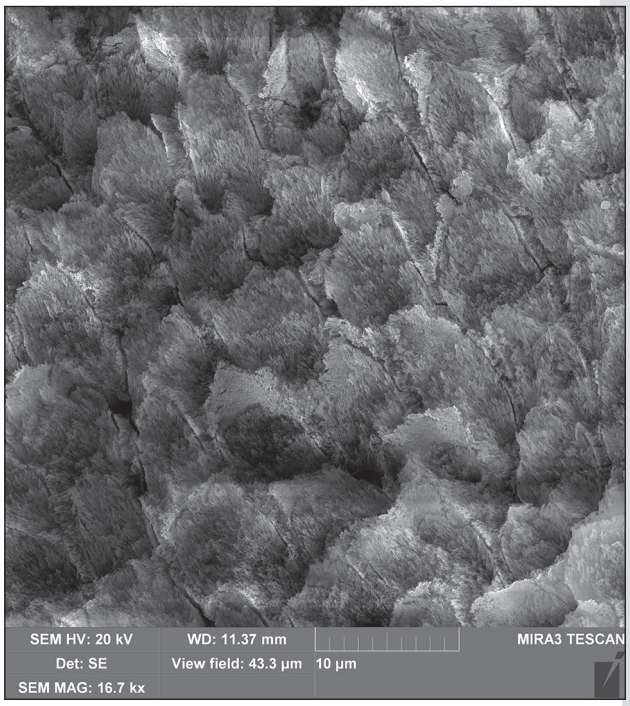


Figure 4. and Figure 5. SEM of treated enamel with ER: YAG laser followed by phosphoric acid



Discusion

Laser systems are used more commonly in dentistry in recent years. Because the main purpose of orthodontics is to preserve maximum tooth structure while treating anomalies, this study was designed to determine whether laser systems can be used in orthodontics with minimal tooth structure destruction and optimal bracket retention. The SEM evaluation helped us to inspect the amount of destruction on the etched enamel surfaces. Laser ablation has become available as an alternative to acid etching of enamel and dentin. After being exposed to laser energy, enamel showed physical changes including melting and recrystallization thus forming numerous pores and small bubble-like inclusions [20]. Investigation of enamel surface roughness showed that laser irradiation yielded a comparable [21] or similar [22] amount of surface roughness as that seen with acid-etch. At the end of the orthodontic treatment the main concern is to turn the enamel surface back to its original state with minimal enamel loss and to return its original roughness. If this is not achieved, there is a great possibility of potential plaque traps and poor aesthetics.

In the literature, there are conflicting reports about the use of lasers for enamel etching. Altho-

ugh some researchers have reported that the mean shear bond strength resulting from laser etching is lower than that from acid etching [23-25] others have reported more favorable results with laser irradiation [26-28].

In the present study, the combined treatment group (conditioned by Er: YAG laser and followed by phosphoric acid etching) yielded the highest tensile bond strength values, but statistically similar to the samples conditioned only by phosphoric acid and only by the Er: YAG laser. Combined method can be considered as successful alternatives to the conventional methods, but on the other hand, laser ablation may be more practical than combined method.

Most of the specimens had bond failure sites at the bracket- resin interface (Chart 1). This indicated that these three kinds of treatment could give good surface wetting. The laser group had more failure rate at the enamel-resin interface.

After acid etching the enamel, demineralization and susceptibility to caries around brackets are complications of orthodontic treatment. Er: YAG laser ablation might overcome this drawback and offer other benefits like reduction in clinical time, a reduced susceptibility to moisture during etching, and bond strength similar to that of acid etching.

Conclusion

We found no statistically significant differences among the etching methods discussed here. This implies that The Er: YAG laser can be an alternative tool to conventional acid etching. The laser may be preferred for etching.

References

1. *Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955; 34: 849-53.*

2. *Newman GV. First direct bonding in ortodontia. Am J Orthod Dentofacial Orthop. 1992; 101: 190-2.*

3. *Battes D, Retief DH, Jamison HC, Denys FR. Effects of acid etch parameters on enamel topography and composite resin-enamel bond strength. Pediatric Dentistry. 1982; 4: 106-10.*

4. *Retief DH. A comparative study of three etching solutions; effects on enamel surface and adhesive-enamel interface. J Oral Rehabil. 1975; 2: 75-96.*

5. *Silverstone LM. The Acid Etch Technique; In Vitro Studies with Special Reference to the Enamel Surface and the Enamel-Resin Interface. St. Paul, USA: North Central Publishing Co; 1975.*

6. *Denys FR, Retief DH. Variations in enamel etching patterns produced by different concentrations of phosphoricacid. J Dent Assoc South Africa. 1982; 37: 185-9.*

7. *Mitić V, Mitić A, Čemerikić Lj, Nišić M. SEM izgled nagriženih bukalnih površina gleđi zuba ortofosfornom kiselinom u različitom vremenskom intervalu. Acta Stomatologica Naissi. 2005; 51: 507-15.*

8. *Ogaard B, Bishara SE, Duschner H. Enamel effects during bonding – debonding and treatment with fixed appliances. In: Graber T, Eliades T, Athanasiou A, editors. Risk Managment in Orthodontics. Experts Guide to Malpractice. New Malden, Surrey, UK: Quintessence Publishing Company Ltd; 2004. 19-47.3-8.*

9. *Retief DH, Busscher HJ, de Boe, P, Jangebloed WL, Arends J. A laboratory evaluation of three etching solutions. Dent Mater. 1986; 2: 202–206.*

10. *Borsatto MC, Corona SA, Dibb RG, et al (2001) Microleakage of a resin sealant after acid-etching, Er: YAG laser irradiation and air-abrasion of pits and fissures. J Clin Laser Med Surg. 2001; 19 (2): 83–87*

11. *Sungurtekin E., Oztas N. The effect of erbium, chromium: yttrium-scandium-gallium-garnet laser etching on marginal integrity of a resin-based fissure sealant in primary teeth. Lasers Med Sci 2010; 25(6): 841–847*

12. *Drummond JL, Wigdor HA, Walsh JT Jr, Fadavi S, Punwani I.Sealant bond strengths of CO(2) laser-etched versus acid-etched bovine enamel. Lasers Surg Med 2000; 27(2): 111–118*

13. *Visuri SR, Gilbert JL, Wright DD, Wigdor HA, Walsh JT Jr. Shear strength of composite bonded to Er: YAG laser-prepared dentin. J Dent Res 1996; 75 (1): 599–605*

14. *Wigdor HA, Walsh JT Jr, Featherstone JD, Visuri SR, Fried D, Waldvogel JL. et al Lasers in dentistry. Lasers Surg Med 1995; 16(2): 103–133*

15. *Maiman, T.H. Stimulated optical radiation in ruby. Nature. 1960; 187, 493–494.*

16. *Goldman, L., Gray, J.A., Goldman, J,. Goldman, B., and Meyer, R. Effect of laser beam impacts on teeth. J. Am. Dent. Assoc. 1965; 70, 601–606.*

17. *Keller U, Hibst R. Experimental studies of the application of the Er: YAG laser on dental hard substances: II. Light microscopic and SEM investigations. Lasers Surg Med 1989; 9(4): 345–351*

18. *Souza-Zaroni WC, Chinelatti MA, Delfino CS, et al Adhesion of a self-etching system to dental substrate prepared by Er: YAG laser or air abrasion. J Biomed Mater Res B Appl Biomater 2008; 86B(2): 321–329.*

19. *Craig RG, Powers JM. Bonding to dental substrates. In: Restorative Dental Materials. 11th ed. St Louis, Mo: Mosby Inc; 2002: 266.*

20. *Walsh, L.J., Abood, D., and Brockhurst, P.J. Bonding of composite resin to carbon dioxide laser-etched human enamel. Dent. Mater. 1994; 10, 162–166.*

21. *Hess, J.A. Scanning electron microscopic study of laserinduced morphologic changes of a coated enamel surface. Lasers Surg. Med. 1990; 10,458–462*

22. *Arcoria, C.J., Lippas, M.G., and Vitasek, B.A. Enamel surface roughness analysis after laser ablation and acid-etching. J. Oral Rehabil. 1993; 20, 213–224.*

23. *Hossain M, Nakamura Y, Tamaki Y, Yamada Y, Murakami Y, Matsumoto K. Anatomic analysis and knoop hardness measurement of the cavity flor prepared by Er,Cr: YSGG laser irridation in vitro. J Oral Rehabil. 2003; 30: 515–21.*

24. *Martinez-Insua A, Da Silva Dominguez L, Rivera FG, sanata- Penin UA. Differences in bonding to acid-etched or Er: YAG-laser-treated enamel and dentin surfaces. J Prosthed Dent. 2000; 84: 280–8.*

25. *Lee BS, Hsieh TT, Lee YL, Lan WH, Hsu YJ, Wen PH, Lin CP. Bond strengths of orthodontic bracket after acid-etched, Er: YAG laser-irradiated and combined treatment on enamel surface. Angle Orthod. 2003; 73: 565–70.*

26. *Driessens FC. Chemical adhesion in dentistry. Int J Dent. 1977; 27: 317–23.*

27. *Walsh LJ, Abood D, Brockhurst PJ. Bonding of composite resin to carbon dioxide laser-etched human enamel. Dent Mater. 1994; 10: 162–6.*

28. *Whitters CJ, Strang R. Preliminary investigation of a novel carbon dioxide laser for applications in dentistry. Laser Surg Med. 2000; 26: 262–9.*

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Neuro-protective effects of Ginkgo Biloba extract on rats with pentylenetetrazol-induced epilepsy

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Abstract

Purpose: To explore the influence of Ginkgo biloba extract (GBE) on the epileptic seizure of rat model with chronic epilepsy induced by pentylenetetrazol (PTZ) and neuro-protective effects. **Methods:** 40 healthy adult male SD rats were randomly divided into four groups: PTZ-induced epilepsy group, PTZ + valproic acid (VPA) group, PTZ + VPA + GBE group and normal saline (NS) control group (n = 10). The changes of behavior, electroencephalogram (EEG), correct rate of electrical maze and the expression of T-type Ca^{2+} channel protein Cav3.2 in temporal lobe and hippocampus were observed in each group.

Results: The onset level of PTZ + VPA group, PTZ + VPA + GBE group and NS control group was 0-II, with normal EEG or a small amount of spike waves. The differences of EEG total power and Cav3.2 level in temporal lobe and hippocampus were significantly lower than those in PTZ group ($P < 0.05$). Compared with PTZ + VPA group, the Cav3.2 expression in PTZ + VPA + GBE group was significantly decreased, between which the difference was statistically significant ($P < 0.05$). **Conclusion:** GBE can reduce Cav3.2 expression, significantly lower the onset level of epileptic rats, decrease cerebral paradoxical discharge and improve cognitive function with auxiliary antiepileptic and neuro-protective effects.

Key words: Ginkgo biloba extract, pentylenetetrazol, neuro-protective effect, Cav3.2.

Introduction

Epilepsy is a chronic disease featured in repeated and transient central nervous system dysfunction caused by sudden and excessive abnormal discharge of brain neurons, which seriously endangers human health [1, 2]. It features complicated pathogenesis and diverse therapeutic targets. One of the current research trends is searching for new antiepileptic drugs with neuro-protective effects [3, 4]. In recent years, a significant neuro-protective effect of Ginkgo biloba extract (GBE) has been widely applied in the prevention and treatment of cardiovascular and cerebrovascular diseases, only a small amount involved in epilepsy intervention [5, 6]. In this study, GBE and VPA were used to treat PTZ-induced epileptic rats so as to explore the influence of GBE on epileptic seizure and neuro-protective effects by observing the seizure, cognitive function and expression of T-type Ca^{2+} channel protein Cav3.2.

Materials and methods

Reagents and instrument

PTZ, Cav3.2 and β-actin antibodies were purchased from Sigma, US. Trizol total RNA extraction kit was bought from Tiangen Biotech (Beijing) Co., Ltd. RT-PCR kit was obtained from TaKaRa (Dalian) Limited. GBE (containing 24% flavonoid glycoside and 6% terpene composed of 3.1% ginkgolide and 2.9% bilobalide) was purchased from Beaufour Ipsen Pharmaceutical Industry Co., Ltd., France. 4371F EEG machine was bought from Nihon Kohden. MG-20/3C maze stimulator was obtained from Zhangjiagang Biomedical Instrument Factory.

Animal grouping and modeling

40 adult male SD rats weighed about 250g (provided by the Experimental Animal Center of Henan Province) were divided into four groups according to the random number table: PTZ-induced epilepsy group, PTZ + valproic acid (VPA) group, PTZ + VPA + GBE group and normal saline (NS) control group, labeled as Groups A, B, C and D respectively, 10 for each group. Rats kindling model was established in Groups A, B and C in accordance

with the method of Diehl [7], in which 10g/L PTZ NS was injected intraperitoneally (ip) at 35mg/kg, once a day; in Group B, 80mg/kg VPA gavage (ig) was given before PTZ ip [8]; in Group C, 100mg/kg GBE ig was given at 1.5 hours before PTZ ip [9], and 80mg/kg VPA ig at 1 hour before PTZ ip, and GBE was given only three days before PTZ ip; Group D was given intraperitoneal injection of 3.5ml/kg saline once a day. Administration for each group was given at 15:00 daily, with an experimental period of eight weeks.

Rat behavioral evaluation indicators

According to Racine grading [10], epileptic seizure are graded as follows: level 0: no convulsive seizure; I: facial spasm; II: facial spasm + rhythmic nod; III: facial spasm + rhythmic nod + forelimb clonus; IV: facial spasm + rhythmic nod + forelimb clonus + hindlimb standing; V: facial spasm + rhythmic nod + forelimb clonus + hindlimb standing + falling down; the occurrence of level IV or V seizure for three consecutive days was determined to be full kindling. The behavior changes of each rat were observed and recorded within 1 hour after PTZ ip or NS.

EEG

40 experimental rats were applied with EEG at the end of the 8th week. The steps were taken as follows: four feet of rat were snared with thick cotton threads and fixed on board. Three pin electrodes designed specially were inserted into the rat's forehead, left and right temporal lobes respectively, to make the three insertion points an isosceles triangle, with the top the reference electrode. Finally, electrodes and wires were fixed with tape.

Accuracy measurement of Y-type electric maze

Electric maze test was conducted on 40 SD rats in the 8th week. For Y-type electric maze, also called trisection radial labyrinth box, the voltage was set as 50-100V and the delay time 5 seconds. At the beginning of the experiment, the power was not connected and one arm was randomly selected as the starting area. The rat was put in the starting area. Due to curiosity, the rat explored for 3 to 5 minutes within the three-arm area. After the rat became stable, it was put back to the starting area. Any area outside the starting one was selected as the safety zone and its button was pressed correspondingly. Electrical stimulation started 5 seconds after the light of the safety zone was turned on. The rat finally escaped to the safety zone with the light on. The light was turned off 30 seconds later and the test ended.

Detection of Cav3.2 expression in temporal lobe and hippocampus

The gene sequence of Cav3.2 in SD rat was found in Genbnak. Primers were designed by Primer 5.0 software. The sequence of Cav3.2 (AF290213) gene primer is as follows: upstream 5'-CTCATCATTATGGGCTCCTT-3' and downstream 5'-CGTGGCTAAAGTGGTAATGG-3', and the amplification product fragment was 400bp. The sequence of internal reference β-actin (NM031144) gene primer is as follows: upstream 5'-ATGCCATCCTGCGTCTGGACCTGGC-3' and downstream 5'-AGCATTTGCGGTGCACG ATGGAGGG-3', and the amplification product was 606bp.

After EEG, rats in each group were injected with 10% chloralic hydras (3.5ml/kg) for celiac anesthesia and conducted systemic perfusion with 10% formaldehyde for fixation. Then, their heads were removed and brains collected. Total cellular RNA and protein were extracted according to instructions of total protein preparation kit and RNA extraction kit. Nucleic acid quantification analyzer was used to measure the output and purity of total cellular RNA. After it was confirmed that there was no degradation through 1% agarose gel electrophoresis, RT-PCR amplification was conducted according to the instructions of reverse transcription kit. Then, the following steps were taken: BCA protein kit quantification, conventional electrophoresis, transfer and immunoassay (40μg total protein in each group was sampled; Cav3.2 and β-actin antibodies were diluted as per 1:500 and secondary antibodies labeled with horse radish peroxidase as per 1:1000), chemiluminescence, development and fixation. The gray value was measured by Image J and its ratio to the gray value of internal reference β-actin was taken for statistical analysis.

Statistical analysis

SPSS 13.0 statistical software was adopted for analysis, and data were presented as (mean ± standard deviation) (±s). Single-factor analysis of

variance or Kruskal-Wallis rank sum test was used for comparison of multiple sets of measurement data among the groups, and Bonferroni test or Mann-Whitney U rank sum test for pairwise comparison between groups, P <0.05 for statistically significant difference.

Results

Behavior observation

After 8 weeks, all rats in Group A were completely kindled, who entered the incubation period 1 to 3 minutes after injection of PTZ, and gradually changed from the state of free activities to quietness or restlessness, and then had myoclonus in the head and on the face, accompanied by nods and "wet dog" shakes of the whole body, which was followed by forelimb clonus, hindlimb standing and head turned to one side. Finally, generalized tonic-clonic seizures occurred, accompanied by jump, bump into cage, tumble and tongue bitten, which could be remitted after lasting for minutes and then recurred recurrently.

EEG results

The rats in Group D had a normal EEG with α and β waves as main manifestations. In Group A, epileptiform discharges appeared, i.e. spike wave, sharp wave, spike-slow and sharp-slow combination waves. Compared with Group A, the EEG epileptiform discharges of the rats in Group C were reduced significantly, mainly manifested in the decrease of epileptiform wave frequency and amplitude. Compared with Group C, the EEG epileptiform discharges of the rats in Group B were increased significantly (Figure 1). Analysis on the difference of total power before and after injection among groups (Table 1): skewed distribution, heterogeneity of variance, multi-group quantitative data, Kruskal-Walls test, P<0.05. The difference was statistically significant. Analysis on total power before and after intra-group injection: t test for paired data. The difference in the total power of EEG were statistically significant between Groups B, C, D and A (P<0.05) and between Groups B and D (P<0.05). There was statistical significance for the difference in total power of Group A (P<0.05), that is, obvious difference was shown in EEG power before and after seizures of PTZ-induced epileptic rats. Epileptic EEG was affected by single VPA and combination of VPA with GBE; but compared with the normal group, no significant change was found in EEG by single treatment with VPA.

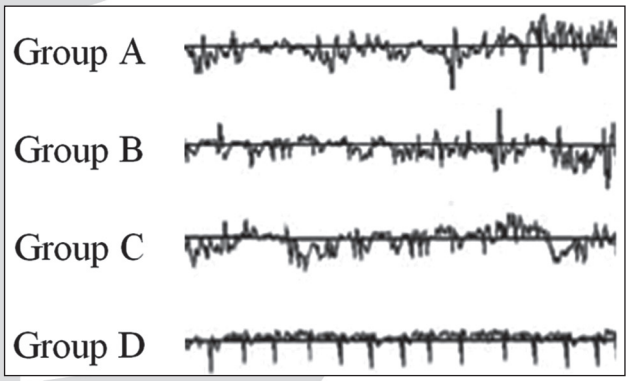

Figure 1. EEG results of each group

Y-maze test results

At the end of the 8th week, the data of correct rate of behavior in rates of each group were measured for Kruskal-Wallis rank sum test of multi-group quantitative data (Table 2). The difference among Groups A, B, C and D was statistically significant (P<0.05). No significant difference was found between Groups of A, B and C (P > 0.05), which indicated that epilepsy can cause cognitive impairment, and VPA and GBE cannot improve cognitive function PTZ-induced epileptic rats.

Table 1. Total power before and after injection of each group

Grouping	Total power before injection	Total power after injection	Difference
Group A	70.97±15.98	4379.13±591.38#	4309.29587.2[5]±Δ
Group B	89.87±10.37	1410.25±130.41	1322.34±128.48*Δ
Group C	91.64±11.45	299.63±31.57	208.22±25.92*
Group D	101.76±9.57	105.88±15.29	4.22±2.19*

*Comparison between groups: compared with Group A, *P<0.05; compared with Group D, ΔP<0.05. Comparison before and after injection between two groups: # P <0.05*

Table 2. Y-maze test results

Grouping	Correction rate
Group A	0.498±0.109
Group B	0.521±0.098
Group C	0.552±0.065
Group D	0.982±0.096

*Compared with Group A, *P<0.05; compared with Group D, #P<0.05.*

Western blot and RT-PCR detection

The expression levels of Cav3.2 in temporal lobe and hippocampus were detected by Western blot and RT-PCR. As shown in Figures 2-4, the level in Group A was higher than that in Groups B, C and D, with the difference statistically significant (P <0.05); the level in Group B was higher than that of Groups C and D, between which the difference was statistically significant (P <0.05); the level in Group D was lower than that in Group C, showing a statistically significant difference (P <0.05); this suggests that the Cav3.2 expression in temporal lobe of epileptic rat was significantly increased, and the combination of GBE with VPA can significantly reduce Cav3.2 level with the effect better than that of VPA single agent.

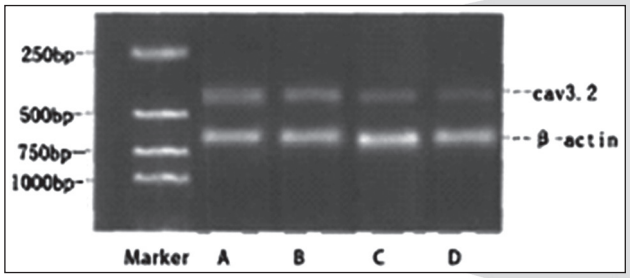

Figure 2. RT-PCR detection of Cav3.2 expression

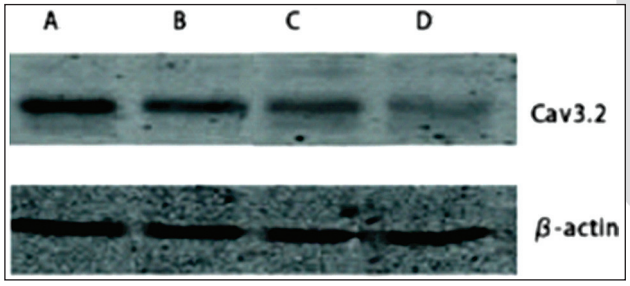

Figure 3. Western blot detection of Cav3.2 expression

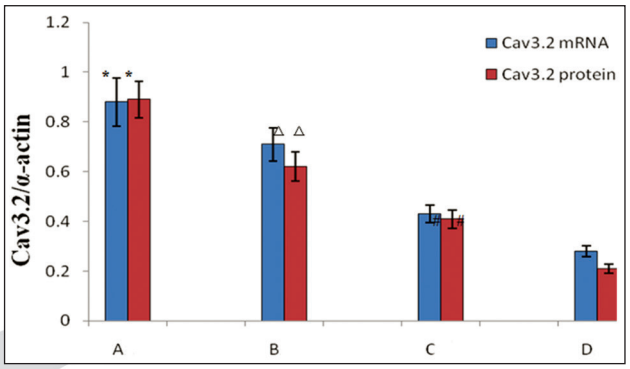

*Figure 4. *Group A was significantly higher than Group B, C and D (P<0.05); △Group B was significantly higher than Group C and D (P<0.05); #Group C was significantly higher than Group D (P<0.05).*

Discussion

The chronic PTZ-induced epileptogenic model used in this study was to conduct periodical reinforce through repeated stimulation of subconvulsive amount and eventually induce epileptic seizure in ethology and epileptic discharge on EEG. The tonic-clonic seizures of PTZ-induced rats are similar to those of humans, and once it is kindled successfully, the sensitivity of rats to this stimulus will maintain a relatively long period of time, so it is an ideal model of epilepsy. And PTZ, without neurotoxic effects itself, is a perfect epileptogenic drug for the study of the relationship between epileptic seizure and neuronal injury [11-14].

One of the common pathogenic mechanisms of epilepsy is the excessive discharge of neurons. The synaptic endogenous plasticity includes excitement of voltage-gated ion channels generating electrical signals [15]. This spontaneous discharge is the neuronal depolarization induced by transient and rapid influx of Ca^{2+} and slow Ca^{2+} influx, in which Ca2 + influx is the aetiological agent for epilepsy [16, 17]. Furthermore, a large amount of Ca^{2+} influx also serves as one of the main factors of brain neuronal damage secondary to epilepsy [18, 19]. Kong et al. [9] found that obvious overload of Ca^{2+} in nerve cells in CA1 and CA3 areas of hippocampus in PTZ-induced epileptic rats by inverted fluorescence microscope. Aamno et al. [20] also confirmed the abovementioned result in the experimental method of cytochemistry, and in addition, they found that this Ca^{2+} influx could be

blocked by L-type calcium channel blocker. The occurrence of early epileptic symptoms caused by epileptogenic factors may be the result of Ca^{2+} influx which on the one hand leads to large release of GLU in neurons, and meanwhile the amount of GABA reduces, thus resulting in epileptic seizure [21]. On the other hand, Ca^{2+} combines with calmodulin as the second messenger to activate protein kinase and immediate early genes in proto-oncogenes, such as c-fos and c-jun to play a biological role, and at the same time, it results in a significant increase of IL-1β in hippocampus which as a nerve excitatory regulating neurotransmitter, mediates the increase of intracellular Ca^{2+} concentration, leading to the aggravation and maintenance of epileptic behaviors [22-24]. However, as IL-1β has the characteristics of short-term self-limited secretion, symptoms of epilepsy also are terminated with the reduction in the amount of IL-1β and resolution of epileptogenic factors, but its specific mode of action and mechanism remains to be further studied.

T-type calcium channel has a relatively high content in the site that releases neurohormone, so it is of great significance in its excitation conduction of current. Epileptic seizure is accompanied by abnormal synchronous discharge of neurons, but the pathogenesis at the molecular level is unclear. With the domestic and overseas studies on calcium channels, it is found that mutation of T-type calcium ion channel gene-CACNA1H is closely related to childhood absence epilepsy [25-27]. Machdonald et al. [28] confirmed the presence of T-type calcium channels in the relay neurons of rat hypothalamus by the whole-cell voltage-clamp technique, and found that T-type calcium channels were correlative with the kindling effect of epilepsy. One of the proteins expressed by T-type calcium channels is Cav3.2 [29]. This study found that chronic epilepsy might cause significant increase of expression of T-type Ca^{2+} channel protein cav3.2 by observing the expression changes of Cav3.2 in temporal lobe and hippocampus of chronic PTZ-induced epileptic rats or those under the intervention of different drugs, which proved T-type calcium channels were involved in epileptic seizure, and also found that GBE drug may have an ancillary antiepileptic effect. The combination of GBE with VPA may significantly lower the Cav3.2 level, reduce the number and level of epilepsy seizures and inhibit abnormal discharge of cerebral neurons, with the treatment effect of epilepsy better than that of single medication with VPA, which provides a theoretical basis for the GBE treatment of epilepsy clinically.

However, through the statistical analysis on the accuracy of electric maze for all the rats, we learn that VPA and GBE cannot improve cognitive function of PTZ-induced epileptic rats. However, it is found in clinic that GB improves quality of life to a certain extent, which has been endorsed by the majority of patients. Ilhan et al. [30] found that compared to the NS treatment group and VPA treatment group, the response of mice treated with EGb761 to PTZ was significantly weakened. PTZ can significantly prolong the incubation period of epileptic seizure. In the EGb761 pre-treatment group, PTZ-induced spasm was significantly exempted. EGb761 and valproic acid can significantly reduce the oxidative damage of brain tissue caused by PTZ. In all the substances studied, EGb761 is the most effective one for the protection of brain oxidative damage induced by PTZ. 30 times of maze were detected in this study to measure the correct rate of behavior, with simple processes, however, there may be limitations for the incubation period, total reaction time and learning indicators were not measured.

References

1. *Ferrier CH, Aronica E, Leijten FS, et al. Electrocorticography discharge patterns in patients with a cavernous hemangioma and pharmacoresistent epilepsy. J Neurosurg, 2007; 107(3): 495-503.*
2. *Curtis M, Tassi L, Lo Russo G, et al. Increased discharge threshold after an interictal spike in human focal epilepsy. Eur J Neurosci, 2005; 22(11): 2971-2976.*
3. *Abou-Khalil BW. Lacosamide: What can be expected from the next new antiepileptic drug? Epilepsy Curr, 2009; 9(5): 133-134.*
4. *Hixson JD, French JA. Guidelines for new antiepileptic drug evaluation. Rev Neurol Dis, 2004; 1(18-26).*
5. *Jung IH, Lee YH, Yoo JY, et al. Ginkgo biloba extract (gbe) enhances the anti-atherogenic effect of cilostazol by inhibiting ros generation. Exp Mol Med, 2012; 44(5): 311-318.*
6. *Szasz BK, Lenkey N, Barth AM, et al. Converging effects of ginkgo biloba extract at the level of transmitter release, nmda and sodium currents and dendritic spikes. Planta Med, 2008; 74(10): 1235-1239.*

7. *Diehl RG, Smialowski A, Gotwo T. Development and persistence of kindled seizures after repeated injections of pentylenetetrazol in rats and guinea pigs. Epilepsia, 1984; 25(4): 506-510.*

8. *Zhu WW, Ding SL, Lu XF, et al. [Effect of different dosage sodium valproate on the neuronal apoptosis of epilepsy model rats]. Chinese Journal of Neuroscience, 2004; 20(6): 437-440.*

9. *Qingxia Kong, Shenggang Sun, Xianzhang Li, et al. [Protective effect of Ginkgo biloba extract on brain injury of PTZ-induced seizures rats]. Nervous Diseases and Mental Health, 2008; 8(2): 118-121.*

10. *Racine RJ. Modification of seizure activity by electrical stimulation. Ii. Motor seizure. Electroencephalogr Clin Neurophysiol, 1972; 32(3): 281-294.*

11. *Pence S, Erkutlu I, Kurtul N, et al. Antiepileptogenic effects of glutathione against increased brain ada in ptz-induced epilepsy. Int J Neurosci, 2009; 119(5): 616-625.*

12. *Li X, Yang Q, Kuang H, et al. Involvement of scn1b and kcna1 ion channels in audiogenic seizures and ptz-induced epilepsy. Epilepsy Res, 2005; 66(1-3): 155-163.*

13. *Cherubini E, Gonella J, Mancia D, et al. Effects of ptz-induced generalized epilepsy on intestinal activity in cats. Epilepsia, 1981; 22(3): 309-314.*

14. *Emami S, Kebriaeezadeh A, Ahangar N, et al. Imidazolylchromanone oxime ethers as potential anticonvulsant agents: Anticonvulsive evaluation in ptz-kindling model of epilepsy and sar study. Bioorg Med Chem Lett, 2011; 21(2): 655-659.*

15. *Vossler DG, Rostad SW, Haltiner AM, et al. Increased ictal discharge frequency and neocortex gliosis in lateral temporal lobe epilepsy. J Clin Neurophysiol, 2012; 29(5): 449-457.*

16. *Yang J, Krishnamoorthy G, Saxena A, et al. An epilepsy/dyskinesia-associated mutation enhances bk channel activation by potentiating Ca^{2+} sensing. Neuron, 2010; 66(6): 871-883.*

17. *Broicher T, Seidenbecher T, Meuth P, et al. T-current related effects of antiepileptic drugs and a ca2+ channel antagonist on thalamic relay and local circuit interneurons in a rat model of absence epilepsy. Neuropharmacology, 2007; 53(3): 431-446.*

18. *Schlichter LC, Kaushal V, Moxon-Emre I, et al. The ca2+ activated sk3 channel is expressed in microglia in the rat striatum and contributes to microglia-mediated neurotoxicity in vitro. J Neuroinflammation, 2010; 7: 7.*

19. *Chao CC, Huang CC, Lu DY, et al. Ca2+ store depletion and endoplasmic reticulum stress are involved in p2x7 receptor-mediated neurotoxicity in differentiated ng108-15 cells. J Cell Biochem, 2012; 113(4): 1377-1385.*

20. *Amano H, Amano T, Matsubayashi H, et al. Enhanced calcium influx in hippocampal ca3 neurons of spontaneously epileptic rats. Epilepsia, 2001; 42(3): 345-350.*

21. *Silve C, Petrel C, Leroy C, et al. Delineating a Ca^{2+} binding pocket within the venus flytrap module of the human calcium-sensing receptor. J Biol Chem, 2005; 280(45): 37917-37923.*

22. *Sarkisova KY, Midzianovskaia IS, Kulikov MA. Depressive-like behavioral alterations and c-fos expression in the dopaminergic brain regions in wag/rij rats with genetic absence epilepsy. Behav Brain Res, 2003; 144(1-2): 211-226.*

23. *Hsieh CL, Lin JJ, Chiang SY, et al. Gastrodia elata modulated activator protein 1 via c-jun n-terminal kinase signaling pathway in kainic acid-induced epilepsy in rats. J Ethnopharmacol, 2007; 109(2): 241-247.*

24. *Vezzani A, Balosso S, Maroso M, et al. Ice/caspase 1 inhibitors and il-1beta receptor antagonists as potential therapeutics in epilepsy. Curr Opin Investig Drugs, 2010; 11(1): 43-50.*

25. *Jianmin Liang, Yuehua Zhang, Juli Wang, et al. [Screening and analysis on variants in CACAN1H gene in childhood absence epilepsy]. Journal of Jilin University(Medicine Edition), 2007; 33(3): 533-537.*

26. *Juli Wang, Chongyang Han, Yuhong Jing, et al. [The effect of CACNA1H gene G773D mutation on calcium channel function]. Chinese Journal of Medical Genetics, 23, 2006; 4(369-373).*

27. *Heron SE, Khosravani H, Varela D, et al. Extended spectrum of idiopathic generalized epilepsies associated with cacna1h functional variants. Ann Neurol, 2007; 62(6): 560-568.*

28. *Macdonald RL, Kelly KM. Mechanisms of action of currently prescribed and newly developed antiepileptic drugs. Epilepsia, 1994; 35(4): 41-50.*

29. *Liao YF, Tsai ML, Chen CC, et al. Involvement of the cav3.2 t-type calcium channel in thalamic neuron discharge patterns. Mol Pain, 2011; 7: 43.*

30. *Ilhan A, Iraz M, Kamisli S, et al. Pentylenetetrazol-induced kindling seizure attenuated by ginkgo biloba extract (egb 761) in mice. Prog Neuropsychopharmacol Biol Psychiatry, 2006; 30(8): 1504-1510.*

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Impact of plasma fetuin-A levels to the presence and severity of coronary artery disease

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Abstract

Purpose: Fetuin- A is a negative acute phase reactant and an antiinflammatory glycoprotein which is synthesized in the liver. We aimed here in the present study to evaluate the association between plasma fetuin- A levels and the extent of coronary arterial disease (CAD).

Method: Plasma fetuin- A levels were measured in 127 consecutive patients who underwent coronary angiography. The relationship with the presence and extent of coronary artery as assessed by Gensini method and plasma fetuin levels were evaluated.

Findings: Of all case, 44.1% (n:56) were detected to have significant coronary arterial disease. Incidence of older age, male gender and tobacco use was significantly higher in the group with CAD. The mean fetuin- A level was found to be significantly lower in CAD group (244.1±117.8μg/ml vs 315.7±124.9μg/ml, p:0.001). Gensini score was documented to be negatively correlated with the fetuin- A level (rho:-0.363, p<0.001). While respective relationships between fetuin- A level and age (r:-0.292, p:0.001), total cholesterol (r:0.196, p:0.028) and LDL- cholesterol (r:0.204, p:0.021) was revealed, it was not the case with body mass index, HDL-cholesterol, triglyceride and fasting blood glucose level. A 1μg/ml decrease in plasma fetuin- A level was related to 1.1-fold increase in the incidence of CAD.

Conclusion: Serum fetuin- A levels were significantly low in the patients with documented coronary artery disease. Moreover, decreasing levels may act as a predictor of the extent and severity of coronary arterial disease.

Key words: Coronary, fetuin- A, atherosclerosis.

Introduction

As a progressive inflammatory disease with underlying atherosclerosis behind the etiology, coronary arterial disease (CAD) is the leading cause of morbidity and mortality in the developed countries. Age, tobacco usage, hypertension, diabetes mellitus, hyperlipidemia and positive family history for CAD are assigned as the main risk factors in larger epidemiological studies. However, these traditional risk factors prove insufficient in elucidating the prevalence of CAD in the community and occurrence of premature CAD in some cases. In some recent studies, roles of inflammation, oxidative stress and vascular calcification have begun to be determined in the onset and progression of this complicated process.[1-4]

Fetuin- A, an anti- inflammatory glycoprotein playing a pivotal in distinct phases of atherosclerosis is accepted as an inhibitor of vascular calcification.[5,6] Upon evaluating the role of fetuin- A in the cardiovascular events, many studies documented low fetuin- A levels in the patients with chronic kidney failure were correlated with increased rates of mortality and morbidity.[7,8] Likewise, low fetuin- A level was shown to be associated with mortality in the cases with acute myocardial infarction.[9,10] The aim in our present study was to determine the role of fetuin- A in the development and extent of coronary atherosclerosis.

Methods

Study population

127 consecutive patients who underwent coronary angiography with the complaint of chest pain were included in the study. Patients with previous history of revascularization or CAD, significant valvular or congenital heart disease, impaired renal and hepatic functions, admission with acute coronary syndrome, diabetes mellitus, any documented systemic disorder and those with active infection were excluded from the study.

Clinical Evaluation

Cardiovascular risk factors were determined in all patients. The length, weight and waist cir-

cumference were measured in each patient. The body mass index (BMI) was calculated in kg/m^2. The cases on antihypertensive medication or those with systolic/diastolic blood pressure to be ≥ 140/90 mmHg in three consecutive measurements were accepted to be hypertensive cases. Insulin and oral antidiabetic medication usage or fasting blood glucose ≥ 126 mg/dl were accepted as presence of diabetes mellitus and subsequently excluded from the study. Statin or other antihyperlipidemic drug usage was regarded as presence of hyperlipidemia.

Coronary angiography and evaluation of the severity of coronary atherosclerosis

Judkins technique was used through femoral approach in the implementation of selective coronary angiography. Left anterior descending (LAD), circumflex (Cx) coronary arteries were evaluated in at least 4 angiographic views, while RCA being evaluated in at least two angiographic views. The coronary angiograms were examined by two specialists blinded to the clinical and laboratory findings of the cases. Coronary arterial disease was defined as presence of at least 50% luminal obstruction in an epicardial coronary artery. Gensini score was utilized in the determination of the severity of CAD. The former score relies on the following scoring criteria: 1 point for luminal stenosis ranging between 0- 25%; 2 points for luminal stenosis between 26- 50%; 4 points for stenosis between 51- 75%; 8 points for stenosis between 76- 90%; 16 points for luminal stenosis between 91- 99%; and, 32 points for total obstruction. Then, the ultimate results were reached by multiplying the scores with a coefficient predetermined for each coronary artery and each segment. The coefficients were as follows: 5 for left mean coronary artery; 2.5 for LAD and Cx; 1.5 for mid LAD; 1 for distal LAD, mid-distal Cx, right coronary artery (RCA) and 1st diagonal branch; 0.5 for 2nd diagonal branch.

Laboratory

Using standard techniques, fasting blood glucose level, blood urea level, creatinin, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL)- cholesterols and triglyceride levels were measures from the blood samples drawn. The blood samples for measurement of fetuin- A was taken just before the onset of coronary angiography. The samples which coagulated after 20- 30 minute-long waiting in a room temperature were centrifuged for 15 minutes at 2000- 3000 rpm in 4 ± 2º C using a refrigerated centrifuge. The supernatant serum samples were transferred into ependorf safe lock tubes and stored at -70º C. fetuin- A levels were measured using human fetuin- A ELISA kit (brand name, Biven-dor Laboratory Medicine).

Statistical Analysis

The statistical analysis in this study was performed using SPSS 11.5 packet software. The categorical variables were expressed in number and ratio (%). ‘’ki- square” test was utilized in order to make comparison of categorical measurements between the groups. Continuous variables were expressed in mean ± standard deviation. Non-normally distributing variables were compared by using Mann Whitney U test, whereas those continuous variables showing normal distribution were compared using Student t test. In an attempt to evaluate the effect of parameters in single- variate analysis with p value< 0.25 on CAD, multivariate logistic regression analysis was performed. Spearman correlation analysis was performed so as to establish the relationship between Gensini score and the continuous variables, whereas Pearson correlation test was utilized in order to evaluate the relationship between fetuin- A level and continuous variables. The effects of the variables with p< 0.25 in single- variate analysis on fetuin- A level and Gensini score were evaluated using multivariate regression analysis. The data not complying with normal distribution were evaluated using logistic regression analysis following logarithmic transformation.

Results

A total of 127 patients, 75 of whom were males and 52 females, with mean age of 58.3 ± 9.6 years, were included in the study. Patients’ age were ranging between 36 and 84 years. The cases were further assigned into two groups as those with CAD and those without CAD. Table 1 exhibits the demographic features and cardiovascular risk factors of the groups. 44.1% of all cases (n: 56) were diagnosed with CAD. The males were more numerous in the CAD group (p: 0.001), and again, the cases

assigned into this groups were older than the other groups (p: 0.002). No significant difference was detected between the groups with regard to waist circumference and BMI (p> 0.05). As for the traditional cardiovascular risk factors, no difference was observed between the groups in regard to hypertension, hyperlipidemia and positive family history (p> 0.05), while smoking was significantly higher in CAD group compared to the group without CAD (p: 0.042). Fasting blood glucose levels, total cholesterol levels and LDL and HDL- cholesterol levels were similar between the two groups (p> 0.05). The mean fetuin- A level was detected to be significantly lower in CAD group (244.1 ± 117.8µg/ml vs 315.7 ± 124.9µg/ml, p: 0.001) (Figure 1).

When the effects exerted by such parameters as age, gender, positive family history, LDL and HDL- cholesterol, triglyceride level and fetuin- A level was evaluated on the basis of backward logistic regression analysis in the CAD group, it was found that age, gender, positive family history and fetuin- A levels were independently correlated with CAD. A 1µg/ml decrease in plasma fetuin- A level was related to 1.1 fold increase in the incidence of CAD.

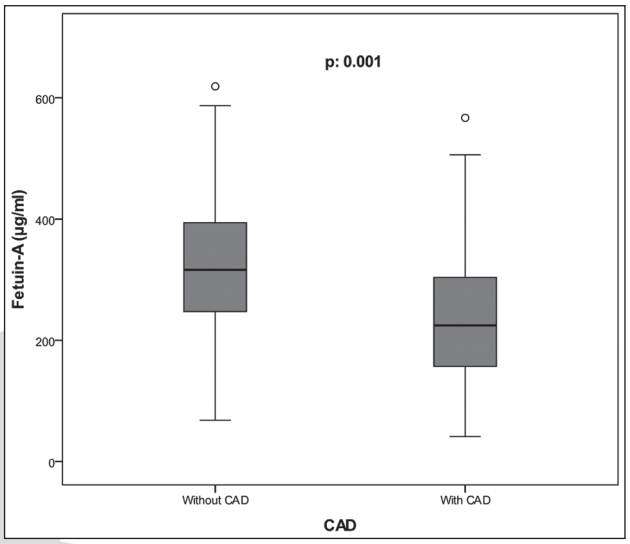

Figure 1. Comparison of the plasma Fetuin- A levels between the patients with and without CAD.

Evaluation of the severity of coronary arterial disease

Gensini score, a scoring system to evaluate the severity of coronary arterial disease, was calculated to be higher in the female patients (20.4 ± 27.6 vs 6.2 ± 11.8, p< 0.001). Gensini score did not differ according to presence or absence of hypertension, smoking, hyperlipidemia and positive family history for CAD (p> 0.05).

Table 1. Demographic, clinical and biochemical characteristics of patients divided by the absence/ presence of significant CAD

Parameters	Without CAD n: 71	With CAD n: 56	*p* Value
Age (years)	56.0±9.2	61.1±9.4	0.002*
Male/Female n (%)	33(46.5%)/38(53.5%)	42(75.0%)/14(25.0%)	0.001*
Waist circumference	100.3±11.4	98.4±12.3	0.357
BMI (kg/m²)	27.0±3.8	26.9±4.9	0.938
Cardiac risk factors, n (%)			
Hypertension	33(46.5%)	23(41.1%)	0.542
Hyperlipidemia	8(11.3%)	8(14.3%)	0.611
Cigarette smoking	15(21.1%)	21(37.5%)	0.042*
Family history	21(29.6%)	23(41.1%)	0.177
Laboratory findings (mg/dl)			
Fasting glucose	100.0±10.3	100.3±10.3	0.870
Total Cholesterol	202.1±37.8	205.9±44.9	0.610
LDL- Cholesterol	127.1±32.1	129.5±36.9	0.697
HDL- Cholesterol	44.9±12.4	41.2±10.7	0.080
Triglyceride	149.6±75.4	176.1±89.3	0.072

BMI, body mass index; CAD, coronary artery disease; HDL, high density lipoprotein; LDL, low density lipoprotein
** statistically significant*

In the correlation analysis, plasma fetuin- A level was found to be negatively correlated with Gensini score (rho: -0.363, p< 0.001) with decreasing plasma fetuin- A level in response to increase in Gensini score (Figure 2). In addition, Gensini score was detected to be significantly correlated with age (rho: 0.382, p< 0.001) and HDL- cholesterol level (rho: -0.208, p: 0.019). No association was revealed between Gensini score and BMI, waist circumference, fasting blood glucose level, total cholesterol level, LDL- cholesterol level and triglyceride level (p> 0.05).

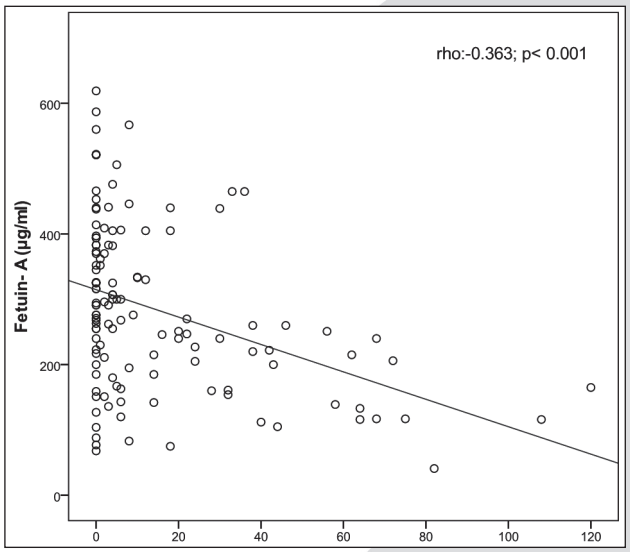

Figure 2. Relationship between plasma fetuin- A levels and the severity of CAD evaluated by Gensini score

Possible independent contributions of age, HDL- cholesterol, gender and fetuin- A level on logarithmic Gensini score were evaluated by multivariate regression model. The analysis revealed that age (β: 0.320, 95%CI: 0.013- 0.040, p< 0.001), HDL- cholesterol level (β: -0.170, 95%CI: -0.023- 0.000, p: 0.044) and fetuin- A level (β: -0.252, 95%CI: -0.003 0.001, p: 0.002) were identified as significant predictors of logarithmic Gensini score.

The correlation between plasma fetuin- A levels and patients' characteristics

The fetuin- A levels measured in the males and the females included in the present study were found to be similar (286.6 ± 128.3µg/ml vs 280.6 ± 125.0µg/ml, p: 0.795). Fetuin- A level did not exhibit significant difference with regard to history of hypertension, tobacco use and positive family history (p> 0.05). While a significant relationship between plasma fetuin- A level and age (p: 0.001), total cholesterol (p: 0.028), LDL- cholesterol (p: 0.021), no correlation was revealed between fetuin- A and BMI, waist circumference, HDL– cholesterol, triglyceride level and fasting blood glucose level (p> 0.05). In the retrospective multivariate regression analysis, age (β: -0.286, 95%CI: -5.939- -1.567, p: 0.001) and LDL- cholesterol (β: 0.196, 95%CI: 0.109- 1.340, p: 0.021) were observed to be significant predictors of plasma fetuin- A levels, whereas the former was not the case for total cholesterol (p> 0.05). Respective fetuin- A levels in the metabolic syndrome group (n: 60) and the group without metabolic syndrome were (282.9 ± 134.2µg/ml and 285.2 ± 120.1µg/ml, p> 0.05), while respective Gensini scores in the aforementioned groups were (15.3 ± 21.1 and 14.0 ± 25.5, p> 0.05), which did not reach the level of significance.

Discussion

The fetuin- A levels in the present study were found to be significantly lower in the group with the diagnosis of CAD established on coronary angiography compared with the group without CAD. Age, gender, positive family history for premature CAD and fetuin- A levels were found to be independently associated with the presence of CAD in multivariate regression analysis. Moreover, a statistically significant negative correlation was revealed between fetuin- A levels and Gensini score. According to these findings, it can be suggested that decreased plasma fetuin- A level is correlated with increased risk for CAD.

Atherosclerosis is a chronic inflammatory disease characterized by progressive accumulation of lipids, macrophages, T- lymphocytes, smooth muscle cells and extracellular matrix in the arterial wall. The inflammation persists during various stages of the atherosclerosis and is a robust indicator of plaque destabilization and thrombosis.[11] Vascular calcification, on the other hand, is known as precipitation of calcium and phosphate crystals, and contributes to the various stages of both the passive and degenerative processes of atherosclerosis and inflammation.[12,13] In our study, the role of the fetuin- A, an important and multifunctional glycoprotein for endothelial dysfunction, vascular calcification

and atherosclerosis, in the genesis of coronary arterial disease was investigated. Fetuin- A, secreted by the liver and found in all extracellular fluids, is glycoprotein characterized by anti-inflammatory effects which has a strong affinity toward calcium and phosphate ions.[5,14] The results with regard to the effect of fetuin- A in the coronary arterial disease yielded by the previous studies are contradicting. In vivo fetuin- A insufficiency in rats were associated with spontaneous dystrophic soft-tissue calcification in the kidneys, liver, aorta and heart.[6] Mehrota et al. reported a positive correlation between serum fetuin- A level and the score for coronary arterial calcification during pre-dialysis period in patients with diabetic nephropathy.[15] Interestingly, Ix et al reported a negative correlation between fetuin- A level and mitral and aortic calcification in the patients without severe renal failure but with coronary arterial disease, and that dystrophic calcification inhibitory effect exerted by fetuin-A was not dictated by the presence of renal disease or other cardiovascular risk factors.[16] Likewise, Mori et al. announced an inverse correlation between plasma fetuin- A level and severe calcific coronary arterial disease in the cases without any diabetes and renal dysfunction.[3] However, Roos et al. documented, in their study where multislice computed tomography was used, revealed no association between fetuin- A level and coronary arterial calcification.[17] A study by Ketteler et al. conducted on 312 stable patients with chronic kidney failure undergoing hemodialysis revealed a correlation between low levels of plasma fetuin-A and cardiovascular deaths.[8] The aforementioned data suggest the presence of a complex and a controversial relationship between fetuin- A level and cardiovascular clinical characteristics in the presence of diabetes mellitus and chronic hemodialysis. In this regard, patients with diabetes and renal dysfunction were excluded from the study. Moreover, coronary arterial calcification could not be evaluated due to the fact that patients underwent coronary angiography on the sole basis of stress tests or presence high pre-test probability for CAD. However, plasma fetuin- A levels were measured to be significantly lower in the cases with wide-spread, multivessel and chronic calcific lesions.

Fetuin- A, by mimicking the receptors for TGF-β II, act as TGF-β antagonist. In case of fetuin- A insufficiency, TGF-β expression increases, thus giving rise to increase in the fibrinogenesis.[18] Merx et al. reported increase in cardiac fibrosis and calcification in their study conducted on the hearts of fetuin- A-deprived rats and disruption in especially the diastolic functions and tolerance to ischemia and development of resistance to catecholamines.[19] Accordingly, fetuin- A was suggested to be a possible protector against cardiovascular outcomes. A study by Lim et al. conducted on patients with ST elevation myocardial infarction patients documented that fetuin- A level measured on the 3rd day after index event was lower compared to the control group, which was associated with a higher cardiovascular mortality rates compared to the patients with high levels of plasma fetuin- A.[9] Weikert et al detected in their 8-year-long follow-up study conducted on the general population that high serum fetuin- A level was associated with increased risk for myocardial infarction and ischemic stroke.[20] Basar et al and Bilgir et al observed in their studies that low levels of plasma fetuin- A in the patients with acute myocardial infarction was correlated with adverse cardiovascular events and mortality.[10,21] The aforementioned findings can be explained based on the suggestions that fetuin- A, which is likely to be used as a novel risk indicator for the prognosis of acute myocardial infarction is a negative acute phase reactant and it exhibits an ‘’U-shaped’’ correlation with the cardiovascular outcomes.[10,20] On the basis of those data, we excluded the patients with acute coronary syndrome from our study, thereby attempting to direction our focuses on its effects on atherosclerosis, rather than its being an acute phase reactant.

Recently, the association between CAD, cardiovascular events and the inflammation markers like serum fetuin- A has been debated. To our knowledge, our study is the first on the basis of the population characteristics to report that low levels of fetuin– A is associated with CAD. Fetuin- A was an independent prognostic indicator for CAD along with age, gender and positive family history in the evaluation based on the traditional cardiovascular risk factors. At the same time, we documented a statistically significant negative correlation between serum fetuin- A levels and Gensini score, a score to evaluate the extent of the coronary arterial disease. This translates into the

suggestion that serum fetuin- A levels decrease as the severity of coronary arterial disease deteriorates and that the lesions on the patients with low levels of serum fetuin- A are more extensive and severe. Kanbay et al. reported fetuin- A to be an independent risk factor for CAD in patients with mild to moderate renal impairment.[22] The inhibitory effect of fetuin- A on the inflammation process and vascular calcification may help elucidate its role in the pathogenesis in atherosclerosis and acute coronary syndromes. A shift in the balance between the pro-inflammatory and anti- inflammatory in favor of pro-inflammation induced by low levels of fetuin- A gives rise to deterioration of the ongoing inflammatory process, thus facilitating the production of cardiotoxic cytokines, which elucidates the contribution of fetuin- A to the progression of coronary atherosclerosis and calcification.

Our aim in the present study was to evaluate any possible effects likely to be exerted by serum fetuin- A levels on cardiovascular outcomes. One of the limitations in our study was inclusion of small number of subjects. Moreover, as expected, the patients in CAD group were older than their counterparts in the control group. Serum fetuin-A level was negatively correlated with advancing age, which was considered to be also a contributing factor to the development of CAD.

In conclusion, fetuin- A, as many other inflammatory mediator do, plays a role in the underlying pathogenesis behind the coronary atherosclerosis. Lower levels of serum fetuin- A is associated with increased risk for and severity of coronary arterial disease. If supported by further studies to be performed in the future, measurement of serum fetuin- A level in selected cases may act as a risk factor, providing opinion with regard to the presence and the extent of coronary arterial disease.

References

1. *Suliman ME, Garcia-Lopez E, Anderstam B, Lindholm B, Stenvinkel P. Vascular calcification inhibitors in relation to cardiovascular disease with special emphasis on fetuin-A in chronic kidney disease. Adv Clin Chem 2008; 46: 217-262.*

2. *Mazzini MJ, Schulze PC. Proatherogenic pathways leading to vascular calcification. Eur J Radiol 2006; 57: 384-389.*

3. *Mori K, Ikari Y, Jono S, Emoto M, Shioi A, Koyama H, Shoji T, Ishimura E, Inaba M, Hara K, Nishizawa Y. Fetuin-A is associated with calcified coronary artery disease. Coron Artery Dis 2010; 21: 281-285.*

4. *Inoue T, Node K. Vascular failure: A new clinical entity for vascular disease. J Hypertens 2006; 24: 2121-2130.*

5. *Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, Jahnen-Dechent W, Shanahan CM. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. J Am Soc Nephrol 2005; 16: 2920-2930.*

6. *Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest 2003; 112: 357-366.*

7. *Pecovnik Balon B, Knehtl M, Bevc S, Jakopin E, Gorenjak M. Fetuin-A as a risk factor for mortality in hemodialysis patients. Wien Klin Wochenschr 2010; 122 Suppl 2: 63-67.*

8. *Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Bohm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 2003; 361: 827-833.*

9. *Lim P, Collet JP, Moutereau S, Guigui N, Mitchell-Heggs L, Loric S, Bernard M, Benhamed S, Montalescot G, Rande JL, Gueret P. Fetuin-A is an independent predictor of death after ST-elevation myocardial infarction. Clin Chem 2007; 53: 1835-1840.*

10. *Basar N, Sen N, Kanat S, Ozlu MF, Ozcan F, Cay S, Erden G, Cagli KE, Yildirimkaya M, Maden O, Covic A, Kanbay M. Lower fetuin-A predicts angiographic impaired reperfusion and mortality in ST-elevation myocardial infarction. J Investig Med 2011; 59: 816-822.*

11. *Hansson GK. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21: 1876-1890.*

12. *Ross R, Glomset JA. The pathogenesis of atherosclerosis (first of two parts). N Engl J Med 1976; 295: 369-377.*

13. *Jono S, Shioi A, Ikari Y, Nishizawa Y. Vascular calcification in chronic kidney disease. J Bone Miner Metab 2006; 24: 176-181.*

14. *Mizuno M, Farach-Carson MC, Pinero GJ, Fujisawa R, Brunn JC, Seyer JM, Bousfield GR, Mark MP, Butler WT. Identification of the rat bone 60K acidic glycoprotein as alpha 2HS-glycoprotein. Bone Miner 1991; 13: 1-21.*

15. *Mehrotra R, Westenfeld R, Christenson P, Budoff M, Ipp E, Takasu J, Gupta A, Norris K, Ketteler M, Adler S. Serum fetuin-A in nondialyzed patients with diabetic nephropathy: relationship with coronary artery calcification. Kidney Int 2005; 67: 1070-1077.*

16. *Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA. Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. Circulation 2007; 115: 2533-2539.*

17. *Roos M, Lutz J, Salmhofer H, Luppa P, Knauss A, Braun S, Martinof S, Schomig A, Heemann U, Kastrati A, Hausleiter J. Relation between plasma fibroblast growth factor-23, serum fetuin-A levels and coronary artery calcification evaluated by multislice computed tomography in patients with normal kidney function. Clin Endocrinol (Oxf) 2008; 68: 660-665.*

18. *Demetriou M, Binkert C, Sukhu B, Tenenbaum HC, Dennis JW. Fetuin/alpha2-HS glycoprotein is a transforming growth factor-beta type II receptor mimic and cytokine antagonist. J Biol Chem 1996; 271: 12755-12761.*

19. *Merx MW, Schafer C, Westenfeld R, Brandenburg V, Hidajat S, Weber C, Ketteler M, Jahnen-Dechent W. Myocardial stiffness, cardiac remodeling, and diastolic dysfunction in calcification-prone fetuin-A-deficient mice. J Am Soc Nephrol 2005; 16: 3357-3364.*

20. *Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, Haring HU, Boeing H, Fritsche A. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation 2008; 118: 2555-2562.*

21. *Bilgir O, Kebapcilar L, Bilgir F, Bozkaya G, Yildiz Y, Pinar P, Tastan A. Decreased serum fetuin-A levels are associated with coronary artery diseases. Intern Med 2010; 49: 1281-1285.*

22. *Kanbay M, Nicoleta M, Selcoki Y, Ikizek M, Aydin M, Eryonucu B, Duranay M, Akcay A, Armutcu F, Covic A. Fibroblast growth factor 23 and fetuin A are independent predictors for the coronary artery disease extent in mild chronic kidney disease. Clin J Am Soc Nephrol 2010; 5: 1780-1786.*

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Effects of paroxetine on the plasminogen activation inhibitor-1of acute myocardial infarction patients after percutaneous coronary intervention complicated with anxiety and depression

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Abstract

Purpose: To observe the effects of paroxetine on the plasminogen activation inhibitor-1 (PAI-1) of acute myocardial infarction (AMI) patients after percutaneous coronary intervention (PCI) complicated with anxiety and depression.

Methods: 98 AMI patients received emergency PCI, of which 76 were assessed as anxiety and depression by the Hamilton Anxiety Scale. They were then randomly divided into a paroxetine group and a control group, which were observed for 6 months. The serum contents of PAI-1 of the patients were determined before and 15 days, 1 month, 3 months and 6 months subsequent to the treatment, and the results were subjected to repeated measures analysis of variance and multivariate analysis of variance.

Results: The serum levels of PAI-1 changed depending on time. Time and grouping interacted, i.e. the serum PAI-1 levels of the two groups differed with increasing time. The symptoms of the patients were alleviated after being treated with paroxetine for 1 month.

Conclusion: Paroxetine significantly decreased the serum PAI-1 levels of AMI patients complicated with anxiety-depression after PCI.

Key words: Paroxetine, acute myocardial infarction, interventional treatment, plasminogen activation inhibitor-1.

Introduction

Atherosclerotic coronary heart disease (CHD) is one of the common diseases endangering human health and a typical psychosomatic disease that mainly leads to death in developed countries [1]. In recent years, the CHD morbidity and mortality rates are increasing apparently, which induces considerable death in males over 40 and females over 60 in China. Since the beginning of the 21st century, the death of CHD has experienced the fastest increase in young and middle-aged males between 30 and 40 years old, and the involved patients will double by 2020 [2-4]. The severe type of CHD, acute myocardial infarction (AMI), is becoming a common disease seriously threatening human life, which elevates mortality and hospitalization rates consequently. Suffering from AMI is a major mental stimulation for patients, which is often accompanied by varying degrees of anxiety and depression that impact the prognosis. Emergent percutaneous coronary intervention (PCI) is the most effective mean for the patients with AMI to quickly enable vascular recanalization. Different therapies, such as emergency intervention, elective intervention, and conventional drug treatment, can also affect the long-term efficacy on the psychological stress of patients [5-7]. Therefore, finding out an ideal composite intervention with drug therapy combining interventional treatment is spotlighted currently. This study mainly aims to explore the condition of anxiety and depression of AMI patients after undergoing PCI, and to observe the effect of combined treatment with paroxetine on plasminogen activation inhibitor-1 (PAI-1) in AMI patients after emergent PCI.

Materials and methods

Objects

98 patients who were diagnosed as AMI and underwent emergent PCI from January 2012 to December 2012 in our hospital were selected. All patients confirmed to the diagnostic criteria of

AMI developed by the World Health Organization (WHO), who received coronary angiography and PCI after admission. The coronary angiography results of the patients show that thrombus shadow signs (protruding vessel wall filling defect or total occlusion) existed in infarct-related arteries (IRA), the onset of this disease was within 24h, and who were in line with emergent PCI, which included 47 cases of left anterior descending branch (LAD), 27 cases of right coronary artery (RCA) and 24 cases of left circumflex artery (LCX). The patients were confirmed to be complicated by anxiety and depression by the Hamilton Rating Scale for Depression and the Manifest Anxiety Scale.

Exclusion criteria: the patients who received the treatment with other antidepressant and antipsychotic drugs within two months or had serious cardiorespiratory failure.

Grouping and PAI-1 Detection

All hospitalized patients with AMI were drawn blood within 12 h for blood cell analysis, and the detections of blood sugar, blood lipid, hs-CRP and hepatic and renal function. Then they were scored using the Hamilton Anxiety and Depression Scale by two psychiatrists within 48 h. The scoring standard was as follows: HAMA (14 items) score> 14 for anxiety disorder and HAMD (24 items) score> 20 for depressive disorder [8,9]. There were 76 AMI patients combined with anxiety and depression after emergent PCI (37 males and 39 females), aged between 35 and 70 (54.2 ± 14.8) years old. The patients were randomly divided into an anti-anxiety and depression treatment group (n=38) and a control group (n=38). All patients were observed for six months, and 3 ml of the fasting cubital venous blood was drawn in the morning before treatment and on the 15th day, 1st month, 3rd and 6th months after treatment respectively (without access to diet 8 h before blood sampling and sleeping well at night), placed in procoagulant tubes for coagulation at room temperature for 1 h, and then centrifuged at 1,200rpm/min and 4 ℃ for 15 min. After separation, the serum was stored in a -30 ℃ deep cryogenic refrigerator for detection. The serum PAI-1 level was detected by ELISA kit which was provided by Beijing Ruixianghe Biotech Co., Ltd., and the operation was conducted in accordance with the instructions.

Treatment Methods

The two groups of patients were administered with nitrates, β-receptor inhibitors, calcium antagonists, ACE inhibitors, anticoagulants, antiplatelets, statins and other conventional treatments after admission. The patients in the control group received conventional treatment, and those in the treatment group were administered with 20 mg/d paroxetine on the basis of conventional therapy, taken with meals. The course of treatment was 6 months. Paroxetine was purchased from Tianjin Smith Kline Pharmaceuticals Co., Ltd. with the specification of 20 mg × 10 tablets.

Statistical analysis

The transverse data files were subjected to multivariate analysis utilizing 1 to 5 (1 to 5 respectively represent the PAI-1 level before treatment and on the 15th day, 1st month, 3rd and 6th months after treatment) as the dependent variables and grouping as grouping variables. The data were subjected to repeated measures analysis of variance and multivariate analysis of variance using SPSS 12.0 software package, and $P<0.05$ was considered statistically significantly different.

Results

General Clinical Information

There were no significant differences between the ages, genders and risk factors and the Hamilton anxiety and depression scores of the two groups ($P>0.05$) (Table 1).

Box's M Test for Homogeneity

By the Box's M test for homogeneity, PAI-1 ($F = 2.036$, $P = 0.741$) met the prerequisite of repeated measures analysis of variance ($P>0.05$).

Multivariate Analysis of Variance

The PAI-1 results suggest that the 5 repeated measurement data were correlated.

During the repeated measures analysis of variance analysis, the results of multivariate analysis of variance prevailed.

Effects of Time and Grouping

After repeated measures multivariate analysis of variance, the PAI-1 level changed over time ($F = 491.71$, $P<0.01$) and with the changes of group-

Table 1. General Clinical Information

Item	Treatment	Control
Case No.	38	38
M/F	20/18	17/21
Age	51.4±12.8	53.1±13.3
Family history (case)	2	2
Hyperlipemia history (case)	5	4
Hypertension history (case)	7	8
Diabetes history (case)	4	4
Smoking history (case)	13	14
Blood sugar (mmol/L)	5.94±0.36	5.86±0.31
Triglyceride (mmol/L)	1.38±0.67	1.43±0.66
Total cholesterol (mmol/L)	5.62±0.16	5.57±0.11
High density lipoprotein (mmol/L)	1.41±0.31	1.42±0.33
Low density lipoprotein (mmol/L)	3.41±0.61	3.42±0.64
Anxiety score	16.2±2.9	16.8±2.1
Depression score	23.8±3.3	24.1±3.1

Table 2. Effects of Time and Grouping

Factor	Estimation value	*F* value	Assumed degree of freedom	*P* value
Observation time	0.943	491.71	4	0.000
Score	0.926	15.62	1	0.000
Observation time × grouping	0.861	4.841	4	0.038

Table 3. PAI-1 Levels Before and After Treatment (ng/L)

Group	Before	15 d after	1 month after	3 months after	6 months after	F value	P
Treatment	92.6±14.3	82.7±12.6	62.5±12.1	36.4±9.1	24.3±5.6	425.26	0.001
Control	92.4±13.9	83.4±12.7	70.2±11.6	43.6±9.2	28.6±5.8	235.61	0.001
P	0.086	0.073	0.004	0.001	0.000		

ing (F = 15.62, P<0.01). Meanwhile, the time had interaction with grouping, i.e. the serum PAI-1 levels of the two groups depended on time differently (F = 4.841, P<0.05) (Table 2).

PAI-1 Levels Before and After Treatment

The results of multivariate analysis of variance did not differ before treatment (P>0.05), suggesting that the baseline data were consistent in the two groups. No differences were found on the 15th day after treatment (P>0.05), but the differences were statistically significant at each time point in the 1st month, 3rd and 6th months after treatment (P<0.01) (Table 3).

Profiles of Interaction between PAI-1 Level and Time

The profiles of the interaction between PAI-1 level and time in the two groups before and after treatment are shown in Figure 1.

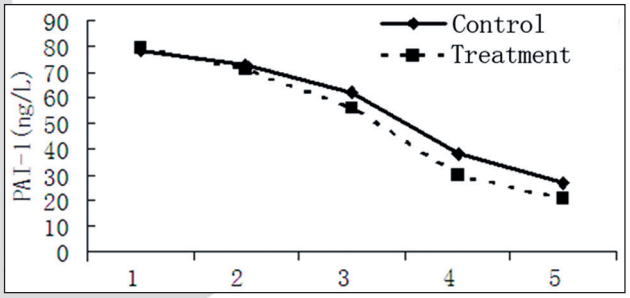

Figure 1. Dependences of PAI-1 levels on time. 1: before treatment; 2: 15 days after treatment; 3: 1 month after treatment; 4: 3 months after treatment; 5: 6 months after treatment.

PAI-1 Levels Before and After Treatment within the Same Group

PAI-1 horizontal data packet split, then the two groups were subjected to repeated measures ANOVA before treatment, 15d, 1 month, and 3 and 6 months after. The results show that the differences in PAI-1 level between the two groups before and

after treatment were statistically significant (F = 425.26 in the paroxetine group and F = 235.61 in the control group, both P<0.01) (Table 3).

Discussion

Epidemiological data show that the prevalence rates of depression in CHD patients range from 16% to 25%, which is almost three times higher than that of ICU patients, and five times higher than that of general population. CHD is one of the medical diseases with serious harm to human health today. Its pathogenesis has not been fully elucidated, but lipid metabolism disorders, hemodynamic changes and changes of arterial wall itself are main direct factors. Psychosocial factors influence these three processes through neuroendocrine intermediaries, thus affecting the occurrence and development of CHD. The impact of depression on the CHD prognosis is equivalent to or even exceeds the cardiovascular risk factors known currently [10,11]. Studies have shown that if cardiovascular diseases are complicated by depression and anxiety disorders, it may affect the compliance with treatment, prognosis and quality of life of patients. Only by the simultaneous treatment of anti-anxiety together with cardiovascular diseases can satisfactory results be achieved, short- and long-term complications be possible to be reduced, and long-term prognosis be improved [12,13]. Current studies have found that: (1) patients with depression complicated by CHD have relatively high platelet activity, increasing the risk of thrombosis; (2) the heart rate variability in patients with depression is significantly reduced. Due to the double impact of disease and mental factors, AMI patients are often accompanied by varying degrees of depression and anxiety symptoms; these negative emotions can cause increased sympathetic activity in vivo, so as to trigger a series of physiological changes, such as excess secretion of catecholamine, lipid metabolic disorder, release of angiotensin II under the action of procoagulant substances and intense vasoconstriction, elevated heart rate and increased blood pressure, etc., the results of which are reduced myocardial blood and oxygen supply, increased myocardial oxygen consumption, precipitating and aggravating angina pectoris, myocardial infarction, arrhythmias and heart failure. (3) Patients with depression complicated by CHD may experience enhanced oxidation in vivo, weakened antioxidant protection and increased oxidized low-density lipoprotein (LDL) [14,15].

The serum PAI-1 level is a good indicator for reflection of the fibrinolytic activity in vivo and clinical determination of the stability and degree of atherosclerotic lesions, which has a certain value on the assessment of prognosis and efficacy of macrovascular diseases [16]. PAI-1 is a major regulatory factor of the fibrinolytic system with multiple sources, including vascular endothelium, adipose tissue and liver. It is one member of serine protease inhibitor family with a relative molecular mass of 50 kD, composed of 379 amino acid residues. It activity is the highest in the morning, which is lower activity in the afternoon and evening [17]. The removal of intravascular fibrin is mainly adjusted by plasmin which is completed by the action of t-PA and u-PA on plasminogen, and PAI-1 can inhibit the activity of t-PA and u-PA. The key link of plasma fibrinolysis is the activation of blood plasminogen which is regulated by the t-PA and PAI-1 balance, therefore, PAI-1 imbalance may cause the occurrence of thrombotic events, and PAI-1 excess is associated with increased cardiovascular events [18].

Paroxetine, as a potent and highly selective 5-hydroxytryptamine (5-HT) reuptake inhibitor, can increase the concentration of 5-HT in the neurosynaptic gap by inhibiting the active transport of 5-HT, strengthen 5-HT nerve conduction to relieve nerve pressure, so as to play the role of anti-depression and anti-anxiety [19]. Due to the rapid onset and good efficacy as well as no receptor affinity of histamine H1, α1 and M, paroxetine has no risk of increasing cardiovascular events, few adverse reactions and good compliance, with mild anti-cholinergic activity, sedation, orthostatic hypotension and other symptoms. In this study, the Hamilton Anxiety and Depression Rating Scale was used to know about the onset of anxiety and depression in AMI patients [20]. The results showed that the level of serum PAI-1 in AMI patients with anxiety and depression after PCI was changed over time, and the time had interaction with grouping, that is, the serum PAI-1 levels were different with the changing trend of time between the two groups. Compared with the patients who did not receive anti-anxiety and depression treatment, the serum PAI-1 level of AMI patients with anxiety and depression was

improved after they received paroxetine treatment for a month. Currently, there is rare report on the research that AMI patients with anxiety and depression receive paroxetine treatment after PCI. The results of this study showed that combined treatment with paroxetine on AMI patients with anxiety and depression after emergent PCI can benefit by improving the level of serum PAI-1.

References

1. *Horvath Z, Csuka D, et al. Elevated C1rC1sC1inh levels independently predict atherosclerotic coronary heart disease. Mol Immunol, 2012; 54(1): 8-13.*
2. *Zhong Y, Wang N, et al. Ischemia-modified albumin in stable coronary atherosclerotic heart disease: clinical diagnosis and risk stratification . Coron Artery Dis, 2012; 23(8): 538-41.*
3. *An X, Yu D, et al. Insulin resistance predicts progression of de novo atherosclerotic plaques in patients with coronary heart disease: a one-year follow-up study. Cardiovasc Diabetol, 2012; 11(3): 71-4.*
4. *Watanabe T, Sato K, et al. Endogenous bioactive peptides as potential biomarkers for atherosclerotic coronary heart disease. Sensors (Basel), 2012; 12(4): 4974-85.*
5. *Koch W, Mueller JC, et al. Two rare variants explain association with acute myocardial infarction in an extended genomic region including the apolipoprotein(a) gene. Ann Hum Genet, 2012; 77(1): 47-55.*
6. *Choi CH, Park CH, et al. Acute type a aortic dissection initially diagnosed with myocardial infarction . Korean J Thorac Cardiovasc Surg, 2012; 45(6): 424-5.*
7. *Zhou C, Wu J, et al. On-admission serum uric acid predicts outcomes after acute myocardial infarction. Croat Med J, 2012; 53(6): 642-4.*
8. *Melzer J, Rostock M, et al. Preliminary data of a HAMD-17 validated symptom scale derived from the ICD-10 to diagnose depression in outpatients . Forsch Komplementmed, 2012; 19(4): 191-6.*
9. *Helmreich I, Wagner S, et al. Sensitivity to changes during antidepressant treatment: a comparison of unidimensional subscales of the Inventory of Depressive Symptomatology (IDS-C) and the Hamilton Depression Rating Scale (HAMD) in patients with mild major, minor or subsyndromal depression. Eur Arch Psychiatry Clin Neurosci, 2012; 262(4): 291-304.*
10. *Pogosova GV. Depression a risk factor for coronary heart disease and a predictor of coronary death: 10 years of scientific research. Kardiologiia, 2012; 52(12): 4-11.*
11. *Barley EA, Haddad M, et al. The UPBEAT depression and coronary heart disease programme: using the UK medical research council framework to design a nurse-led complex intervention for use in primary care . BMC Fam Pract, 2012; 13(1): 119-23.*
12. *Pizzi C, Santarella L, et al. The impact of treatment on the pathophysiologic mechanisms linking coronary heart disease and depression . J Am Coll Cardiol, 2012; 60(23): 2424-5.*
13. *Kiropoulos LA, Meredith I, et al. Psychometric properties of the cardiac depression scale in patients with coronary heart disease . BMC Psychiatry, 2012; 12: 216-8.*
14. *Ye S, Denton EG, et al. Epidemiology and Management of Depression Following Coronary Heart Disease Diagnosis in Women . Curr Cardiovasc Risk Rep, 2012; 6(3): 210-218.*
15. *Leung YW, Flora DB, et al. The impact of premorbid and postmorbid depression onset on mortality and cardiac morbidity among patients with coronary heart disease: meta-analysis . Psychosom Med, 2012; 74(8): 786-801.*
16. *Lima LM, Carvalho Md, et al. PAI-1 4G/5G polymorphism and plasma levels association in patients with coronary artery disease . Arq Bras Cardiol, 2012; 97(6): 462-389.*
17. *Katsaros KM, Kastl SP, et al. Clopidogrel pretreatment abolishes increase of PAI-1 after coronary stent implantation . Thromb Res, 2012; 123(1): 79-84.*
18. *Sarecka B, Zak I, et al. Synergistic effects of the polymorphisms in the PAI-1 and IL-6 genes with smoking in determining their associated risk with coronary artery disease. Clin Biochem, 2012; 41(7): 467-73.*
19. *Snoeren EM, Refsgaard LK, et al. Chronic paroxetine treatment does not affect sexual behavior in hormonally sub-primed female rats despite 5-HT(1)(A) receptor desensitization . J Sex Med,2012; 8(4): 976-88.*
20. *Mongeau R, Martin CB, et al. 5-HT2C receptor activation prevents stress-induced enhancement of brain 5-HT turnover and extracellular levels in the mouse brain: modulation by chronic paroxetine treatment. J Neurochem, 2012; 115(2): 438-49.*

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The use of human fibrin sealant – an example for achalasia surgery

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Abstract

The goal of this study is to present the results of the use of human fibrin sealant in thoracoscopic achalasia surgery. During the period 2002 – 2010 at the 1st surgery clinic of Emergency Hospital "N. Pirogov" were operated 19 patients with achalasia. The surgical method was previously reported and in general it is a video assisted minimally invasive thoracoscopic surgical (VATS) method with application of fibrin sealant Tissucol through pressure.

X-ray examination was applied to all patients preoperatively, as well as fibrogastroscopy. The diagnosis was set up also after careful clinical symptoms evaluation, patient reports and anamnesis. Clinical tests and biochemistry was also applied. After the surgery during the follow up visits a secondary anamnesis was taken, and secondary X-ray examination was performed together with clinical biochemical tests. The age distribution is among 18 and 73 years of age (mean 51.26 years), n=9 are male, and n=10 female. All patients were reporting similar symptoms during the anamnesis and were with clinically proven achalasia. During the follow up visits the X-ray examination shows very good physiology functions without esophagus defects in the common nutrition. No statistically significant correlation among the patients' characteristics before and after the surgery was observed.

The use of fibrin sealant "Tissucol" is the first reported for achalasia surgery. The necessity of additional protective mucosa manipulations declined through its application.

Key words: Achalasia, surgery, fibrin sealant, VATS.

Introduction

Fibrin sealant is a biotechnological tissue adhesive based on the final stage of coagulation process[1]. Human Fibrin Glue (HFG) is made of two components which are storage in separate vials: a freeze dried concentrate of clotting proteins, mainly fibrinogen, Factor XIII and fibronectin (the sealant) and freeze dried thrombin (the catalyst). The first component is reconstituted with an aprotinin solution that inhibits tissue fibrinolysis. The second component (thrombin), available in 500 IU concentration, is dissolved with calcium chloride. The fibrin glue is used in the haemostatic process and in the wound healing. The term fibrin was first introduced by Virhov in 1847 year. In 1909 S. Bergel reports for its haemostatic function in case of injuries[2]. In 1916 S. Harvey used the fibrin powder for haemostasis of parenchyma organs bleeding during abdominal surgery[3]. In 1940 year J. Yong and P. Medawar used the experimental fibrin sealant for joining the peripheral nerves[4]. The vast clinical application of fibrin sealant starts during 70s years of the 20 century with the development of the highly concentrated solutions[5, 6]. Since 1978 in the clinical practice starts the use of the „Tissel„ and „Tissucol„ (Immuno, Viena, Austria)[7,8], as well as and other industrially developed biotechnological fibrin sealants.

The indications for the use of human fibrin sealant are numerous and present in all the surgical branches [9, 10, 11, 12, 13, 14, 15, 16, 17]. A randomized controlled trial of 50 patients undergoing hernia repair according to Lichtenstein's technique under local anaesthesia was performed and showed that human fibrin sealant is effective in preventing local hemorrhagic complications after inguinal hernia repairing in patients with concurrent coagulation disorders[18]. New sphincter-saving approaches have been applied in the treatment of perianal fistula in order to avoid the risk of faecal incontinence [19]. Two articles have been exploring the use of HFG Tussicol in the cardiovascular surgery and

expressed some safety reasons [20, 21]. A review is focussing on bone substitute composites made by mixing ceramic biomaterials with fibrin sealants [22]. In the ophthalmology the use of fibrin sealant was considered as safe and effective approach that is surgeon and patient friendly [23]. The use of fibrin sealant was also explored in the urology [24]. Only one article has been found to present the use of fibrin sealant in one patient with achalasia due to the oesophago-respiratory fistula in a complication of advanced malignant tumours [25].

The goal of this study is to present the results of the use of human fibrin sealant in thoracoscopic achalasia surgery.

Materials and methods

During the period 2002 – 2010 at the 1st surgery clinic were operated 19 patients with achalasia (n=9 were male and n=10 female) admitted at Emergency Hospital "N. Pirogov". The surgical method was previously reported and published [26]. In general it is a video assisted minimally invasive thoracoscopic surgical (VATS) method with application of fibrin sealant Tissucol through pressure [27].

According to the severity of achalasia the patients are separated in 4 groups:

Ist stage – functional spasm;
IInd stage – spasm with moderate dilution of the esophagus;
IIIrd stage - pronounced esophagus dilatation;
IVth stage – cardiostenosis with huge dilatation and S-view of the esophagus.

X-ray examination was applied to all patients preoperatively, as well as fibrogastroscopy. The diagnosis was set up also after careful clinical symptoms evaluation, patient reports and anamnesis. Clinical tests and biochemistry was also applied.

After the surgery during the follow up visits a secondary anamnesis was taken, and secondary X-ray examination was performed together with clinical biochemical tests.

The following patients' characteristics were followed - age, gender, duration of symptoms before the surgery, weigh reduction, availability of previous dilatations, postoperative complications.

Descriptive statistics and correlation analysis were applied towards the above variables. Pearson correlation was applied towards the continuous variables, Spearman for rang variables and Fisher's exact test for dichotomized variables.

Results

The main patients characteristics before and after the surgery are shown on Annex 1. The age distribution is among 18 and 73 years of age (mean 51.26 years), n=9 are male, and n=10 female. All patients were reporting similar symptoms during the anamnesis as are vomiting, stuck of food, firstly observed for liquid and then for solid food, heartburns, which are not common [28] (Table 1).

The symptoms duration is among 3 months to 18 years (mean 3.88 years). All patients were with clinically proven achalasia and 10 passed previous dilatations among n=1 to n=4 with contemporary improvement of symptoms. The mean hospital stay was 8.95 days (SD 2.4). All patients reported considerable weigh reduction (10.58 kg., SD 9.640). Average time for operation is 114.74 minutes (SD 18.06). Three of the patients received post operative complications, as follows. One

Table 1. Descriptive statistic of the patients' characteristics

	N	Minimum	Maximum	Mean	Std. Deviation
Age	19	18	75	51.26	15.800
Previous dilatations	19	0	4	1.21	1.475
Symptoms duration	19	0	18	3.88	4.301
Previous surgeries	19	0	1	.58	.507
Weigh reduction	19	4	40	10.58	9.640
Time to follow up	19	1	92	37.11	24.826
Hospital stay	19	3	13	8.95	2.392
Post operative complications	19	0	1	.16	.375
Time of surgery	19	90	150	114.74	18.064

was transferred to emergency department due to hypertension, 1 was transferred to conventional laparoscopic fundoplication and 1 to conventional thoracotomy.

During the follow up visits the X-ray examination shows very good physiology functions without esophagus defects in the common nutrition.

Availability of possible correlation among the patients' characteristics before and after the surgery was examined statistically and no statistically significant correlation was observed (Table 2).

The time for follow up visits is varying among 1 to 92 months and all 19 patients reported lack of any additional symptoms, nor the disease relapse for this period, instead of their initial characteristics, as well as their postoperative complications (Figure 1).

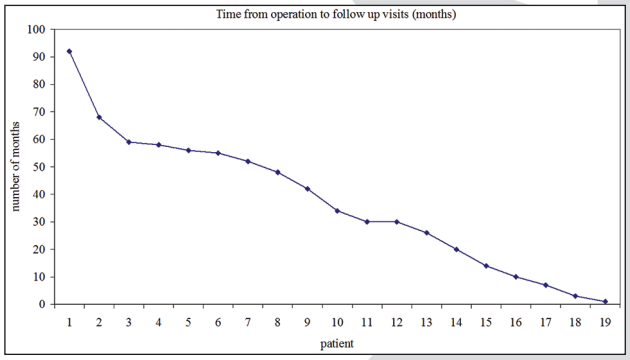

Figure 1. Time from operation to follow up visits (months)

Discussion

The use of fibrin glue "Tissucol" is the first reported for achalasia surgery. The necessity of additional protective mucosa manipulations declined through its application. In our methodology the glue is also applied under pressure that allows uniform distribution on the mucosa surface.

Every surgical technique places a variety of questions among the surgeons. One of them is to describe the patients suitable for its application and their characteristics that could influence its success. The second question refers to the factors that could affect the success of surgery, possible risks, period without relapses after the surgery, survival time etc.

The statistical analysis shows that there is no statistically significant correlation among all patients' characteristics before and after the surgery. Such results could be commented as proving the appropriateness of the surgical techniques for different patient groups, with varying preliminary characteristics as is the stage of the achalasia, weigh reduction, availability of previous complications and/or surgery technique.

Conclusion

The under pressure fibrin sealant usage is safe and efficacious approach for achalasia thoracoscopic surgery with long term asymptomatic period survival period.

Table 2. Correlation analysis of the patients' characteristics

Correlated variables	Correlation method	Coefficient	Statistical significance
Age / Weigh loss	Pearson	p = 0.0899 r = 0.3998	No
Age / Symptoms duration	Pearson	p = 0.4803 r = -0.1724	No
Age / Postoperative complications	Spearman (rang correlation)	p = 1.0000 r = 0.0000	No
Weigh loss / Postoperative complications	Spearman (rang correlation)	p = 1.0000 r = 0.0000	No
Symptoms duration / Postoperative complications	Spearman (rang correlation)	p = 0.4617 r = 0.1740	No
Previous surgery / Postoperative complications	Fisher's exact (for independent dichotomized variables)	p = 0,5459	No
Previous dilatations / Postoperative complications	Fisher's exact (for independent dichotomized variables)	p = 1.000	No

Annex 1. Main characteristics for the observed 19 patients

Patient №	Previous dilatetion №	Gender	Age (years)	Previous surgery procedures (PO)	Duration of symptoms (years)	Weigh reduction (kg)	Date of surgery	Duration of surgery (min)	Anesthesia	Hospital stay (days)	Post-operative therapy	Postoperative complications
1	0	F	63	Gastromyotomy	1	40	18.5.2010	90	General	5	PPIs	No
2	1	F	49	Osteomielitis	3	10	25.3.2009	95	General	8	PPIs	No
3	3	M	73	Hernia	1	7	08.9.2008	120	General	13	PPIs	No
4	0	F	55	Cholecistectomy	2	14	08.3.2008	90	General	9	PPIs	No
5	2	F	49	Ovarial surgery	0.5	5	16.2.2007	105	General	8	PPIs	No
6		M	35	No	0.6	5	18.7.2006	150	General	3	PPIs	24 hours in emergency
7	4	M	51	No	1	6	05.5.2006	150	General	7	PPIs	No
8	1	M	40	Appendectomy	8	8	03.7.2008	145	general	7	PPIs	No
9	3	F	32	Gastromyotomy, Appendectomy	6	4	23.5.2007	100	general	10	PPIs	No
10	0	M	63	Appendectomy	3	7	21.9.2006	105	general	12	PPIs	No
11	1	M	53	Hernia	5	4	29.4.2003	120	general	9	PPIs	Conventional laparoscopic fundoplication
12	0	F	46	No	3	4	13.6.2005	105	general	9	PPIs	No
13	1	M	72	No	1	20	30.5.2008	120	general	10	PPIs	Conventional left thoracotomy
14	0	F	18	No	0.5	6	18.6.2010	105	general	8	PPIs	No
15	0	M	70	No	1	10	01.11.2010	105	general	11	PPIs	No
16	4	F	31	Appendectomy	1	5	01.4.2006	115	general	9	PPIs	No
17	3	F	55	Nephrolytiasis	1	30	18.1.2007	120	general	11	PPIs	No
18	0	F	44	Leg amputation	10	5	04.11.2009	120	general	10	PPIs	No
19	0	F	75	Cholecystectomy	0.25	11	22.10.2010	120	general	11	PPIs	No

References

1. *Saxena S, Jain P, Shukla J. Preparation of two component Fibrin Sealant and its clinical evaluation in skin grafts and flaps. Indian J Plast Surg 2003; 36: 14-7*
2. *Berg PL, Barina W, Born P. Endoscopic injection of fibrin sealant versus policdocanol in peptic ulcer hemorrhage: a pilot study. Endscopy 1994; 6(26); 528-53*
3. *Harvey SC. The use of fibrin paper and forms in surgery. Boston Med Surg J 1916; 174: 658-659*
4. *Young J., Medawar P., Fibrin suture of peripheral nerves. Lancet 1940; 11: 126-129*
5. *Gaunthier L. et al. Use of fibrin sealant Tussicol for treating perforated or preperforated comeal ulcer. J Fr Ophtalmol 1986; 7(12): 469-747*
6. *Sentovic S.M. Fibrin sealant for all anal fistulas. Gastrointest Surg 2001; 2(5): 158 – 161*
7. *Holcomb J.B., Pusateri A.E., Hess J.R. et al., Implications of new dry fibrin sealant technology for trauma surgery. Surg Clin North Am 1997; 77: 943-952*
8. *Knighton D.R., Thoms K.H. Thakral K.K. et al., Role of plates and fibrin in heal in sequence. Ann Surg 1982; 4(196): 379-388*
9. *Buckley RC, Breazeale EE, Edwand JA, Brzezienski MA. A simple preparation of autologous fibrin sealant for skin-graft fixation. Plast Reconstr Surg 1999; 103: 202-6.*
10. *Saltz R, Sierra D, Feldman D, Marcia, Dimick Alan, et al. Experimental and clinical application of fibrin sealant. Plast Reconstr Surg 1991; 88: 1005*
11. *Stechison MT. Rapid polymerizing fibrin sealant from autologous or single donor blood : Preparation and indications. J Neurosurg 1992; 76: 626*
12. *Toma AG, Fisher Eward Cheesman AD. Autologous fibrin sealant in the repair of dural defects in craniofacial resection. J Laryngol Otol 1992; 106: 356.*
13. *Stuart JD, Morgan RF, Kennev JG. Single donor fibrin sealant for hand burns. Ann Plast Surg 1990; 524.*
14. *Mouritzen C, Doromer M, Keinecke HO. The effect of fibrin sealanting to seal bronchial and alveolar leakages after pulmonary resections and decortications. Eur J Cardiothorac Surg 1993; 7: 75*
15. *Nissen AJ, Johnson AJ, Parkins RC et al. Fibrin sealant in otology and neurotology. Am J Otol 1993; 14: 147*
16. *Mandel MA. Closure of blepharoplasty incisions with autologous fibrin sealant. Arch Ophthalmol 1990; 108: 842.*
17. *Kjaergard HK, Weis-Fogh, et al. A simple method of preparation of autologous fibrin sealant by means of ethanol. Surg Gynecol Obstet 1992; 175: 72.*
18. *Canonico S. The use of Human Fibrin Sealant in the surgical operations. ACTA BIO MEDICA 2003; 74, Suppl. 2: 21-25*
19. *Cirocchi R., E. Farinella, F. La Mura, L. Cattorini, B. Rossetti, et al. Fibrin sealant in the treatment of anal fistula: a systematic review Annals of Surgical Innovation and Research 2009, 3:12 doi:10.1186/1750-1164-3-12*
20. *Gorler H, Oppelt P, Abel U, Haverich A. Safety of the use of TissucolW Duo S in cardiovascular surgery: retrospective analysis of 2149 patients after coronary artery bypass grafting. Eur J Cardiothorac Surg 2007; 32: 560—6*
21. *Lamm P, Adelhard K, Juchem G, Weitkunat R, Milz S, Kilger E, Gotz A, Reichart B. Fibrin sealant in CABG operations: casting out the Devil with Beelzebub? Eur J Cardiothorac Surg 2007; 32: 567-72*
22. *Guéhennec L. Le, P. Layrolle, G. Daculsi. A review of bioceramics and fibrin sealant. European cells and materials 2004, 8: 1-11*
23. *Midha A., S. Srivastava, M. Sharma, P. Jain. Fibrin Sealant - A Safe, Effective, Patient and Surgeon Friendly Technique for Attaching Limbal Conjunctival Autografts In Comparison To Conventional Suturing- A Prospective Study. AIOC 2008 PROCEEDINGS: 221-223*
24. *Evans A. L., A. F. Morey. Current Applications of Fibrin Sealant in Urologic Surgery. International Braz J Urol 2006; 32(2): 131-141*
25. *Solt J, Boros S, Zoltán I, Horváth OP, Andics L, Bajor J. Malignant esophageal-respiratory fistula and esophageal stenosis treated with a Gianturco-Z-stent. Orv Hetil. 1998; 139 (41): 2447-53*
26. *Sopotensky St., Al. Cervenjakov. Adapted surgical thoracoscopic Heller's myotomy in the treatment of achalasia . Acta Medica Bulgarica, 2012; 1: 47-55*
27. *Short Product Characteristic of Tissucol Kit, Two component fibrin sealant. www.bda.bg*
28. *Fatin R., Polat, Sabriye Polat. The relationship between grade's of the gastroesophagial reflux disease and hiatal hernia. HealthMed 2012; 6(7): 2268 – 2270*

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Tpeak-end interval /QT interval ratio of ECG maybe predict sudden cardiac death and malignant Ventricular arrhythmias in coronary heart disease

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Abstract

Background: Recent studies suggest that Tpeak-end interval /QT interval (Tp-e/QT) may be more meaningful to predict malignant arrhythmias. To evaluate its value of forecasting sudden cardiac death, we retrospectively analyzed the Tp-e/QT change in ECG before sudden cardiac death.

Methods: Collected complete information of 28 patients in our hospital with sudden cardiac death or malignant arrhythmias and 68 healthy adult individuals, Tp-e interval, QT interval and Tp-e/QT ratio in 12-lead ECG before their sudden cardiac death occurred were measured and analyzed.

Results: The average of Tp-e/QT ratio were 0.21 ± 0.03 in the all healthy adult individuals, less than the patients whose Tp-e/QT ratio were 0.22 ± 0.09 ($p<0.01$); healthy individuals with Tp-e/QT ratio <0.21 were significantly higher than the patients ($p<0.01$, 83.82% vs. 32.14%), and the patients with Tp-e/QT ratio ≥ 0.21 were also observably higher than the healthy adult individuals ($p<0.01$, 67.86% vs.16.18%). 17 patients (17/28, 60.71%) with coronary heart disease had Tp-e/QT ratio ≥ 0.21.

Conclusion: Tp-e/QT ratio can be as an index in predicting sudden cardiac death.

Key Words: T peak-end interval (Tp-e), QT interval (QT), Tp-e/QT Ratio, sudden cardiac death (SCD), Malignent Ventricular Arrhythmia (MVA).

Introduction

Definition of sudden cardiac death (SCD) is unexpected death occurred cardiac causes within 1h. 70%~80% of SCD were caused by the fast Ventricular arrhythmia (such as ventricular tachycardia, ventricular flutter and ventricular fibrillation), 20% caused by the slow arrhythmia (such as severe atrioventricular conduction block, sinus bradycardia, sinus arrest, etc.). Most of ventricular tachycardia or ventricular fibrillation are comprehensive pathophysiology results due to coronary vascular events, myocardial injury, myocardial metabolism and (or) changes in the autonomic nervous tension. Reasons of tachyarrhythmia mainly included triggered ventricular arrhythmia, reduced heart rate variability, QT interval abnormalities, cardiac shock, T-wave alternant and ventricular late potentials and so on.

Tp-e refers to the interval from the peak to the end of the T wave. In recent years, studies have shown that Tp-e can be used as the quantitative indicators reflecting ventricular transmural dispersion of repolarization (TDR) [1-3]. Ventricular TDR increases are the main mechanism of 2 Phase reintrary of ventricular arrhythmia (ventricular tachycardia, ventricular fibrillation). Prasad G[4] recent studies suggest that Tp-e/QT may be more meaningful to predict malignant arrhythmias. To evaluate its value of forecasting sudden cardiac death, we retrospectively analyzed the Tp-e/QT change in ECG before sudden cardiac death.

Materials and methods

General Information

The 28 cases with sudden cardiac death due to ventricular tachycardia or ventricular fibrillation (21 males, 7 females, aged 23-85 years, mean age 66 ± 9 years old) had been reviewed from March 2004 to March 2009 in our hospital, Including coronary heart disease (CHD) 17 cases, dilated cardiomyopathy 3 cases, hypertension 3 cases, sick sinus syndrome (SSS) 2 cases, diabetic nephropathy 1 case, kidney transplantation 1 case and long

QT syndrome 1 case. This case has died 20 cases (71.4%). See Table 1. Healthy adult individuals include 25 males, 5 females, aged 25-83 years, mean age 67 ± 5 years old.

Methods

Synchronous 12-lead ECG (machine from the Japanese optoelectronics of model ECG9130P) has been recorded with speed of 25mm/s, Tp-e intervals of each figure was measured by the three trained staff members back to back (see Fig. 1). Lead V3 were selected as the measurement of lead. Each lead was measured three QRS-T complex wave, and noted the average results.

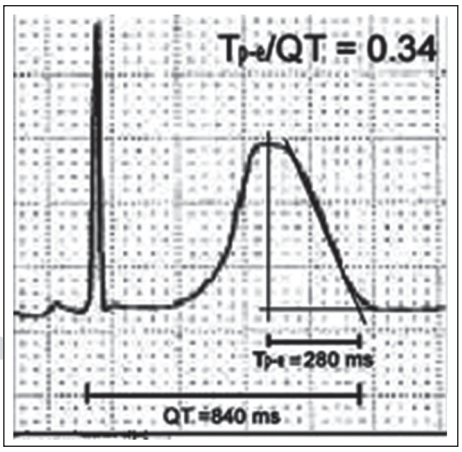

Figure 1. Tp-e interval measurements

Table 1. General information of the patients

No.	Sex	Age	Diagnosis	Prognosis
1	M	85	CHD, OMI(anterior)	death
2	M	65	Diabetic nephropathy	death
3	M	74	CHD, Angina, Alzheimer's disease	death
4	M	80	Hypertension	death
5	M	73	CHD, AMI(anterior)	death
6	M	69	CHD, OMI(Inferior), 2 diabetes mellitus	death
7	M	30	Kidney transplantation	death
8	M	48	CHD, Angina	death
9	M	64	CHD,OMI(anterior)	death
10	M	83	CHD, OMI(Inferior)	death
11	M	72	CHD, AMI(anterior)	death
12	F	70	CHD, Angina, 2 diabetes mellitus	death
13	M	47	CHD, AMI(non-ST elevation)	death
14	F	47	CHD, AMI(anterior), 2 diabetes mellitus	death
15	M	72	Hypertension, Alzheimer's disease	death
16	F	70	CHD, OMI(Inferior and anterior)	death
17	F	72	CHD, Angina	death
18	M	65	CHD, Angina	death
19	M	73	SSS	living
20	M	53	Hypertension	living
21	M	66	CHD, AMI(Inferior)	living
22	F	69	SSS	death
23	M	69	CHD, Angina	death
24	F	68	Dilated cardiomyopathy	living
25	F	61	Dilated cardiomyopathy	living
26	M	71	Dilated cardiomyopathy	living
27	M	39	CHD, AMI(Inferior)	living
28	M	23	LQT syndrome	living

CHD=coronary heart disease; AMI=acute myocardial infarction; OMI=old myocardial infarction; SSS=sick sinus syndrome; LQT=long QT interval.

Statistical Analysis

Data of all patients are entered into the computer, using SPSS17.0 for statistical analysis.

Results

Tp-e interval, QT interval and Tp-e/QT in ECG measurement results of all patients: (see Table 2)

Tp-e interval, QT interval and Tp-e/QT in ECG measurement results of healthy individuals and the patients: In 68 healthy individuals, the average of Tp-e/QT of all healthy adult individuals were 0.21 ± 0.03, with 0.21 as the boundary, Tp-e/QT≥ 0.21 were 11 individuals (16.18%), Tp-e/QT<0.21 were 60 individuals (83.82%). The average of Tp-e/QT of all patients were 0.22 ± 0.09, with 0.21 as the boundary, Tp-e/QT≥ 0.21 were 19 cases (67.86%), Tp-e/QT<0.21 were 9 cases (32.14%). see Table 3.

Discussion

Yan GX et al[5] found that there were potential gradient between two-phase of three layers of myocardial cell action potential, M cells had the maximum potential of the platform, so the potential difference form the opposite direction among middle of the M cells, subendocardial and subepicardial ventricular muscle. Among 3 layers of myocardial cells, the action potential duration of M cells was longest, action potential duration of the epicardium (Epi) was shortest, action potential duration of endocardial (Endo) ranged between M cells and Epi. So the order of ventricular depolarization from Endo to Epi and repolarization from Epi to Endo had become the order of ventricular depolarization from Endo to M sells to Epi and repolarization from Epi to Endo to M sells.

The studies of cardiac electrophysiological characteristics suggest that QT interval is mainly reflecting the excitement time of M cells (including time of depolarization and repolarization), Tp-e interval reflects the size of Transmural dispersion which caused by inconsistency myocardial repolarization of 3 layers of myocardial cells[6]. Therefore, Tp-e/QT ratio will reflect the size of the transmural dispersion time in the M-cell activation time. Based on electrophysiological theory, If the ratio is large, the ventricular potential difference formed among platform potential of M cells, subendocardial and subepicardial ventricular muscle will increase, and it is possible to form two-phase reentry to produce ventricular fibrillation.

Table 2. Measurement results of the patients

No	QT (s)	Tp-e (s)	Tp-e/QT	No	QT (s)	Tp-e (s)	Tp-e/QT
1	0.32	0.08	0.25	15	0.40	0.09	0.225
2	0.35	0.08	0.23	16	0.44	0.12	0.27
3	0.36	0.09	0.25	17	0.30	0.04	0.133
4	0.40	0.08	0.20	18	0.34	0.05	0.14
5	0.39	0.08	0.21	19	0.31	0.04	0.129
6	0.40	0.09	0.225	20	0.40	0.12	0.30
7	0.31	0.05	0.16	21	0.44	0.12	0.27
8	0.40	0.08	0.20	22	0.44	0.11	0.25
9	0.36	0.08	0.222	23	0.40	0.10	0.25
10	0.44	0.12	0.27	24	0.50	0.10	0.20
11	0.39	0.05	0.128	25	0.48	0.12	0.25
12	0.36	0.08	0.22	26	0.44	0.12	0.27
13	0.32	0.04	0.125	27	0.44	0.12	0.27
14	0.42	0.05	0.119	28	0.44	0.12	0.27

Table 3. Comparison of the measurement results of healthy adult individuals and the patients

Group	N	Tp-e/QT(s)	Tp-e/QT≥ 0.21(%)	Tp-e/QT<0.21(%)
Control group	68	0.21 ± 0.03	16.18	83.82#
Patient group	28	0.22 ± 0.09*	67.86*	32.14

**p<0.01 vs. healthy adult individuals; #p<0.01 vs. the patients*

Our study demonstrated that the average of Tp-e/QT ratio were 0.21 ± 0.03 in the all healthy adult individuals, less than the patients whose Tp-e/QT ratio were 0.22 ± 0.09 ($p<0.01$); healthy individuals with Tp-e/QT ratio <0.21 were significantly higher than the patients ($p<0.01$, 83.82% vs. 32.14%), and the patients with Tp-e/QT ratio ≥ 0.21 were also observably higher than the healthy adult individuals ($p<0.01$, 67.86% vs.16.18%). We also fund that 17 patients (17/28, 60.71%) with coronary heart disease had Tp-e/QT ratio ≥ 0.21. This result descript that Tp-e/QT ratio may be a new indicator in predicting sudden cardiac death, especially in the patients with coronary heart disease. Next a large number of clinical studies need to prove it because of limited number of cases observed in this paper.

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References

1. *Zipes DP, Wellens HJ. Sudden cardiac death. Circulation, 1998; 98: 2334-2339.*
2. *Zipes DP, Camm AJ, Borggrefe M, et al. ACC / AHA / ESC 2006 Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: a report of the ACC/AHA Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. Circulation, 2006; 114: e385.*
3. *Antzelevitch C. T peak-Tend interval as an index of transmural dispersion of repolarization. Eur J Clin Invest, 2001; 31: 555-559*
4. *Prasad G, Chinmay P, Harsh P. Tp-e/QT ratio as an index of arrhythmogenesis Journal of Electrocardiology. 2008, (41): 567-574.*
5. *Yan GX, Antzelevitch C. Cellular basis for the normal T wave and the electrocardiographic manifestations of the long - QT syndrome. Circulation, 1998, 98: 1928 - 1936.*
6. *Cheng Hongyong, Zhang Zheng. Tp-e in Electrocardiogram and its clinical application.Journal of Clinical Electrocardiology, 2006; 15(3): 208-210.*

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Impact of spinal anesthesia on accurrence of postoperative hematomas after hernioplasty according to Lichtenstein

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Abstract

Introduction: Hernia repair is one of the most frequent surgeries in general surgery, and it appertains to the most frequent surgeries in general. According to the surgical technique, hernia surgeries can be classified as conventional and laparoscopic, and conventional surgeries can be 'tension' and tension-free ones. The present-day trend, which started some twenty years ago, is that the most frequent surgical technique in hernia repairs in adults is the one according to Lichtenstein, which is the golden standard in all the international good practice guides for hernioplasty (4).

Objective of the paper: To determine the incidence of postoperative hematomas after hernioplasty according to Lichtenstein in relation to the type of the anesthesia used and in relation to the age of patients, in the groups of over 65 and under 65 years of age, in the Medical System Belgrade in the period from 01/01/2003 to 31/12/2012.

Materials and methods: The study is of retrospective-prospective character and covers 1460 patients operated on for inguinal hernia in the Medical System Belgrade. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012 and, as retrospective, in the period from 01/01/2003 to 31/12/2007. In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covers patients operated on for inguinal hernia, either direct or indirect, while the data on femoral and incisional ones were not processed. The data from case histories were used and, in patients who were operated on in the prospective part of the study, in addition to case histories, questionnaires were used, which were filled in by patients at admission. Patients were classified according to the localization of hernias**,** according to the type of the anesthesia used, and according to the age, whereby they were divided into two groups: of over 65 and under 65 years of age.

Results: Out of the total number of those operated on (1420), 696 (49%) had suffered from inguinal hernias and 724 (51%) patients from inguinoscrotal hernias. Out of 1420 patients, 80% (1136) were operated on under spinal anesthesia, 12% (170) under general, and 8% (113) under local anesthesia. In the study period, there were 839 (59.03%) patients operated on in the group of over 65 years of age with past medical history, out of which number, 596 (71%) of them had suffered from inguinoscrotal hernias and 243 (29%) from inguinal ones. A statistically significant difference between the type of anesthesia applied and development of postoperative hematomas was not established. Considerably higher is the percentage of postoperative hematomas present in the group of patients over 65 years old (13.84%) in relation to the other group, where there were 8 % of them. If a patient is in the group of over 65 years of age and suffers from an inguinoscrotal hernia, the incidence of postoperative hematomas is considerably higher than in patients in the same group suffering from inguinal hernias, same as it is higher in relation to the group of patients under 65 years of age.

Conclusions: A statistically significant correlation between the type of anesthesia used and the occurrence of postoperative hematomas was not established. A considerably higher percentage of postoperative hematomas appeared in the group of patients of over 65 years of age with past medical history, and the incidence is particularly pronounced in patients in this group suffering from inguinoscrotal hernias.

Key words: Hernioplasty according to Lichtenstein, spinal anesthesia, postoperative hematoma.

Introduction

A rupture (Hernia) designates a bulging of tissue, most often intestines or fats under the skin through a natural or acquired opening in the abdominal wall. It occurs due to congenital weaknesses of soft-tissue structures or develops in the course of one's lifetime. It is usually located in the groin, around the navel or at the place of a former surgical intervention. The location determines the name of a hernia (1).

Inguinal hernia constitutes 75% of all hernias in the abdominal wall and it is around 25 times more frequent in men than in women. Those hernias are divided into two separate groups, direct and indirect ones. Both are located in the groin but have different origins. Both types of hernias look similarly as a bulge in the groin, and sometimes it is difficult to distinguish what form is in question. An indirect inguinal hernia develops due to an innate weakness of the internal inguinal ring and may develop any time in one's lifetime. They are discovered immediately after birth in around 1% of boys and they are connected with the migration of testicles into the scrotum. An inguinal hernia is located in the lower parts of the abdomen, specifically above the groin close to the pubic region. A direct inguinal hernia is the consequence of weakness of the posterior wall of the inguinal canal and generally develops in older patients because, with the ageing, the anterior abdominal wall grows weaker. At that spot, the abdominal wall is naturally thin. It seldom descends into the scrotum (1).

Symptoms of an inguinal hernia differ among patients. Patients do not have to have any symptoms and may, on the other hand, have polymorphic difficulties in the form of discomforts and pain in the groin, prolapse of the abdominal contents into the region under the skin in the groin or of descend of the contents into the scrotum. Sometimes patients feel difficulties only when their hernia gets jammed and sometimes an inexperienced surgeon opens the abdomen because of the pain not having seen that the patient has a strangulated inguinal hernia. When strangulation takes place, the patient experiences pain in the groin or in the abdomen, has nausea, sickness, and very often even vomits. Sometimes a patient notices a swelling up in the groin and sometimes not. At any rate, strangulation is a serious condition that can be life-threatening to a patient (1).

Hernias are diseases that can only be treated by surgery. There are two types of surgeries: conventional and laparoscopic ones. Conventional surgeries may be 'tension' and tension-free ones. Tension-free surgeries started being performed with the introduction of prosthetic materials in the surgery of hernias. The actual term 'tension-free' means that, with the aid of meshes, the tension in the wound is reduced by which we achieve that, postoperatively, there is less pain, that a patient very early, i.e. after several hours, can be mobilized, and that the percentage of relapses is drastically lower. Today, 'tension' techniques constitute a lower percentage than in the past. They are performed on children and younger people and are more often performed by older surgeons and those who are skeptical about novelties. Out of the 'tension' techniques, the ones most frequently performed are Bassini, Halsted, Shouldice, Ferrari, etc. It should be stressed that, in all the international good practice guides, Lichtenstein one (2) is proposed as the technique of choice for hernia repairs.

Lichtenstein technique is performed in such a way that the inguinal canal is opened in the usual way and funiculolysis is performed during which the hernia sac is prepared if an indirect hernia is in question. Thereafter the prepared hernia sac is resected and ligated. On the other hand, if a direct hernia is in question, the posterior wall plasty is performed by connecting torn ends of the transversus abdominis and internus abdominis muscles. After that, a mesh is placed in such a way that it is stitched along ligamentum inguinale Pouparti with continuous sutures (nylon 20). Contrary to the conventional wisdom, the first suture should never be placed on tuberculum pubicum as in 'tension' techniques but on tendo conjuctivus instead, whereby we avoid postoperative pains that used to be frequent in the past. After the suture on Pouparti, a slit on the mesh is made, which serves to place funiculus spermaticus into it. On its extreme part, it should have a circular extension because it is necessary to avoid squeezing of the funiculus, which could postoperatively result in a hydrocele or a severe atrophy of testicles.

The subsequent sutures that are placed are individual ones. They are made using the same suture

material (nylon 20) and are placed on musculus internus abdominis whereby the mesh is practically adhered to that muscle. Please note that it is not necessary to place the mesh under a great tension or for sutures to be very deep as it was done in the old days. Naturally, sutures are of mattress type so that the mesh does not fold, i.e. wrinkle. The last suture is placed so that the two tails of the mesh that encircle the funiculus are connected. Thereafter it is necessary to check the size of the space around the funiculus before suturing fascia, i.e. aponeurosis, externus abdominis, and thereafter of subdermis and skin (3).

Objective of the paper

Several objectives were set in this paper. Objectives of this paper are to:

- Determine the incidence of postoperative hematomas after hernioplasty according to Lichtenstein in relation to the type of anesthesia used, and
- Determine the incidence of postoperative hematomas after hernioplasty according to Lichtenstein in relation to the age of the patients, in the groups of over 65 and under 65 years of age, in the Medical Center Belgrade in the period from 01/01/2003 to 31/12/2012.

Materials and methods

The study is of retrospective-prospective character, covering 1420 patients operated on for inguinal hernia in the Medical System Belgrade. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012, and as retrospective in the period from 01/01/2003 to 31/12/2007.

In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covered patients operated on for inguinal hernia, either direct or indirect, while the data on femoral and incisional ones were not processed.

The data were used from case histories and, in patients who were operated on in the prospective part of the study, in addition to case histories, questionnaires were used, which were filled in by the patients at admission.

Patients were classified according to the localization of hernias, according to the type of anesthesia used, and according to their age, whereby they were divided into two groups: of over 65 and under 65 years of age. The data were processed applying modern methods of descriptive and analytical statistics. The results are shown in tabular form and graphically.

The questionnaire, which was drawn up for the purpose of the prospective part of the study:

Name and family name .....
.....
Place and year of birth .....
.....
Have you had any diseases and which ones?.....
.....
.....
.....
Do you suffer from diabetes (if yes, since when)?
.....
Do you suffer from asthma (if yes, since when)?....
.....
Have you undergone any surgeries (which ones and when)?.....
Since when have you had hernia? .....
.....
How did it develop (suddenly or gradually)? .....
.....
Has it been jammed and how many times? .....
.....
Can the hernia you have got be reduced or it is always present without the possibility of reduction?
.....
.....
Is there anyone with a hernia in the family? .....
.....
Have you been operated on for hernia before? .....
.....
When were you operated on and were you operated on on the same side and have you hernia now?
.....
.....
Who was the surgeon that operated on you? .....
.....
Roughly, how old was your surgeon? .....
.....
Did you have an infection or suggillation in the wound then? .....
On what postoperative day did you leave the hospital? .....

Results

Out of the total number of surgeries, 4380, surgeries on hernias were performed on 1560 patients, which accounts for 35.6% of the cases. Applying the method of chi-squared test for pair-matched samples, we tested to see whether the incidence of hernia is significant in the total number of those operated on.

Table 1. Number of those operated on in the 2003-2012 period

Type of surgery	Incidence	%
Hernia surgeries	1560	35.6
Other surgeries	2820	64.4
Total surgeries	4380	100

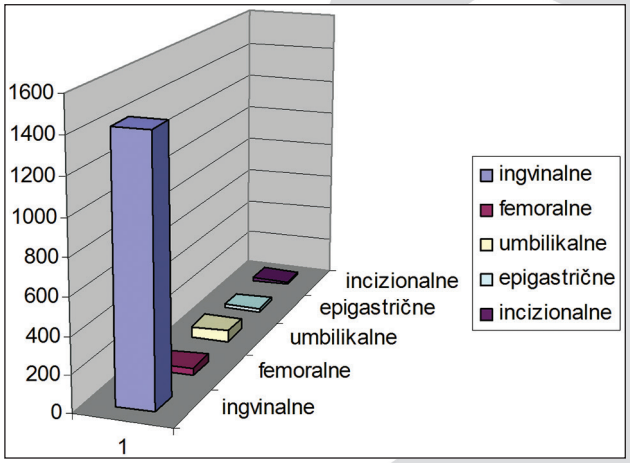

Diagram 1. Number of those operated on for hernias by the localization of hernias in the 2003-2012 period

In the 2003-2005 period, 452 patients were those operated on and, in surgical management of inguinal hernias, 'tension' and tension-free techniques were almost equally applied. In that period, 'tension' surgical techniques constituted 46%, specifically most frequently Halsted and Bassini ones, and tension-free accounted for 54%, specifically most frequently Lichtenstein.

Table 2. Share of surgical techniques in inguinal hernias in the 2003-2006 period

Type of surgical technique	Number of those operated on	%
'Tension' techniques	244	46
Tension-free techniques	208	54
Total surgeries	452	100

In the 2006-2012 period, 968 patients were operated on and, in relation to type of surgical technique, statistically highly significant were surgeries of tension-free type. They constitute 95% of surgical techniques involving inguinal hernias (ANOVA for proportions $F= 8.786$; $p < 0.01$). Within this group, Lichtenstein surgical technique ($F = 4.245$; $p< 0.05$) statistically significantly stands out.

Table 3. Share of surgical techniques in inguinal hernias in the 2006-2012 period

Type of surgical technique	Number of those operated on	%
Tension-free techniques	920	95
'Tension' techniques	48	5
Total surgeries	968	100

In 80% (1136) patients suffering from hernia, spinal anesthesia was applied, in 12% (170), general and, in 8% (113), local anesthesia.

Table 4. Share by the type of anesthesia applied in surgeries of inguinal hernias

Total number of hernias operated on	Spinal anesthesia	General anesthesia	Local anesthesia
1420	1136 (80%)	170 (12%)	113 (8%)

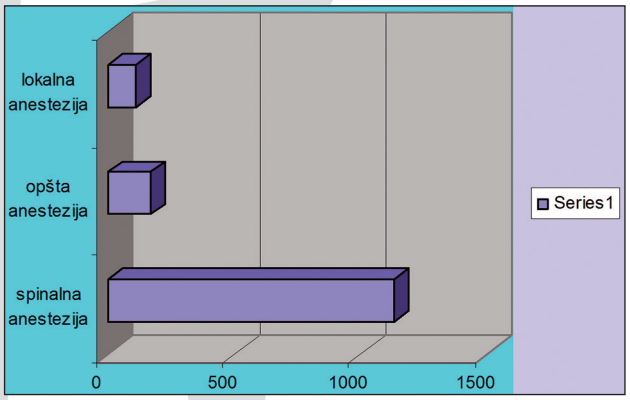

Diagram 2. Share by the type of anesthesia applied in surgeries of inguinal hernias

In the study period, there were 839 (59.03%) patients operated on in the group of over 65 years of age with past medical history, and 581 (40.97) of those under 65 years of age.

Table 5. Share of patients operated on for inguinal hernia by age

Total number of patients	Over 65 years of age with past medical history	Under 65 years of age
1420	839 (59.03%)	581 (40.97%)

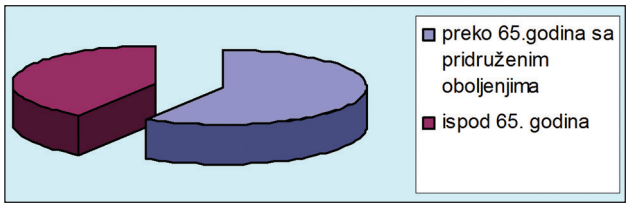

Diagram 3. Share of patients operated on for inguinal hernia by age

Among the studied sample, 696 (49%) patients had got inguinal and 724 (51%) had got inguinoscrotal hernias.

Table 6. Share of patients by the type of inguinal hernia

Total number of patients	Inguinal hernia	Inguinoscrotal hernia
1420	696 (49%)	724 (51%)

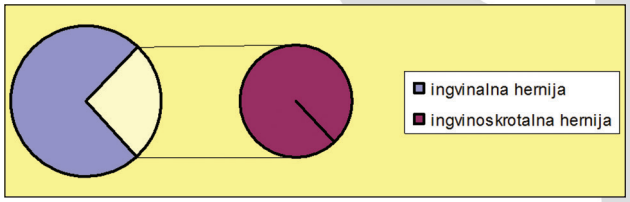

Diagram 4. Share of patients by the type of inguinal hernia

A statistically significant correlation between the type of anesthesia used and development of postoperative hematomas was not established.

Table 7. Share of postoperative hematomas in relation to the type of anesthesia

Type of anesthesia	Number of patients	Number of postoperative hematomas
Spinal	1136	105 (8.46%)
General	170	14 (7.60%)
Local	113	9 (7.37%)

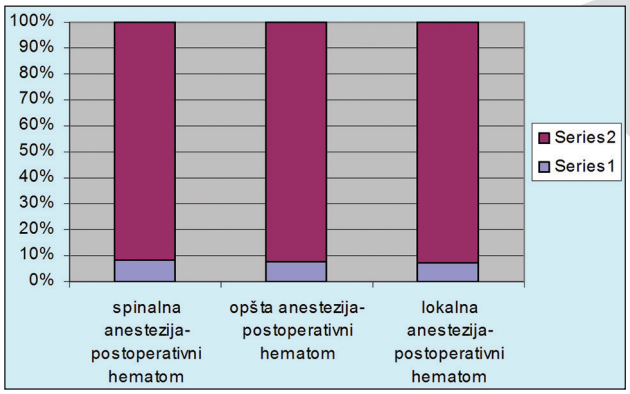

Diagram 5. Share of postoperative hematomas in relation to the type of anesthesia

In the group of patients over 65 years old with past medical history, there was a higher percentage of postoperative hematomas than in the group of patients under 65 years of age.

Table 8. Share of postoperative hematomas in the groups of patients over and under 65 years of age

Group of patients	>65 years of age with past medical history	< 65 years of age
Total number	839	581
Number of hematomas	134 (13.84%)	51 (8%)

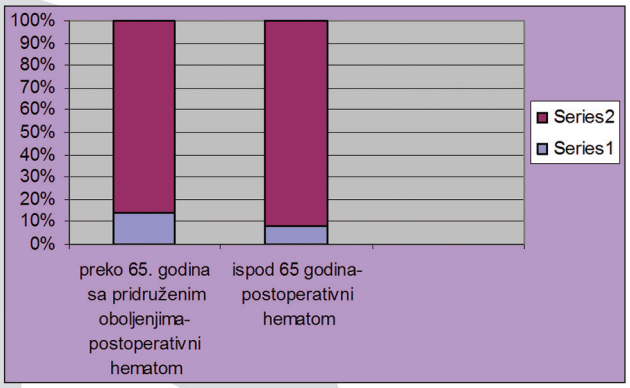

Diagram 6. Share of postoperative hematomas in the group of patients over and under 65 years of age

Table 9. Share of patients by the type of inguinal hernias in the group of over 65 years of age

Total number of patients	Inguinal hernia	Inguinoscrotal hernia
839	596 (71%)	243 (29%)

In the group of patients over 65 years old with past medical history and inguinoscrotal hernias, there is a high percentage of postoperative hematomas, 30.8%, which is statistically significant in relation to all the other groups.

Table 10. Share of postoperative hematomas in the group of patients over 65 years of age suffering from inguinoscrotal hernias and in the group under 65 years of age

	Group > 65, suffering from inguinoscrotal hernias	Group < 65 years of age suffering from inguinoscrotal hernias
Number of patients	243	581
Number of postoperative hematomas	75 (24%)	51 (8 %)

Table 11. Share of postoperative hematomas in the group of over 65 years of age suffering from inguinal and inguinoscrotal hernias

	Group of over 65 years old - inguinoscrotal hernias	Group over 65 years old - inguinal hernias
Number of patients	243	596
Number of postoperative hematomas	75 (24%)	59 (9%)

There is a statistically significant difference in the percentage of development of postoperative hematomas in patients in the group over 65 years old suffering from inguinal and inguinoscrotal hernias.

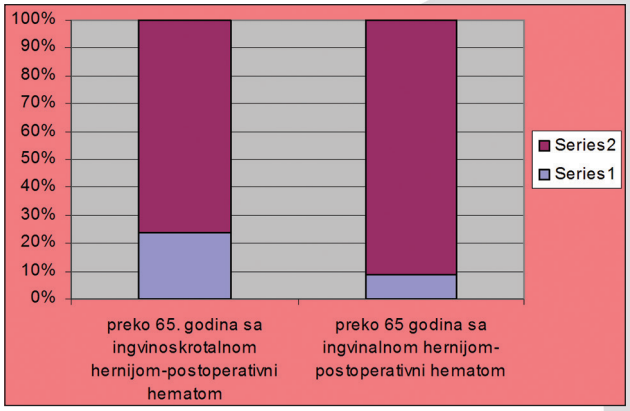

Diagram 7. Share of postoperative hematomas in the group of over 65 years of age suffering from inguinal and inguinoscrotal hernias

Discussion

Within our study, 1420 patients were operated on due to inguinal hernias, out of which number, 696 (49%) of them had suffered from inguinal hernias and 724 (51%) from inguinoscrotal ones. In relation to the total number of those operated on in the study period, it is a significant percentage and points to the fact that repair of hernias is one of the most frequent surgeries in surgery, which corresponds to the data from literature (3).

Although surgeons, at their professional meetings, constantly point out that spinal anesthesia increases the risk of development of postoperative hematomas, particularly due to the hypotensive side-effect it causes, we, in our study, when comparing the two groups of patients, specifically the one suffering from inguinal hernias of over 65 years of age and with past medical history and the group under 65 years of age, did not get a statistically significant difference in the incidences of postoperative hematomas in relation to the use of a specific type of anesthesia, spinal, general or local one, which corresponds to the data from literature (6).

A statistically significant difference in the development of postoperative hematomas after hernioplasty according to Lichtenstein was found in the group of patients over 65 years old with past medical history and suffering from inguinoscrotal hernias in relation to the patients from the same group suffering from inguinal hernias, as well as in relation to the group of patients under 65 years of age, which corresponds to the data from literature (2).

Conclusions

Out of the total number of those operated on (1420), 696 (49%) had got inguinal hernias and 724 (51%) patients had got inguinoscrotal ones.

Out of 1420 patients, 80% (1136) were operated on under spinal anesthesia, 12% (170) under general, and 8% (113) under local anesthesia.

Within the study period, there were 839 (59.03%) patients operated on in the group of over 65 years of age with past medical history, out of which number, 596 (71%) of them had got inguinoscrotal hernias and 243 (29%) inguinal ones.

A statistically significant difference between the type of anesthesia used and the development of postoperative hematomas was not established.

A considerably higher percentage of postoperative hematomas is present in the group of patients over 65 years old (13.84%) in relation to the other group, where there were 8 % of them.

If a patient is in the group of over 65 years of age and suffers from inguinoscrotal hernia, the incidence of postoperative hematomas is considerably higher than in the patients in the same group suffering from inguinal hernias, same as it is higher in relation to the group of patients under 65 years of age.

References

1. *Amid PK. Hernia, 1999; 3(S12): 47.*

2. *Davidov M, Ognjenović A, Popov D. Uporedna analiza hernioplastike po Lichtensteinu i Gilbertu. (Comparative Analysis of Hernioplasty According to Lichtenstein and Gilbert). Medicina danas. 2009; 8(1-3), 35-38.*

3. *Milić DJ, Pejić MA. Beztenzione procedure u hirurškom lečenju preponskih kila (Tension-free Procedures in Surgical Treatment of Inguinal Hernias). Srpski arhiv za celokupno lekarstvo.2003; 131(1-2): 82-91.*

4. *Dabić D, Cerović S, Azanjac B, Marić B, Kostić I. Prolene Hernia System, Ultrapro Hernia System and 3D Patch Devices in the Treatment of Inguinal, Femoral, Umbilical and Small Incisional Hernias in Outpatient Surgery.*

5. *Jovanović D, Veljković R, Gluhović A, Ivanov D, Protić M. Ambulantna hirurgija (kila) (Outpatient Surgery (Hernia)). Medicina danas.2012; 11(4-6), 113-118.*

6. *Young DV. Comparison of Local, Spinal, and General Anesthesia for Inguinal Herniorrhaphy. Am J Surg, 1987; 153(6): 560-3.*

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Repair of urethrocutaneous fistulas secondary to urethroplasty: Retrospective analysis of 54 patients

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Abstract

Aim: To investigate the correlations between the size, location, and number of urethrocutaneous fistulas (UCF) and the effects of surgical therapy, as well as assess the surgical methods of UCF and compare patient prognosis following different treatment protocols.

Methods: Data were collected on 54 patients who underwent UCF after urethroplasty of hypospadias and other urethral malformations, such as urethral stricture, circumcision, urethral diverticulum, chordee penis, and urinary incontinence, from 2003 to 2011. Patients underwent simple suturing, advancement of skin flap, pedicle penile skin flap urethroplasty or oral mucosa urethroplasty, depending on the size, location and number of UCF and the presence of urethral strictures.

Results: Initial UCF repair was successful in 46 patients (85%), with 4 patients (7.5%) cured after secondary surgery in our hospital and 4 who planned to undergo repair surgery 6 months after discharge. The overall success rates of UCF in the coronary sulcus, the penis, the junction between the penis and scrotum, and the junction between the perineum and scrotum were 100%, 84%, 86%, and 80%, respectively. The success rates of UCF repair were 89% and 67% for single and multiple fistulas, respectively, 89% and 70% for small and large fistulas, respectively, 85% and 100% for first and second surgery, respectively, and 84% and 88% for one and several rounds of urethroplasty, respectively.

Conclusions: Reasonable choices of the surgical methods may decrease the complications and improve the success rates of UCF repair. Interposition of a waterproofing layer may have some benefits in the repair for the UCF.

Key Words: Urethrocutaneous fistula, urethroplasty, operative therapy.

Introduction

Urethrocutaneous fistula (UCF) is one of the most common complications of urethroplasty, especially of the hypospadias with incidence rates ranging from 4% to 25% [1]. UCF have been associated with long-term local infection, urethral distal obstruction, paraurethral unhealthy tissue, ischemic skin flap, large wound closure tension, and poor drainage after surgery [2]. The most common locations of UCF are anastomoses of urethral surgery and the coronary sulcus. The recurrence of UCF may be reduced by better experience with urethroplasty and skin flap (which means long-years of surgical clinical experience, some basis of plastic surgery, as well as a large number of urethrocutaneous fistula repair success cases), and the improvements in suture materials. We assessed the repair of UCF in 54 patients using simple suturing, advancement of skin flap, pedicle penile skin flap urethroplasty , and oral mucosa urethroplasty. Additionally, we assessed the relationships between surgical method and the size, location and number of UCF and whether urethral stricture was present.

Materials and Methods

Patient details

We assessed 54 male patients who underwent repair surgery of UCF in our in-patient department following urethroplasty between 2003 and 2011. There is no patients who have lichen sclerosus or other skin disorders Patients with coexisting causes of UCF, including urinary tract infection within a month, severe hypertension, diabetes mellitus with poor glycemic control, and coronary heart disease like myocardial infarction, some taboos of these operations, were excluded. The mean age for the patients was 26 years and the mean stay in the in-patient department was 11

days. The average time from previous urethral surgery was 41 months.

Of the 54 patients, 34 underwent UCF repair after hypospadias and 20 after other types of urethroplasty, including urethral stricture, circumcision, urethral diverticulum, chordee penis, and urinary incontinence. Of the 54 patients, 38 patients had undergone urethroplasty once, while the other 16 had undergone urethroplasty at least twice. Six patients had UCF in the coronary sulcus, 31 in the penis, 7 at the junction of the penis and scrotum, and 10 at the junction of the perineum and scrotum, while 44 patients had small (diameter $<$0.5cm) fistulas and 10 had large (diameter $\geq$0.5cm) one. At the time of UCF repair, 45 patients had one fistula and 9 had two or more.

Operative methods

All patients were examined to determine whether a stricture was present in the distal urethra and the number, size and position of fistulas, especially fistulas hidden subcutaneously by urethrography before surgery. We utilized four methods for UCF repair: simple suturing; advancement of skin flap; pedicle penile skin flap urethroplasty; and oral mucosa urethroplasty. Suturing was performed using 6-0 Dexon for in-turn sutures of the fistula on the urethra and 5-0 absorbable sutures for the interrupted suture of the subcutaneous tissue and the skin. All patients required interpositioning of a waterproofing layer. Subcutaneous tissue was used to build the interposition layer of the UCF in the perineum and scrotum, whereas subcutaneous tissue, the fascia of the corpus cavernosum or nearby pedicle penile skin flap was used for the interpositioning of a waterproofing layer of the UCF in the penis.

The 6 patients with UCF in the coronary sulcus were repaired using simple sutures. In the 14 patients with single small (diameter <0.5cm) fistulas in the penis and healthy skin nearby, we used simple suturing to repair the fistula after isolating surrounding skin. In the 17 patients with similar fistulas but unhealthy skin and tissues nearby, we used advancement of skin flap after removing unhealthy tissues.

In the 11 patients with fistulas in the penis and the perineum and scrotum, we first remove the unhealthy surrounding tissue. The skin, subcutaneous tissue and fascia were cut gradually, choosing suitable skin flaps based on the size of fistula. Each skin flap was inverted free-tension with good preservation of the fascia pedicle. Suturing of the fistula should be parallel to the urethra, and the skin flap should be larger than the fistula. We used vertical mattress suture to close the skin. If there was still high tension in the pedicle penile skin flap urethroplasty, we performed relaxation suturing in the dorsal penis. Cystostomy was performed following surgery. The 6 patients with large (diameter $\geq$0.5cm) and complicated fistulas with urethral strictures underwent suitable oral mucosa urethroplasty for UCF repair, with the type of urethroplasty depending on the condition of the surrounding skin of fistula and the length and location of urethral stricture. Vertical mattress suture was used to close the skin, and cystostomy was performed following surgery. The wound was covered with pressure dressing.

Surgery was not performed in any patient with UCF and urinary infection until the infection had been cleared.

Postoperative management

All patients received antibiotics to prevent infections. If necessary, the bladder was flushed through the bladder fistula, and the indwelling catheter was maintained for at least 3 days. Each wound dressing was uncovered on the first day postoperatively and blood crust was removed. Dressing was regularly changed to keep the wound clean and dry.

Results

The success of the surgery is defined as there is no recurrence in six months after the surgery. Initial UCF repair was successful in 46 of the 54 patients (85%), with 4 patients cured after second surgery in our hospital and 4 planning to undergo repair surgery 6 months after discharge. The success rates of repair of UCF in the coronary sulcus, the penis, the junction of the penis and scrotum and the junction of the perineum and scrotum were 100%, 84%, 86%, and 80%, respectively. The success rates of UCF repair were 89% and 67% for single and multiple fistulas, respectively, 89% and 70% for small and large fistulas, respectively, 85% and 100% for first and second surgery, respective-

ly, and 84% and 88% for one and several rounds of urethroplasty, respectively. None of these patients experienced complications such as flap necrosis or postoperative infection that led to poor wound healing, bleeding or urethral stricuures. Postoperative recurrence was observed in only one patient in those 46 successful cases in one year after the surgery, with a recurrence rate of 2%.

Table 1 shows success rates by position of UCF. The highest rate was observed for repair of UCF in the coronary sulcus, with other success rates being somewhat lower. Success rates of UCF repair, however, were not affected by the numbers or sizes of UCF (Table 2) or the history of urethroplasty (Table 3). Simple suturing at initial surgery yielded a higher success rate than other surgical methods (Table 4). At repeat surgery, however, both methods used were completely successful. UCF repair was less successful in patients who underwent UCF for hypospadias than for other kinds of urethroplasty (Table 5).

Discussion

Factors related to the success of UCF repair surgery

Past failed operations can increase penile urethral malformation, reduce the skin flap to be chosen, and damage blood supply. We observed no obvious difference in success rates between the

Table 1. Association between UCF position and repair success rates

Position of UCF	Success/Total	Ratio of position (%)	Success rates (%)
coronary sulcus	6/6	11	100
penis	26/31	57	84
junction of penis and scrotum	6/7	13	86
perineum and scrotum	8/10	19	80
Total	46/54	100	85

Table 2. Associations between UCF number, size and repair success rates

	Success/Total	Success rates (%)
Number of UCF		
One	40/45	89
More than one	6/9	67
UCF size		
Small (<0.5cm)	39/44	89
Large (≥0.5cm)	7/10	70

Table 3. Association between the history of urethroplasty and repair success rates

History of urethroplasty	Success/Total	Success rate (%)
One	32/38	84
More than one	14/16	88

Table 4. Association between surgical method and repair success rates

Stage of Surgery	Surgical methods	Success/Total	Success rate (%)
First Surgery	Simple suturing	19/20	95
	advancement of skin flap	13/17	76
	pedicle penile skin flap urethroplasty	9/11	82
	oral mucosa urethroplasty	5/6	83
Second Surgery	Simple suture	1/1	100
	advancement skin flap	3/3	100

Table 5. Association between UCF etiology and repair success rates

Etiology of UCF	Success/Total	Success rate (%)
hypospadias	27/34	79
other kinds of urethroplasty	19/20	95

patients who underwent UCF repair following one (84%) or several (88%) rounds of urethroplasty. UCF repair in patients who had undergone multiple operations was performed using either pedicle penile skin flap or oral mucosa urethroplasty, yielding good prognosis.

Many factors can affect the prognosis of patients who have undergone UCF repair. The position, size, and number of UCF were found to have little effect on surgical success rates [3]. Although we found that success rates were not influenced by the number and size of UCF, we found that success rates differed for UCF at different sites. The highest success rate was observed for UCF repair in the coronary sulcus, with lower rates observed for UCF in the penis, the junction between the penis and scrotum, and the junction between the perineum and scrotum. Almost all UCF in the coronary sulcus were very small (≤0.2 cm). We were able to remove unhealthy tissues completely and utilize single suturing without tension, reducing the UCF recurrence rate. A possible reason that the distal strictures had the best outcome was because they were less severe patients with better develop blood supply, better tissue, and less scarring. Although the condition of the surrounding skin flap and tissues can affect patient prognosis, this condition cannot be analyzed objectively.

The basic principles of UCF repair include inverting sutures of the fistula, isolating normal tissue completely, using multi-layer free-tension sutures to remain a good blood supply, complete hemostasis during surgery, treatment with antibiotics to prevent infection, and maintaining the distal urethra in an unlocked condition [4]. The success rates of UCF repair after hypospadias were reported to be 77% with single suturing and 90% with all kinds of skin flap urethroplasty [5]. When using skin flaps to repair UCF in our study, we were able to acquire enough suitable subcutaneous tissue with wide pedicles and good blood supply and good transposition to build a waterproofing layer, which improved the success rate. We found that the success rates were 95% using single suturing and 79% using advancement of skin flaps, with the deeply sub-divided into three types, 76% for advancement of skin flaps alone, 82% for pedicle penile skin flap urethroplasty and 83% for oral mucosa urethroplasty. All patients repaired with single suture had small (<0.5 cm) and/or single fistulas, whereas the patients who underwent skin flap urethroplasty had large (≥0.5cm) and/or several complicated fistulas, which are harder to repair and frequently require wound healing after surgery. Since building a subcutaneous waterproofing layer was found to improve the success rate of UCF repair and reduce the recurrence rate [6,7], we used a waterproofing layer interposed between subcutaneous tissue and fascia for UCF repair in all patients. This resulted in successful repair rates and low recurrence rate.

Perioperative management of UCF repair surgery

The optimal time for UCF repair was reported to be 6 months after the last urethroplasty, when the local tissue is healthy and there is no obvious signs of urinary infection [8]. Of our patients, Most of the patients underwent repair surgery 6 months after the last urethroplasty, with good results.

In patients with recurrent UCF, the possibility of distal urethral obstruction or urethral diverticulum should be excluded before repair surgery [9]. Distal urethral obstruction can cause high pressure in the proximal urethra, and urethral diverticulum can result in turbulent flow in the local area, increasing the likelihood of UCF recurrence. Thus, any urethral obstruction should be relieved prior to UCF repair.

Cystostomy after surgery can reduce UCF recurrence in patients with large or multiple fistulas and those who have gone urethroplasty for several times, but may not affect patients with small single fistulas [10, 11]. Cystostomy through the perineum has been found to result in better drainage than traditional cystostomy [12]. We therefore performed cystostomy after surgery in all patients with large and multiple complicated fistulas and those who had undergone urethroplasty several times. We found that drainage was patent and bladder flush was easy, resulting in good effects.

Conclusion

Reasonable choices of the surgical methods may decrease the complications and improve the success rates of UCF repair. Interposition of a waterproofing layer may have some benefits in the repair for the UCF.

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References

1. *Belman AB. The de-epithelialized flap and its influence on hypospadias repair. J Urol. 1994; 152: 2332-4.*
2. *Hinman F Jr. Urological Surgery Maps, Beijing: People Health Press. 1996:27-8.*
3. *Retik AB, Keating M, Mandell J. Complications of hypospadias repair. Urol Clin North Am. 1988; 15: 223-36.*
4. *Duckett JW. Hypospadias. In: Walsh PC, Retik AB, Vaughan ED, et al. Campbell's Urology . 7th ed, Harcourt Asia. WB Saunders, 2001, 2114-5.*
5. *Elbakry A. Management of urethrocutaneous fistula after hypospadias repair: 10 year's experience. BJU Int. 2001; 88: 590-5.*
6. *Srivastava RK, Tandale MS, Panse N, Gupta A, Sahane P. Management of urethrocutaneous fistula after hypospadias surgery – An experience of thirty-five cases. Indian J Plast Surg. 2011; 44: 98-103.*
7. *Handoo YR. Role of tunica vaginalis interposition layer in hypospadias surgery. Indian J Plast Surg. 2006; 39: 152-6.*
8. *Latifoglu O, Yavuzer R, Unal S, Cavuşoğlu T, Atabay K. Surgical treatment of urethral fistulas following hypospadias repair. Ann Plast Surg. 2000; 44: 381-6.*
9. *Richter F, Pinto PA, Stock JA, et al. Management of recurrent urethral fistulas after hypospadias repair. Urology. 2003; 61: 448-51.*
10. *Santangelo K, Rushton HG, Belman AB. Outcome analysis of simple and complex urethrocutaneous fistula closure using a deepithelialized or full thickness skin advancement flap for coverage. J Urol. 2003; 170: 1589-92.*
11. *Shankar KR, Losty PD, Hopper M, Wong L, Rickwood AM. Outcome of hypospadias fistula repair. BJU Int. 2002; 89: 103-5.*
12. *Luo HH, He XH, Li QR. Y-V skin flap urethroplasty for the UCF. Chin J Urol. 1997; 18: 84.*

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Effects of biomass fuel smoke on lung gas diffusion capasity in healthy women

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Abstract

Background: It is estimated that half of the world population and more than 90 % of the rural population in developing countries use biomass fuels. Biomass smoke is a mixture of complex particles and gases that are harmful to human health, especially the lungs. We aimed to investigate the Lung involvement by use of diffusing capacity for carbon monoxide (DLCO) of healthy women exposed to biomass smoke.

M**ethods**: The participants' history of exposure to tobacco and biomass smoke was investigated, and respiratory function tests (including a DLCO) were performed. The inclusion criteria for the study were exposure to biomass smoke and no history of smoking and no history of previous disease that it can effects of DLCO.

Results: The study group consisted of 91 women, and the control group consisted of 35 women. The mean length of exposure to biomass smoke was 18.6 ± 6.7 hours/year. The mean age of the study group was 51 ± 9 years, while the mean age of the control group was 47 ± 5 years ($p < 0.05$). The FEV1/FVC ratio of the study and control groups were 87.5 ± 7.9% and 93 ± 8.3%, respectively ($p = 0.006$). All of the patients exhibited FEV1/FVC rates above 70 % and FVC values above 80 %. The mean DLCO values for the study and control groups were 24.6 ± 0,7 (111±4.5%) ml/min/mmHg and 27,1±9 (118.5±5.7%) ml/min/mmHg, respectively ($p>0.05$). Fifteen percent of the study group and 0% of the control group exhibited abnormal DLCO values below 80% ($p=0.001$).

Conclusion: Women exposed to biomass smoke can exhibit lower DLCO despite normal respiratory function tests. The reduced DLCO of these women may be the first sign of future respiratory problems.

Key Words: Biomass, lung, diffusion capacity.

Introduction

Exothermic biological sources are called biomass. About 50 % of the world population and 90 % of the rural communities in developing countries currently use biomass fuels as a source of energy (1). The smoke emitted from the burning of biomass contains a large number of pollutants (2). Different studies have reported that biomass smoke causes lung diseases (3-5). Tobacco smoking (TS) is responsible for more than 80 % of the COPD cases in industrialized countries (6, 7). Many studies have observed the presence of COPD in the rural areas of developing countries, where it predominately affects non-smoking women who are exposed to biomass smoke (BS) during cooking (1, 2, 8). Studies have shown that the DLCO tests of healthy cigarette smokers are abnormal several years before their FEV_1 becomes abnormal (9, 10). We aimed to determine the lung involvement in women had normaly who have been exposed to biomass smoke using DLCO and other spirometric parameters.

Methods

Patients

One hundred and ten consecutive healthy female volunteers were selected between September 2007 and October 2010. Volunteers were randomly chosen among women with biomass smoke exposure history or with no biomass smoke exposure living in peripheral areas of our town where biomass is commonly used. All participants had similar socio- economic status and diet. Ninety-one of the volunteers had been exposed to biomass smoke, while 35 were non-exposed. The study group inclusion criteria included.

Following

At least 10 years of biomass smoke exposure, no history of previous disease that it can effects of DLCO and no history of smoking. The control group inclusion criteria included no history of biomass smoke exposure, no history of smoking, and no history of previous disease that it can effects of DLCO. Those females who smoked and had a history of biomass smoke exposure were excluded from the study. The potential role of atmospheric air pollution was excluded because all of the volunteers were from the same region van, Turkey.

Demographic characteristics

The age, height, and body weight of the volunteers were recorded. The Pulmonary Function Tests (PFT) included forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC, and DLCO measurements, which were expressed as percentages of the normal predicted values. The biomass exposure and smoking histories were collected as part of a routine prospective clinical protocol and were retrospectively collated. The biomass smoke exposure time was defined in years, and the biomass smoke exposure intensity was defined in hour-years, with 1 year of exposure for 1 hour a day being 1 hour-year. Former biomass exposure was defined as having been exposed for at least 10 years.

Pulmonary Functional Measurements

The PFT measurements were made using a SpiroLab-2 device and the Viasys Respiratory Care Inc. Encore 22D BEBC1800 device, respectively. The DLCO was measured by the single-breath method, following the American Thoracic Society guidelines (11) and adjusting for standard hemoglobin concentration (12). The measurements were considered adequate if the subject's inspiration reached at least 90% of her forced vital capacity within 2 seconds and her breath could be

held for 10 seconds. The testing was continued until two measurements falling within 10% of each other were obtained or a maximum of four tests was reached. The subjects were allowed a 10-minute interval between tests. The mean of the two technically acceptable measurements was used for our analysis. The units for the DLCO measurements are mL/min/mmHg. Those volunteers with FEV1/FVC values below 70% and FVC values below 80% were excluded from the study.

Statistics

The Statistical analysis were performed using SPSS 15.0 (Statistical package for social sciences). The volunteers with and without biomass smoke exposures was compared using an independent sample t test for continuous variables or Chi-squared test for the qualitative variables. Since age distribution between the two groups was not even, covariance analysis was performed for age affected factors. As a consequence of covariance analysis performed, while the impact of age was found to be significant for DLCO and DLCO% values. Age-corrected values were used in age affected factors. The results are presented as the mean ± standard deviation (SD), and the frequency is expressed as a percent. A "p" value of < 0.05 was considered statistically significant.

Results

This study included 126 subjects. The mean age of the participants with biomass smoke exposure was 51 ± 9 years, and the mean age of the control group was 47 ± 5 years ($p < 0.05$). The mean biomass exposure intensity was 18.6 ± 6.7 year-hours in study group. The FEV1/FVC values of the biomass exposure and control groups were 87.5 ± 7.9 % and 93 ± 8.3%, respectively. While the FEV1/FVC ratios were higher than 70% in both groups, there was a statistically significant difference in the FEV1/FVC ratios between the groups ($p=0.006$). The FVC percentage was above 80% in both of the groups, measuring 91.2 ± 11 % in the biomass smoke exposure group and 93.2 ± 9.8 % in the control group. There was no statistically significant difference in the FVC percentages of the groups. The DLCO percentage was 111±4.5 % (24,6 ± 0.7 ml/min/mmHg) in the biomass smoke exposure group and 118.5±5,7 % (27.1±0.9 ml/min/mmHg) in the control group ($P > 0.05$).

A marked statistical difference was found when the number of subjects with expected DLCO percentages below 80 % was compared using the Chi-squared test. While the expected DLCO percentage was below 80 % in 14 (15%) of the biomass exposure subjects, none of the subjects in the group without biomass smoke exposure exhibited an expected DLCO percentage below 80% ($p= 0.003$).

Table 1. The pulmonary function measurements and general characteristics of the biomass exposed and control groups

Variable	Control (n = 35)		Biomass (n = 91)		*p* value
	Mean	SD	Mean	SD	
Age, yr	47	5	51	9	0.04
Height, cm	152.3	7.3	153.1	6.0	0.68
Body mass index, kg/m^2	28.0	4.4	26.8	5.4	0.05
FEV_1, L	2.7	0.38	2.5	0.54	0.15
FEV_1, % predicted	97.9	10	96	12	0.35
FVC, L	3.2	0.55	2.9	0.54	0.3
FVC, % predicted	99.4	12.3	97.7	17.3	0.81
FEV_1/FVC, %	93	8.3	87.5	7.9	0.006
Exposure to biomass smoke, hour-years			18.6	6.7	
DLCO (ml/min/mmHg)	27.1	0.9	24.6	0.7	0.06
DLCO, %	118.5	5.7	111	4.5	0.07
DLCO < 80 % of predicted, (n)	0		14		0.003

Discussion

Biomass fuels are composed of complex organic matter. In our region generally women use biomass to cook in closed ovens which have no ventilation except for the entry door and a small chimney. Substances in biomass smoke are harmful to human health because they act as irritants, are toxic to cilia and have coagulant and carcinogenic effects (13, 14). The relationships between biomass smoke and respiratory function, the structure of the lungs, and numerous pulmonary pathologies, such as chronic obstructive pulmonary disease (COPD), interstitial pulmonary disease, and lower respiratory tract infections have already been established, and some studies have also suggested a relationship between biomass smoke and cancer and asthma, (3-5).

Chronic exposure to biomass smoke was found to be strongly associated with chronic bronchitis in women who are involved in cooking in rural areas (15-21). Continual exposure to biomass smoke may cause physiological and structural anomalies to develop in the lung (3, 22). Numerous studies have suggested that exposure to biomass smoke is a risk factor for COPD (4, 20, 23, 17). Some studies have found an association between lung fibrosis, progressive massive fibrosis, pneumoconiosis-like lung disease and biomass exposure (3, 24-26). Some another studies and meta-analyses have suggested that there is an increased risk of lung cancer related to biomass fuel use (27-30). These effects are associated with the duration and magnitude of exposure. Majority of the women's living in the rural areas of our region use biomass fuels as household energy sources and consequently are exposed to high levels of in-house air pollution. Our practice frequently encounters female patients who have similar characteristics as a result of biomass smoke exposure.

The effects of biomass smoke exposure on the pulmonary airway, vessels and interstitium have also been shown in experimental and autopsy studies. Fidan et al. found that biomass smoke caused macrophage infiltration into the alveolar and bronchial lumen, alveolar destruction, emphysematous changes and bronchoalveolar hemorrhaging in rabbits, although these effects were less severe than the effects of cigarette smoke (31). Rivera et al. found that lengthy exposure to BS caused emphysema and other lesions typically observed in cigarette smokers; however, there were vascular changes were more severe in the BS group (32). Ozbay et al. found that the long-term exposure of rats to biomass smoke was associated with chronic inflammatory and premalignant alterations in different regions of the respiratory tract and intimal thickening of the pulmonary vessels (33). Tesfaigzi and colleagues found that the length of exposure to wood smoke was associated chronic mild inflammation, squamous metaplasia and the thickening of the alveolar septae (34). These studies suggest that biomass may effect on pulmonary structure and function as tobacco.

Many studies have investigated the effect of biomass smoke on pulmonary function. A study comparing those cooking with gas found that the active use of a biomass fuel was associated with a reduced FEV1/FVC value but failed to find a difference between the FEV1 % and the FVC %. The authors have stated that the average impact of exposure on lung function was mild, despite an expectation of more substantial adverse effects (35). Our previous study demonstrated that pulmonary hypertension was more frequent in healthy women who were exposed to biomass smoke than a control group of women (36). Similar results have been reported in other studies (37-39). These studies suggest that biomass smoke may affect before the pulmonary vasculature system, than airway system. Arslan and colleagues compared the pulmonary function (including DLCO) of healthy non-smoking women and women exposed to biomass smoke, finding significant differences between the two groups' FVC, FEV1, FEV1/FVC and DLCO parameters. However, when these values were compared with the expected percentage values, only the FEV1/FVC parameter exhibited a significant difference. In addition, the authors did not state whether there was a difference in the frequency of abnormal DLCO values between the groups (40). Behera et al. showed that lung function, particularly the FVC, was affected by indoor air pollution caused by cooking with biomass fuel (41). These findings are similar to our pulmonary function data. However, our study did not include patients with an airway obstruction or respiratory symptoms.

Buist et al. and Clark et al. found that the single breath N_2 and CO diffusion tests of smokers are usually abnormal for several years before the FEV1 becomes abnormal (9,10). We found similar results in women exposed to biomass smoke. Tesfaigzi and colleagues have found a significant reduction in the FEV1/FVC values between the wood smoke exposed and nonexposed groups in rats. There was a modest but meaningful reduction in DLCO that demonstrated an impairment of gas exchange in the wood smoke exposure group. We found that while 14 (15 %) of the subjects with biomass smoke exposure exhibited an abnormal DLCO %, none of the subjects in the control group exhibited abnormal DLCO values ($p= 0.09$). However, there was not a significant difference in the DLCO % between the groups ($p>0.05$). One limitation of our study is that there might be some factors which we couldn't detected other than age effecting results.

In conclusion, our study has suggested that diffusion capacity measurements may be the first clue showing the early disorder of pulmonary function in female patients with biomass smoke exposure, even if the patients do not have restrictive or obstructive pulmonary disease. Studies have suggested that the necessity of protection strategies for women and children, which they are natural victims of indoor air pollution.

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Authors' contributions

1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published.

AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References

1. *World Resources Institute, UNEP, UNDP, World Bank. 1998–99 world resources: a guide to the global environment. New York USA; Oxford University Press, 1998.*

2. *Koning HW, Smith KR, Lost JM. Biomass fuel combustion and health. Bull World Health Organ 1985; 63: 11-26.*

3. *Özbay B, Uzun K, Arslan H, Zehir İ. Functional and radiological impairment in women highly exposed indoor biomass fuels. Respirology 2001; 6: 255-258.*

4. *Demirtaş N, Seyfikli Z, Topçu S. Sivas bölgesinden hastanemize başvuran kadın hastalarda biomass kullanımı ile KOAH arasındaki ilişki. Solunum hastalıkları. 1999; 10: 148-55.*

5. *Delgado J, Martinez LM., Sanchez TT., Ramirez A, Iturria C, Gonzalez G. Lung cancer pathogenesis associated with wood smoke exposure. Chest 2005; 128: 124-131.*

6. *Environmental Protection Agency. Respiratory health effects of passive smoking; lung cancer and other disorders. The report of the US Environment Protection Agency. USA: 1993.*

7. *Bolue JSM. Domestic air pollution from biomass burning in Kenya. Atmospheric Environment 1989; 23: 1677-81*

8. *Pandey MR, Regmi HN, Neupane RP, Bhandari DP. Domestic smoke pollution and respiratory function in rural Nepal. Tokai j exp Clin. Med 1985; 10: 471-81.*

9. *Buist S, Nagy J. A longitudinal study of smokers and nonsmokers. 5-6 year follow-up using spirometry and the single breath N_2 test. Chest. 1980; 77(2 Suppl): 259.*

10. *Clark KD, Wardrobe-Wong N, Elliott JJ, Gill PT, Tait NP, Snashall PD. Cigarette smoke inhalation and lung damage in smoking volunteers. Eur Respir J. 1998; 12: 395-9.*

11. *Single breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique. Statement of the American Thoracic Society. Am Rev Respir Dis 1987; 136: 1299-1307.*

12. *Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993; 6: Supp. 1541-52*

13. *Tuthill RW. Woodstoves, formaldehyde, and respiratory disease. Am J Epidemiol 1984; 120: 952-55.*

14. *Fick RB, Paul ES, Merril WW, Reynolds HY, Lake JSO. Alterations in the antibacterial properties of rabbit pulmonary macrophages exposed to woodsmoke. Am Rev Respir Dis 1984; 129: 76-81.*

15. *Samet MJ, Tielsch J. Commentary: could biomass fuel smoke cause anemia and stunting in early childhood. Int J Epidemiol.epidemiology 2007; 36: 130-31*

16. *Abalak R, Frisancho AR, Keeler GJ. Domestic biomass fuel combustion and chronic bronchitis in two rural Bolivian villages. Thorax 1999; 54: 1004-8.*

17. *Pandey MR. Domestic smoke pollution and chronic bronchitis in a rural community of the Hill Region of Nepal. Thorax 1984; 39: 337-39.*

18. *Cetinkaya F, Gülmez I, Aydin T, Oztürk Y, Ozesmi M, Demir R. Prevalence of chronic bronchitis and associated risk factors in a rural area of Kayseri, Central Anatolia, Turkey. Monaldi ArcMonaldi Arch Chest Dis. 2000; 55: 189-93.*

19. *Albalak R, Frisancho AR, Keeler GJ. Domestic biomass fuel combustion and chronic bronchitis in two rural Bolivian villages. Thorax 1999; 54: 1004-8.*

20. *da Silva LF, Saldiva SR, Saldiva PH, Dolhnikoff M. Impaired lung function in individuals chronically exposed to biomass combustion. Environ Res 2012; 112: 111-7.*

21. *Tasleem Akhtar, Zahoor Ullah, Mir Hassan Khan, Rubina Nazli. Chronic Bronchitis in Women Using Solid Biomass Fuel in Rural Peshawar, Pakistan. Chest 2007; 132: 1472-1475*

22. *Grobbelaar JP, Bateman ED. Hut lung. A domestically acquired pneumoconiosis of mixed aetiology in rural woman. Thorax 1991; 46: 334-40.*

23. *Fang X, Wang X, Bai C. COPD in China: the burden and importance of proper management. Chest 2011; 139: 920-29.*

24. *Mohsenifar Z, Brown HV, Schnitzer B, Prause A, Koerner SK. The effect of abnormal levels of hemotocrit on the single breath diffusing capacity. Lung 1982; 160: 325-30.*

25. *Gupta A, Shah A. Bronchial anthracofibrosis: an emerging pulmonary disease due to biomass fuel exposure. Int J Tuberc Lung Dis 2011; 15: 602-12.*

26. *Sigari N, Mohammadi S. Anthracosis and anthracofibrosis. Saudi Med J 2009; 30: 1063-6.*

27. *Lim WY, Seow A. Biomass fuels and lung cancer. Respirology 2012; 17: 20-31.*

28. *Hosgood HD, Boffetta P, Greenland S, Lee YC, McLaughlin J, Seow A, Duell EJ, Andrew AS, Zaridze D, Szeszenia-Dabrowska N. In-home coal and wood use and lung cancer risk: a pooled analysis of the International Lung Cancer Consortium. Environ Health Perspect 2010; 118: 1743-7*

29. *Lissowska J, Bardin-Mikolajczak A, Fletcher T, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, Fabianova E, Cassidy A, Mates D, Holcatova I. Lung cancer and indoor pollution from heating and cooking with solid fuels: the IARC international multicentre case-control study in Eastern/Central Europe and the United Kingdom. Am J Epidemiol 2005 15; 162: 326-33.*

30. *Behera D, Balamugesh T. Indoor air pollution as a risk factor for lung cancer in women. J Assoc Physicians India 2005; 53: 190-92.*

31. *Fidan F, Unlu M, Sezer M, Sahin O, Tokyol C, Esme H. Acute effects of environmental tobacco smoke and dried dung smoke on lung histopathology in rabbits. Pathology 2006; 38: 53-7.*

32. *Rivera RM, Cosio MG, Ghezzo H, Salazar M, Pérez-Padilla R. Comparison of lung morphology in COPD secondary to cigarette and biomass smoke. Int J Tuberc Lung Dis. 2008; 12: 972-77.*

33. *Ozbay B, Yener Z, Acar S, Kanter M. Histopathological alterations in respıratory tractus of rats exposed to bıomass smoke. J Med Sci 2009; 29: 877-83.*

34. *Tesfaigzi Y, Singh SP, Foster JE, Kubatko J, Barr EB, Fine PM, McDonald JD, Hahn FF, Mauderly JL. Health effects of subchronic exposure to low levels of wood smoke in rats. Toxicol Sci 2002; 65: 115-25.*

35. *Relagado J, Perez Padilla R, Sansores R, Ramirez J, Brauer M. The effect of biomass burning on respiratory symptoms and lung function in rural Mexican women. Am J Respir Crit Care Med 2006; 174: 901-90*

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Correlation between interleukin-33 gene polymorphism and female primary knee osteoarthritis

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Abstract

Objective: To observe the correlation between interleukin-33 (IL-33) gene polymorphism and female primary knee osteoarthritis (PKO).

Methods: 56 PKO patients whose Kellgren/Lawrence scores were higher than 2 as well as 59 venous blood samples from female healthy controls were collected for the genotyping of rs20541, rs1800925 and rs4599536 polymorphic sites in two groups of IL-33 using the polymerase chain reaction-restriction fragment length polymorphism, aiming to calculate the distribution differences in two groups of genotype and allele and analyze the correlation between IL-33 gene polymorphism and PKO genetic susceptibility.

Results: All gene frequencies of three SNP loci in the two groups were distributed following the Hardy-Weinberg equilibrium without significant differences between the alleles. The non-conditional logistic regression analysis on dominant mode of single locus shows that through the comparison between the three genotypes of rs20541, the onset risk of PKO in T/G genotype was significantly reduced compared with that in T/T genotype (OR = 0.38, 95% CI = 0.19 ~ 0.64, P = 0.024). In rs1800925, the onset risk in C/T genotype was significantly higher than that in C/C genotype (OR = 1.94, 95% CI = 1.04 ~ 3.51, P = 0.038).

Conclusion: In female population, the genetic variation of IL-33 is correlated with the susceptibility of PKO.

Key words: Knee osteoarthritis, interleukin-33, gene, single nucleotide polymorphism, histological construction.

Introduction

Epidemiological investigations on primary knee osteoarthritis (PKO) have indicated that the incidence of PKO is much higher in female than that in male, which is more common in the middle-aged and elderly people. In the elderly patients older than 60, symptoms and radiographic evidence show that the female/male ratio in PKO patients is up to 3/1. Its main pathological changes are stiff deformation and collapse of knee articular surface caused by normal weight-bearing, accompanied by secondary synovitis with movement disturbance and joint swelling as main clinical syndromes [1-3].

Currently, many researchers hold that PKO may be a multi-gene heredopathia, but its pathogenesis has not been fully clarified yet. In recent years, finding PKO-related genes has gradually drawn the attention of scholars. The current genetic studies have shown that the incidence of PKO is correlated with interleukin-1 (IL-1), IL-6 and IL-10, etc., which plays an important role in inflammatory mediation. IL-33 is a multifunctional cytokine, whose gene polymorphism has been spotlighted. The primary pathological changes include the generation of inflammatory cytokines [4-7].

In view of this, this study assumes that there might be some correlation between IL-33 gene and the incidence of PKO, aiming to analyze IL-33 gene polymorphism and its genotypes by polymerase chain reaction-restriction fragment length polymorphism as well as the distribution frequency of allele to explore its correlation with female PKO.

Materials and methods

Materials

DNA Marker and dNTP Mixture (Beijing Dingguo Changsheng Biotech Co., Ltd.); Ahd I, Nde I and Bsm A I (Shanghai DEMO Medical Tech Co., Ltd.); PCR amplification primers (Beijing DeLiLai Technology Development Co., Ltd.); automatic gel imaging system (Shanghai Bioshine Scientific Instrument Co., Ltd.)

Subjects

Osteoarthritis group: 56 female patients with PKO who were treated in our hospital from January 2012 to December 2012 were collected. Inclusion criteria: patients whose medical history, physical examination and imageological examination met with the diagnostic criteria of PKO, and Kellgren/Lawrence score of knee joint X-ray film ≥ 2 points, body mass index (BMI) ≤ 28 kg/m2 and age ≥ 40 years old. Exclusion criteria: 1) the patients with unclear clinical history; 2) the patients who had histories of knee infection, knee deformity, leg length discrepancy, knee trauma, knee tumor, metabolic bone disease or recent use of metabolic drugs [8,9].

Normal control group: 59 female healthy controls with non-PKO who were treated in the department of orthopedics were selected in the same period. Inclusion criteria: healthy subjects with non-PKO whose age, gender, BMI and labor categories were matched with the osteoarthritis group over the same period. Exclusion criteria: females who were clinically suspected or diagnosed with histories of knee deformity, knee tumor, autoimmune diseases and metabolic bone disease before adulthood, or surgical history of knee trauma, any symptom and sign of articular inflammatory diseases.

All the experimental subjects had no genetic relationship. According to the "Administrative Regulations on Medical Institutions" issued by the State Council of the People's Republic of China, the experimental program was approved by the ethics committee of our hospital, the subjects were informed of experimental program and risks and signed informed consent before experiment.

Sample collection and DNA extraction

3mL fasting venous blood was drawn from the subject, immediately injected into 0.1 mol/L EDTA-Na_2 anticoagulant sterile vacuum tube, fully mixed to prevent coagulation, and then stored in refrigerator at -8 ℃ for extracting genomic DNA. Conventional phenol-chloroform extraction method was adopted to extract DNA which was reserved in refrigerator at -20 ℃. All specimens were detected centrally within 7d.

RT-PCR reaction system and procedure: the sequence amplification primers of loci of rs20541, rs1800925 and rs4599536 of IL-33 gene were designed with reference to the relevant literature [10]. The sequences are shown in Table 1.

PCR reaction system

The RT-PCR reaction system contains 5.0 μL of 10 × PCR digestion buffer (Mg^{2+} Plus), 4.0 μL of dNTP, 3.0 μL of 20.0 μmol/L Forward Primer, 3.0 μL of 20.0 μmol/L Reverse Primer, 4.0 μL of genomic DNA template, 1.0 μL of Takara Taq (10U/μL) and 30 μL of sterile distilled water, with a total volume of 50 μL. Reaction procedure of rs20541T/G locus: initial denaturation at 95℃ for

Table 1. Primer sequences of the three loci and the corresponding reaction conditions

Polymorphisms	Primer sequences (5'-3')		
rs20541T/G	F: AGA GTG GGT CCA AGC TTG CAG TA		
rs1800925C/T	R: AAT CAC AGC AGG TTG CCT TTC CTA G		
	F: GAT CCC GTC GCT TTC CAG GTT CAC		
	R: TGT GAC TAC AAA CCC AGC CTT CTC C		
rs4599536T/C	F: CTC CAC ACT CAA AAC GGT AGC TGA		
	R: CAC TGT CCA TGT CAA AAG TTT CCA C		
Polymorphisms	Annealing temperature (°C)	Endonuclease	Product size (bp)
rs20541T/G	65	Nde I	171
rs1800925C/T	70	Bsm A I	334
rs4599536T/C	65	Ahd I	280

10 min first, then 10 cycles according to the procedures (denaturation at 95℃ for 1 min, annealing at 60℃ for 1 min, extension at 70 ℃ for 2 min), and extension at 70℃ for 10 min after the last cycle. The annealing temperatures of rs1800925C/T and rs4599536T/C are shown in Table 1. The other reaction steps were the same as those of rs20541 T/G.

Amplification product digestion system

The rs20541 digestion system contains 20.0 μL of amplification product, 4.0 μL of 10 × buffer, 3.0 μL of restriction enzyme Nde I and 23 μL of sterile double distilled water, with a total volume of 50.0 μL.

Detailed procedure: 20.0 μL of PCR amplification product, 4.0 μL of 10 × digestion buffer, 3.0 μL of restriction enzyme Nde I and 23.0 μL of sterile double distilled water were added into sterilized PCR tube, mixed sufficiently, then centrifuged at high speed for a few seconds, and then placed in water bath at 37℃ for incubation for 12 h. The digestion products were stored in a -20 ℃ refrigerator, or directly used for electrophoresis. The endonucleases of the other two loci are listed in Table 1. The reaction steps were the same as those of rs20541 [11].

Electrophoresis and sequencing of amplification and digestion products

The final reaction products were subjected to 2% agarose gel electrophoresis, and the results were read by an automatic gel imaging system, recorded and saved. To verify the genotypes PCR amplification and digestion products were sent to the Chinese Academy of Medical Sciences for DNA sequencing. The genotype gel strips of three loci of IL-33 gene were analyzed and summarized in Table 2.

Statistical analysis

All data were analyzed by SPSS 13.0 and compared by the t test. The Hardy-Weinberg (H-W) genetic equilibrium test was performed by SNPstats, and the genotypes and risks of knee osteoarthritis were evaluated by a non-conditional Logistic regression model. The odds ratio (OR, 95% confidential interval, CI) and PI values were calculated with a=0.05.

Results

Number of subjects

All the 115 subjects were included in the analysis of results.

Baseline data of included subjects

The patients in the PKO group were aged between 43 and 71 years old with an average age of (57.5 ± 8.4) years old, and the age range of the control group was 42 to 73 years old, with mean age of (59.8 ± 8.6) years old, the difference between which was not statistically significant (P> 0.05).

IL-33 genotype frequency distribution

The DNA concentration extracted was about 120 ~ 150μg/L, and the absorbance value $A_{260\ nm/280\ nm}$ was 1.9 ~ 2.0, which met the requirements of PCR experiment. The electrophoresis results are consistent with the DNA sequencing results. The experimental results of rs20541, rs1800925 and rs4599536 showed the genotypes of CC, TG, TT, TT, CT, TT, GG, CC and TC. The results indicated that the distribution frequencies of all genotypes of three loci accorded with H-W equilibrium (P> 0.05), no significant difference found in allele (P> 0.05) (Table 3).

Table 2. Genotype gel strips of three loci of IL-33 gene

Polymorphisms of IL-33	Type Shorthand	Number of gene fragments	Fragment size
rs20541T/G	CC	2	182, 31
	TG	2	163, 22
	TT	1	173
rs1800925C/T	TT	3	183, 142, 29
	CT	2	191, 34
	TT	1	158
rs4599536T/C	GG	1	184
	CC	2	173, 26
	TC	1	178

Table 3. H-W equilibrium test results and Goodness of Fit of allelic genes (χ^2 test)

	rs20541		rs1800925		rs4599536	
	OA	Control	OA	Control	OA	Control
Observe value						
1/1a	21	33	33	29	34	39
1/2a	31	21	20	28	19	18
2/2a	4	5	3	2	3	2
Estimate value						
1/1a	25	31	34	34	33	35
1/2a	26	24	19	21	21	22
2/2a	5	4	3	4	2	2
χ^2	3.34	4.01	2.67	3.86	0.76	2.17
P	0.14	0.07	0.19	0.09	0.84	0.26
Allele						
1a	72	98	89	95	82	109
2a	39	38	19	35	26	22
χ^2	3.88		6.04		2.72	
P	0.29		0.57		0.18	

a: rs20541: 1-T, 2-G; rs1800925: 1-C, 2-T; rs4599536; 1-T, 2-C; SNP: single-nucleotide polymorphisms; H-W: Hardy-Weinberg

Correlation between IL-33 genotypes and primary knee osteoarthritis

The codominant model of single locus in non-conditional logistic regression was optimal. The results showed that through the comparison among the three genotypes of rs20541, the onset risk of PKO in T/G genotype was significantly reduced compared with T/T genotype (OR = 0.38, 95% CI = 0.19 ~ 0.64, P = 0.024); in rs1800925, the onset risk in C/T genotype was significantly higher than that in C/C genotype (OR = 1.94, 95% CI = 1.04 ~ 3.51, P = 0.038); no significant differences were found among the genotypes of rs4599536 (Table 4).

Discussion

Currently, studies have shown that the onset risk of PKO is associated with interleukin gene polymorphisms [12]. Genetic studies have found that interleukin genes are identified candidate genes of osteoarthritis, whose linkage mutation is located on chromosome 2q12-q13. While IL-33 gene is located at district 2 and belt 6 of long arm, which is included in the above chromosome fragment, so there is a certain correlation between the two in the respect of genetics [13,14]. In terms of cytology, IL-33, as a proinflammatory cytokine,

Table 4. Non-conditional Logistic regression analysis of the correlation between IL-33 genotypes and knee osteoarthritis

Site	Genotype	Genotype frequency [n (%)]		OR (95%CI)	P
		Osteoarthritis group	Control group		
rs20541	C/C	20 (35.71%)	36 (61.02%)	1.00[a]	0.024
	T/G	31 (55.36%)	16 (27.12%)	0.38 (0.19～0.64)	
	T/T	5 (8.93%)	7 (11.86%)	1.05 (0.42～2.36)	
rs1800925	T/T	32 (57.15%)	29 (49.15%)	1.00[a]	0.038
	C/T	18 (32.14%)	28 (47.46%)	1.94 (1.04～3.51)	
	T/T	6 (10.71%)	2 (3.39%)	0.56 (0.28～1.25)	
rs4599536	G/G	27 (48.21%)	35 (59.32%)	1.00[a]	0.154
	C/C	24 (42.86%)	22 (37.29%)	0.57 (0.22～0.98)	
	T/C	5 (8.93%)	2 (3.39%)	0.49 (0.20～1.05)	

a: Reference Standards

can induce chemotactic responses of many immune cells, including monocytes, eosinophils, T lymphocytes and dendritic cells, etc., and promote secretion of many other cytokines [15]. Although knee osteoarthritis is not an autoimmune disease, but the joint contains many cytokines synthesized by chondrocytes and synovial cells that are involved in the process of cartilage metabolism. The metabolic imbalance of intraarticular cytokines may affect the anabolic and catabolic balance of articular cartilage to cause structural damage of normal cartilage extracellular matrix proteins, eventually leading to the destruction of articular cartilage and the occurrence of osteoarthritis [16,17]. Currently, studies have shown that knee osteoarthritis is associated with IL-1, IL-6, IL-10 and other genes [18].

Epidemiological studies have indicated that there are significant differences in gender distribution in PKO, in which the incidence ratio of female is much higher than that of male, and its incidence is affected by their different living habits, work environment, hormone metabolism and other factors, and besides, it may be related to different genetic factors [19,20]. This experiment included only female study groups, increasing the clinical homogeneity; the restrictions on the body mass index also helps to reduce the influence of body weight factor on PKO morbidity. However, due to the limitations of the experimental conditions and subjects, multivariate stratified analysis was not conducted on the study group, it is required to collect more complete clinical data in the follow-up study for more systematic exploration of the correlation between PKO genetic susceptibility and IL-33 gene polymorphism by stratification and correction.

In this study, 56 cases of PKO and 59 healthy controls were included to explore the correlation between the three polymorphic sites of rs20541, rs1800925 and rs4599536 of IL-33 gene and the onset risk of PKO using the method of "case-control study". The results showed that the osteoarthritis and control groups were well-matched in age; the distribution of all gene frequencies of the three SNP loci in the two groups accorded with H-W equilibrium, no significant difference found in allele, indicating that the samples selected are representative and comparable. The non-conditional logistic regression analysis on single locus showed that, through the comparison among the three genotypes of rs20541, the onset risk of PKO in T/G genotype was significantly reduced compared with T/T genotype; in rs1800925, the onset risk in C/T genotype was significantly higher than that in C/C genotype, suggesting that there may be susceptible correlation between IL-33 genotypes and PKO.

In summary, the T/G genotype of the IL-33 allele rs20541 may be a protective factor for the incidence of PKO, and the gene polymorphism of allele-rs1800925 locus may a risk factor that affects the occurrence of PKO. Therefore, for female population, the genetic variation of IL-33 may be correlated with the susceptibility of PKO.

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References

1. *Jamsen E, Peltola M, et al. Comorbid diseases as predictors of survival of primary total hip and knee replacements: a nationwide register-based study of 96 754 operations on patients with primary osteoarthritis. Ann Rheum Dis, 2012; 35(6):15-23.*

2. *Van Manen MD, Nace J. Management of primary knee osteoarthritis and indications for total knee arthroplasty for general practitioners. J Am Osteopath Assoc, 2012; 112(11): 709-15.*

3. *Hootman JM, Albohm MJ. Anterior cruciate ligament injury prevention and primary prevention of knee osteoarthritis.J Athl Train, 2012; 47(5): 589-90.*

4. *Jamsen E, Nevalainen P, et al. Obesity, diabetes, and preoperative hyperglycemia as predictors of periprosthetic joint infection: a single-center analysis of 7181 primary hip and knee replacements for osteoarthritis. J Bone Joint Surg Am, 2012; 94(14): 101-5.*

5. *Fang X, Shi X. Expression of beta-catenin in articular cartilage of knee primary osteoarthritis. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2012; 26(5): 532-5.*

6. *Dowsey MM, Dieppe P. The association between radiographic severity and pre-operative function in patients undergoing primary knee replacement for osteoarthritis. Knee, 2012; 19(6): 860-5.*

7. *Kelly S, Dunham JP, et al. Spontaneous firing in C-fibers and increased mechanical sensitivity in A-fibers of knee joint-associated mechanoreceptive primary afferent neurones during MIA-induced osteoarthritis in the rat. Osteoarthritis Cartilage, 2012; 20(4): 305-13.*

8. *Leskinen J, Eskelinen A. The incidence of knee arthroplasty for primary osteoarthritis grows rapidly among baby boomers: a population-based study in Finland. Arthritis Rheum, 2012; 64(2): 423-8.*

9. *Lowe CJ, Barker KL. Comparison of postdischarge physiotherapy versus usual care following primary total knee arthroplasty for osteoarthritis: an exploratory pilot randomized clinical trial. Clin Rehabil, 2012; 26(7): 629-41.*

10. *Sabatino G, Nicoletti M. Impact of IL -9 and IL-33 in mast cells. J Biol Regul Homeost Agents, 2012; 26(4): 577-86.*

11. *Lopetuso LR, Scaldaferri F, et al. Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. Fibrogenesis Tissue Repair, 2012; 15(1): 18-23.*

12. *Honsawek S, Yuktanandana P, et al. Correlation between plasma and synovial fluid basic fibroblast growth factor with radiographic severity in primary knee osteoarthritis.Int Orthop, 2012; 36(5): 981-5.*

13. *Blom L, Poulsen LK. IL-1 family members IL-18 and IL-33 upregulate the inflammatory potential of differentiated human Th1 and Th2 cultures. J Immunol, 2012; 189(9): 4331-7.*

14. *Arshad MI, Piquet-Pellorce C, et al. TRAIL but not FasL and TNFalpha, regulates IL-33 expression in murine hepatocytes during acute hepatitis. Hepatology, 2012; 56(6): 2353-62.*

15. *Hsu CL, Bryce PJ, et al. Inducible IL-33 expression by mast cells is regulated by a calcium-dependent pathway. J Immunol, 2012; 189(7): 3421-9.*

16. *Williams NH, Amoakwa E, et al. Activity Increase Despite Arthritis (AIDA): phase II randomised controlled trial of an active management booklet for hip and knee osteoarthritis in primary care. Br J Gen Pract, 2012; 61(589): 452-8.*

17. *Tanavalee A, Honsawek S, et al. Inflammation related to synovectomy during total knee replacement in patients with primary osteoarthritis: a prospective, randomised study. J Bone Joint Surg Br, 2012; 93(8): 1065-70.*

18. *Borgonio-Cuadra VM, Gonzalez-Huerta C. Analysis of estrogen receptor alpha gene haplotype in Mexican mestizo patients with primary osteoarthritis of the knee. Rheumatol Int, 2012; 32(5): 1425-30.*

19. *Yan CH, Chiu KY, et al. Total knee arthroplasty for primary knee osteoarthritis: changing pattern over the past 10 years. Hong Kong Med J, 2012; 17(1): 20-5.*

20. *Al-Jarallah KF, Shehab DK, et al. Prevalence of the Pro12Ala missense mutation in the PPARG2 gene in Kuwaiti patients with primary knee osteoarthritis. Ann Saudi Med, 2012; 31(3): 35-9.*

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Serum macro - micro element responses to acute maximal physical exercise

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Abstract

Purpose: In this study, it was aimed to examine the effect of acute maximal physical exercise on some macro - micro element levels in women.

Method: Blood samples of subjects were drawn 2 times in total; shortly before and after the exercise. The flame atomic absorption spectrophotometric method used for analysis of zinc, calcium, magnesium and copper levels. Serum sodium and potassium concentrations were measured using ion selective electrodes.

Results: It was found that there was significant increases for the zinc, calcium, magnesium, potassium and significant decrease for copper ($p<0.01$) while non-significant difference was found for the sodium parameter ($p>0.01$).

Conclusion: As result our findings show that acute maximal physical exercise can lead to some changes on mineral metabolism in human. Such a diversity of results in the literature, indicate the importance of factors such as duration and intensity of exercise, fitness status of the participants, pre-exercise levels of mineral etc.

Key Words: Physical exercise, macro element, calcium, magnesium, sodium, potasium, micro element, copper, zinc, mineral.

Introduction

Minerals are divided into two groups depending on body weight; those which weigh more than 0.1% of total body weight are called macro - elements while those weigh less than 0.1% of body weight are called micro-elements (trace elements)[1].

In human body, many trace elements take part in numerous physiological and biochemical events. The changes in the element levels changes depending on the type, length and intensity of the exercise, as well as the nutritional behavior[2].

Fourteen essential trace minerals have been identified, but only six are related to exercise, and these are iron, zinc, copper, selenium, chromium and vanadium[3].Carbohydrate, iron, calcium, B-vitamins (B6. B12, folate), vitamin D, magnesium, iron, and zinc are nutrients particularly affected by energy restriction among athletes. All of these nutrients are critical for optimal health and performance[4].

Over the past few years, a sustained interest has developed in trace element nutrition and metabolism as it relates to athletic performance[5].

The aim of the present study was to examine the effect of acute physical exercise on some macro - micro element levels in women.

Material and Method

Selection of Participants

The study included 18 - 23 age group 10 female subjects who did exercise only at fitness level. The present study was caried out after approval of the local ethic committee. All subjects were informed about the purpose and procedures of the study. None of the subject were in the period of menstruation.

Study Design

The heights, weights, resting hearth rates and 5 cc. venous blood samples of subjects were taken before the exercise.

The subjects warmed up for ten minutes before they had 20 m shuttle run. The purpose of this test was to tire the subjects to the exhaustion. The heart beat rates were followed during the test in order to determine the exhaustion levels of the participants.

The 5 cc. venous blood samples were taken again just after the exhaustion exercise.

It is important to note that runs of all subjects were short duration, lasting 9 - 13 min.

Physical and Physiological Measurements

Measurement of height and weight

The height and weight measurement were measured 1 hour before the exercise. The weight of subjects were meausered by an electronic scale without shoes and wearing only short and t shirt. The height measurements were determined with metric scale.

Measurement of resting hearth rate

The resting herth rate of subjects were taken on sitting position with stethoscope and chronometer after 20 min. resting period before 1 hour exercise.

Collection of the blood samples

The blood samples of subjects were taken from left arms two times. The first measurements were taken on resting and sitting position before 15 min. the start of exercise. The second measurements were taken just after the fatiguing exercise on sitting position. In these measurements were used heparinized plastic syringes and 5 cc. venous blood samples were taken. After the labeled all samples were centrifuged and put in a deep freeze.

Biochemical Analyses

The flame atomic absorption spectrophotometric method used for analysis of zinc, calcium, magnesium and copper levels[6]. Serum sodium and potassium concentrations were measured using ion selective electrodes.

Statistics

Statistical evaluation was conducted using SPSS 15 software program. The differences between first and second results were determined by wilcoxon signed ranks test method at a significance level of $p<0.01$.

Results

The average age, body weight, height, resting hearth rate and Max VO2 values of the subjects were determined to be 19.80±1.31 years, 56.89±6.81 kg and 162.00±2.82 cm, 77.40±6.80 beat/min and 33.25±3.90 ml.kg/min, respectively.

Table 2 shows the comparisons in micro element levels. According to findings, serum Zn concentration increased while Cu levels decreased shortly after exercise ($P<0.01$).

Table 1. Descriptive Statistics of the Subjects

Parameters	N	Mean (X)	Min-Max
Age (year)	10	19.80±1.31	18-23
Height (cm)	10	162.00±2.82	158-167
Body Weight (kg)	10	56.89±6.81	48.70-68.40
Resting Hearth Rate(beat/min.)	10	77.40±6.80	64-88
Max VO_2 (ml.kg/min)	10	33.25±3.90	26.43-39.86

Table 2. The comparisons of the participants some micro element levels

Parameters	N	Pre exercise	Post exercise	X_1-X_2	Z	P
		Mean (X_1)	Mean (X_2)			
Zn (umol/L)	10	14.92±1.98	18.12±1.26	-3.20	-2.80[a]	0.005*
Cu (umol/L)	10	18.86±2.38	17.10±2.21	1.76	-2.70[b]	0.007*

$P<0.01$ a. Based on negative ranks b. Based on positive ranks*

Table 3. The comparisons of the participants some macro element levels

Parameters	N	Pre exercise	Post exercise	X_1-X_2	Z	P
		Mean (X_1)	Mean (X_2)			
Ca (mg/dl)	10	9.31±0.39	10.30±0.24	-0.99	-2.80[a]	0.005*
Mg (mg/dl)	10	1.73±0.25	2.80±0.10	-1.07	-2.80[a]	0.005*
Na (mmol/L)	10	135.40±0.69	134.30±1.63	1,10	-1.76[b]	0.077
K (nmol/L)	10	4,21±0.38	5.54±0.62	-1.33	-2.80[a]	0.005*

$P<0.01$ a. Based on negative ranks b. Based on positive ranks*

Table 3 shows the comparisons in macro element levels. It can clearly be seen from the table that calcium, magnesium and potasium levels increased with statistically significant extent (P<0.01). As for sodium levels, there was no remarkable difference between the times before and shortly after exercise (P>0.01).

Discussion

Exercise is a potent stressor that influences circulating Zn concentrations in the blood. In general, short-duration, high-intensity activities induce an immediate increase in plasma and serum Zn concentrations[5]. In our study was found significant increase post exercise zinc levels (P<0.01).

In parallel with findings, large number of studies indicated increase in zinc level after exercise[7-8-9-10-11-12]. However, there are some studies suggesting a decrease in zinc level after exercise[13].

The increase in plasma Zn levels can be explained as result of release of Zn from erythrocytes or from skeletal muscle, since both intravascular hemolysis and muscle damage can ocur during strenuous exercise[14].

Copper is another divalent cation with important biological functions including modulation of enzyme activity and also a role in the synthesis of hemoglobin, catecholamines, and some peptide hormones[15]. However the studies showed no appreciable correlation between the copper level in blood and physical exercise. Although some workers claim that this ratio increases as a result of physical activity there is no consensus here as well, as it is in zinc[16].

In this study, it was determined that copper level significantly decreased after exercise (P<0.01).

The different results were reported in the literature about the copper levels after acute strenuous exercise. Some studies indicated that copper levels increased after exercise[7-10-11-13-17]; however, others reported that these levels decreased after exercise[8-18].

The mechanism of Cu metabolism is unclear. Further research is required to clarify the effect of exercise.

Magnesium participates in many events such as cellular glucose, fat and protein metabolisms; regulation of membrane stability, neuromuscular, cardiovascular and hormonal functions[19]. Authors that have summarized the literature are in agreement that, in general, high-intensity exercise induces an increase in plasma Mg, while submaximal exercise has the opposite effect. However, there have been many exceptions to the general trend. Drops in plasma Mg have been reported after high-intensity exercise routines[20].

This study supports that high-intensity exercise leads to an increase in plasma Mg (P<0.01).

There are some studies that showing increases in paralel to ours in literature[10-20]. Similarly, Hazar et al. found a non-significant rise in plasma magnesium after acute maximal physical exercise ($p<0.05$)[21].

The increase in serum Mg concentration during high-intensity but short time exercise depends on the decrease in plasma volume[21].

Calcium is found in cell membranes and subcellular organelles. It participates in blood coagulation process and contraction of transversely striated muscles. It activates multiple enzymes (e.g. phospholipase, phosphocreatin kinase)[22].

Our findings show that strenuous exercise leads to a statistically increase post exercise calcium plasmatic concentrations (P<0.01).

In many previous studies, the calcium levels in serum were shown to increase after exercise[10-12-23-24].

The changes in serum calcium concentrations may not be clear in general sense because calcium is primarily under homeostatic control. Parathyroid hormone (PTH) acts to increase the concentration of calcium in the blood by increasing the calcium secretion by bones and the calcium absorption in intestine and kidney tubules[21]. The data of the literature concerning the relation between parathyroid hormone and exercise have shown that physical exercise is an important modifier of parathyroid hormone concentrations depending on intensity and duration of exercise[25].

Sodium and potassium play an important role on a series of metabolic process such as formation of membrane potential, regulation of water balance and distribution in body, stabilizing osmotic pressure, acid-base balance, and maintaining normal cardiac rhythm[19].

In the present study, the exercise performed up to the point of exhaustion was seen to increase the plasma potassium levels (P<0.01).

In some studies, the increase in the potassium levels are generally determined immediately after the exercise and are in paralel to the findings obtained in our study regarding the exhaustion[10-12-26-27-28].

In our study, sodium levels were not affected by the exercise ($p>0.01$) .

It is reported in the literature that long-term exercise can result in significant sodium losses through sweating[29].

A possible explanation for the non-significant changes in sodium level could be short duration exercise in our study.

The results of this study show that acute maximal physical exercise can lead to some changes on mineral metabolism in human. According to the results of the abovementioned studies, it can be said that the levels of many minerals show variation depending on the kind of exercise, fitness status of the participants, pre-exercise levels of mineral etc.

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References

1. *Wildman REC. The Nutritionist Food, Nutrition, and Optimal Health. 2nd Ed. New York: Routledge;2009.*
2. *Pourvaghar MJ, et al, The Changes of Serum Magnesium on Nano Scale After an Exhaustion Exercıse in Male Students, Digest Journal of Nanomaterials and Biostructures. 2010; 5(1): 141-145.*
3. *Maughan RJ. Nutrition in Sport Volume VII of the Encyclopaedia of Sports Medicine An IOC Medical Commission Publication. London : Blackwell Science Ltd; 2000.*
4. *Wolinsky I, Driskell JA, Sports Nutrition : Energy Metabolism and Exercise. 1st ed. Newyork : CRC Press Taylor & Francis Group; 2008.*
5. *Wolinsky I, Driskell JA. Sports Nutrition : Vitamins and Trace elements, 2nd ed. Newyork: CRC Press Taylor & Francis Group; 2006.*
6. *Elmer, P. Analytical Methods for Atomic Absorptions Spectrophotometry. Norwalk, CT: Perkin Elmer;1982.*
7. *Anderson RA, et al. Acute Exercise Effects on Urinary Losses and Serum Concentrations of Copper and Zinc of Moderately Trained and Untrained Men Consuming a Controlled Diet. Analyst. 1995; 120: 867-870.*
8. *Bordin D. High Intensity Physical Exercise Induced Effects on Plasma Levels Of Copper And Zinc, Biological Trace Element Research. 1993; 36(2): 129-134.*
9. *Cordova A, Navas FJ. Effect of Training on Zinc Metabolism: Changes in Serum and Sweat Zinc Concentrations in Sportsmen. Ann. Nutr. Metab. 1998; 42: 274-282.*
10. *Cinar V, Baltaci AK, Mogulkoc R. Effect of Exhausting Exercise and Calcium Supplementation on Potassium, Magnesium, Copper, Zinc and Calcium Levels in Athletes. Pak J Med Sci. 2009; 25(2): 238-242.*
11. *Deuster PA. Exercise-Induced Changes in Blood Minerals, Associated Proteins and Hormones in Women Athletes. The Journal of Sports Medicine and Physical Fitness. 1991;31(4): 552-560.*
12. *Meludu SC, Nishimuta M, et al. Anaerobic Exercise – Induced Changes in Serum Mineral Concentrations. African Journal of Biomedical Research 2002; 5: 13-17.*
13. *Savaş S, et al. Effect of Acute Maximal Aerobic Exercise upon the Trace Element Levels in Blood. Neuroendocrinology Letters. 2007; 28(5): 675-680.*
14. *Clarkson PM, Haymes EM, Trace Mineral Requirements for Athletes, Int. J Sports Nutr 1994; 4: 104-19.*
15. *Maughan RJ, Burke LM. Handbook of Sports Medicine and Science Sports Nutrition. London : Blackwell Science Ltd; 2002.*
16. *Savaş S, et al. Effect of Six Weeks Aerobic Training upon Blood Trace Metals Levels, Neuroendocrinology Letters, 2006; 27(6): 822-827.*
17. *Ohno H, et al. Changes in Dopamine-Beta-Hydroxylase and Copper and Catecholamine Concentrations in Human Plasma with Physical Exercise. J Sports Med Phys Fitness. 1984; 24: 315–320.*
18. *İri R. A Comparison of Certain Trace Elements In Wrestlers Before and After Aerobic and Anaerobıc Exercises. South African Journal for Research in Sport, Physical Education And Recreation. 2011; 33(3): 51-58.*
19. *Kara E. The Effects of Acute Submaximal Exercise on Trace Element Metabolism. HealthMED. 2011; 5(6) : 1580-1585.*

20. *Westmoreland D, et al. Effects of Maximal and Submaximal Exercise on Plasma Electrolyte Shifts. Trace Elements and Electrolytes. 2006; 23(4) : 312-317.*

21. *Hazar M, et al. Physiological Responses of Macro-Elements to Maximal Aerobic Exercise Among Elite Women and Men Field Hockey Players. HealthMED. 2012; 6(9): 3084-3090.*

22. *Wochyński Z, et al. Changes in Concentration of Macroelements in the Blood Serum of Long Distance Runners Before and After the Preparatory Period. Medsportpress. 2008; 3(6): 177-188.*

23. *Brahm H, Piehl-Aulin K, et al. Bone Metabolism During Exercise and Recovery: The Influence of Plasma Volume and Physical Fitness. Calcified Tissue International. 1997a; 61: 192-198.*

24. *Cunningham J, Segre GV, et al. Effect of Heavy Exercise on Mineral Metabolism and Calcium Regulating Hormones in Humans. Calcified Tissue International. 1985; 37(6): 598-601.*

25. *Bouassida A, et al. Parathyroid Hormone and Physical Exercise: A Brief Review. Journal of Sports Science And Medicine. 2006; 5: 367-374.*

26. *Marcos E, Ribas J. Kinetics of Plasma Potassium Concentrations During Exhausting Exercise in Trained and Untrained Men. European Journal of Applied Physiology & Occupational Physiology 1995; 71(2/3): 207-214.*

27. *Tenan MS, Mcmurray RG, et al. Changes in Plasma Potassium During Graded Aerobic Exercise and Two Hours of Recovery, Journal of Human Kinetics. 2010; 26: 51-P55.*

28. *Zoladz JA, Duda K, et al. Effect of Different Cycling Frequencies during Incremental Exercise on the Venous Plasma Potassium Concentration in Humans. Physiological Research. 2002; 51(6): 581-586.*

29. *Stachenfeld NS. Acute Effects of Sodium Ingestion on Thirst and Cardiovascular Function. Current Sports Medicine Reports (American College of Sports Medicine). 2008; Supp, 7(4): S7-S13.*

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Gene polymorphisms of non-small-cell lung carcinoma ERCC1 and XRCC1 and the sensitivity to gemcitabine/cisplatin chemotherapy

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Abstract

Objective: To explore the polymorphisms of DNA repair enzyme genes ERCC1 118C/T and XRCC1 Arg194Trp as well as the sensitivity of non-small-cell lung carcinoma (NSCLC) patients to gemcitabine/cisplatin (GP) chemotherapy.

Methods: 73 patients diagnosed as NSCLC from March 2009 to March 2012 were selected. Their venous bloods were sampled before chemotherapy, and the DNA of the leukocytes therein was extracted and the ERCC1 118C/T and XRCC1 Arg194Trp genotypes were subjected to PCR-RFLP detection. The patients all underwent GP chemotherapy.

Results: In the NSCLC patients, the genotype frequencies of ERCC1 118 C/C, C/T and T/T were 47.95% (35/73), 52.05% (38/73) and 0 respectively, and those of XRCC1 Arg194Trp Arg/Arg, Arg/Trp and Trp/Trp were 53.42% (39/73), 39.73% (29/73) and 6.85% (5/73) respectively. The overall effective rate was 32.88% (24/73) after chemotherapy. The chemotherapy effective rates of the patients with ERCC1 118C/T and C/C genotypes were not significantly higher than those with C/T. The chemotherapy effective rates of the patients with XRCC1 Arg194Trp Arg/Arg, Arg/Trp and Trp/Trp genotypes were 15.38%, 51.72% and 60.00% respectively, which did not differ significantly. The effective rate of the patients with Trp allelic genome (Trp/Trp + Arg/Trp) was significantly higher (52.94%) than that of those with Arg/Arg genotype ($\chi 2>5$, $P<0.05$). XRCC1 Arg194Trp and ERCC1 118C/T polymorphisms were sensitive to chemotherapy interactively. The effective rates of the patients with XRCC1 Arg194Trp Arg/Arg and ERCC1 118C/T and C/C genotypes were merely 10.00%, whereas those of the patients with XRCC1 Arg194Trp and ERcc1 118C/T, C/C and C/T genotypes were apparently higher.

Conclusion: The polymorphisms of DNA repair enzyme genes ERCC1 118C/T and XRCC1 Arg194Trp are associated with the sensitivity of NSCLC to GP chemotherapy. The latter may be predicted by detecting the genotypes of patients.

Key words: Non-small-cell lung carcinoma, chemotherapy, gene, polymorphism.

Introduction

Non-small cell lung cancer (NSCLC) is the most common lung cancer, accounting for about 80% of all lung cancers. It mainly includes three categories, i.e. adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma, with poor sensitivity to traditional radiotherapy and chemotherapy [1]. Currently, gemcitabine (GEM)/cisplatin (DDP) regimen (GP) is one of the preferred clinical chemotherapies for the treatment of advanced NSCLC [2]. Studies have shown that the repair capabilities of individuals differ evidently after DNA damage [3]. These drugs barely function in case of strong cell DNA repair. Nucleotide excision repair pathway dominates the DNA damage induced by platinum drugs, in which excision repair cross-complementation 1 (ERCC1) is a key enzyme. Besides, X-ray repair cross-complementation 1 (XRCC1) is an important component in the DNA base excision repair/single-strand break repair system [4]. Studies have found that single nucleotide polymorphisms Asn118Asn (C→T) and Arg194 Trp exist in ERCC1 and XRCC1 genes respectively, which affect the mRNA expression and enzyme activity in vivo [5]. Therefore, studying the effects of the polymorphisms of ERCC1 and XRCC1 on NSCLC chemosensitivity may be of clinical significance in improving the safety and effectiveness of drug use. In this study, we investigated the relationship between the polymorphisms of ERCC1

118C/T and XRCC1 Arg194Trp and the chemosensitivity of NSCLC to GP regimen.

Materials and methods

Sample source

73 NSCLC patients pathologically confirmed in the Respiratory Medicine Department of our hospital from March 2009 to March 2012 were selected, including 56 males and 17 females, aged between 35 and 75 years old, with the median of 60 years old. There were 50 cases of adenocarcinoma, 15 cases of squamous cell carcinoma and 8 cases of other cancers. According to the international lung cancer staging system in 1988, there were 2 cases in phase II, 28 cases in phase III and 43 cases in phase IV. All cases were confirmed by CT diagnosis with measurable tumor lesions. Before chemotherapy, the blood routine and liver and kidney functions of the patients were normal, the electrocardiogram had indiscernible abnormality, and all functional status scores were higher than 60. 2 mL of venous blood was drawn from all cases before chemotherapy, and placed in a sodium ethylene diamine tetracetate anticoagulant tube to separate the leukocytes layer. Leukocyte DNA was extracted with QIAamp DNA extraction kit and stored in a -30 ℃ cryogenic refrigerator.

Genotype analysis

Under the informed consent of patients, 5 mL of venous blood was drawn before chemotherapy and blood clot was taken after standing to extract DNA which then was stored in a -30 °C cryogenic refrigerator for standby. ERCC1 and XRCC1 genotypes were detected by the PCR-RFLP method. For ERCC1 118C/T gene, the PCR primers were F: 5'-GGTGCAAGAAGAGGTGGAG-3', R: 5'-TCAGATCCCCAGGAGTCC-3', product 471 BP and endonuclease BsrD I; for XRCC1 Arg194Trp gene, the PCR primers were F: 5'-GCCAGGGCCCCTCCTTCAA-3', R: 5'-TACCCTCAGACCCACGAGT-3', product 485 BP and endonuclease Pvu II.

Treatment and efficacy evaluation standards

All patients were treated with DDP and GEM. 25 mg/m^2 DDP (Shandong Luoxin Pharmacy Stock Co., Ltd., National Medicine Permit No.) was infused intravenously on d1~d3. 1,000 mg/m^2 GEM (Harbin Gloria Pharmaceuticals Co., Ltd., State Drug Approval No.: H20063675, dosage form: powder injection, Specification: 1g) was infused intravenously on d1 and d8. For the above chemotherapies, every 3 weeks were considered as a period. The efficacy was evaluated two cycles after chemotherapy. With reference to the WHO evaluation standard for solid tumors in 1981, it is divided into complete remission (CR), partial remission (PR), no change (SD) and no progress (PD), CR + PR for effectiveness.

Statistical analysis

SPSS 16.0 software was used for statistical analysis. X^2 test or Fisher exact probability test (when the expected value <5) was adopted for the difference in the chemotherapy efficacy between different genotypes.

Results

General information

In the 73 patients with NSCLC after GP chemotherapy, there were 24 effective cases and 49 ineffective cases, with the total effective rate of 32.88%. No statistically significant difference was found in gender, age, cytological type, as well as chemotherapy effective rate of the patients with different clinical stages (Table 1).

Relationship between ERCC1 and XRCC1 genotypes and chemotherapy results

In all cases, the frequencies of ERCC1 118C/T, C/C, C/T and T/T genotypes were 47.95% (35/73), 52.05% (38/73) and 0 respectively, and the genotype frequencies of XRCC1 Arg194Trp Arg/Arg, Arg/Trp and Trp/Trp were 53.42% (39/73), 39.73% (29/73) and 6.85% (5/73) respectively. The chemotherapy effective rates of the patients with ERCC1 118C/T and C/C genotypes were not significantly higher than those with C/T. The chemotherapy effective rates of the patients with XRCC1 Arg194Trp Arg/Arg, Arg/Trp and Trp/Trp genotypes were 15.38%, 51.72% and 60.00% respectively, which did not differ significantly. The effective rate of the patients with Trp allelic genome (Trp/Trp + Arg/Trp) was significantly higher (52.94%) than that of those with Arg/Arg genotype ($\chi 2 > 5$, $P < 0.05$) (Table 2).

Relationship between ERCC1 118C/T and XRCC1 Arg194Trp polymorphic combination and efficacy

The chemosensitivities of ERCC1 118C/T and XRCC1 Arg194Trp polymorphisms interacted obviously. The efficient rate of the patients carrying ERCC1 118C/T, C/C and XRCC1 Arg194Trp Arg/Arg simultaneously was 10.00%, while that of the patients carrying both XRCC1 Arg194Trp and ERCC1 118C/T, C/C and C/T genotype was significantly elevated (Table 3).

Discussion

The action mechanism of platinum drugs is mainly that guanine, adenine and cytosine on DNA forms Pt-DNA adducts, resulting in DNA interstrand cross-linking or intrastrand cross-linking, causing DNA replication obstacle, thereby inhibiting tumor cell division [6]. GEM, with a chemical name of 2 ‘, 2’-difluoro deoxycytidine, is an antimetabolic anticancer drug [7]. In addition to the anti-tumor effect of itself, GEM also has synergistic effect with DDP, the mechanism of which may be related to that cytidine analogues can enhance the chimeric stability of DDP with DNA and GEM can inhibit DNA repair after DDP-induced damage [8]. Nevertheless, there is still a great difference in the chemotherapy sensitivity of different individuals to the GP regimen. XRCC1 can repair DNA damage caused by a variety of physical and chemical factors, including DDP through the action with poly ADP-ribose polymerase, DNA ligase III and DNA polymerase β [9].

Nucleotide C → T change takes place in codon of nucleotide excision repair enzyme ERCC1 118C /

Table 1. General information and treatment efficacy of NSCLC patients

Item		Case No.	Effective	Ineffective	χ^2_{MH}	*P* value
Gender	Male	56	19	37	<5	>0.05
	Female	17	5	12		
Age	<60	36	12	24	<5	>0.05
	≥60	37	12	25		
Cytological type	Adenocarcinoma	50	16	34	<5	>0.05
	Squamous-cell carcinoma	15	8	7		
	Others	8	0	8		
Clinical staging	II	2	0	2	<5	>0.05
	III	28	10	18		
	IV	43	14	29		
Total		73	24	49		

Table 2. Relationship between ERCC1 and XRCC1 genotypes and chemotherapy results

Item		Case No.	Effective (%)	Ineffective (%)	χ^2_{MH}	P value
ERCC1 118C/T	C/C	35	8 (22.86)	27 (77.14)	<5	>0.05
	C/T	38	16 (42.11)	22 (57.89)		
	T/T	0	0	0		
XRCC1 194	Trp/Trp	5	3 (60.00)	2 (40.00)	>5	<0.05
	Trp/Arg	29	15 (51.72)	14 (48.28)		
	Arg/Arg	39	6 (15.38)	33 (84.62)		

Comparison between the group with XRCC1 194 Trp and that with 194 Arg/Arg: $\chi^2>5$, $P<0.05$.

Table 3. Relationship between ERCC1 118C/T and XRCC1 Arg194Trp polymorphic combination and efficacy

ERCC1 118C/T	XRCC1 Arg194Trp	Case No.	Effective (%)	Ineffective (%)	χ^2	P value
C/C	Arg/Arg	20	2 (10.00)	18 (90.00)		
C/T	Arg/Arg	15	5 (33.33)	10 (66.67)		>0.05
C/C	Trp/Trp+Trp/Arg	11	6 (54.55)	5 (45.45)		<0.05
C/T	Trp/Trp+Trp/Arg	20	11 (55.00)	9 (45.00)	>5	<0.05

T, changing nucleotide ordering in codon from AAC to AA T. Although both are encoded aspartate, the change leads to reduced expression of ERCC1 gene, thus weakening the repair activity [10]. XRCC1 is an important component of the base excision repair/ single-strand break repair system, involved in the repair process of DDP-induced DNA damage [9]. The gene coding region has single nucleotide polymorphisms leading to amino acid substitutions, which affect XRCC1 activity [11].

It is found in researches on the susceptibility of a variety of tumors, such as breast cancer, gastric cancer and nasopharyngeal cancer that XRCCI genetic polymorphism is related to the increasing of tumor risk [12,13]. Strong DNA repair capacity of individual can reduce the risk of the occurrence of cancers, but the strong DNA repair capacity of cancer patients might reduce the susceptibility of chemotherapy [14]. Therefore, in recent years, there have been a lot of researches and reports at home and abroad on the relation between DNA repair enzyme genetic polymorphism and the susceptibility, toxicity and prognosis of cancer chemotherapy [15]. This study analyzed the relationship between ERCC1 118C/T codon, XRCC1 Arg194Trp codon polymorphisms and the sensitivity of NSCLC patients to GP chemotherapy, and found that the chemotherapy effective rate of patients carrying ERCC1 118C/T and C/T genotype is one time higher than that of patients carrying C/C genotype and the chemotherapy effective rate of patients carrying at least one XRCC1 Arg194Trp Trp allele was obviously higher compared with those carrying Arg/Arg genotype. In the genotype conjoint analysis, it was found that the chemotherapy effective rate of patients carrying ERCC1 118C/T and C/C and XRCC1 Arg194Trp Arg/Arg genotypes simultaneously was obviously reduced. This result suggests that there might be a certain joint action in the chemosensitivity between ERCC1 118C/T and XRCC1 Arg194Trp polymorphisms, which is consistent with the literature report [16]. In the study on the chemotherapy of advanced colorectal cancer, Noda et al. [17] found that the effective rate of oxaliplatin/52FU chemotherapy for patients carrying ERCC1 118C/T, T/T, C/T and C/C genotypes is partially the same with the result of this study. However, the research in foreign literature [18,19] on the white race show that the patients carrying ERCC1 118C/T T/ T genotype account for about 30-40%, while no T/T genotype was detected in this study, suggesting that there exists racial difference in ERCC1 118C/T polymorphism and that the distribution of ERCC1 118C/T T/ T genotype in the Chinese might be relatively low [20]. Osawa et al. [21] found that the genetic polymorphism of XRCC1 gene is associated with the sensitivity to platinum-based chemotherapy, which is consistent with the result of this study. Nevertheless, the object of this study only included the NSCLC patients receiving GP chemotherapy, which can rule out the interference of differences in efficacy of different chemotherapy regimens.

Thus, their findings may be more reliable and more practical in terms of the choice of chemotherapy regimens. In this study, PCR-RFL P technique was used to detect ERCC1 and XRCC1 genotypes in peripheral blood leukocyte DNA, with the simple and rapid characteristics. In the meantime, it does not require the collection of cancer tissue, which can overcome the difficulty that it is unable to obtain the cancerous tissue from patients not receiving surgical treatment, especially advanced cancer patients. Therefore, the study herein is of high clinical value.

References

1. *Liang S, Galluzzo P, Sobol A, Skucha S, Rambo B, Bocchetta M. Multimodality Approaches to Treat Hypoxic Non-Small Cell Lung Cancer (NSCLC) Microenvironment. Genes Cancer 2012; 3: 141-151.*

2. *Ren S, Zhou S, Wu F, Zhang L, Li X, Zhang J, et al. Association between polymorphisms of DNA repair genes and survival of advanced NSCLC patients treated with platinum-based chemotherapy. Lung Cancer 2012; 75: 102-109.*

3. *Wang JL, Wang PC. The effect of aging on the DNA damage and repair capacity in 2BS cells undergoing oxidative stress. Mol Biol Rep 2012; 39: 233-241.*

4. *Zhang X, Zhang L, Chen Q, Yang Z, Yu J, Fu H, et al. XRCC1 Arg399Gln was associated with repair capacity for DNA damage induced by occupational chromium exposure. BMC Res Notes 2012; 5: 263.*

5. *Liang J, Jiang T, Yao RY, Liu ZM, Lv HY, Qi WW. The combination of ERCC1 and XRCC1 gene polymorphisms better predicts clinical outcome to oxaliplatin-*

based chemotherapy in metastatic colorectal cancer. Cancer Chemother Pharmacol 2010; 66: 493-500.

6. Wu Z, Liu Q, Liang X, Yang X, Wang N, Wang X, et al. Reactivity of platinum-based antitumor drugs towards a Met- and His-rich 20mer peptide corresponding to the N-terminal domain of human copper transporter 1. J Biol Inorg Chem 2009; 14: 1313-1323.

7. Bergman AM, Adema AD, Balzarini J, Bruheim S, Fichtner I, Noordhuis P, et al. Antiproliferative activity, mechanism of action and oral antitumor activity of CP-4126, a fatty acid derivative of gemcitabine, in in vitro and in vivo tumor models. Invest New Drugs 2011; 29: 456-466.

8. Ledermann JA, Gabra H, Jayson GC, Spanswick VJ, Rustin GJ, Jitlal M, et al. Inhibition of carboplatin-induced DNA interstrand cross-link repair by gemcitabine in patients receiving these drugs for platinum-resistant ovarian cancer. Clin Cancer Res 2010; 16: 4899-4905.

9. Della-Maria J, Hegde ML, McNeill DR, Matsumoto Y, Tsai MS, Ellenberger T, et al. The Interaction between Polynucleotide Kinase Phosphatase and the DNA Repair Protein XRCC1 Is Critical for Repair of DNA Alkylation Damage and Stable Association at DNA Damage Sites. J Biol Chem 2012; 287: 39233-39244.

10. Zhang YW, Regairaz M, Seiler JA, Agama KK, Doroshow JH, Pommier Y. Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells. Nucleic Acids Res 2011; 39: 3607-3620.

11. Hanssen-Bauer A, Solvang-Garten K, Sundheim O, Pena-Diaz J, Andersen S, Slupphaug G, et al. XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage. Environ Mol Mutagen 2011; 52: 623-635.

12. Chen B, Zhou Y, Yang P, Wu XT. Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis. Mol Biol Rep 2012; 39: 1305-1313.

13. Dai L, Duan F, Wang P, Song C, Wang K, Zhang J. XRCC1 gene polymorphisms and lung cancer susceptibility: a meta-analysis of 44 case-control studies. Mol Biol Rep 2012; 39: 9535-9547.

14. Xu C, Wang X, Zhang Y, Li L. [Effect of the XRCC1 and XRCC3 genetic polymorphisms on the efficacy of platinum-based chemotherapy in patients with advanced non-small cell lung cancer]. Zhongguo Fei Ai Za Zhi 2011; 14: 912-917.

15. Adhikari S, Choudhury S, Mitra PS, Dubash JJ, Sajankila SP, Roy R. Targeting base excision repair for chemosensitization. Anticancer Agents Med Chem 2008; 8: 351-357.

16. Parker KA, Glaysher S, Polak M, Gabriel FG, Johnson P, Knight LA, et al. The molecular basis of the chemosensitivity of metastatic cutaneous melanoma to chemotherapy. J Clin Pathol 2010; 63: 1012-1020.

17. Noda E, Maeda K, Inoue T, Fukunaga S, Nagahara H, Shibutani M, et al. Predictive value of expression of ERCC 1 and GST-p for 5-fluorouracil/oxaliplatin chemotherapy in advanced colorectal cancer. Hepatogastroenterology 2012; 59: 130-133.

18. Khrunin A, Ivanova F, Moisseev A, Khokhrin D, Sleptsova Y, Gorbunova V, et al. Pharmacogenomics of cisplatin-based chemotherapy in ovarian cancer patients of different ethnic origins. Pharmacogenomics 2012; 13: 171-178.

19. Jones NR, Spratt TE, Berg AS, Muscat JE, Lazarus P, Gallagher CJ. Association studies of excision repair cross-complementation group 1 (ERCC1) haplotypes with lung and head and neck cancer risk in a Caucasian population. Cancer Epidemiol 2011; 35: 175-181.

20. He SY, Xu L, Niu G, Ke PQ, Feng MM, Shen HW. Predictive value of excision repair cross-complementing rodent repair deficiency complementation group 1 and ovarian cancer risk. Asian Pac J Cancer Prev 2012; 13: 1799-1802.

21. Osawa K. Gene Polymorphisms and Chemotherapy in Non-small Cell Lung Cancer. Zhongguo Fei Ai Za Zhi 2009; 12: 837-840.

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Effects of different aerobic programs on manifestation of strength

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Abstract

The research problem is to determine the differences between the effects of two popular aerobic programs for students of the Faculty of Sport and Physical Education, with regard to certain aspects of the manifestation of strength. The appropriate sample consisted of 149 male students of the first year of the Faculty of Sport and Physical Education in Novi Sad, who regularly attended classes of Anthropomotorics, average age 19 years. The participants were divided into two experimental groups (one, N = 52, which performed physical practices following the model of high-low aerobics and the other, N = 54, which has practiced following the model of step aerobics) and a control group (N = 43), which followed the regular curriculum for the course of Anthropomotorics on the first year of studies. The changes that have occurred in students of the experimental and control groups, under the influence of the experimental program of aerobics and the program of control group, in duration of six weeks (three times weekly), were monitored through fourteen measuring instruments for assessment of strength, as follows: five for the assessment of explosive strength, five for the assessment of repetitive strength and four for assessment of the static force.

The research results showed that there were no statistically significant differences between the two experimental groups, while there were significant effects found in the transformation of all aspects of strength manifestation in both experimental groups compared to the control group, noting that the experimental program of step aerobics had more adequate effects on the improvement of the lower extremities explosive strength. All this indicates that the experimental programs of high-low and step aerobics had a significant impact on all aspects of the strength manifestation.

Key words: Aerobics, students of the Faculty of Sport and Physical Education, strength, high-low aerobics, step aerobics.

Introduction

Physical activity in the form of a designed physical exercise has a exceptional positive effect on human mental and physical health. In searching for solutions to accumulated problems of modern lifestyle, fitness movement offers three groups of programs. One group is based primarily on dealing with stress and therefore emphasizes the strength in the broadest sense. The second group consists of various toning and anti-stress programs based on flexibility of body, while the third consists of programs that are based on working on aerobic endurance. This third group of programs has different forms, but they all can be summed under a generally accepted term – *aerobics*.

Today there are many types of aerobics, and certainly the two most popular types are high-low and step aerobics. The experimental programs, high-low and step aerobics, could be defined as programs for developing of aerobic abilities, through various movement structures that affect coordination (walking, running, hoping and jumping in all directions, and their integration into the dance steps and different coordinating-rhythmic units), while satisfying the esthetic criteria [1-5]. This definition should be complemented with strength and flexibility development, since the work on aerobic endurance and coordination on the aerobics classes continues through strengthening exercises and exercises for flexibility development (stretching).

By analyzing the previous research on aerobics one can conclude what was the most common research subject. First, research investigated the impact of aerobic exercise on body image dissatisfaction and physical self-perceptions [6, 7]. Then, its effects on body composition and bone density were studied [8-10]. Furthermore, there are studies and functional and motor abilities [11-17], which are probably the most numerous. In studying the effects of exercise on motor skills a number of papers dealt with the effects of aerobic exercise on strength [18, 19]. All such studies were performed on women. A smaller number of papers in the sample combine both women and men [20-22] or only men [23-26]. The smallest number of papers dealt with the effects of different aerobic programs in men [27-29]. It is the effects of two different aerobic programs in men which are subject of interest in this paper and the results are supposed to contribute to the knowledge about them and aerobics in general.

The subject of the research are the effects of two aerobics programs on strength transformation in the students of the Faculty of Sport and Physical Education.

Method

The research was operational, longitudinal and quasi-experimental (since the sample was not random) with parallel groups. When the randomization is not possible… the solutions are in the quasi-experimental designs [30]. The procedure of measurement was conducted on a sample (N=149) of male students of the first year of the Faculty of Sport and Physical Education in Novi Sad, mean age 19 years. Students were divided into three groups. During the application of the experimental program the first experimental group (E1) was working on high-low aerobics exercise program (N=52), the second experimental group (E2) was working on step aerobics exercise program (N=54) and control group (K) had regular classes according to the curriculum of the Faculty of Physical Education's I (first) year studies for the subject of Anthropomotorics (N = 43). In programes of high-low and step aerobics, which are operated by the experimental groups, were elements of the syllabus of first year studies for the subject of Anthropomotorics, but, essentially, experimental groups were not working according to the program of Anthropomotorics. The participants, male students, were involved in the experiment without direct systematic examination, because the same was done during the enrolment on the Faculty.

Starting from the mentioned subject of research, the following assessment tests were performed: explosive strength tests (Standing broad jump, Standing high jump (vertical jump), Standing triple broad jump, Lying medicine ball throw and Seated medicine ball throw), repetitive strength tests (Pushups, Pull-ups, Lifting the trunk for 30 seconds, Correcting the trunk and Deep squat for 30 seconds) and the static force tests (Endurance in pushup, Hang in pull-up (bent arm hang), Back extension endurance test and the Half-squat endurance). Tests for strength assessment were applied according to the proven standardization and measurements techniques [31-34].

Strength testing was organized using the principle of workplaces. In one workplace there were no more than 10 participants. Workplace change was always performed on a sign given by the testing coordinator. Prior to commencement of any task, each group of participants was familiarized with the manner of its execution. The entire survey was organized by a team of trained measurers during the period from February-May 2006, and lasted 8 weeks. The experiment itself lasted 6 weeks. During that period, experimental programs, and a program performed by a control group, were carried out three times a week (Mondays, Wednesdays and Fridays) lasting one school class, during the morning hours and in a rhythmic hall of the Faculty. Rhythmic hall was desirable because it had mirrors, which are usually necessary for the aerobic exercises to music. The total number of 18 classes was completed in the experimental programs, and 18 hours of regular classes for the control group (K). The realization of the experimental programs involved a number of trained associates – instructors of high-low and step aerobics. Basic features of the experimental groups curriculum were as follows:

- at the beginning of each lesson a kind of music that is used during the lesson was chosen,
- each educational unit was divided into three parts – introduction (I), main (II) and the final part of the class (III),

- in the introductory part of the class (I) the basic steps and simple movement structures were elaborated, designed for the preparation of those muscle groups that will be most involved in the main part of the class,
- in the aerobic part of the main part of the class (II) a prepared choreography for a given class was practiced; choreography record contains the number of music blocks, the number of musical phrases (eights), description of the steps (leg movements), the direction of movement, the focus (i.e. the body position of the exerciser relative to the direction of movement) and movements of the hands,
- within the final part of the class (III) stretching exercises were performed - stretching by Andersen method,
- work on programs of high-low and step aerobics has been intensified from class to class, going from the beginning to the end of the program.

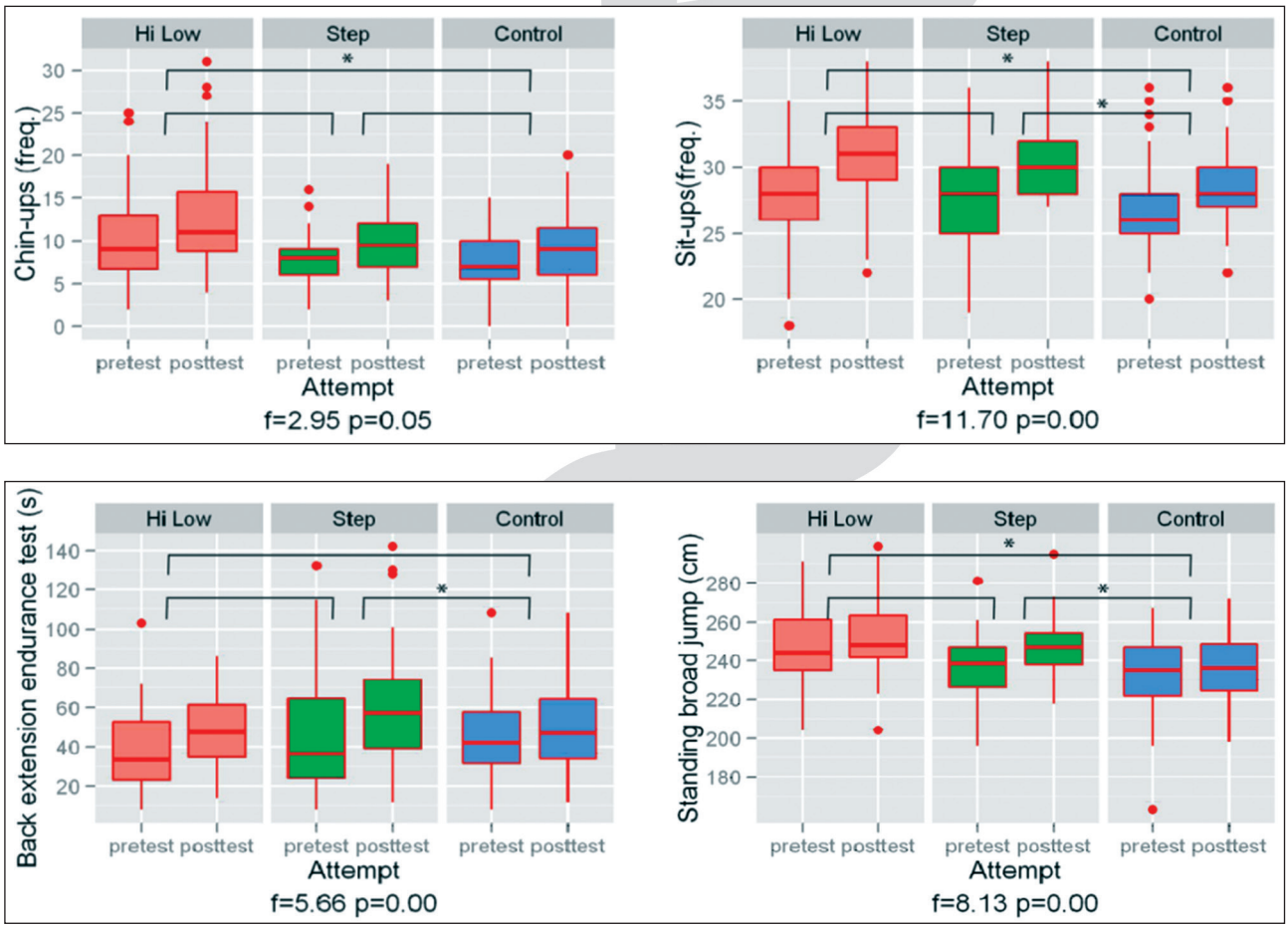
Data analysis

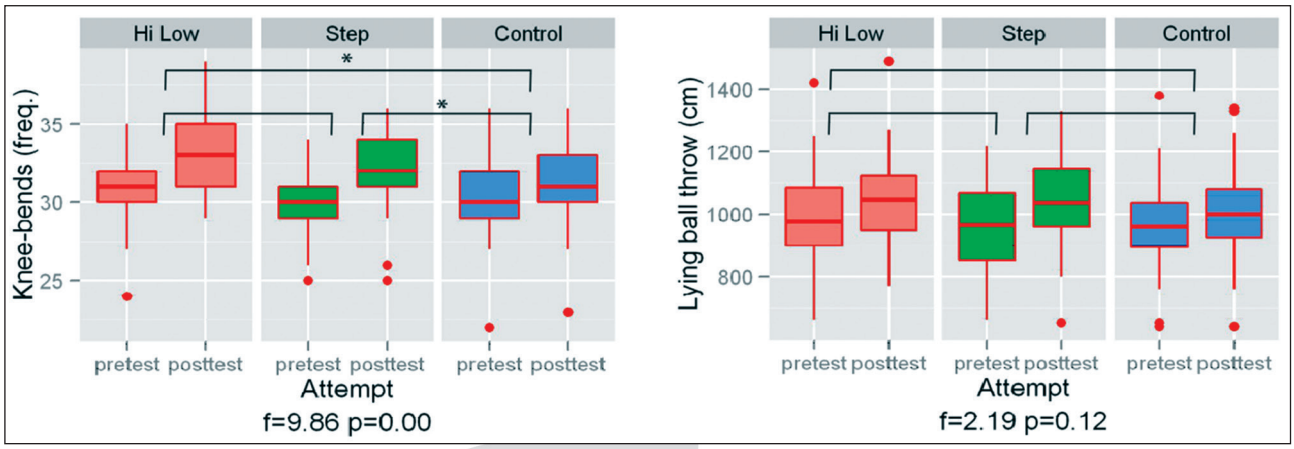
Differences between groups after the neutralization possible pretested differences were calculated by univariate analysis of covariance (ANCOVA). In addition, Bonferroni corrected t-tests were used as post hoc tests in cases when analysis of covariance (ANCOVA) f ratios were significant. All results were obtained with "R" statistic software, open source stat program, which could be downloaded completely free from: http://www.r-project.org/. Because the authors were chosen to present results on a little bit unusual way (not on classic – in tables), box-plot form as a part of graph gallery were very appropriate for solving these problem. ggplot2, as a one of the famous library in "R", was used for making box-plots.

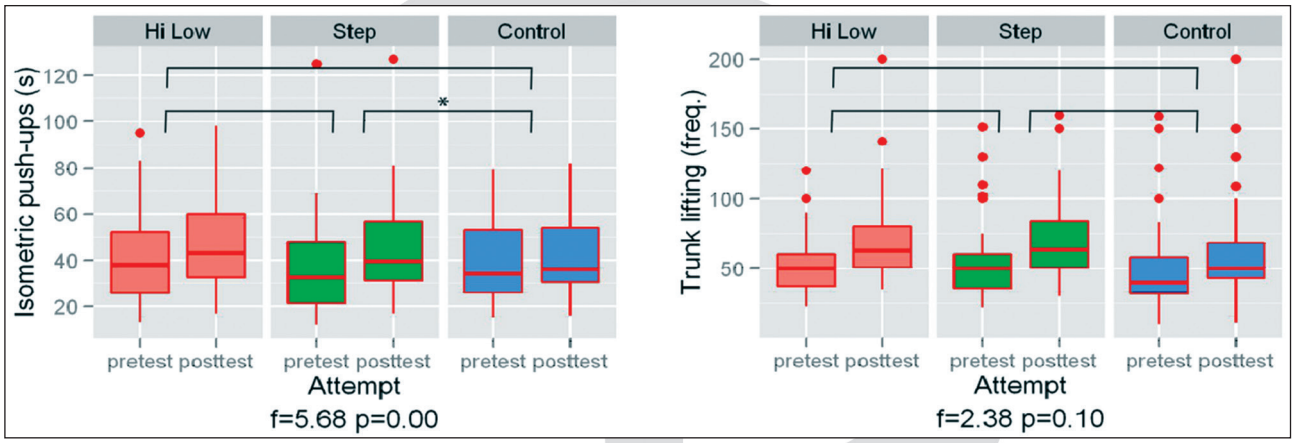
Results

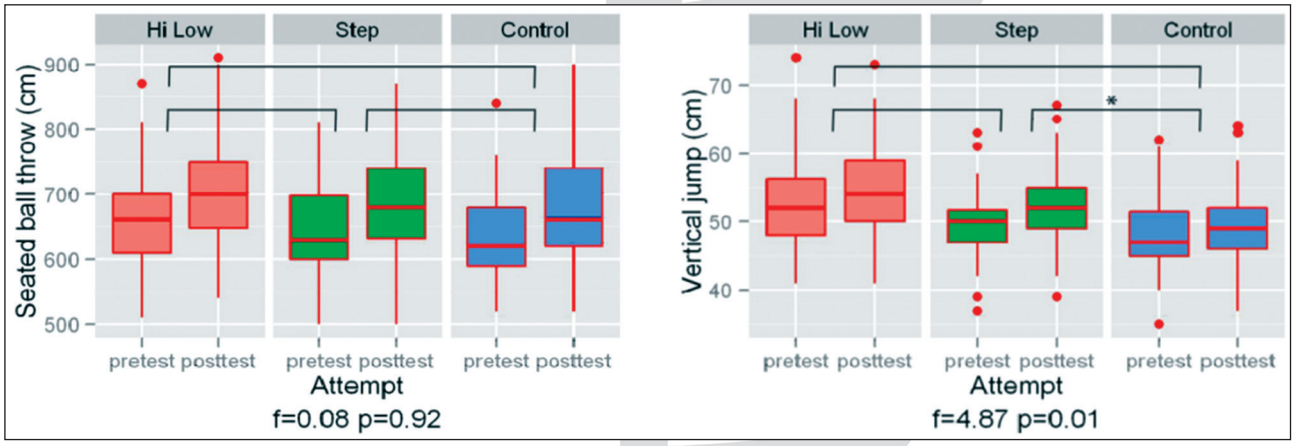
All calculated results are presented with box-plots just as follows (Figure 1):

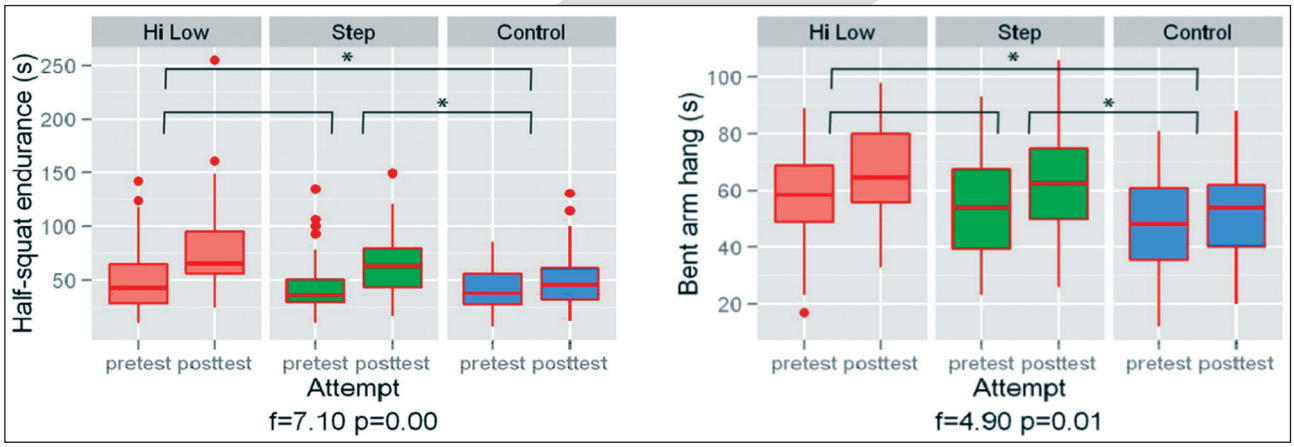
Comparing the presented box-plots one can observe that there were no statistically signifi-




Hi Low
Step
Control
Knee-bends (freq.)
35
30
25
pretest posttest
Attempt
f=9.86 p=0.00
Lying ball throw (cm)
1400
1200
1000
800
f=2.19 p=0.12


Hi Low
Step
Control
Isometric push-ups (s)
120
100
80
60
40
20
pretest posttest
Attempt
f=5.68 p=0.00
Trunk lifting (freq.)
200
150
100
50
f=2.38 p=0.10


Hi Low
Step
Control
Seated ball throw (cm)
900
800
700
600
500
pretest posttest
Attempt
f=0.08 p=0.92
Vertical jump (cm)
70
60
50
40
f=4.87 p=0.01


Hi Low
Step
Control
Half-squat endurance (s)
250
200
150
100
50
pretest posttest
Attempt
f=7.10 p=0.00
Bent arm hang (s)
100
80
60
40
20
f=4.90 p=0.01

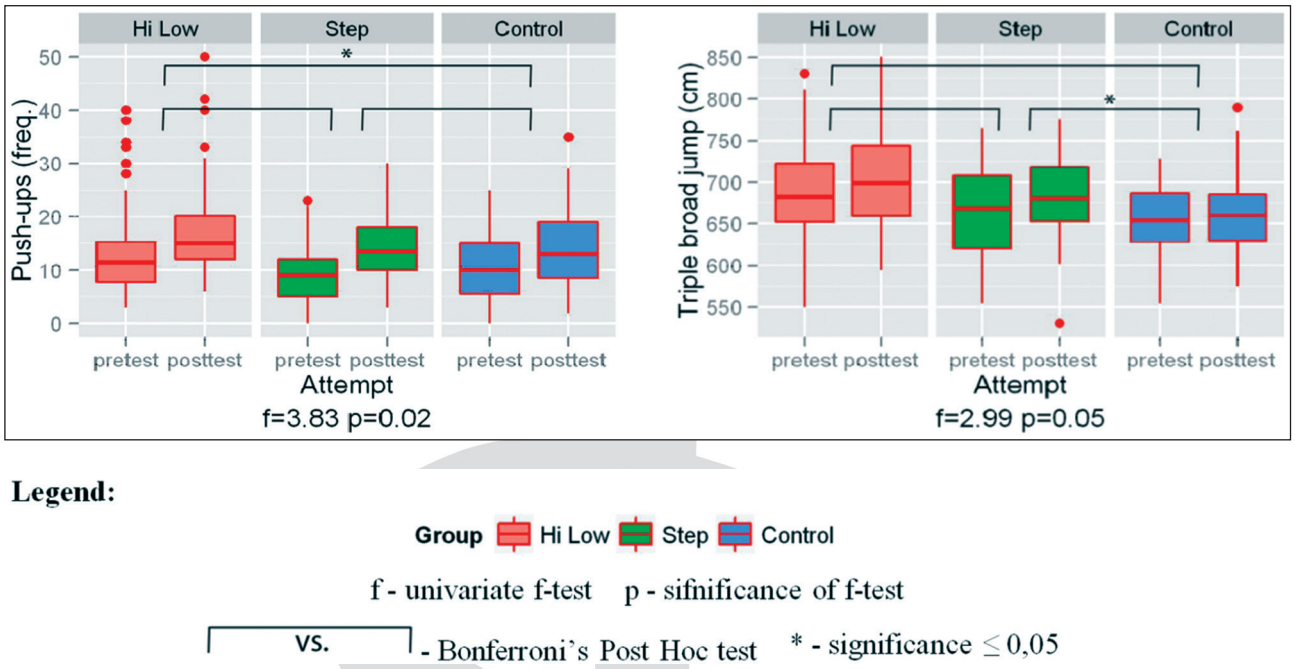


Figure 1. Differences between groups after the treatment

cant differences between the experimental groups which followed different aerobics programs.

Differences between the groups of participants who exercised following the model of high-low aerobics and control group were found in favor of high-low program in the following tests for assessment of: explosive strength (Broad jump), repetitive strength (Pull-ups, Trunk lifting for 30 seconds, Deep squat for 30 seconds, Push-ups) and the static force (Half-squat endurance and Bent arm hang).

On the other hand, differences between groups of subjects who trained following the model of step aerobics and control group were found in favor of step aerobics program in the following tests for assessment of: explosive strength (Broad jump, Vertical jump, Triple broad jump), repetitive strength (Deep squat for 30 seconds, Trunk lifting for 30 seconds) and the static force (Bent arm hang, Half-squat endurance, Pushup endurance, Back extension endurance).

Discussion

Based on the interpreted research results, significant effects in the transformation of strength in both experimental groups were observed, but not in the control group (K).

The experimental program, using high-low aerobics, which was followed by the first experimental group (E1), had adequate effects on improving all aspects of strength compared to the control group (K). High-low aerobics is an exercise program that is based on dance aerobics. Movements and motions within this program can be done standing or moving (forward, backward, sideways, with a twist), in different planes and different amounts of time [13, 28]. Previous research [18, 22] determined the effect of high-low aerobics to all forms of strength, which was confirmed by this study. Some studies exclude the impact of high-low aerobics on a static force [19, 24], but this can be attributed to not-using a sufficient number of tests that cover this kind of strength. The complexity of movement structures of high-low aerobics, involving the use of steps of low (one foot is in constant contact with the ground) and high (presence of the flight phase) intensity, steps in endurance, as well as dance steps, certainly influenced the improvement of all aspects of strength.

Experimental program of the step aerobics, which was followed by the second experimental group (E2), also had an adequate impact on improving all aspects of strength compared to the control group (K). Step aerobics is characterized by the use of a stepper (of 15, 20 or 25cm in height), with alternately climbing up and down from it, with the use of various movement structures. During these movements, the muscles of the gluteal region and legs are primarily engaged, and to a lesser extent,

the muscles of arms and shoulders. This is the kind of plyometric training. If we take into account all of the above mentioned, the results on the improvement of explosive strength of lower limbs become logical and expected, which was confirmed by numerous studies [3, 4, 13, 23, 28, 35]. Some authors also mention step aerobics as a means to improve the repetitive force of leg muscles and abdominal muscles [20, 24], which was confirmed in this study. The movement structures in the step aerobics, which involve climbing up and down the stepper, require constant flexion and extension in the joints of the legs, leading to improvements in repetitive strength of the legs and hip joints, leading to improvements in repetitive strength of abdominal muscles. Variability of the repetitive strength and static force dimensions falls under the same mechanism for regulating the duration of excitation [31], which explains the improvement in all static force assessment tests for the experimental group which followed the model of step aerobics in comparison to control group.

The results on the impact of high-low and step aerobics on improvement of all aspects of strength can be seen in papers whose authors dealt with similar studies [13, 19, 22, 28, 35]. During the aerobics class explosive strength is the most dominant form of strength manifestation. This is because the class involves numerous jumps, hops, turns and other similar forms of movement. During the class there is a need for repetitive and static strength of all body segments. The higher strength level in all three forms of its manifestation guarantees a higher quality of work.

Conclusion

Generally, the proven impact of high-low and step aerobics programs on all aspects of strength, recommends these models of training also in the work with the male population, where it can be effectively implemented, as well as with women. Also, aerobics as an activity used primarily in work with amateurs, can be applied in the same way in the work with individuals of above-average motor skills, and students of physical education. Finally, we should note better results in all tests of explosive strength for the group which followed the step aerobics program compared to the control group, which recommends this program as more adequate, compared to the high-low aerobics, if we want to influence the development of explosive strength in male population with above-average motor skills.

References

1. *Bergoč Š, Zagorc M. Metodika učenja pri aerobiki. Ljubljana: Fakulteta za šport, Inštitut za šport, 1999.*
2. *Bergoč Š, Zagorc M. Metode poučevanja v aerobiki. Ljubljana: Fakulteta za šport, Inštitut za šport, 2000.*
3. *Brick LG. Fitness Aerobics. Champaign, IL: Human Cinetics, 1996.*
4. *Pillarella D, Roberts S. Fitness Stepping. Champaign, IL: Human Cinetics, 1996.*
5. *Zagorc M, Zaletel P, Ižanc N. Aerobika. Ljubljana: Fakulteta za šport, Inštitut za šport, 1998.*
6. *Asci FH. The effects of physical fitness training on trait anxiety and physical self-concept of female university students. Psychology of Sport and Exercise, 2003; 4: 255-264.*
7. *Burgess G, Grogan S, Burwitz L. Effects of a 6-week aerobic dance intervention on body image and physical self-perceptions in adolescent girls. Body Image: An International Journal of Research, 2005; 3: 57-67.*
8. *Arslan F. The effects of an eight-week step-aerobic dance exercise programme on body composition parameters in middle-aged sedentary obese women. International SportMed Journal, 2011; 12 (4): 160-168.*
9. *Behestani MM. Effects of a 12-week aerobic exercise on back spine and thigh bone mineral density in heavy women after menopause. HealthMED, 2012; 6 (5): 1667-1673.*
10. *Kostic R, Djuraskovic R, Miletic Dj, Mikalacki M. Changes in the cardiovascular fitness and body composition of women under the influence of aerobic dance. Facta Universitatis, Series: Physical Education and Sport, 2006; 4 (1): 59 – 71.*
11. *Clary S, Barnes C, Bemben D, Knehans A, Bemben M. Effects of ballates, step aerobics and walking on balance in women aged 50-75 years. Journal of Sports Science and Medicine, 2006; 5: 390-399.*
12. *La Forge R. World current trends in exercise science research 2000. California, Anaheim: World 2000 Idea, Convention guide, 2000.*
13. *Mandarić S. Efekti programiranog vežbanja uz muziku kod učenica sedmih razreda osnovne škole. Doktorska disertacija. Beograd (Srbija). Univerzitet u Beogradu, Fakultet sporta i fizičkog vaspitanja, 2003.*

14. *Obradović J. Motorička analiza nekih motoričkih struktura sportskog aerobika. Doktorska disertacija. Novi Sad (Srbija). Univerzitet u Novom Sadu, Fakultet fizičke kulture, 2004.*

15. *Srhoj Lj, Katic R, Kaliterna A. Motor abilities in dance structure performance in female students. Collegium Antropologicum, 2006; 30 (2): 335-341.*

16. *Zivanović N, Andrasic S, Cirić M, Cosić V. The difference in VO2max between soccer and handball federation rank players as shown in the cooper test. Research in kinesiology, 2009; 37 (2): 355-357.*

17. *Zivanović N, Palic R, Stankovic V, Ciric M, Andrasic S. Effects of speed endurance test on the levels of cortisol and testosterone in handball players. Journal of Physical Education and Sport, 2011; 13 (1): 31-34.*

18. *Mandaric S, Sibinovic A, Stojiljkovic S. Affects of a high-low aerobic program on the morphological features, functional and motor abilities of female elementary school eighth graders. Facta Universitatis, Series: Physical Education and Sport, 2011; 9 (3): 307-319.*

19. *Viskic-Stalec N, Stalec J, Katic R, Podvorac F, Katovic D. The impact of dance-aerobics training on the morpho-motor status in female high-schoolers. Collegium Antropologicum, 2007; 31 (1): 259-266.*

20. *Chia-Lin L, Hsu-Min T, Fang-Rowe T, Shwn-Jen L. The effectiveness of an aerobic exercise intervention on worksite health-related physical fitness – A case in a high-tech company. Chang Gung Medical Journal, 2006; 29: 100-106.*

21. *Pitsavos C, Panagiotakos DB, Tambalis KD, Chrysohoou C, Sidossis LS, Skoumas J, Stefanadis C. Resistance exercise plus to aerobic activities is associated with better lipids' profile among healthy individuals: the ATTICA study. Q J Med, 2009; 102: 609–616.*

22. *Sibinovic A, Mandaric S, Mikalacki M, Stojiljkovic S. Effects of high and low aerobics programme on motor abilities of the eighth grade elementary school students. 6Th Fiep European Congress, 2011.*

23. *Cvetkovic M, Kovacevic R, Jaksic D, Matic R. The effects of step-aerobics on the explosive strength. 4. Conditional preparation of the athletes, Zagreb: Faculty of Kinesiology, University of Zagreb, the Association of fitness trainers of Croatia, 26-27 February, 2010; 439-442.*

24. *Okuneye R, Adeogun J, Idowu I. The effects of a six-week aerobic dance programme on selected fitness components and waist-hip-ratio in adult males. Sierra Leone Journal of Biomedical Research, 2010; 2(1): 17-22.*

25. *Radjo I, Talovic A, Solakovic E, Kudumovic A, Mujakovic A, Mahmutovic I, Celik D, Manic G. Effects of static and dynamic training on the cardiovascular system. HealthMED, 2011; 5 (4): 965-970.*

26. *Šebić-Zuhrić L, Manić G, Bonacin D, Hmjelovjec I. Relacije bazično motoričkih sposobnosti i stilizovanih kretnih struktura u muškoj ritmičkoj gimnastici. Homo Sporticus, 2008; 1: 18-21.*

27. *Cvetković M. Efekti različitih programa aerobika kod studenata fakulteta sporta i fizičkog vaspitanja. Doktorska disertacija. Novi Sad (Srbija). Univerzitet u Novom Sadu, Fakultet sporta i fizičkog vaspitanja, 2007.*

28. *Cvetković M. Efekti aerobika u transformaciji morfoloških karakteristika kod studenata fakulteta fizičke kulture. Glasnik Antropološkog društva Srbije, 2009; 44: 109-121.*

29. *Cvetković M. Efekti aerobika u transformaciji karakteristika telesnog sastava kod studenata fakulteta fizičke kulture. Glasnik Antropološkog društva Srbije, 2009; 44: 123-132.*

30. *Savić M. Implementacija statističkih metoda u planiranju eksperimenata u ekonomskim istraživanjima. Doktorska disertacija. Subotica (Srbija). Univerzitet u Novom Sadu, Ekonomski fakultet u Subotici, 2003.*

31. *Kurelić N, Momirović K, Stojanović M, Šturm J, Radojević Đ, Viskić-Štalec N. Struktura i razvoj morfoloških i motoričkih dimenzija omladine. Beograd: Institut za naučna istraživanja fakulteta za fizičko vaspitanje, 1975.*

32. *Metikoš D, Prot F, Hofman E, Pintar Ž, Oreb G. Mjerenje bazičnih motoričkih dimenzija sportaša. Zagreb: Fakultet za fizičku kulturu, 1989.*

33. *Oja P, Tuxworth B. Eurofit for adults – Assessment of health-related fitness. Finland, Tampere: Counsil of Europe, Committee for the development of sport and UKK Institute for health promotion research, 1995.*

34. *Zaciorski VM. Fizička svojstva sportiste. Beograd: NIP Partizan, 1975.*

35. *Sekulić D. Mogućnost primjene modificiranog programa u treningu eksplozivne snage tipa skočnosti (Zbornik radova). Zagreb: Fakultet fizičke kulture i Zagrebački športski savez, 1997.*

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Dynamic changes of biochemical indices and prognosis of insulin therapy in burn shock resuscitation

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Abstract

Purpose: This study aims to conduct a preliminary exploration on the application value and prognosis of the insulin therapy in the burn shock resuscitation by observing the impact of insulin on the shock resuscitation fluid requirement, urine output and organ function of patients with major burns during the shock stage.

Methods: Fifty-eight patients with major burns including 31 males and 27 females admitted to the Burn Department of our hospital during the period from February 2012 to December 2012 were randomly arranged in an insulin treatment group and a control group, each consisting of 29 patients. The initial resuscitation fluid replacement was calculated based on the "Ruijin formula", i.e., the total volumes of the colloid (plasma) and crystal (lactated Ringer's solution) were calculated on a 1.5ml/kg/1% TBSA basis, and the ratio of crystal to colloid was between 1:1 and 2:1. The shock resuscitation indicators and platelet changes were tested.

Results: The unit colloid volume of the treatment group in the first 24h was (0.98±0.29) ml/kg/1%TBSA, which was significantly lower than (1.16±0.32) ml/kg/1%TBSA in the control group. Meanwhile, the unit urine outputs during the shock stage for the treatment group and the control group were (1.90±0.68) ml/kg/h and (1.37±0.61) ml/kg/h respectively. The treatment group showed significantly higher unit urine output than the control group. The differences between the two groups were statistically significant (P<0.05). The TBIL, DBIL, BUN and Cr levels between the patients in the two groups had no significant difference one day after burn. Since day 2 after burn, the treatment group showed lower levels of these indicators than the control group at various time points, and the difference had statistical significance (P<0.05) at most time points. Platelet counting of the control group was higher than the treatment group on days 3, 6, 9 and 12. The platelet counting between the two groups showed statistical significance (P<0.05). The APACHE II score of the control group (6.49 ± 2.18) was significantly lower than that of the treatment group (16.25 ± 3.81) in the first 24h after the admission. The APACHE II scores of the two groups had statistical significance (P<0.05).

Conclusion: The application of insulin during the shock stage can reduce the colloid / crystal fluid requirement for resuscitation and increase the urine output of the patient with major burns. This therapy can also extenuate tissue damage and protect organ function. The study shows potential promise of the application of insulin in the shock resuscitation from severe burns.

Key Words: Insulin, burn shock, biochemical index, dynamic change, clinical analysis.

Introduction

Systemic inflammatory response syndrome (SIRS) is a common complication occurring on severely burned patients. This syndrome can further develop into multiple organ dysfunction syndrome (MODS) or even lead to multiple organ failure and shock, which are the main cause for death in patients with severe burns. Burn shock is one of the causes of early death in the burn patients, and the treatment during the shock stage of the severe burn is of vital importance. The smooth transition of this stage will create good conditions for future wound treatment and reduce the occurrence of sepsis or multiple organ failure.[1-3]

Since the non-diabetic efficacy of insulin was discovered in early 1920s, there have been many studies exploring the regulation and mechanism of insulin on various cytobiological behaviors throu-

gh non-metabolic means. Multiple stages of wound healing are involved in the role of insulin in regulating the wound repair. Insulin can stimulate the proliferation and migration of the epidermal keratinocytes and vascular endothelial cells so as to promote re-epithelialization and angiogenesis and the wound healing. Insulin can also directly regulate systemic and wound local inflammatory response.[4-6] It has been found by some researchers that insulin is also involved in the regulation of vascular permeability. The role of insulin in regulating the wound inflammatory response and vascular permeability may be helpful in the shock resuscitation of patients with major burns.[7]

By observing the impact of insulin application on the fluid volume required for resuscitation and the urine output and organ function of the patients with major burns during the shock stage, this study aims to conduct a preliminary exploration on the application value of insulin therapy in the resuscitation from burn shock, determine the severity of the major burns and its significance in the prognosis.

Materials and Methods

Subjects

Fifty-eight patients aging between 16 and 60 with major burns including 31 males and 27 females admitted to the Burn Department of our hospital during the period from February 2012 to December 2012 were enrolled for this study. Their total burn areas were between 10% and 80% TBSA, and their third degree burn areas ranged between 30% and 70% TBSA. The 58 patients were randomly arranged in an insulin treatment group and a control group, each consisting of 29 patients.

Excluded cases: patients who had severe primary cardiac, hepatic, renal or endocrine diseases (including diabetes); burns combined with inhalation damage and had been subject to tracheotomy; or burns due to electric shock, explosion, chemical poisoning and other special causes; and delayed resuscitation patients with evident shock symptom upon admission.

Fluid replacement

The initial resuscitation fluid replacement was calculated based on the "Ruijin formula", i.e., the total volumes of the colloid (plasma) and crystal (lactated Ringer's solution) were calculated on a 1.5ml/kg/1% TBSA basis, and the ratio of crystal to colloid was between 1:1 and 2:1. The initial resuscitation fluid replacement was calculated based on the "Ruijin formula", i.e., the total volumes of the colloid (plasma) and crystal (lactated Ringer's solution) were calculated on a 1.5ml/kg/1% TBSA basis, and the ratio of crystal to colloid was between 1:1 and 2:1. During the resuscitation stage, the volumes of the colloid and crystal fluids were adjusted according to the vital signs and urine output of the patients. The target was to ensure that the patients were conscious and quiet with warm limbs, well filled peripheral circulation and urine output of 1-2 ml/kg. The crystal and colloid fluid volumes during the second 24 h were half of the actual intake during the first 24 h; and the colloid of the same volume as the second 24 h was given and no crystal was infused in the third 24 h.[8]

In addition to the above crystal and colloidal fluids, 3,000 ml/d of water should be supplemented on a daily basis. The water supplementation may be increased to 5,000 ml/d according to the body weight of the patient, the ambient temperature and humidity and other conditions. The treatment group was given water supplementation in the form of GIKC solution, which was prepared with 1,000 ml of 0.9% NaCl solution + 30u of regular insulin + 10 ml of 10% KCl solution + 1 g of vitamin C; while the water supplementation of the control group was given as 0.9% NaCl solution with the same amount of vitamin C, i.e., 1 g of vitamin C per 1,000 ml of NaCl solution. There was no difference in other aspects of the treatments for the two groups.

Shock resuscitation indices

Record the volumes of the actually infused colloid and crystal of the patient in the first 24 h, and calculate the unit colloid (or crystal) volume of the first 24 h according to the body weight and burn area: unit colloid (or crystal) volume of the first 24 h (ml/kg/1% TBSA) = volume of the actually infused colloid (or crystal) in the first 24 h (ml)/body weight (kg)/1% TBSA.

Record the urine output of the first 24 h and the total urine output in the shock stage (72 h after burn), and calculate the unit urine output: unit urine output of the first 24 h (ml/kg/h) = urine output of the first 24 h (ml)/body weight (kg)/24 (h); unit uri-

ne output in the shock stage (ml/kg/h) = urine output in the shock stage (ml)/body weight (kg)/72 (h).

Biochemical indices

Record the hematocrit indicators in the routine blood tests as well as liver and kidney function related biochemical indicators of the two groups on days 1, 2 and 3, including: albumin, prealbumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin (TBIL), direct bilirubin (DBIL), blood urea nitrogen (BUN), and creatinine (Cr).

Platelet counting

Test the platelet counting of the peripheral venous blood on the day of admission, and monitor the change of platelet counts at 5 time points, i.e., on days 1, 3, 6, 9 and 12 after the admission. Record as well the poorest value of the physiological indicators in the first 24 h after admission (the higher the score, the worse the illness state), and calculate the APACHE II score accordingly.

Statistical analysis

All the data were analyzed by SPSS 12.0. The measurement data were expressed as (±s). The groups were compared by the non-paired t-test. $P<0.05$ was considered statistically significant.

Results

General data

The two groups had no statistically significant difference ($P>0.05$) in sex ratio, age composition, body weight, burn area and third degree burn area (Table 1).

Comparison between shock resuscitation indices

The treatment group showed a first 24 h unit crystal volume of (1.02±0.37) ml/kg/1%TBSA, which was lower than that of the control group (1.08±0.41) ml/kg/1%TBSA. Meanwhile, the treatment group showed a first 24 h unit urine output of (1.30±0.59) ml/kg/h greater than that of the control group (1.26±0.62) ml/kg/h. No significant difference ($P>0.05$) was found between the two groups. The unit colloid volume of the treatment group in the first 24h was (0.98±0.29) ml/kg/1%TBSA, which was significantly lower than (1.16±0.32) ml/kg/1%TBSA in the control group. Meanwhile, the unit urine outputs during the shock stage for the treatment group and the control group were (1.90±0.68) ml/kg/h and (1.37±0.61) ml/kg/h respectively. The treatment group showed significantly higher unit urine output than the control group. The differences between the two groups were statistically significant ($P<0.05$) (Table 2). It is suggested that the insulin therapy can reduce the colloidal dosage and increase the urine output in the shock resuscitation.

Hematocrit results of the two groups

On days 1, 2 and 3 after burn, the hematocrit of the treatment group were lower than that of the control group, but no significant difference ($P>0.05$) was found between the two groups (Table 3).

Table 3. Hematocrit results of the two groups (%)

Group	1st day	2nd day	3rd day
Control	0.56±0.07	0.53±0.05	0.50±0.05
Insulin treatment	0.51±0.06	0.47±0.07	0.45±0.06

Table 1. General data of the two groups

Group	Case No.	Male (%)/female (%)	Age (years old)	Body weight (kg)	Total burn area	Third degree burn area
Control	29	17 (58.63)/12 (41.38)	46.7±14.6	67.5±12.5	43.9±24.9	51.3±18.4
Insulin treatment	29	14 (48.28)/15 (51.72)	48.2±15.1	65.9±14.9	44.8±26.2	49.8±17.9

Table 2. Comparison between shock resuscitation indices

Group	First 24 h unit colloid volume (ml/kg/1%TBSA)	First 24 h unit crystal volume (ml/kg/1%TBSA)	First 24 h urine output (ml/kg/h)	Urine output during shock (ml/kg/h)
Control	1.16±0.32	1.08±0.41	1.26±0.62	1.37±0.61
Insulin treatment	0.98±0.29*	1.02±0.37	1.30±0.59	1.90±0.68*

*Compared with the control group, *P<0.05.*

Liver and renal functions-relating biochemical indices

On the first 3d after burn, two groups had no statistically significant difference (P>0.05) in prealbumin, albumin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels.

The TBIL, DBIL, BUN and Cr levels between the patients in the two groups had no significant difference one day after burn. Since day 2 after burn, the treatment group showed lower levels of these indicators than the control group at various time points, and the difference had statistical significance (P<0.05) at most time points (Table 4).

Dynamic changes of platelet count after admission

The platelet counts of the treatment group and the control group on day 1 after admission had no statistically significant (P>0.05) difference with each other. The platelet counts of the two groups dropped significantly on day 4 compared with day 1 and the differences were statistically significant (P<0.05). On days 6, 9 and 12, the platelet counts of the two groups grew from low levels to the normal range (100×10^9/L~300×10^9/L) or even became higher than the normal value. The platelet count of the control group was higher than the treatment group on days 3, 6, 9 and 12. The platelet counting of the two groups showed statistical significance (P<0.05) (Table 5).

First 24 h APACHE II scores after admission

The APACHE II score of the control group (6.49 ± 2.18) was significantly lower than that of the treatment group (16.25 ± 3.81) in the first 24h after the admission. The APACHE II scores of the two groups had statistical significance (P<0.05).

Correlation between prognosis and clinical indices of the insulin treatment group

For severely burned patients in the treatment group, logistic regression is done with survival or death prognosis after the admission as the dependent variable and age, sex, APACHE II score, presence of platelet counting reduction (defined as the presence of platelet count $<100\times10^9$/L at two or more time points, 29 cases in total) and the length of hospital stay as the independent variables (Table 6).

It is shown in the logistic regression analysis that APACHE II score and presence of platelet counting reduction were closely related to the prognosis after severe burn (the OR values were 1.484 and 0.013

Table 4. Liver and renal functions-relating biochemical indices

Group	Time	TBIL (μmol/L)	DBIL (μmol/L)	BUN (mmol/L)	Cr (μmol/L)
Control	1st day	31.25±12.67	6.28±3.45	6.18±2.16	117.15±29.14
	2nd day	29.44±11.94	6.16±3.48	5.68±2.19	104.52±24.8
	3rd day	28.62±11.61	5.82±2.49	5.62±2.17	97.81±22.91
Insulin treatment	1st day	30.58±12.53	6.24±3.28	6.09±2.17	115.9±28.45
	2nd day	27.49±11.78	4.25±1.81*	3.15±1.05*	71.39±16.81*
	3rd day	18.41±9.15*	4.16±1.62*	2.98±0.98*	65.91±17.61*

*Compared with the control group, *P<0.05.*

Table 5. Dynamic changes of platelet count after admission ($\times10^9$/L)

Group	1st day	3rd day	6th day	9th day	12th day
Control	172.2±38.1	99.4±26.8	183.6±49.5	216.9±54.8	273.3±61.9
Insulin treatment	169.1±40.2	82.5±28.4*	161.5±42.9*	198.4±51.6*	254.5±62.8*

*Compared with the control group, *P<0.05.*

Table 6. Correlation between prognosis and clinical indices of the insulin treatment group

Variable	Value set method
Age (X1)	Actual age
Sex (X2)	Male=1, female=2
APACHE II score (X3)	Actual score
Platelet decrease or not (X4)	Decrease=1, not decrease=2
Length of hospital stay (X5)	Actual length of hospital stay

respectively; and the P values were 0.026 and 0.041 respectively), and the regression equation was Logit(p) =1.281+0.519X3-3.956X4. It is suggested that the non-presence of platelet counting reduction existed as a protective factor, and the APACHE II score was the risk factor.

Discussion

After burn, the organism was in a stringent state and might have glucose metabolism disorders and insulin resistance. The stringency could lead to hyperglycemia, but insulin secretion and function in the organism were still relatively insufficient due to the reduction of insulin/pancreatic glucagon ratio. The early application of insulin with glucose after burn could increase the utilization ratio of glucose to maintain stable blood glucose and overcome the impact of insulin resistance.[9] Insulin has been widely known as a synthetic hormones involved in the regulation of carbohydrates, fat and protein metabolism after severe burns. In recent years, researchers have discovered insulin receptor-mediated and non-metabolism dependent regulation capacity of the insulin for various cells of the organism. For instance, insulin can promote the proliferation and migration of the epidermal keratinocytes, vascular endothelial cells and other wound repair cells. Insulin can also regulate the time phases and function of the appearance and extinction of the neutrophils and wound macrophages on the wound surface.[10,11] By exploring the non-diabetic application of insulin, the clinical application indications of insulin were broadened and new solutions were provided for some difficult clinical problems.

Increased vascular permeability is the main pathophysiological basis of burns and septic shock. It is mediated by thrombin, interleukin-8, tumor necrosis factor-α and other inflammatory mediators, and there has been so far no targeted therapy. Although there are reports on the application of the signaling molecule Src inhibitor against the change of the vascular permeability, but Src can be widely involved in cell function, which limits its clinical application as an inhibitor.[12,13] The current therapies, such as burn shock resuscitation or septic shock early goal-directed therapy (EGDT), are all "stopgap" measures after the circulatory body fluid has been greatly leaked, lost or transferred to the interstitial space. Rabiee discovered that early insulin intervention could reduce damage to endothelial cells and reduce granulocyte infiltration, which suggested indirect regulatory capacity of insulin on vascular permeability.[14] These findings suggest that with its capacity mediated by the antagonistic inflammatory mediators to increase the vascular permeability and reduce damage to the vascular endothelial cells, insulin used in the shock stage can reduce body fluid leakage for patients with major burns in shock stage and their fluid requirements in the shock resuscitation. This is conducive to fundamentally improving or correcting the burn shock.[15,16] The group of patients treated with insulin (treatment group) had first 24h unit colloid volume lower than that of the control group. However, the treatment group had significantly increased unit urine output in the shock stage. This suggested that the insulin therapy can reduce the colloidal dosage and increase the urine output in the shock resuscitation. The relatively high hematocrit in the control group also suggested that the control group had more concentrated blood and more evident circulatory fluid loss than the treatment group.

Under the stimulation of the infection, trauma, shock and other risk factors, macrophages were activated and a large number of pro-inflammatory cytokines were released, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and etc. These cytokines promoted the further release of other inflammatory cytokines, and induced the synthesis of tissue factor (TF) and its expression on the cell surface, causing damage to endothelial cells and activation of the platelets. The activated platelets aggregated and had abnormal distribution on the damaged endothelial cells.[17] The inflammatory cells and damaged endothelial cells were activated at the same time, releasing a large number of platelet-activating factors (PAFs) and von Willebrand factors (vWFs) and further increasing the aggregation of platelets. Massive destruction of platelets can result in the release of the high concentrations of 5-hydroxytryptamine (5-HT) and prostaglandin (PGH), further mediating the massive release of inflammatory mediators. Platelet-activating factors play an important role in the inflammation of the organism and damage to important organs after burn, especially damage to the lungs, gastrointestinal tracts and kidney.[18]

Furthermore, the activation of platelets was positively correlated with the high expression of the proinflammatory cytokines. Both of them were related to the disease condition and prognosis. More severe disease condition would increase the platelet activation and shorten the life expectancy of the platelets. The platelet counting would decrease as a result of excessive consumption.[19]

Dynamic monitoring of the peripheral blood platelet counting of severely burned patients can reflect the change of disease condition of the patients with high sensitivity, and can, combined with the APACHE II score after admission, accurately reflect the prognosis. It can serve as a relatively reliable indicator to determine the severity and prognosis of the patients with severe burns. For patients with significantly decreased platelet counting, in addition to the treatment of the primary disease, thrombopoietin drugs should be applied promptly, and platelets should be transfused to improve the prognosis.

The application of insulin therapy together with the colloid and crystal resuscitation in the shock stage can reduce the volume of resuscitation fluids required in the shock stage. The insulin therapy can also protect the function of the cells, tissues and organs, and is conducive to the treatment of patients with severe burns.[20] Besides, randomized and controlled experiments with larger sample sizes and testing of more indicators including the blood sugar and urine sugar will be conducive to the comprehensive and clear illustration of the function and working mechanism of insulin. They are what we are going to do in the near future.

References

1. *Samuelsson A, Farnebo S, et al. Implications for burn shock resuscitation of a new in vivo human vascular microdosing technique (microdialysis) for dermal administration of noradrenaline. Burns, 2012; 38(7): 975-83.*
2. *Goertz O, Lauer H, et al. Extracorporeal shock waves improve angiogenesis after full thickness burn. Burns, 2012; 38(7): 1010-8.*
3. *Fioramonti P, Cigna E, et al. Extracorporeal shock wave therapy for the management of burn scars. Dermatol Surg, 2012; 38(5): 778-82.*
4. *Wang Z, Liu L, et al. Protective effect of glucose-insulin-potassium (GIK) on intestinal tissues after severe burn in experimental rats. Burns, 2012; 38(6): 846-54.*
5. *Lee J, Fortlage D, et al. Computerized insulin infusion programs are safe and effective in the burn intensive care unit. J Burn Care Res, 2012; 33(3): 114-9.*
6. *Sugita M, Sugita H, et al. Inducible nitric oxide synthase deficiency ameliorates skeletal muscle insulin resistance but does not alter unexpected lower blood glucose levels after burn injury in C57BL/6 mice. Metabolism, 2012; 61(2): 127-36.*
7. *Carter EA, Bonab AA, et al. Evaluation of the antioxidant peptide SS31 for treatment of burn-induced insulin resistance. Int J Mol Med, 2012; 28(4): 589-94.*
8. *Arrandale L, Ng L, et al. Superficial burn caused by a Hotline fluid warmer infusion set. Anaesthesia, 2009; 64(1): 101-2.*
9. *Zhang WF, Zhu XX, et al. Intensive insulin treatment attenuates burn-initiated acute lung injury in rats: role of the protective endothelium. J Burn Care Res, 2012; 32(3): 51-8.*
10. *Chen XL, Xia ZF, et al. Escharectomy and allografting during shock stage reduces insulin resistance induced by major burn. J Burn Care Res, 2011; 32(3): 59-66.*
11. *Tuvdendorj D, Zhang XJ, et al. Intensive insulin treatment increases donor site wound protein synthesis in burn patients. Surgery, 2011; 149(4): 512-8.*
12. *Xin LC, Zhao FX, et al. mTOR partly mediates insulin resistance by phosphorylation of insulin receptor substrate-1 on serine(307) residues after burn. Burns, 2011; 37(1): 86-93.*
13. *Gauglitz GG, Halder S, et al. Post-burn hepatic insulin resistance is associated with endoplasmic reticulum (ER) stress. Shock, 2011; 33(3): 299-305.*

14. *Rabiee A, Andreasik RN, et al. Numerical and clinical accuracy of a continuous glucose monitoring system during intravenous insulin therapy in the surgical and burn intensive care units. J Diabetes Sci Technol, 2010; 3(4): 951-9.*

15. *Ballian N, Rabiee A, et al. Glucose metabolism in burn patients: the role of insulin and other endocrine hormones. Burns, 2010; 36(5): 599-605.*

16. *Gauglitz GG, Toliver-Kinsky TE, et al. Insulin increases resistance to burn wound infection-associated sepsis. Crit Care Med, 2010; 38(1): 202-8.*

17. *Maciel FB, DeRossi R, et al. Scanning electron microscopy and microbiological evaluation of equine burn wound repair after platelet-rich plasma gel treatment. Burns, 2012; 38(7): 1058-65.*

18. *Kostina OV, Peretiagin SP. The platelet hemostatic system and prooxidant and antioxidant potentials in the dynamics of burn disease. Klin Lab Diagn. 2011; 4: 33-5.*

19. *Chen W, Zheng JS, et al. Advance in the research of platelet-rich plasma in burn treatment. Zhonghua Shao Shang Za Zhi, 2012; 28(4): 288-90.*

20. *Kasper SO, Phillips EE, et al. Blockade of the Renin-Angiotensin system improves insulin receptor signaling and insulin-stimulated skeletal muscle glucose transport in burn injury. Shock, 2011; 35(1): 80-5.*

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Ultrasound diagnostic and comparative analysis of conservative treatment of popliteal cyst in children

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Introduction

Term popliteal (Baker's) cyst or hygroma of popliteal region is widely used to describe benign cystic formation, filled with gelatinous substance in popliteal region in children and adults (4,11).

Backer's cyst is usually located in the posteromedial aspect of the popliteal fossa, and the majority extended between the deep fascia and the medial head of the gastrocnemius muscle, occasionally the cyst extended towards the lower thigh or between the soleus and the medial head of the gastrocnemius (38). Popliteal cyst (PC) are usually single, simple cyst and they can be found in all ages, but there is little data on rate of occurrence in adults as well as children (16).

Wolfe and Colloff (36) have said that in order cyst to be formed, there are two prerequirements: anatomical communication and chronic effusion which opens this potential communication. Also, Wigley (37) stated that "any pathology that can cause knee effusion can cause cyst". The pathophysiology of cyst formation has been attributed to trauma, arthritis and infection (38).

Although any of these processes can be involved, formation of the cyst in adults has been usually associated with previous trauma of intra and extracapsular structures, rheumatic disease and inflammation of joint, meniscal lesion, and with intra articular surgery (27). Recent papers indicate that there is difference in tumefactions etiology between adults and children.

Popliteal cyst in children seldom communicates with joint space, it is not associated with intra articular abnormalities and trauma, and is often referred as bursa of gastrocnemius and semimembranosus muscle (9, 31), or as neoplasma of idiopathic etiology.

Popliteal cyst in children seldom associated with joint effusion, meniscus tear, or anterior cruciate ligament tear (35), it rarely communicates with joint space, it is not associated with intra articular abnormalities, and is often referred as bursa of gastrocnemius and semimembranosus muscle (9, 31), or as neoplasma of idiopathic etiology.

Typically, there is no history of previous knee injury or related symptoms and signs for children with Baker's cyst. The natural history of true juvenile Baker's cysts appears to be more favorable and these cysts warrant conservative treatment thereafter (35).

In our standard practise, diagnosis of popliteal cyst is based on anamnestic data and clinical examination. It is found as clearly defined, oval and smooth tumefaction, firmly elastic consistency (10), in popliteal region in children. There is distinct difference between PC and other lesions (traumatic, benign, malign, inflammatory and vascular). With ultrasound imaging(US) becoming widely available it has become a number one diagnostic tool in diagnosis of popliteal cyst (10,14). Computerised tomography (CT) and Magnetic resonance(MR) is not routinely used (51).

Materials and methods

This was prospective study that had been conducted between 1.2.2009. and 1.2.2012. on Clinic for paediatric surgery, Institute for health of children and Youth of Vojvodina (IZZZDIOV), Novi Sad, Serbia.

Methodological procedure included clinical examination, US examination, clinical observation, and use of punction-sclerosation therapy with or without direct US control.

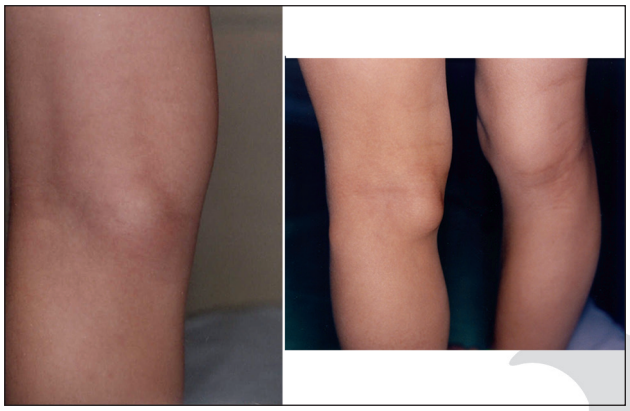

Figure 1. Popliteal cyste in fully extended knee

US examination

Popliteal cyst was identified like weak ehogen or anehogen oval cyst formation, clearly defined, not in contact with joint space of the knee, and with no other pathological lesion of knee and adjacent structures. In six participants we verified communication between cyst and joint space. In these participants we could not administer punction-sclerosation therapy, so they were put in control group. After verification, dimension of the cyst were measured by measuring distance between two farthest point in longitudinal (D1 diameter) and transversal (D2 diameter) direction, with US sonde in longitudinal and transversal position in respect to axis of extremity, and values were shown in mm.

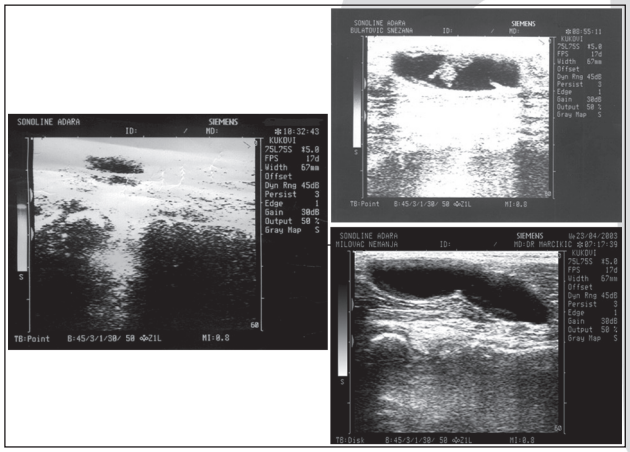

Figure 2. Intracystic anatomical variants (transversal fibrotic barrier, longitudinal fibrotic barrier and L cyst)

Participants

Study included 90 participants, from 0 - 15 years, who came to IZZZDIOV because of tumefaction in popliteal region, and in whom had been clinically and US verified Baker's cyst.

They were divided in three groups (30 participants in each) depending on the treatment procedure and the use of US control:

Group I - Therapeutic procedure using punction-sclerosation therapy without US control

Group II - Therapeutic procedure using punction-sclerosation therapy with direct US control

Control group - No therapeutic procedure

Table 1. Research protocol

Follow up	1. month	2. month	5. month
Clinical measurment	D1= D2=	D1= D2=	D1= D2=
US mesurment with sonde in longitudinal position	D1= D2=	D1= D2=	D1= D2=
US mesurment with sonde in transversal position	D1= D2=	D1= D2=	D1= D2=
Other			

Therapeutic procedure

After clinical and US verification of popliteal cyst, and exclusion of communication with knee joint space, as well as exclusion of all others differential diagnosis, therapeutic procedure consisted of punction of the cyst and aspiration of gelatinous content, which was followed by instillation of 2 - 4 ml of sclerosing agent - 2% Aetoxisclerol, depending on the size of the cyst.

Therapeutic procedure without US control

Patient was in prone position, with knee fully extended. Popliteal region was thoroughly cleaned using betadine foam, afterwards betadine solution was applied and sterile compress with a window was placed above the tumefaction. In caudal part of the cyst 2 ml local anesthetic (Lidocaine chloride) was instilled. Through the same opening, where local anesthetic was instilled, needle for cyst puncture was placed, holding syringe (10 cm^3) parallel to extremity. After overcoming the resistance of cyst wall and entering in it, content of the cyst was aspirated. Holding needle in place syringe with content of the cyst was replaced with syringe with sclerosing agent, which was instilled through the same needle. At the end of procedure betadine gauze and elastic bandaging was placed.

Therapeutic procedure under US control

Patient was in prone position, with knee fully extended. Popliteal region was thoroughly cleaned using betadine foam, afterwards betadine solution was applied and sterile compress with a window was placed above the tumefaction. In caudal part of the cyst 2 ml local anesthetic (Lidocaine chlorid) was instilled. Under direct control of US (linear sonde with sterile rubber cover was used), through the same opening, where local anesthetic was instilled, needle for cyst puncture was placed, holding syringe (10 cm^3) parallel to extremity. US verified the position of a needle in the cyst, after which content was aspirated. Holding needle in place syringe with content of the cyst was replaced with syringe with sclerosing agent, which was instilled through the same needle. At the end of procedure betadine gauze and elastic bandaging was placed.

Follow up

First follow up exam was scheduled one month after the first procedure. Exam consisted of verifying absence or presence of popliteal cyst by means of US and clinical examination. In case of persistence of cyst, morphometric measurements were taken and therapeutic procedure (second act) was repeated.

Second follow up was scheduled one month after previous, in case of persistence morphometric measurements were taken and therapeutic procedure (third act) was repeated.

Third follow up was scheduled three months after when absence or presence of popliteal cyst was verified and morphometric measurements were taken.

We used standard statistical analysis for biological sample. Complete research material was processed numerically and graphically, using absolute and relative numbers, mean values and bivariant correlation Person and Sigmund.

Results

Study included 90 participants, from 0 - 15 years, and in whom had been clinically and US verified Baker's cyst.

We found that majority of patients are within group age 8-11 years, 43%, a that most of the patients had been 8 years old, 16,67%.

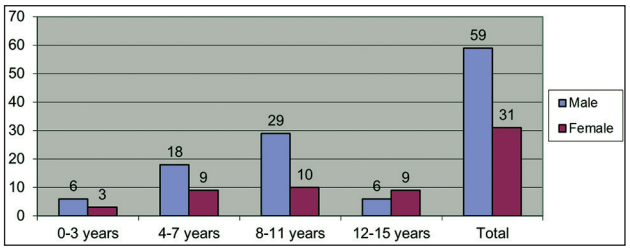

Figure 3. Sex structure in age groups (crosstabulation)

They were divided in three groups (30 participants in each) depending on the treatment procedure and the use of US control : Group I, Group II and Control group.

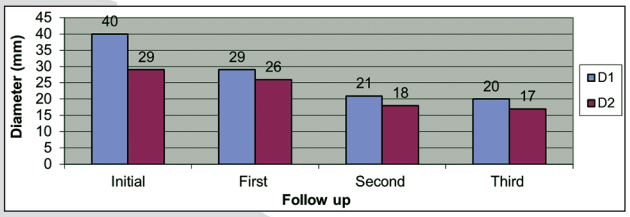

Figure 4. Clinically measured values of D1 and D2 diameter, initially and on follow ups (mm)

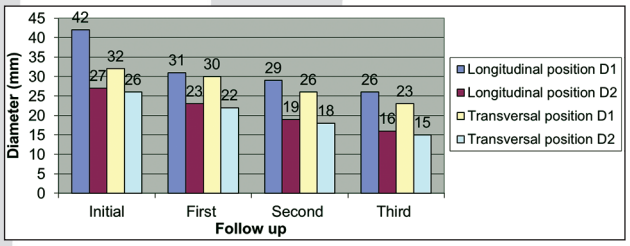

Figure 5. US measured values of D1 and D2 diameter, in longitudinal and transversal position of sonde initially and on follow ups (mm)

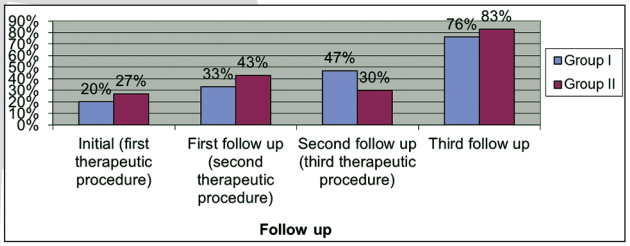

Figure 6. Rate of normalization of clinical and US finding after therapeutic procedures, initially and on follow ups (mm)

Discussion

In everyday practise diagnosis of these tumefaction was by means of clinical examination, where smooth, oval tumefaction, firmly elastic in consi-

stency, above and medial of tibial plateau had been identified, with no pathological lesion to the skin above tumefaction (2,10,31). In contemporary literature this is often referred as bursa of gastrocnemius and semimembranosus muscle, seldom as true synovial cyst or herniation of knee joint space.

Popliteal cyst occurs in all ages, but there is little data on frequency in children and adults.

In recent years US imaging is used as standard and routine procedure in diagnosing popliteal pathology, because of its non invasiveness, quickness and objectiveness, without any consequences. It has replaced or pushed out from diagnostic algorithm other radiological or aggressive procedures (13, 26, 31).

Anatomical, that is sonoanatomical features of tumefactions in popliteal regions that should be visualised are: shape, size, structure, relationship with surrounding tissue and communication with knee joint space.

In the past treatment of popliteal cyst meant surgical excision, with all possible postoperative complication that go with surgery and return of tumefaction (relapse), which in literature go as high as 40%. Recently, as an alternative to surgery, punction of the popliteal cyst and instillation of sclerosing agents are used. Before the procedure is administered, communication between the cyst and knee joint space must be excluded by US (10, 14, 32). This type of treatment, in our experience, and as in experiences of other authors (Fucumoto, Pemberton Nemeth, Soslow), results in more than 75% of patients cured (10, 21, 25, 31).

US exam clearly shows position, shape, size, anehogen or weak ehogen cystic formation, as well as relationship with surrounding soft tissues, and possible communication with the knee joint space.

In clinically ambiguous cases, and also in cases where intracystic anatomical variants had been detected, originally modified punction technique with the injection of sclerosing agent and direct control of US had been used.

Clear morphological and morphometrical parameters were created by which indication for type of therapeutical procedure had been made.

We found that majority of patients are within group age 8-11 years, 43%, a that most of the patients had been 8 years old, 16,67%. Two thirds of patient were male gender. Right leg was affected in 54% of cases, compared to left leg, in 46% of cases. We had not found cases with both legs affected.

By comparing values of D1 and D2 diameter which were clinically measured initially and on follow up exams with values of the same parameters measured with US imaging, we found there is statistically significant difference ($p<0,05$; bivariant correlation by Pearson and Sigmund) in objectifying precise size of lesion, and we found that US imaging is more precise technique than clinical measurement.

In twelve participants intracystic anatomical variants had been detected. Regarding whole sample, in 81% of cases simple cyst was found, in 7% of cases communication we knee joint was found, in 4% fibrotic transversal barrier had been detected, 2% of participants had multilocular cysts, and "L" shaped cyst was found in 2% of children.

In control group we clinically observed 24 patients (26,67%).

Follow ups were conducted in precisely specified intervals, during which clinically and by US imaging cystic tumefaction had been verified or excluded, and if popliteal cyst persisted, morphological measurements were taken and effect of administered therapy was observed.

During follow up exams we found that there is decrease in value of D1 and D2 diameter measure clinically and by US imaging, that is the result of gradual decrease in content volume of the cyst and successful therapeutic procedure. This data reflect values found in participant group I and group II. In control group we found that values of D1 and D2 parameter shown a growth tendency.

Mean values of D1 and D2 diameter measured using US imaging with sonde in longitudinal position were: initially D1 42mm, D2 27mm; first follow up D1 30mm, D2 23mm, second follow up D1 29mm, D2 19mm, third follow up D1 26mm, D2 16mm. Mean values of D1 and D2 diameter measured using US imaging with sonde in transversal position were: initially D1 32mm, D2 26mm; first follow up D1 30mm, D2 22mm, second follow up D1 26mm, D2 18mm, third follow up D1 23mm, D2 15mm.

In first experimental group (punction-sclerosation therapy without US control) results were: in therapeutical act complete clinical and US normalisation was in 20% of group participants, after second

therapeutical act 33,33% participants were cured, and after third act 46% participants were cured. On the last follow up, five months after the initial treatment, 76,7% of patients shown complete clinical and US normalisation, and presence of the popliteal cyst was verified in 23,3% of patients.

In second experimental group (punction-sclerosation therapy using direct US control) results were: in therapeutical act complete clinical and US normalisation was in 26,67% of group participants, after second therapeutical act 43,33% participants were cured, and after third act 30% participants were cured. On the last follow up,five months after the initial treatment, 83,7% of patients showed complete clinical and US normalisation, and presence of the popliteal cyst was verified in 16,3% of patients.

In third experimental group, six patient underwent punction of popliteal cyst without injecting sclerosing agent and without US control in first therapeutic act, however it did not show clinical nor US normalisation. Second therapeutical act was done in one patient, 16,67% (this date should be taken with caution, because patient did not show up on next follow up exam). Third therapeutical act was conducted on five participants, 83,33%. On the last follow up, five months after the initial treatment popliteal cyst was detected in all of patients.

US imaging has been accepted as primary, routine and standard imaging technique in diagnosis of popliteal cyst in development period of a child, and as a quick i reliable way, without adverse effects on organism, by which administration and the effects of punction-sclerosation therapy could be followed.

Conclusion

US imaging has been accepted as primary, routine and standard imaging technique in diagnosis of popliteal cyst in development period of a child

Introduction of US imaging in diagnostic algorithm of popliteal cyst has minimised or pushed out other invasive, and hazardous diagnostic procedures.

Using US imaging, and measuring morphological characteristics of popliteal cyst, more adequate treatment could be selected.

Morphological and morphometrical criteria had been identified and clearly established in US imaging of popliteal cyst in the development period of a child, as a factor for administration of punction-sclerosation therapy.

Punction-sclerosation treatment is a procedure of choice for treating popliteal cysts.

US imaging is a quick, reliable and reproducible method that helps in effective administration and tracking of effects of punction-sclerosation therapy of popliteal cyst.

Use of originally modified technique punction-sclerosation therapy under the direct control of US is an optimal therapeutic procedure for treatment of popliteal cyst, with low per cent of relapses.

References

1. *Fukumoto K, Kojima T, Tomonari H, et al. Ethanol injection scletotherapy for Baker's cyst thyreoglossal duct cyst and brachial cleft cyst. ANN Plastic Surg 1994; 33(6): 615-9*
2. *E. Jeff Jusis Jr. in Campbell operative orthopaedics VII edition 2256-57, L. H. Creenshav. Washington 1987 Vol III.*
3. *Gebest HJ, Muller-Buhl U. Therapy of Baker's cysts. Dtsch Med Wochenschr 1989; 114 (47): 1845-7.*
4. *Grasso A, Pellicano G. Ultrasonography and computerized tomography in giant Baker's cyst. Radiol Med 1991; 82(1-2): 60-3.*
5. *Hughston JC, Baker CL, Mello W. Popliteal cyst: a surgical approach. Orthopedics 1991; 14(2): 147-50.*
6. *Helbich TH, Breitenseher M, Trattnig S, Nehrer S, Erlacher L, Kainberger F. Sonomorphologic variants of popliteal cysts. J Clin Ultrasound 1998; 26(3): 171-6.*
7. *Hašpl M, Pećina M. Ultrazvučna dijagnostika koljena i podkolenice u Matasović i sar.*
8. *Ultrazvučna dijagnostika sustava za kretanje. Zagreb: Školska knjiga, 1988.*
9. *Jerosch J, Castro WH, Sons HU, Winkelmann W. The value of sonography in injuries of the knee joint. Ultraschall Med 1989; 10(5): 275-81.*
10. *Johnson LL, Van Dyk GE, Johnson CA, Bays BM, Gully SM. The popliteal bursa (Baker's cyst): an arthroscopic perspective and the epidemiology. Arthroscopy 1997; 13(1): 66-72*
11. *Kalke S, Jankharia B, Balakrishnan C, Mangat G, Khomani R, Chinoy RJ, Agarwala S, Deshpande RB, Joshi VR. Evaluation of the knee pathology by ultrasonography. J Assoc Physicians1998; 46(7): 623-4*

12. *Kohnke J. Baker's cyst. Chirurg 1999; 70(2): 217*

13. *Kurjak A. i sar. Ultrazvuk u kliničkoj medicini. Zagreb 1989.*

14. *Kruger T, Niedermanner I, Hube R, Hein W. Recurrence of Baker's cysts with regard to operation procedure and intraarticular pathology. Zentralbl Chir 2002; 127(10): 905-8*

15. *Krome J, de Araujo W, Webb LX. Acute compartment syndrome in ruptured Baker's cyst. J South Orthop Assoc 1997; 6(2): 110-4*

16. *Lukač I, Kovačevič N. Dijagnostički ultrazvuk u gastroenterologiji i nefrologiji. Dunaj 1994.*

17. *Maffulli N, Regine R, Carrillo F, Minelli S, Beaconsfield T. Ultrasonographic scan in knee pain in athletes. Br J Sports Med 1992; 26(2): 93-6.*

18. *Maloch J.D. Popliteal cyst in in children. Brit J Surg 1970; 69: 629.*

19. *Mannino M, Marino C, Chawla K. Ruptured pyogenic Baker's cyst. J Natl Med Assoc 1988; 80(9): 1018-22.*

20. *Matasović T. i sar. Ultrazvučna dijagnostika sustava za kretanje. Zagreb: Školska knjiga, 1988.*

21. *Muller-Buhl U, Gebest HJ. Diagnosis of Baker's cysts. Dtsch Med Wochenschr 1989; 114 (47): 1842-4.*

22. *Nemeth P, Fuzesi K. Sclerotising therapy an alternative tretment for popliteal cyst in children. Ped Surgg Int 1990; 5: 134-5.*

23. *Petković L. i sar. Ultrasonografska dijagnostika poplitealnih cisti kod dece. Ultrazvuk 1995; 1-2: 69-72.*

24. *Pemberton DJ, Mackie IG, Maheson M. A new surgical drainage procedure for Baker's cysts. Ann R Coll Surg Engl 1990; 72(6): 373-4.*

25. *Rader L, Worsdorfer O. Arthroscopic therapy of Baker's cyst. Zentralbl Chir 2000; 125(9): 776-9*

26. *Samoilovich EF, Alekseenko AA. Baker's cyst in children. Khirurgiia (Mosk) 1995 (4):74-6.*

27. *Scherf F.G, Hillejani L, Krawzak HW et al. Baker's cyst.a follow up study after surgical therapy. Unfallchir 1994; (97)2: 85-8.*

28. *Szer IS, Kleinn-Gitelman M, De Nardo BA et al. Ultrasonography in the study of prevelance and clinical evolution of popliteal cysts in children with knee effusions. J.Rheumat 1992; 19 (3): 458-62.*

29. *Tachdjijan M.O. Popliteal cyst in Pediatric orthopedics. Vol I. Philadelphia: W B Saunders, 1990: 735-47.*

30. *Van Rhijn LW, Jansen EJ, Pruijs HE. Long-term follow-up of conservatively treated popliteal cysts in children. J Pediatr Orthop B 2000; 9(1): 62-4 .*

31. *De Maeseneer M, Debaere C, Desprechins B, Osteaux M. Popliteal cysts in children: prevalence, appearance and associated findings at MR imaging. Pediatr Radiol 1999; 29 (8): 605-9.*

32. *Seil R, Rupp S, Jochum P, Schofer O, Mischo B, Kohn D. Prevalence of popliteal cysts in children. A sonographic study and review of the literature. Arch Orthop Trauma Surg 1999; 119(1-2): 73-5*

33. *Tschauner Ch, Klapsch W, Graf R. Eandel der Behandlugsstrategien und behandlungsergebnisse im Zeitat. Parbhoo A, Govender S. Acute pyogenic psoas.lter des sonographischen Neugeborenscreenininges. Ortoped.Praxis 1990; (26): 693-698.*

34. *Ward EE, Jacobson JA, Fessell DP, Hayes CW, van Holsbeeck M. Sonographic detection of Baker's cysts: comparison with MR imaging. AJR Am J Roentgenol 2001; 176(2): 373-80.ž*

35. *Jian-Chih C, Cheng-Chang L, Yen-Mou L, Chung-Hwan C, Yin-Chih F, Peng-Ju H, Yin-Chun T. A modified surgical method for treating Baker's cyst in children. The Knee 2008; (15): 9 – 14.*

36. *Wolfe RD, Colloff B. Popliteal cysts. An arthrographic study and review of the literature. J Bone Joint Surg Am 1972; (54): 1057–1063.*

37. *Wigley RD. Popliteal cysts: variations on a theme of Baker. Semin Arthritis Rheum 1982; (12): 1–10.*

38. *Labropoulos N., Shifrin D. A. and Paxinos O. New insights into the development of popliteal cysts. British Journal of Surgery 2004; (91): 1313–1318.*

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Osteonecrosis in an ankylosing spondylitis and myasthenia gravis diagnosed patient

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Abstract

Ankylosing spondylitis is a chronic, progressive rheumatic disease characterized by inflammation and ankylosis of the axial skeleton, especially sacroiliitis, which is regarded as the hallmark of the disease. Myasthenia gravis is a neuromuscular disorder characterized by fluctuating, painless muscle weakness. It typically starts in the extraocular muscles and remains purely ocular in 15% of patients. In the remaining 85% of patients, myasthenia gravis becomes generalized. We will present a patient with osteonecrosis who had been both diagnosed with ankylosing spondylitis and myasthenia gravis.

Key words: Osteonecrosis, Ankylosing Spondylitis, Myasthenia Gravis.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder that affects the sacroiliac joints, thoracic cage and axial skeleton (1). Major symptoms are pain, joint stiffness, and a progressive loss of spinal mobility and these symptoms lead severe functional limitations (2). Myasthenia gravis (MG) is an autoimmune disorder caused mostly by antibodies (Abs) against the acetylcholine receptor (AChR) in neuromuscular junctions (3).

Steroids, being first introduced in 1935, have been widely used for the treatment of myasthenia gravis (MG) since the 1950s (4). Steroids are often considered as an alternative for immunotherapy because other immunologic treatments such as plasma exchange, intravenous immunoglobulins, or immunosuppressive agents are expensive and associated with potentially serious adverse effects (5).

Besides, steroids also have important side effects such as immunsupression, Cushing syndrome, glucose intolerence, potassium loss, osteoporosis, tendon rupture, osteonecrosis (5). Osteonecrosis, also known as aseptic necrosis, avascular necrosis, ischemic necrosis and osteocondritis dissecans, has been associated with corticosteroid usage, alcoholism, infections, hyperbaric events, storage disorders, marrow-infiltrating diseases, coagulation defects, and some autoimmune diseases (7). With this case we aimed to evaluate an osteonecrosis patient diagnosed with MG and AS before.

Case

A twenty nine year old male patient admitted to our clinic with left hip pain walking difficulty. He had been diagnosed with AS at the age of 17, when he admitted to hospital with left hip pain, lumbalgie and morning stiffness complaints. He had used Salisilazosulfapiridin 2*2 ve non steroidal antiinflamatory drug treatment but after a short treatment period he stopped taking his drugs voluntarily. 6 months after he was diagnosed with MG because of his speaking disorder, difficulty in swallowing and pytosis. He was hospitalised and treated with Azathioprine 100mg 1*1 po, Pridostigmin 60 mg 3*1 po and prednisolon 56mg po daily during hospitalisation. He has stil been using prednisolon 20 mg 1*1 po.

In his physical examination left hip range of motion was opened and painfull. He showed mild pain and limitation of lumbar spine movement and lumbar lordosis. His Modified Schober test was 4,5 cm and chest expansion was 5 cm, Occiput to wall distance was 0 cm. Patrick–Fabere and sacroiliac compression tests were bilateral positive, suggestive of sacroiliitis.

He underwent some laboraty tests. WBC: 9,74 K/ul Hb: 16,4 K/ul, Plt: 286,3 K/ul, ALT: 24 IU/L, AST: 18 IU/L, BUN: 12,4 mg/dl Cr: 0,84 mg/dl, ESR: 6 mm/hr CRP: 0,2 mg/dl.

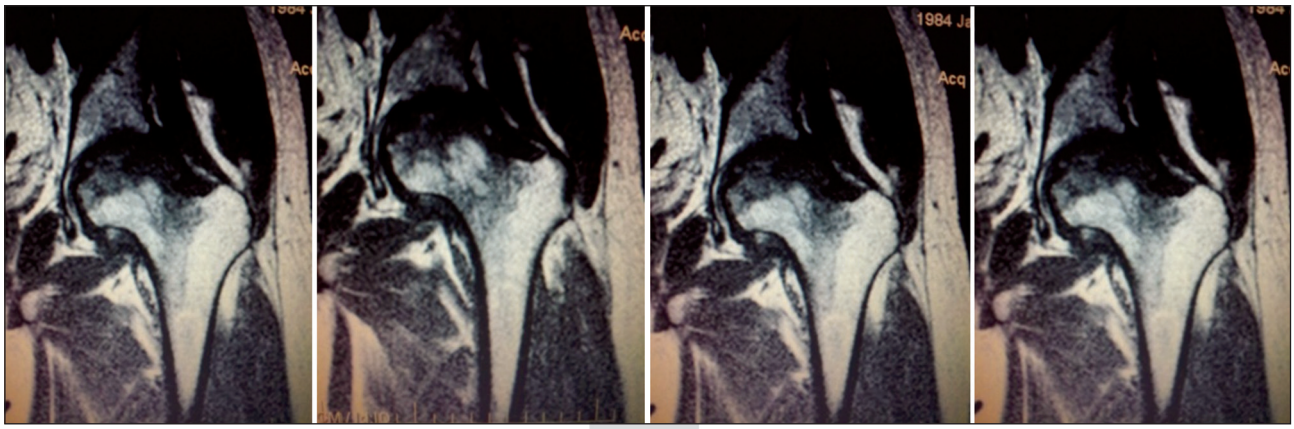

Figure 1. MRI image of avascular necrose of the patient

In the radiological evaluation magnetic resonance imaging of his left hip avascular necrosis was seen (Figure 1). The patient was consulted to orthopaedics and traumatology clinic for surgery. The patient underwent core decompression operation.

Discussion and conclusion

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disorder with unknown etiology, mainly affecting the sacroiliac joints and spine, with possible involvement of other joints, entheses, and extra-articular structures. The major symptoms in patients with AS are pain, stiffness and progressive loss of spine mobility which may result in physical limitations (8-10). Myasthenia gravis (MG) is a neuromuscular disorder characterized by fluctuating, painless muscle weakness. Mechanistically, MG is an autoimmune disorder of neuromuscular transmission, usually caused by antibodies to postsynaptic nicotinic acetylcholine receptors (AChRs) (11).

MG commonly accompanies most autoimmune diseases such as Rheumatoid Arthritis, Systemic Lupus Erythematosus, Polymyositis/Dermatomyositis. In the literature. James et al reported review of 857 patients having myasthenia gravis who were seen at the Mayo Clinic from 1934 to July 1964. In his review three cases diagnosed both with MG and AS (12). Our case was diagnosed with both AS and MG, and the infrequency of this togetherness made difficult to evalute his hip pain. It might be a symptom of AS or complication of steroid usage. The use of corticosteroids in the immunologic treatment of ocular myasthenia gravis (OMG) is controversial primarily because of the potential for significant side effects (13). Some have suggested that steroids should be used only "when absolutely necessary" in the treatment of myasthenia gravis (14), whereas others have shown favorable outcomes with long-term low-dose regimens of prednisone (15). The incidence of developing osteonecrosis as a consequence of steroid therapy is low. One possible etiology of osteonecrosis secondary to steroid use involves the formation of microemboli in the arteries supplying the bone due to changes in circulating lipids. Other theories suggest that steroids cause venous endothelial modification, leading to stasis and an increase in intraosseus pressure. The greatest risk lies in patients treated with long-term, high-dose corticosteroids; however, reports have noted osteonecrosis development in physiological glucocorticoid replacement (16). Tauchmanov`a et al reported either a period of medication greater than 216 days or a cumulative steroid dose of 6 g as a risk factor (17). In our case, patient's MRI image showed avascular necrosis and he undewent operation. However his pain could just be explained with his AS disease but the MRI image provided us to be alert about steroid usage. In literature no cases have been reported with avascular necrosis who both had been diagnosed with AS and MG. Side effects of steroids shouldn't be forgotten in these patients. And further evaluation should be performed in these patients.

References

1. *Russell AS. Ankylosing spondylitis: History. In: Klippel JH, Dieppe PA. Rheumatology. 2nd edn. London: Mosby, 1998, pp. 1-2.*

2. *Zink A, Braun J, Listing J, Wollenhaupt J; German Collaborative Arthritis Centers. Disability and handicap in rheumatoid arthritis and ankylosing spondylitis-results from the German rheumatological database. J Rheumatol 2000 Mar; 27(3): 613-622.*

3. *Takamori M, Motomura M, Kawaguchi N, et al. Anti-rynodine receptor antibodies and FK506 in myasthenia gravis. Neurology 62: 1894-1896, 2004.*

4. *Simon HE: Myasthenia gravis: effect of treatment with anterior pituitary extract. JAMA 1935; 104: 2065–2066.*

5. *Muscle Study Group: A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. Neurology 2008; 71: 394–399.*

6. *Sghirlanzoni A, Peluchetti D, Mantegazza R, Fiacchino F, Cornelio F: Myasthenia gravis: prolonged treatment with steroids. Neurology 1984; 34: 170–174.*

7. *Assouline-Dayan Y, ChangC,Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Sem Arthritis Rheum. 2002; 32: 94–124.*

8. *Forejtova´ S, Mann H, Stolfa J, Vedral K, Fenclova´ I, Ne´methova´ D (2009) Factors influencing health status and disability of patients with ankylosing spondylitis in the Czech Republic. Clin Rheumatol 27: 1005–1013 2.*

9. *Karapolat H, Akkoc Y, Sari I, Eyigor S, Akar S, Kirazli Y, Akkoc N (2008) Comparison of group-based exercise versus home-based exercise in patients with ankylosing spondylitis: effects on Bath Ankylosing Spondylitis Indices, quality of life and depression. Clin Rheumatol 27: 695–700.*

10. *Lim HJ, Lim HS, Lee MS (2005) Relationship between selfefficacy and exercise duration in patients with ankylosing spondylitis. Clin Rheumatol 24: 442–443.*

11. *Drachman DB (1994) Myasthenia gravis. N Engl J Med 330: 1797–1810.*

12. *James B. Carter, MD; G. Roy Diessner, MD; Frank M. Howard, MD Myasthenia Gravis and Rheumatoid Spondylitis: Coexistence in Three Cases JAMA. 1965; 194(8): 913-914. doi: 10.1001/jama.1965.03090210077028.*

13. *Peppa M, Krania M, Raptis SA. Hypertension and other morbidities with Cushing's syndrome associated with corticosteroids: a review. Integr Blood Press Control. 2011; 4: 7–16.*

14. *Kaminski HJ, Daroff RB. Treatment of ocular myasthenia: steroids only when compelled. Arch Neurol. 2000; 57: 752–753.*

15. *Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteroid treatment of myasthenia gravis: report of 116 patients. Ann Neurol. 1984; 15: 291–298.*

16. *Vreden SG, Hermus AR, van Liessum PA, Pieters GF, Smals AG, Kloppenborg PW. Aseptic bone necrosis in patients on glucocorticoid replacement therapy. Neth J Med. 1991; 39: 153–157.*

17. *Tauchmanov`a L, De Rosa G, Serio, B, et al. Avascular necrosis in long-term survivors after allogeneic or autologous stem cell transplantation: a single center experience and a review. Cancer. 2003; 97: 2453–2461.*

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Healthy Turkish adults' perception of good death

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Abstract

To determine Turkish community adults' opinions regarding the best definition of good and bad death, 896 volunteers answered objective and open-ended questions about good and bad death during a home visit. The most popular definition of a good death was dying without feeling any pain, the most popular definition of a bad death was dying due to the loss of physical body integrity. The high ratio of people wanting to die instead of feeling pain, despite a ban in Turkey on euthanasia, was an important finding.

Key Words: Death, Dying, Advance Directives, End of Life, Ethics

Introduction

In Western culture, according to the results of several studies in Europe[1-4] and in the USA and Canada [3,5,6] good death is painless ; in sleep; fast; without knowing it is coming; after saying goodbye to family members; at home; in comfort and without being a burden to loved ones. In addition, good death is not prolonged and not alone.

In Eastern culture, good death is the death of a person whose time had come and who had done what he or she should in life. In addition, the cause of death and the place of death are also important[4] Studies that were carried out in India [7], South Korea [8] and Japan [9] found that good death involves the absence of physical or spiritual pain, the opportunity to say goodbye to family and loved ones, not being a burden to family, being able to prepare for death, dying in faith, and/or dying at the requested place (especially at home and with the support of family during the process). Thus, in both Western and Eastern culture, painless death is the most commonly mentioned characteristic of a good death. When it comes to bad death, according to two studies in Europe [10] involves being alone, murdered, buried in an unmarked grave, too young ,and following living in vegetative state for a long time.

According to the results of two studies, one in Korea [8] and one in India[7], the people in these two cultures list the types of bad death as being in a vegetative state, sudden (unexpected) death, painful death, dying alone, being a burden to family members, and dying as a result of suicide, traffic accident or murder. There was also a consensus on timely death in Eastern culture. For example, being elderly (over 90) meant that life was fully lived and complete; death at this age is a death that should be celebrated [7] .

Death and the process of death are taboo subjects that are generally not open for discussion in Turkish society. However, Turkish people take seriously and participate in postmortem rituals, comprised mainly of Islamic rules. In Islam, the main belief that affects the perception of death is that all creatures are entrusted by God and one day, God will take his charge back. The belief that "Nobody can take the life God gave, other than God himself" bans suicides in Islam. There are also concepts of heaven and hell in Islam, including a belief in an afterlife. In Turkish belief, when praying to God, requests are made for the timely, orderly death for the person himself and for his loved ones [11]. In Turkish society, it is unacceptable to give up life. In this context, the application of advance directives is not in practice in Turkey, where Human Rights and Biomedicine Conventions are approved. Currently, the number of deaths in hospitals is increasing and dying patients are being kept in the intensive care units for long periods of time. Additionally, there is no consensus on brain death or on withdrawing or terminating treatment. It is known that health professionals have different opinions and applications on these issues. While the number of studies on rights of patients is increasing, there is no study on public opinions about the rights and demands of dying patients and their families.

In Turkey, where the majority of the population is Muslim, the existence of various religions and beliefs from past to present are also known. Being the only Muslim country with a secular go-

vernment within its geographical region distinguishes Turkey from other countries in the East.

In Turkish society, which contains elements of Eastern and Western culture and has an increasing number of young people, the general attitudes of individuals may vary. These differences can influence the perceptions of death and the dying process. This is the purpose of this study: to determine the best description of good and bad death, their distinctive elements, and views on end of life decisions and advance directives in Turkish society.

Method

Participants

Participants were 896 people (497 women) living in Muğla,, an important tourist city in western part Turkey, on the Aegean Sea that has many coastal residential areas, including Bodrum, Marmaris, Datça, Göcek, and Ölü Deniz. Nowadays, many people from European countries, led by the British, choose to live in this city permanently because of its natural beauty. The city also attracts attention with its historical inheritance. This city, where the first settlement started around 3000 BC, has the remains of Karia, Byzantium, Seljuk and Ottoman civilizations; the heritage of these different cultures are kept alive in the social life.

The Saburhane District of Muğla, which was selected as the study area, was previously declared a heritage area; around 400 houses were put under protection because of the architecture and social structure in the district. Until 90 years ago, it was a typical settlement where two different cultures, namely Turkish-Greek or Muslim-Christian cultures lived together Muğla University Health College students make two home visits per week in this area for public health practices. Every student has certain families that he/she is responsible for. This study was carried out between April 22, 2012 and June 30, 2012. Students scheduled appointments beforehand; willing participants were visited in their homes. If individuals were not able and/or willing to participate at home, interviews were done in a popular community café

Survey

Validity studies had been done and Cronbach's alpha was found as 0.793 and in line with that the study has been conducted. Face-to-face interviews with the all participants as a pilot study were done by the first and the second coder. In this application, the place of the interviewers was changed and the same questions were asked to the respondents again and the answers were compared. After necessary revisions, the questionnaire was finalized. The questionnaire that was developed by the researchers as a result of the literature review had five main sections. The first section was comprised of eight questions which asked for personal information including age, sex, education level, number of children, the effect of religious beliefs on daily decisions, having a chronic disease, losing a relative and having given care to a terminally ill patient. The second section had two open-ended questions about perceptions of good death and bad death. The third section included two questions having eleven statements about the dying process in terms of pain-agony feelings and addiction to something or somebody. The first of the three questions in the fourth section was based on the assumption that a fatal sickness is diagnosed sometime in the future. There were six statements about medical treatment options under that question. In the fifth section, there were two open-ended questions, one of which asked the choices about advanced directive applications and the reason for the choice. The other asked about the identity of end-of-life treatment decision makers. The researchers spoke face-to-face with each of the participants and, taking the ages of the subjects and their literacy levels into consideration, the answers were marked on the study form by the researchers.

Ethical Responsibility

The study was carried out with the permission of Muğla University Research Ethics Board, number 20/2/8/3, dated the 20th of April, 2012. In addition, the compulsory legal permission from Muğla Provincial Directorate of Health was also obtained in order to carry out a study in the area. After providing information during time periods that were suitable for the families, willing volunteers were included in the study. If, after the participants agreed to answer the questions, they were disturbed by the questions and did not want to continue, they were removed from the study, as their voluntary status had come to an end.

Results

The youngest of the participants was 18 years old and the oldest was 89 years old. As the age distribution of the participants was over a wide spectrum, the statistical evaluation was made according to the median value (mean=43.01±16.05, mode=42, median=41). More than half of the participants were female (55.5%) and 38.3% had 5 years of education, 82% had children. 34.6% of the volunteers reported a chronic disease. 55.1% of the participants stated that religious beliefs had no effect on their daily life decisions (Table 1).

Table 1. Distribution of Individuals According To Their Identifying Characteristics

Characteristics	n (%)
***Age**	
18-41 years	451 (50.3)
42-89 years	445 (49.7)
Sex	
Female	497 (55.5)
Male	399 (44.5)
Education Level	
Illiterate	81 (9.0)
5 years	343 (38.3)
12 years	287 (32.0)
More than12 years	185 (20.7)
Children	
Yes	735 (82.0)
No	161 (18.0)
Having Chronic Disease	
Yes	310 (34.6)
No	586 (65.4)
The Effect of Religion on Daily Decisions	
Yes	402 (44.9)
No	494 (55.1)
Total	**896 (100.0)**

**Median=41 age*

The loss of a relative was reported in 85.8% of the participants, while 28.9% expressed that they provided care for a relative in the process of death. Most of the care providers were female (63.3%). The difference between women and men was statistically significant (X^2=9.093, p=0.000). In addition, most people (67.1%) who had provided care for dying relatives were over the age of 41 (X^2=45.396, p=0.000). Statistically, a positive relationship between age and providing care for a dying relative was determined (r=0.584, p=0.005). Care giving roles were more prevalent in those with lower education levels (X^2=49.331, p=0.000).

Perception of death: In order to evaluate the death perceptions of the participants, *"good death"* and *"bad death"* perceptions were questioned. The participants described *good death* firstly as pain free death (32.1%), and secondly as sudden death in sleep (31.6%). More than half of the participants (54.1%) described *bad death* as death by destruction of the physical integrity of the body in an accident (by burning, freezing, drowning, dismemberment) followed by dying in pain (29.7%) (Table 2).

Table 2. Perceptions about death

Death Perception	n	%
Good death		
Painless death without agony	288	32.1
Sudden death in sleep	283	31.6
Dying without being a burden to anybody	124	13.8
Dying when the time comes	86	9.6
Dying in faith	78	8.7
Dying at home by saying farewell to the loved ones	24	2.7
No death is good	13	1.5
Bad death		
Dying in an accident with damage to body integrity	485	54.1
Dying in pain	266	29.7
Dying young	76	8.5
Dying without faith	24	2.7
Dying alone	20	2.2
Dying in need of someone's care	15	1.7
Suicidal death	9	1.0
Not leaving anything behind after death	1	0.1
Total	**896**	**100.0**

Opinions on the process of death: When the participants were asked about feeling any pain-agony in the period before death, 38.7% said "I'd rather die than suffer"; while 37.6% responded "I'd suffer if it is my destiny". On the subject of being dependent on someone during the process of death, 33.4% didn't want to be a burden to their children, while 25.3% preferred death to being dependent (Table 3). Characteristics such as age, education level, having children and the effect of religion on daily decisions had statistically significant effects on the opinions on being in pain-agony and

being dependent during the period before death (Table 3). Most of the people who had 12 or more years of education (X^2=32.589, p=0.000) and the ones who said religious beliefs didn't affect their daily decisions (X^2=7.977, p=0.046) preferred to die instead of feeling pain during the period before death. Most of the people who had 5 years of education (X^2=64.744, p=0.000) and who had children (X^2=17.277, p=0.001) said that if pain was in their destiny, they would suffer. Most of the people under the age of 41 (X^2=36.304, p=0.000) and people who had 12 years or more of education (X^2=36.197, p=0.000) preferred dying to being dependent.

Table 3. The opinions of Muğla province residents on the process of death

Opinions on feeling pain-agony in the period before death	n (%)
I'd rather die than suffer *	347(38.7)
I'd suffer if it is my destiny ≠	337(37.6)
I'm very afraid of feeling pain	197(22.0)
Other	15 (1.7)
Opinions on being dependent to somebody in the period before death	
I wouldn't want to be a burden to my children	299(33.4)
I'd rather die than be dependent ∞	227(25.3)
My children will take care of me, I won't be a burden ¥	112(12.5)
I've looked after my parents	106(11.8)
I'd have myself looked after by paying	77(8.6)
I wouldn't want to be a burden economically	51(5.7)
Other	24 (2.7)

** For education level, parental status and for religion p<.05*
≠ For age and parental status p<.05
¥ For education level p<.0 5
∞For age and for education level p<.0 5

Medical treatment preferences in the case of a potentially fatal disease: When the participants were asked about their preferences for treatment if they were diagnosed with a fatal disease at some point in the future for which nothing could be done medically, half (51.2%) said they would want to be at home and be relieved of pain and would not request any other treatment. 18% would request to be relieved of pain and placed in a special care facility. However, the remainder of the participants (30.9%) said they could suffer if needed for any treatment, definitely wanted to be in intensive care, and would want to be provided with all the life support that is possible (Table 4).

Table 4. Medical treatment preferences in case of a fatal disease

Let's assume you are diagnosed with a fatal disease some day in the future If there's nothing that can be done about your disease and death is inevitable which of the following would you want?	n (%)
I'd want to be at home and relieved of pain, I wouldn't want any other treatment I'd want to be relieved of pain and in a special care facility I'd suffer if I need to for any treatment I'd want to stay in intensive care at the hospital even if I am unconscious I'd want all life supporting treatments	459 (51.2) 161 (18.0) 128 (14.3) 91 (10.2) 57(6.4)
Total	**896**

***Opinions on Advance Directives*:** As an answer to question "If one day in the future you are unconscious and diagnosed with a fatal disease, would you like to leave a request or a directive beforehand today about your treatment" 42.9% said that they wanted to leave a directive. 25.4% of these individuals expressed this request as "I haven't thought of this before but I'd like to". 30.7% of those who didn't want to leave a directive said they had doubts about this subject. When the participants were asked if one day they are diagnosed with a fatal disease and they are incapable of making this decision, who would they want to give the end-of-life treatment decisions, 33.4% stated that they would like it to be their spouse (Table 5).

Discussion

The top choice among good death definitions was dying without feeling any pain, while the leading choice among bad death definitions was dying due to loss of physical body integrity as a result of an accident. On the subject of agony during the period before death, the number of people who preferred dying to suffering was also high. When death was inevitable, two thirds of the participants did not want intensive care treatments; instead they wanted to be at home or in a special care institution provided they

Table 5. Advance Opinions regarding Advance Directives

Advance Directives (N=896)	n (%)
Yes	384 (42.9)
No	512 (57.1)
Yes, why (N= 384)	
No answer	228 (59.4)
I wouldn't want to be miserable being sick	120 (31.3)
I wouldn't want to suffer pain-agony	20 (5.2)
I'd like to decide on my own destiny	12 (3.1)
I wouldn't want to hurt my family	4 (1.0)
No, why (n= 512)	
I have doubts about this subject, I don't know it very well	275 (53.8)
I believe in miracles	101(19.7)
I didn't think about it, it doesn't suit me	91(17.7)
Everything possible should be done until the last moment	45 (8.8)
Who would you want to give end of life treatment decisions for you? (n=896)	
Spouse	299 (33.4)
Parents	281(31.4)
Offspring	174 (19.4)
Doesn't matter	129 (14.4)
Physician	8 (0.9)
Sibling	5(0.6)

could be relieved of pain, although such institutions do not exist in Turkey. Similarly, although Advance Directives are not a part of healthcare applications in Turkey, more than forty percent of the participants said yes to leave Advance Directives for end-of-life treatments. This positive attitude was remarkable. Less than one percent of the participants considered the physician to be a suitable decision maker for their end-of-life decisions. According to our study, although good and bad death elements showed similarities to Eastern and Western cultures, preferences associated more with autonomy than the paternalistic approach stood out. For example, requests for applications such as palliative care or hospice, which do not currently exist in the Turkish healthcare system; the high numbers of people preferring to die instead of feeling pain, although there is a ban on euthanasia; and the possibility of a growing acceptance of advance directives despite limited knowledge of them, were seen.

According to the results of the study, pain-free death was the leading choice for the elements of good death. In studies that were carried out in other countries, freedom from pain was similarly one of the most commonly cited elements of a good death [1, 4, 12-15].

In our study, destruction of body integrity in an accident (by burning, freezing, drowning, or dismemberment) was the most common choice of bad death characteristics. This opinion suggests that the participants may have been affected by unfortunate events that have occurred in and around Turkey. Traffic accidents and terror attack deaths find a dramatized place in the press. The nurses who participated in the study of Kim and Lee (2003) listed the elements of a bad death as persistent vegetative state, sudden death, pain and agony, dying alone, and being a burden[8] .

While some studies have seen a relationship of good and bad death elements with ethnicity and age [16], in our study, no relation was seen between the independent variables and the characteristics of good and bad death. Similar numbers of participants expressed preference for one of the two opposite views "I'd suffer if it is my destiny" and "I'd rather die than suffer" about suffering in the period before death. It was seen that these preferences were influenced by independent variables such as age, education level, having children and the effect of religion on daily decisions. Previous studies have shown that ethnicity and religion have a strong influence on beliefs about death and dying; these

effects are greater than those of sex and education level" [17] . It is known that the attitudes towards death and the death process are affected by age [16]. In our study, the education level and age made more of a difference than the other independent variables. Additionally, despite the ban of euthanasia in Turkey, the high level of people saying "I'd rather die than suffer" (38.7 %) attracted attention.

One third of the participants in our study did not want to be a burden to their children during the period before death. There is a tradition of patient care being done at home, especially with elders being looked after by their children. Indicative of this tradition, nearly one third of the participants had provided care for a dying relative; these individuals were more likely to be female and over 41 years old. Women often take the role of caregiver in Turkish society. Similarly, it is seen that the care of terminally ill patients is done at home by women in Japan [18]. The participants in this study indicated that, in the event of a fatal, untreatable disease diagnosis some day in the future, they preferred to be at home, relieved of the pain of inevitable death. Dying at home is a desirable death in Japanese tradition, too. Nagamine (1988) reports, as a result of his study on 150 Japanese families, that the majority prefers dying at home to a hospital [18]. Similarly, Chinese people also say that dying at home in the company of family has a special meaning [19]. In the study of Got *et al.*, home was again chosen by elderly people as the best place to die [1]. In other studies, home is reported as the ideal place to die[7, 20-22]. It is interpreted that what is meant by death at home is dying in the presence of family members and with provided care, as dying alone at home is an unwanted death type. Seale (2004) states in his study that the British, like other societies see dying alone at home as a bad death type [10]. According to the results of our study, when death is inevitable, people want to be in a place where pain control treatments rather than aggressive treatments are used, where they can receive special care, is the second most common request, after those who want to die at home. In a country where there are no palliative care centers, the high percentage of participants requesting this type of death is thought provoking. There are not any such care facilities in Turkey, other than a few beds in some private health institutions. However, home care is supported. Especially in the last five years, the state has begun supporting home care. In addition, studies on financial and technical support and expanding home care countrywide are on the way. In our study, the total number of people who said they would suffer if needed for any treatment during the period before death, who wanted to stay in intensive care at the hospital and who would want all life supporting treatments even if they were unconscious, consisted of roughly one third of the participants. The majority, with two thirds of the participants, preferred palliative treatment rather than such aggressive treatments. This ratio is very important in Turkey, where it is common to reject the removal of life support even in the event of brain death. Increasing numbers of terminal patients are kept alive in intensive care units for months, only to die in these units.

In our study, it was remarkable that more than forty percent of the participants said yes to having an advance directive for end-of-life treatments, as advance directives are not a part of health applications in Turkey. Turkey has signed the Oviedo Convention. In addition, there is a Patient Rights Directive that was issued and came into effect in 1998; studies on expanding its application are still ongoing [23] . It is also known that the public does not have any information about this. Although these change the paternalistic approach of the physician which was more common in previous years and although emphasis is being made on autonomy, advance life directives are not yet put into use [24]. However, in countries where there is a use of advance directives, it is reported that the number of people who define advance directives in practice is low [25]. According to the results of our study, no relevance was seen between the opinions on advance life directives and independent variables. But there are different results in the studies that are carried out in other countries. For example, Doorenbos and Nies (2003) found in their study that sex is determining advance directive decisions of Asian Indians. According to the results of the study, Asian Indian women were more willing than men for advance directives applications [25]. According to the study results of Wijmen *et al.* (2010), education and the presence of religious convictions that play an important role in one's life increases the chance of not wanting to draw up

an advance directive [26]. According to the study of Matsui *et al.* (2008), the use of advance directives is influenced more by the society in which a person lives than by their ethnicity. They found that end-of-life attitudes would change between Japanese people living in the USA and those living in Japan. For example, while in Japan, no Japanese person took any action on advance directives, a large percentage of Japanese people living in the USA had given such a directive [27]. In our study, more than half of the people who said yes to the use of advance directives did not state any reason for this opinion. The majority of people who stated a reason for saying yes to their use had said that they did not want to suffer while being sick. Half of the people who said no stated that they did not have any information on this subject and had doubts. These results led us to believe that, while there is a positive attitude towards advance directives, which are still a legally and ethically controversial subject in many countries, the participants might have some confusion about them, due to limited knowledge.

In our study, the participants chose spouse, parents and children as the top three choices for making end-of-life decisions for them. Contrary to the paternalistic approach, only 8 people wanted their physician to make the decision. In other studies, family members led by spouses were preferred for end-of-life decisions as well.

With this study, we discovered important results clarifying a subject which is not discussed in Turkey. However, it was observed that participants felt emotionally uncomfortable and distanced themselves from the subject because death and dying are not something openly spoken about in public. An additional limitation is that the study can only give an opinion of the city in the western part of Turkey where the study was carried out, rather than the whole country.

It seems important that these subjects, which do not currently appear in the Turkish healthcare and legal systems, are discussed by the legal field and other relevant disciplines and be shared with the public for discussion. According to this study, it was discovered that the perception of a good death in Turkish adults is similar to that of both the Eastern and Western world, just as Turkey's geographic, historic and social structure is.

References

1. *Gott M, Seymou J, Bellamy G, Clark D, Ahmedzai S. Older people's views about home as a place of care at the end of life. Palliative Medicine, 2004; 18: 460. doi: 10.1191/0269216304*

2. *Ariès P, Murchland B. Death inside out. Hastings Center Studies, 1974; 2(2): 3-18.*

3. *Kring DL. An exploration of the good death. Advances in Nursing Science, 2006; 29(3): 12–24.*

4. *Mak JMH, Clinton M. Promoting a good death: an agenda for outcomes research - a review of the literature. Nursing Ethics, 1999; 6(2): 97-107.*

5. *Singer PA, Martin DK, Kelner M. Quality end-of-life care. JAMA, 1999; 281(2): 163–168.*

6. *Beckstrand R, Callister C, Kirchhoff KD. Providing a "good death": critical care nursing' suggestions for improving end of life care. American Journal of Critical Care, 2006; 15(1): 38–47.*

7. *Gupta R. Death beliefs and practices from an Asian Indian American Hindu perspective. Death Studies, 2011, 35: 244–266. doi: 10.1080/07481187.2010.518420.*

8. *Kim S, Lee Y. Korean nurses' attitudes to good and bad death, life-sustaining treatment and advance directives. Nursing Ethics, 2003; 10(6): 624-639. doi: 10.1191/0969733003*

9. *Hirai K, Miyashita M, Morita T, Sanjo M. Good death in Japanese cancer care: a qualitative study. Journal of Pain and Symptom Management, 2006; 31(2): 140–147.*

10. *Seale C. Media constructions of dying alone: a form of 'bad death'. Social Science & Medicine, 2004; 58: 967–974.*

11. *Sarhill N, LeGrand S, Islambouli R, Davis MP, Walsh D. The terminally ill Muslim: Death and dying from the Muslim perspective. American Journal of Hospice Palliative Care, 2001; 1: 18, 251. doi: 10.1177/104990910101800409*

12. *Kehl KA. Moving toward peace: an analysis of the concept of a good death. American Journal of Hospice Palliative Care, 2006; 23: 277. doi: 10.1177/1049909106290380.*

13. *Clark D. Between hope and acceptance: the medicalisation of dying. BMJ, 2002; 324: 905-907.*

14. *Kellehear A. On dying and human suffering. Palliative Medicine, 2009; 23: 388-397. doi: 10.1177/0269216309104858*

15. *Payne SA, Langley-Evans A, Hillier R. Perceptions of a 'good death': a comparative study of the views of hospice staff and patients. Palliative Medicine, 1996; 10: 307–12.*

16. *Kalish RA. Reynolds DK. The role of age in death attitudes. Death Education, 1977; 1(2): 205-230. 1977*

17. *Cicirelli VG. Religious and nonreligious spirituality in relation to death acceptance or rejection. Death Studies, 2011; 35: 124–146. doi: 10.1080/07481187.2011.535383.*

18. *Nagamine T. Attitudes toward death in rural areas of Japan. Death Studies, 1988; 12(1): 61-68. doi: 10.1080/07481188808252220*

19. *Hsu CY, O'Connor M, Lee S. Understandings of death and dying for people of Chinese origin. Death Studies, 2009; 33(2): 153-174.*

20. *Brazil K, Howell D, Bedard M, Krueger P, Heidebrecht C. Preferences for place of care and place of death among informal caregivers of the terminally ill. Palliative Medicine, 2005; 19: 492-499.*

21. *Higginson IJ, Sen-Gupta GJ. Place of care in advanced cancer: a qualitative systematic review of patient preferences. Journal Palliative Medicine, 2000; 3: 287-300.*

22. *Ratner ER, Song JY. Ethics and dying at home. Home Health Care Management Practice. 2003; 5: 123. doi: 10.1177/1084822302239300*

23. *Türkiye Cumhuriyeti Sağlik Bakanliği [Turkish Ministry of Health] Hasta Haklari Yönetmeliği (HHY). (Bill of Patients' Rights.) 1998. Date 01.08.98; no. 23420 (in Turkish).*

24. *Guven T, Sert G. Advance directives in Turkey's cultural context: examining the potential benefits for the implementation of patient right. Bioethics. 2010; 24(3): 127–133. doi:10.1111/j.1467-8519.2009.01789.x*

25. *Doorenbos AZ, Nies MA. The use of advance directives in a population of Asian Indian Hindus. Journal of Transculture Nursing, 2003; 14: 17-24. doi: 10.1177/1043659602238346*

26. *Wijmen MPS, Rurup ML, Pasman HRW, Kaspers PJ, Philipsen BDO. Advance directives in the Netherlands: an empirical contribution to the exploration of a cross-cultural perspective on advance directives. Bioethics, 2010; 24(3): 118–126. doi:10.1111/j.1467-8519.2009.01788.x*

27. *Matsui M, Braun KL, Karel H. Comparison of end-of-life preferences between Japanese elders in the United States and Japan. Journal of Transcultural Nursing, 2008; 19: 167-176. doi: 10.1177/1043659607312969*

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Therapeutic approach in treatment of Methicillin Resistent Staphylococcus Aureus in female newborn caused by Nosocomial infection

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Abstract

Nosocomial infections represent a significant problem in the development of morbidity and mortality among hospitalized patients. The article presents a case of a female infant which immediately after birth was infected by umbilical cord and after receiving the BCG vaccine in hospital conditions developed very high fever with general weakness and fatigue.

Key words: hospital infections, Staphylococcus aureus, BCG vaccine.

1. Introduction

Staphylococcus is one of the most common pathogenic bacteria which cases infections of the skin, soft tissue, systemic infections, pneumonia, sepsis, endocarditis and meningitis (1).

In recent decades all over the world is increasing the resistance of clinically important bacteria to antibiotic therapy. Health institutions are the source and reservoir of pathogenic bacteria with high resistance level. Mistakes in controlling infections can lead to transmission of infection, even by highly resistant pathogens such as Methicillin-Resistant Staphylococcus Aureus (MRSA).

Shortly after birth staphylococcus colonizes the skin and nose vestibule of a newborn, nasopharynx mucosa, often the digestive system and vagina, which in a large number of patients remain as permanent colonization (2).

Staphylococcus from the local focus can spread by lymph or through the blood throughout the body and thus cause sepsis and metastatic purulent inflammation anywhere in the body, pneumonia, respiratory infections and purulent meningitis.

Diseases caused by Staphylococcus aureus are the result of its ability to reproduce and spread in the tissue and to produce numerous enzymes and toxins (3).

The enzyme catalase significantly slows the eradication of Staphylococcus aureus in phagocytes, followed by coagulase which staphylococcus excreted into the environment, thus creating layers of fibrin around the bacterial cells which makes staphylococcus cells out of reach of defensive host factors (2).Approximately 50% of human strains of Staphylococcus aureus produce one or more of the six types of known antigenic enterotoxins (A-F) in about 75% the secreted is enterotoxin A as well as TSST-1, with other enterotoxins of super antigen capability. Staphylococcus enterotoxins have emetic action primarily in the central nervous system and cause profuse vomiting, but also accelerate bowel mobility (3).

Primary immune bacteria eradication during opsonization takes place in the liver (1). Staphylococcal infections may be transmitted by touch, by air, and they are particularly important in hospitals where are located risk group for the development of staphylococcal infections in neonates, surgical patients in intensive care departments, undernourished elderly persons, diabetics and immune compromised patients (1).

Sources of infection are usually healthy people who are colonized with staphylococcus.

It is well known that over 50% of people carry Staphylococcus aureus in the nose, especially among hospital staff (2).

The best protection in the hospital is attentive and proper hygiene, washing hands before and after each contact with sensitive hospitalized population (2).

2. Case report

Female newborn T.A. born on June 18, 2008, born in term, TT 4400 grams.

According to the program of regular immunizations received after one day BCG vaccine and HEB vaccine.

Immediately after the vaccine, develops fever, general weakness and fatigue, poor level of reflexes and poor positional varus (5). Newborn's umbilical cord infection was detected after birth.

ECHO examination of the brain in standard approaches and planes did not identified focal changes or hydrocephalus.

Dg.Omphalitis acuta, granuloma umbilicale

Laboratory findings: Complete blood tests: **Leucocytes: 22.3** (elevated) reference values: up to 18x10/L, **Erythrocytes: 4.4**, Hb: 16g reference value14.5 to 22.5), thrombocytes: 253 L: 16.5 (reference 12)

AST: 48 (15-60), ALT: 39 (reference 5-25) LDH: 1071 (reference 160-450), CK: 225 (reference value 68-580) (5).

After this was started the therapy by infusion, oxygen, Lendacin amp.(ceftriakson), ampicillin ampule. Baby was discharged with a recommendation Zinat syrup(cefuroksim-axetil syrup, Rivanol solution, and control of leukocytes, CK and LDH in seven days.

3. Disease course

Control medical examination after seven days showed physical findings of the heart and lungs as normal, abdomen was soft and insensitive, liver and spleen are not palpable. Leukocytes remained elevated and slightly elevated LDH with a much better motor skills, with a recommendation to perform laboratory urine analysis.

During the following control transaminases were within normal limits, but the leukocytes are still elevated, stuffy nose (microbiological analysis of nasal swab was not performed). Introduced in the treatment is a recommendation of Amoxibos (amoxicillin) syrup and Aqua Maris isotonic solution as the nose spray several times a day. At the following control examination leukocytes level decreased with a slightly elevated LDH. Since the child is still not well with poor clinical presentation, fewer, general weakness it was proposed to perform analysis of a nasal swab from which was isolated Staphylococcus aureus.

Upon the recommendation to a given therapy clinically the newborn was not better.

Once again nasal swab was taken and re-isolated from nasal swabs was Staphylococcus aureus. On the basis of antibiogram in treatment is included macrolide antibiotic erythromycin on which the infant responded well clinically and was better.

After ten days, the control microbiological finding of nose swab once again isolated Staphylococcus aureus and isolated a large number of colonies.

Newborn has been clinically well.

In continuation of antibiotic therapy included Bactrim (trimetoprim-sulfometoksazol), for seven days.

From then after two months during medical control examination was also isolated Staphylococcus aureus from nasal swab, with plenty of attenuated strains but remains isolated culture of a large number of colonies.

During the therapeutic break of fifteen days without antibiotic therapy, made is control swab which again from the nose isolated Staphylococcus aureus.

On the isolated strain of Staphylococcus aureus was performed antibiogram by disk-diffusion method where only Cefazoline was in the zone of sensitivity and other discs were resistant to isolated strain. In therapeutic approach for eradication of isolated strain started local actions where it was made a solution of 1/3 of cefazoline vial of 250 mg and a bottle of solution "Sunce moje malo" (My little sunshine). Introduced is local therapy in the form of nose drops that lasted about five days.

After five days the control microbiological evaluation of nasal swab showed that the nasal flora is sterile. After about a month again at the control examination Staphylococcus aureus was isolated from nasal swab with the large increase in the number of colonies. Antibiogram was done and the only antibiotics in the zone of sensitivity were Cefazoline and Gentamycin.

Consulted is infectious disease specialist due to isolated Staphylococci.

The child is in a couple of days developed clinical presentation with high fever, diarrhea,

vomiting and refusing food with enterocolitis and dehydration. The child was hospitalized and immediately was introduced antibiotic Cefazoline in forms of vials, Linex˙(probiotic) and symptomatic therapy. During the fifth day child reacted well to a given therapy with better clinical presentation and discharged with the recommendation to take Bactrim (trimetoprim-sulfometksazol) and control in agreement with the responsible pediatrician. With better clinical presentation the child was again included in a regular vaccination program which was delayed because of the constantly present Staphylococcus aureus.

4. Material and methods

Control of nasal swabs was performed at the microbiological laboratory of Cantonal Hospital in Travnik and microbiological laboratory of the Public Health Institute.

In determining the sensitivity of isolated Staphylococcus aureus to antimicrobial agents was used a disk diffusion method: paper disc saturated with standardized amounts of antibiotics, is placed on the surface of a solid substrate (Muller-Hinton agar) which was previously inoculated by pure cultures of the test bacteria and incubated at 35 -37 ^{o}C. Test is set according to a standard McFarland suspension 0.5 (9).

5. Discussion and conclusion

Staphylococcus infections are serious, severe and long lasting. Local use of antibiotics in the nose is usually ineffective in eradication of MRSA in the throat and sputum (1).

During the follow up, the findings of staphylococci that for a long time was isolated from nasal swabs, and the child for certain period of time did not expressed any clinical symptoms, at one point was a resistant strain that is resistant to all disks except cefazoline that belongs to the group of the first generation cephalosporins which is administer per enteral. Given that the child must be covered with antibiotics, we decided on the local cefazoline therapy in order to spare the organism of the newborn from any adverse side effects due to excessive coverage with antibiotic therapy. So control nasal swab findings were sterile.

Further considering that about one month was waiting period for control, because it was thought that the strain was eradicated (persistent are fractions of bacterial cells resistant to that concentration of antibiotic that destroys most of the bacterial population) (6) it had time to breed again in the one month pause in a small and enough matured immune system so the control smear showed a large increase of these bacteria in the nose of the newborn. Bacterial resistance occurs or as a result of selection of resistant strains or as a result of changes in the genetic material of microorganisms.

Antimicrobials create very potent selective pressure of antimicrobial drugs on the population of bacteria and contribute to the growth of those who can resist its effect. Nature of Staphylococcus aureus will continue to change or to be genetically modified and this bacterium is specific, as it will keep a step ahead with the development of new antibiotics, because by now to even those antibiotics that were drug of choice it is becoming resistant These infections will require new strategies and modification of management in the prevention as well as therapeutic approach (7).

References

1. *Damani NN, Emmerson AM. Prijevod drugog izdanja, (urednici) Kalerić S, Horvatić J, Priručnik o postupcima kontrole infekcija: Zagreb, 2004; 117-125.*

2. *Kalenić S, Plečko V, Nemet D. Mikrobiologija sepse: Medicinska naklada: Zagreb 2003; 4-7.*

3. *Kalenić S, Mlinarić-Missoni E, i suradnici: Medicinska bakteriologija i mikologija, Zagreb Merkur A.B.D. 2001: 161-168.*

4. *Roitt I. Osnove imunologije, Medicinska naklada Zagreb, 1974; 131-134.*

5. *Marešić D. i suradnici: Pedijatrija Zagreb – Školska knjiga 2003: Referentne vrijednosti laboratorijskih nalaza u djece. 1110-1111.*

6. *Vraneš J, Marić S. Biofilm-patogeneza i uloga u infekcijama povezanim s intravaskularnim kateterima, 14 poslijediplomski tečaj stalnog medicinskog usavršavanja prve kategorije iz kliničke mikrobiologije (sprečavanje infekcija povezanih s intravaskularnim kateterima) KBC Zagreb, Edukacijski centar – Rebro. Zagreb 2005; 11.*

7. *Društvo mikrobiologa, Šesti simpozij o kontroli bolničkih infekcija Bosne i Hercegovine, Tuzla. 2008; 1.*

8. *Opća medicinska bakreriologija, (antimikrobni lijekovi) Sarajevo, 1997; 25.*

9. *Uzunović-Kamberović S. Medicinska mikrobiologija, Zenica, 2009; 248-249.*

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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.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

Table 1. Page layout description

Paper size	A4
Top margin	20 mm
Bottom margin	20 mm
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Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledge-ment) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

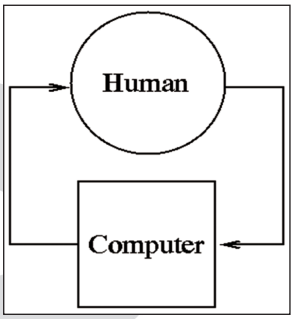

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

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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