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Potential iatrogenic risk factors in periodontitis

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Abstract

Dental iatrogenic complications can initiate disorders in oral cavity and indirectly influence whole organism. Current studies present the complexity of periodontitis etiology. Recent research concentrates on the influence of local and systemic factors on periodontitis development. The aim of the following study was to answer this question: Do local factors like overhanging, interproximal fillings and overcontured crowns are responsible for local epithelial attachment loss and destruction of the alveolar bone?

Results of the conducted study let us to draw following conclusions:

1. Improper dental fillings predispose to the local loss of the epithelial attachment.
2. Size of the overhanging dental filling under the gingival line is responsible for the intensity of the local periodontitis.
3. Time which passed since the application of the overhanging dental filling has a negative influence on periodontium.
4. Time which passed since the application of the overhanging dental filling has a smaller negative effect on the development of local periodontitis than the existing fault size.

Key words: Iatrogeny, periodontium, overhanging filling, overcontured crown, periodontal ligament, alveolar bone loss.

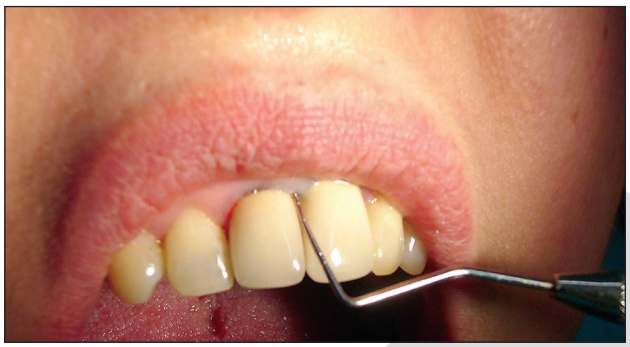
Introduction

Potential risk of performing iatrogenic operations has been known since Hippocrates' time. Etymology of iatrogeny refers to greek language, 'iatros'-means doctor and 'genesis'-origin. Research presented by Offenbacher and Zambon proved the influence of pathogens of periodontitis in systemic diseases such as cardiologic and vascular disorders, strokes and premature birth. In the following study the theory of bacteriological embolism has been presented, which proves that the products of bacterial metabolism and cytokines transferred by bloodstream settle down in tissues and cause their local damages (3). Dental iatrogenic operations can initiate disorders in oral cavity and indirectly influence whole organism. Current study presents the complex etiology of periodontitis and newest research with regard to the influence of local and systemic factors on development of periodontitis.

Material and methods

The studies were preformed on 102 patients with an overhanging filling or an overcontoured crown. Written informed consent was obtained from every patient qualified to enter the study. All patients underwent clinical examination and dental x-ray. Clinically, the length of the filling reaching the gingival line was measured by calibrated periodontal probe. The length of the overhang localized under the line of the gingiva was found by a subtraction of the total length of the filling and filling existing above the gingival line. (Picture 1). The conducted research survey concerned: the accommodation, socio- economic conditions, variety of patients diet, oral hygiene, frequency of dental check-ups and addictions. Presence of the overhang was confirmed by radiographic examination (the straight angle technique). Additionally, the investigation of dental pockets where conducted by calibrated periodontal probe, in 4 points for each tooth: external surface (buccal/ labial);(I), internal (palatal/ lingual);(II), mesial (III), distal (IV). Patients with diabetes mellitus type 1 and 2, Acquired Immune Deficiency Syndrome (AIDS), immunosuppressive therapy of neoplastic diseases, alcoholics, tobacco smokers, pregnant and

feeding women, patients with removable dentures or orthodontic appliances were excluded from the study.



Picture 1. Clinical examination of the dental pockets

Statistical Analysis

All values are expressed as mean and standard deviations (mean±SD). Distributions of the analyzed variables were tested using the Shapiro-Wilk test. Statistical analysis was performed using chi -square test (χ^2), U Manna-Whitney and ANOVA variance. In all test, p-value <0.05 was considered significant. All statistical analyses were conducted using the Statistica 10.0 software.

Results

A research group consist of 102 people aged 32 to 71, including 47 (46.07%) women and 55 men (53.93%). Among the respondents 67.7% graduated high school, 12.75% universities and 9.8% primary school. Social and living conditions of the studied people were "rather good" (76.47%), 22.55% "moderately good" and a few of them described it as "bad". Taking the frequency of teeth brushing of the respondents under consideration, 60.78% clean their teeth twice per day, 18.63 once per day and 11.76% three per day and few of them did not brush teeth at all. It was shown that average highest value of the depth of the periodontal pockets was found next to the distal surface of molars (IV) 3.09±1.26 mm, minimal values were 0 mm and maximal 6 mm. Furthermore, average smallest value of the depth of periodontal pockets was found at the labial surface of canines (I) 1,15±0.36 mm, minimal values were 1 mm and maximal 2 mm, so the pathological periodontal pockets adjacent to these surfaces were not found. We did not find pathological periodontal pockets (>2 mm) next to the incisors in points I, II and canines in points I, II. Moreover, the study population was divided into subgroups according to the materials type of the overhanging filling. It was shown that 25 of overhanging amalgam fillings, 62 of composite fillings and 15 over contoured porcelain crown were incorrect. The study revealed, that among patients with higher percentage of large overhanging filling localized subgingivaly, the SBI (Sulcus Bleeding Index) was significantly higher than among patients with smaller size of subgingival overhang, p=0.03. The highest percentage of subgingival overhangs made of amalgam were discovered among patients with the shortest time to pass since the fillings application, p=0.02. As a matter of fact it was found that the percentage of the overhanging fillings below the gum line differed significantly regarding the type of that filling. Significantly, the highest percentage of the subgingival overhangs was observed among patients with amalgam fillings in relation to the composites. In the conducted study the mean value of SBI was 29.11% and moderate value of API (Aproxima Plaque Index) was 58.34%. The time which passed since the application of the overhanging filling was not longer than 8 years, average was 3.11±1.77 years. The results indicated that significantly higher SBI (p=0.007) and API (p=0.03) were found among patients with shorter time since the application of the overhanging filling. It was proved that the type of the material of the filling has an influence on the sulcus bleeding index. We found significantly higher SBI among patients with overcontured porcelain crowns compared to patients with overhanging composites and amalgams. In addition to that, probing results of teeth with iatrogenic restorations were compared to those of teeth free from iatrogenic faults. It was observed that pathological pockets were significantly more frequent among the teeth of patients with improper fillings or crowns (35.54% vs. 22.55%); (table 1 and 2).

Table 1. Type of the material of the „overhanging filling" versus the % of the „overhang" under the gingivae

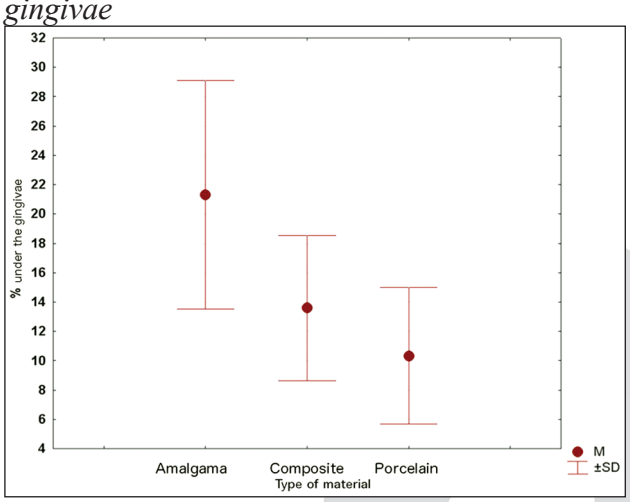
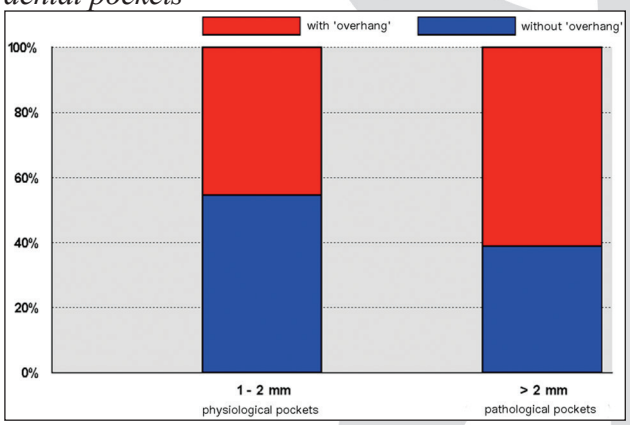


Table 2. Overhanging filling versus pathological dental pockets



Discussion

Periodontal diseases and their etiologies have been a subject of many scientific researches for years. The researchers emphasized that the etiology of periodontal inflammation is complex. Local factors, in addition to mechanical damage to the periodontal structures, may promote plaque accumulations, and change its composition. Dental plaque is considered one of the major risk factors for periodontal disease (2). In recent years publications indicate the existence of more than 600 species of bacteria colonizing dental plaque. Slots and Morrison considered Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides fragilis, Treponema denticola, less often mention as Eikenella corrodens, Camphylobacter rectus, Prevotella intermedia, Fusobacterium nucleatumthe to be the most common bacteria associated with the development of periodontal disease(7). Already in 1912, after introduction of Black's classification of cavities, it was found that improperly reconstructed dental filling is an irritanting factor for the periodontium and causes an inflammation. The study conducted by Hakkarainen et al in 2005 revealed the presence of overhangs in more than 50% of all fillings in molars. Comparing the loss of alveolar bone at the tooth surface with radiographically identified overhanging filling and homologous tooth surface without the overhang, the authors showed a statistically significant difference. Loss of alveolar bone adjacent to the overhang is increasing with the time since its performance (1). Similar results were obtained in the presented study. Schatzle and coauthors also observed the significant loss of periodontal attachment in the first three years after the performing the second class according to Black's classification dental filling. (6). Many scientific reports describe adverse effect of the second class dental fillings to the adjacent periodontium, even without clinical or radiological verification. It is assumed that properly performed dental filling should not be the reason of the periodontal inflammation. Our findings also emphasize the impact of the type of the material of the filling and type of restoration on the severity of periodontal inflammation. The highest rates of SBI index were observed with overcontured porcelain crowns compared to overhanging composites and amalgams, and also statistically significantly higher with amalgams compared to composites. Our results allow to conclude that the greater impact has the size of overhang under the gum line or in the case of crowns exceeding the biological width than the degree of smoothness of the surface of the material and the type of filling material. The lowest rate of SBI was observed in patients with overhanging composite fillings. There are also studies which show no correlation between periodontal inflammation of adjacent tissues to the overhanging fillings or overcontured prosthetic crowns, or dental caries (4,5). 12 months after performing glassionomer and amalgama fillings Paloantonio and co authors have not found any difference in clinical examination or bacteriological tests in tissues adjacent to the treated teeth (4,5). Observations based on present study and studies of other authors evaluating

the effect of defective and properly executed Class II restorations on the adjacent periodontal tissues focus on the correctness and regularity of those restorations. The size of the filling under the gum line, the time since its performance until overhang correction, the type of the material and the use of the oral hygiene products have influence on the adjacent periodontal tissues.

After analyzing carefully the results of the study we suggest further, more circumstantial diagnostics of the dental fillings and oral cavity examination, including:

- type of the restorative material,
- level of smoothness,
- regularity of shape,
- presence of secondary caries,
- time which passed since the application of the restoration,
- relation with the gingival line and biological width,
- conditions of periodontium before applying of the filling,
- oral hygiene indices,
- control dental check ups.

Conclusions

Results of the conducted study let us to draw following conclusions:

1. Improper dental fillings predispose to the local loss of the attachment.
2. Size of the overhanging dental filling under the gingival line is responsible for the intensity of the local periodontitis.
3. Time which passed since the application of the overhanging dental filling has a negative influence on periodontium.

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Clinical process management with use of health information technology focusing on hospital information system

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Abstract

Background: Healthcare organizations faced with coordination challenges. Use of Work Flow Management System (WFMS) is the suitable method for improving performance.

Aim: In this article method of managing workflow for organizational and care process has studied separately. Suitable IT tools for supporting each process with practical work about HIS effects has presented.

Methods: We conducted A cross sectional survey in February- May of 2011 at Mazandaran University of Medical Sciences, Iran, among practitioners. Part one of this study was conducted as a library method and systematic review of literatures with inclusion and exclusion criteria. Using snowballing including Reference tracking and citation, more papers that are valid were retrieved

Results: Sixty nine percent of respondents believed that HIS lead to easy access to information required. Health care information systems such as Hospital Information System (HIS) have to support organizational and treatment process separately. We asked respondents used HIS in therapeutic process to answer the valid questions about effect of HIS**.**

Conclusion: To identify current challenges in healthcare, it is necessary that organizational process be separate of care process. Learning is necessary to use HIS for improving workflow and results of tasks.

Key words: Clinical process management, health information technology, hospital information system.

Introduction

Hospital with different departments, information processing plans and professional healthcare staff from different fields, need inter and intra organizational communication. In recent decades, information technology and communications have changed ways of life in all aspects including health care. Scientific texts show that use of information technology for the best decision in the realm of medical fields is needed (1, 2). In this field, many information and knowledge will be produced and used. However, due to lack of programs and systems and formal structures for conversion, retention and transfer of knowledge and information about health care, with certainty can be said that health care organizations have strong information but from the knowledge aspects, are weak. (3)

Panzarasa in his paper shows that the medical knowledge in current health care system essentially would not be used(4) . Institute of Medicine in Nov. 1999' report announced that medical errors are the third cause of mortality in the United States and annually occur nearly 98,000 mortality due to these errors. Administrative measures and even employees and executive errors are as part of these errors.(5)

These cases represent an inappropriate use, failure to deliver timely information to health care providers and lack of appropriate technology to support the transfer, delivery, presenting, and integration of information. It is also important to note the health care profession with the highest dynamics to maximize coordination is faced with numerous challenges. Most of these difficulties are related to care processes and workflow. The-

refore, to achieve effective treatment, efficiency and quality, it is necessary that the treatment processes are coordinated(6, 7). During the past 20 years, process-oriented information systems for Integrating and coordinating of the patient's treatment always has been discussed(8, 9). IT as a useful tool in managing of health care information is used in workflow management. In fact, WFMS is the process of automating places and points that documents, information and tasks moving between parts and members of an organization on the basis of predefined rules and policies to meet organizational goals(10). Many studies have identified of the positive effects of information technology in health care systems, particularly in the management of adverse drug reactions (1, 11-14). Electronic health records capabilities as a central database for data integration of different systems of health support structured data entry, electronic information exchange, and participate in medical care and risk reduction measures for patients (15). it has been determined inadequate communication and little information of medical staff is the main factors in creating major side effect drugs and treatment(16) . IT prepare correct information and timely access to HealthCare Practitioners, so it supports the processes of health care in the care of patients(11). But despite all the above, there are significant differences between the use of IT and its potential benefits, (17). What could be the reason the limited use of IT to support information flow between processes in health care? To find a suitable response to this question, it is essential to understand complex characteristics of health care. Identify challenges of IT using in health care management, organizational processes need to be separated from the processes of medical care. In integrating of these two processes, we will be faced with major challenges(18). This study aims at, how to manage workflow in the organizational and therapeutic process separately, and the use of appropriate information technology tools to support each process was reviewed.

Part one of this study was conducted as a library method and systematic review of literatures. Systematic review is planned and structured process the right to seek all available sources of information and research to search all available sources of information and research in the field research question. For this purpose, the research strategy was developed to search for all available and related resources in principle; it makes up the structure and main system of study. Using snowballing including Reference tracking and citation, more papers that are valid were retrieved.

Part two explains effect of HIS in improving of hospital workflow.

Organizational process in health care

In this part, the way of supporting the organizational processes hospital without contact with patient processes will be studied. In addition, the question to be answered is why hospitals want to use IT in support of health care processes?.

It is clear that with increasing in different specialties in medicine, nursing, and medical technician, will be increased therapeutic tasks. In addition, methods of treatment will be changed in most cases. Therefore, it is necessary medical measures are planned, appointments scheduled, laboratory and pathology samples of patients could evaluated and transferred electronically. Therefore, coordination and cooperation between people in different parts of the one task is repetitively but very important. These tasks manually coordinated and performed in most centers and often led to the organizational problems and it has increased workload of hospital staff. This can cause many problems and trigger unwanted effects(10).

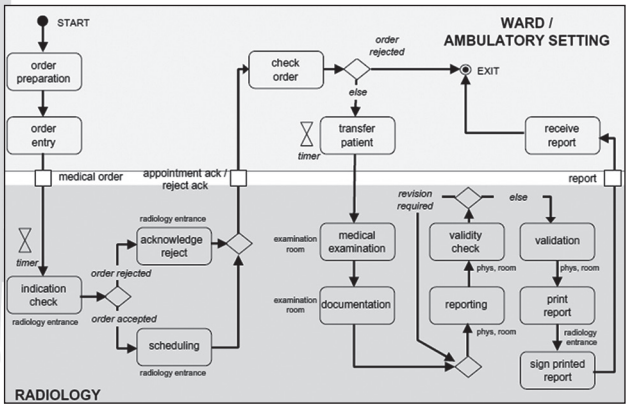

Figure 1. Workflow of radiology unit(18)

The term workflow as "a set of tasks within the organization to produce the final products" and workflow management defines information or tasks based on specific law shall be referred to a person of another person (19, 20). With partial observations and analysis of daily work in health care

domains, new opportunities is opened for applying effective ICT in the administrative processes (19). Organizational process of the ordering physician to report the results of a radiology department and ward (ambulatory setting) is shown in figure 1.

In Lenz's example, a nurse or a physician at the ward or at an ambulatory setting places an order. In the most general case, the indication is checked in the radiology department and depending on the result, the order placer is informed whether the request has been rejected or scheduled. The actual radiological examination and the corresponding documentation are done in the examination room. The radiology report is generated afterwards. If necessary some iteration for corrections are passed until the experienced radiologist can validate the report by his signature. The final report is sent back to the order placer (18) Process mentioned is a part of the basic hospital processes clinical measures and organizational knowledge acquisition for coordination Processes in health care between different individuals and organizational units.

Support of organizational processes using WFMS

Today organizations are faced with globally competitive in many areas. So, for overcoming the challenges it is necessary to use of new technologies such as IT(19). Application of workflow management technology has become easier with increasing role of information technology (18). Hospitals that have a mix of different sections with various computer programs is necessary to support inter and intra department process integrated and coordinated the data produced by different systems and processes (20). It can say firmly that implementation of information technology in an organizational context requires integration of information technology with the organizational workflow. Many organizations use technology such as workflow management systems to the most coordination and consistency between technologies and real work flow are created (21).

In modeling WFMS, usually the following questions was answered; what to do? (Administrative processes and workflow), how (tasks), by whom (actors) and by what means (tools). Workflow management can be defined as systems for specific implementation, monitoring and coordinating of the work flow cases in administrative distributed environments(22). Eshuis and colleagues illustrate in Figure 2. that how WFS interact with actors and a database system. They believe that actors, database system, and WFS together are part of the organization. The most important entities in the environment are the customers to which the organization delivers its products and services(23). WFS inform actors that they may start some new activities. When an actor completes, it notifies the WFS that it has finished its activity. An actor is under the social obligation only to start an activity once the WFS has notified him that he may start the activity. Moreover, an actor should finish an activity he is working on. These may seem obvious constraints or assumptions; nevertheless we state them here explicitly for better understanding of workflow management (23).

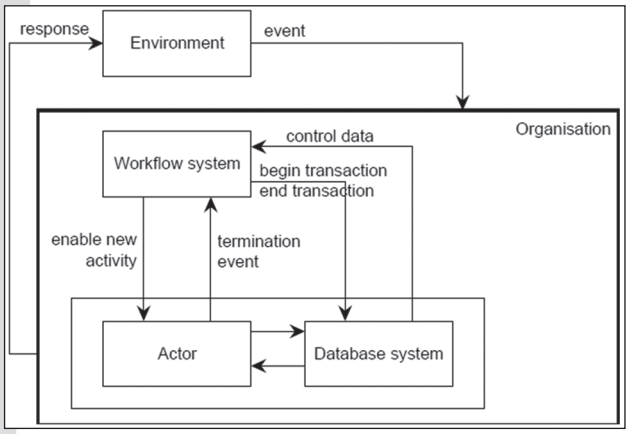

Figure 2. Function of workflow system (23)

As an example of WFM reviewing at the University Hospital of Saarland, it is determined. Wards and service units (e.g., Departments of radiology, endoscopy, or pathology) have to cooperate and to exchange information for the treatment of inpatients. The complete workflow is controlled by a WFMS(24). Tools such as Petri net (they could serve as a conceptual standard for modeling and analysis of workflow), state charts, activity diagrams such as UML (Unified Modeling Language) or also block-structured description language is used to graphically modeling of Workflow system. These tools allow the WF designer to quickly define and modify WF schemes at a higher semantic level, and enable the build time components of the WFMS to detect behavioral inconsistencies and errors in a very early implementation stage(25, 26).

Many of today's available WFMSs provide ad-hoc construct to model workflow procedures.

Some WFMSs provide strange constructs whose semantics sometimes is not very clear. To avoid these problems one could use a Petri-net-based WFMS. The exchange of workflow process definitions between two Petri-net-based WFMSs is easy compared to the exchange of workflow process definitions between two WFMSs based on different concepts (25).

Ngai and colleagues in their paper have reported that use of WFMS in order to improve productivity of primary care services increase the volume of services, income, quality, reduce waiting times and the duration of the visit and also coordinating the responsibilities of employees and health providers. WFMS is important for linking front-end and back-end applications to automate business processes. According to the all items listed will be determined the relationship between organizational structure and information is very high and IT systems support organizational processes strongly(27).

Therapeutic decision-making

Workflow management techniques are required to guarantee the termination of complex distributed process schemes and the logical completion of tasks(7). The medical therapeutic process is often such as cycle figure 3(28) was comprised data collection (observation), diagnostic, and therapy. It starts with the patient History and proceeds with diagnostic procedures that are selected based on available information. It is the job of an (Electronic) Patient Record to assist healthcare personnel in making informed decisions. Consequently, the system should present relevant information at the time of data acquisition and at the time of order entry(18). Each pass of this cycle is aimed at decreasing the uncertainty about the patient's disease or the actual state of the disease process(18).

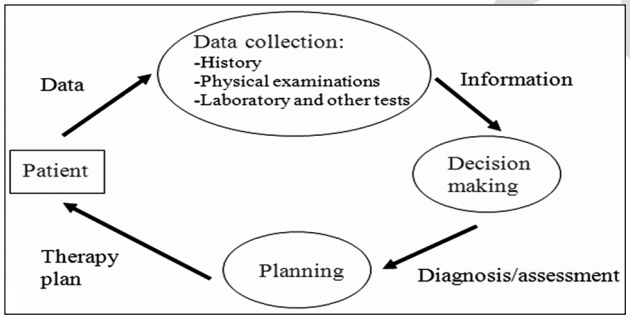

Figure 3. The Diagnostic-Therapeutic Cycle, a Simplified View (18)

Numerous medical software programs on the internet are installed on computers. The clinical reference applications enable the user guidelines and information that can be accessed via the internet(29). Clinical data available at the point of care provide access to complete and accurate clinical information for the care provider (30, 31) . Handheld computers offer significant advantages over desktop computers handheld computers may offer solutions to administrative and information management that are more realistic than those offered are by desktop computers or traditional EMRs. Desktop computers and networks typically require computer information service personnel for maintenance(30).

Clinical process's support with Hospital Information System

There is increasing interest in changing the hospital information system to support clinical processes in a more direct way. Workflow technology is a wonderful candidate to understand process-oriented HIS (32).

Today, health care, with more knowledge, better management and more people in the treatment process can be achieved(4). This means that tasks should be performed to the right persons at the right point in time with the necessary information and the application functions needed.

WFMS can be used to carry out a standardized and defined route through evidence-based clinical processes(33). Such processes are known, as care pathways, care maps, or clinical processes. It has been demonstrated that their implementation reduces the variability in clinical practice and make better outcomes(34). Historically, guidelines, protocols, and clinical pathways are especially helpful when formulating care for costly or complex diagnoses, where many disciplines and a variety of services and interventions are required(35). Guidelines can be individual, local, regional, or national, and often many variants of the same guidelines exist at the same time. Guidelines should be updated regularly. Most importantly, they should be based on the best available evidence. The evidence is that many, perhaps most, are not(36).

As shown in figure 4, the care pathway must involve liaison between primary and secondary care. This is vital to ensure newly prescribed med-

ications are issued (these patients are often used medication system boxes) (37).

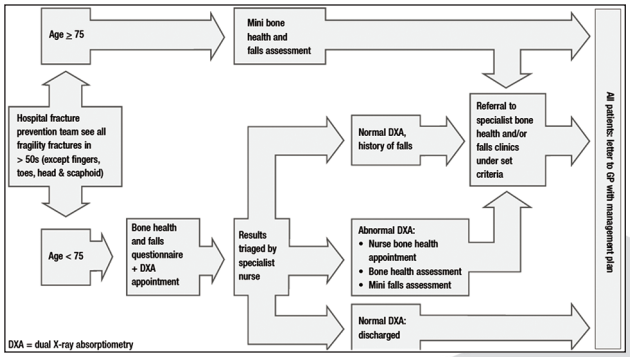

Figure 4. Care pathway for secondary prevention of fragility fracture(37)

Reducing of computer interfaces due to the severe shortage time of physicians is the most important issue in support of clinical processes. One way to reduce documentation and computer interfaces is the use of valid checklist or flowchart. As a result, many subsequent actions are performed automatically(18). Accordingly, Marburg medical center has created an integrated hospital information system. Integration is the most important factors in support of clinical processes(36). As mentioned before, the Marburg Hospital Information System is based on a commercially available holistic system. Extensibility is achieved via a CASE tool, which can be used to implement adapted clinical applications on a rapid prototyping basis. Tool-generated adapted applications are mainly based on workflow-enabled electronic forms which are integrated with the rest of the system via a common database(18) this case clearly demonstrates that the implementation of clinical processes using IT is possible (32, 38, 39). In addition to the Marburg medical center, In Europe, BAZIS, becoming the leading hospital information system in the Netherlands. The Diogene system in Geneva has a distributed architecture and a broad scope of functionality including the use of Intranet technology for accessing and retrieving medical images. In the U.S., the Regenstrief medical record system has been extended to store patient records at more than 30 clinics(40). The Department of Veterans Affairs (VA)'s DICOM1 capabilities are used to interface different PACS2 systems and numerous different radiology image acquisition modalities. Vista HIS is a comprehensive system that supports all the clinical services. All of applications share a distributed Microsoft windows-based client server network architecture, a central database technology, and a common software development environment(41).

According to successful experiences mentioned, IT architecture in supporting of clinical processes should be able to define medical knowledge and to implementation of guidelines and also implementation of guidelines and their relationship with medical records and clinical care systems (42). That is possible but practically difficult. HIS define processes and workflows, send, presentation, distribution of information. The computerized guidelines in information systems are the important tools in improving the quality treatment of patients. It can provide access to information at the point of care despite of growing of medical information and limitations of the medical staff (43-45). From the above, the need for optimally adapting information systems to workflow in healthcare institutions has become evident.

Table 1. The impact of Hospital information system in improving of workflows

Characteristics	Yes/positive effect (%)	No/negative effect (%)	Some what (%)
Easy access to patient data to generate varied records, including classification based on demographic data	69	11	20
Internet-based access improves the ability to remotely access such data	49	30	21
Support automated patient data transfers between departments and institutions	34	15	42
Enhances information integrity in all parts of hospital	74	-	26
Reduces duplication of information entries	56	23	21
Access to lab test results	73	12	15
Patient's admission process	64	18	18

Practical work; HIS and workflows improvement in teaching hospitals

We conducted A cross sectional survey in February- May of 2011 at Mazandaran University of Medical Sciences among practitioners. Interns (22), GP (14), and nurse (18) were selected to study. Their ability to access, use, and interpret of finding information about clinical process is surveyed. Then we asked respondents used HIS in therapeutic process to answer the questions about effect of HIS. Sampling method was based on ease accessible to students (convenience sampling). Our results showed as follows in table 1.

Our results demonstrated that HIS make feasible implementation of workflows, decision-making improvement, and timing reduction. Sixty nine percent of respondents believed that HIS lead to easy access to information required. The mean time of patient's admission process and response to laboratory test reduced.

Discussion

Health care due to the utilization of research findings and new medical technologies is one of the fastest changing areas in the world. The increasing progress in the realm of information technology including in health care large enough to which is for the deployment of IT is a vital identification process flow, why and how performance, control points, areas of process improvement and process owners be identified(1, 7). Given the need to identify health care processes, processes were separated necessary, therapeutics from the organizational processes. To management of organizational processes precise identification of these procedures and identifying information associated with each of the other parts will make the needed coordination and integration be created in the final system(8, 20). The existing experiments indicate that comprehensive study of existing health care provider organizations will be achieved, if only all of the main processes and sub processes investigated using a systematic approach. Moreover, in this regard seems that the pattern of WFMS will be the most systematic method existing to answer project questions (21, 22, 46) . At WFMS, usually the entire workflow is improved and the design of any process in the workflow will be evaluated separately, as a result, no effective actions, and duplication disappears. Following designing the information processing systems of workflow, actual work processes are analyzed and presented, and then the results are computed. Many researchers have identified the potential benefits of workflow technology in the area of health care(19). Webster has shown that the use of WFMS in order to improve efficiency of care increased the volume of services, income, quality, reduce waiting times and reducing the duration of the visit and are caused coordination between health care workers(47). While organizational processes are based on static and fixed organizational models, treatment process is related to the medical knowledge that is changing rapidly(30, 48). The clinical workflow usually is done in parallel according to time and resource constraints and the order of duties should be observed. Computer systems are able to demonstrate tasks as the general as well as if necessary have the ability to display detailed (31, 49-51).

In past years, it is tried using the computer guidelines up to clinical care and resource management to be supported based on of medical information updates. Using Information Technology increases effectiveness and efficiency guidelines in information retrieval (4, 18, 35, 37, 38).

Healthcare institutions need timely patient information from various sources at the point-of-care, and they would like to buy a comprehensive, complete, and fully functional system fulfilling all their needs from one vendor. In order to clarify integration challenges, at least the presentation, functionality, and data layer need to be considered separately(52).

By utilizing HIS organizational processes and patient care is supported separately. This matter will result interconnected network to improve the quality of patient care. As well as process control, standardization of toolbar and work processes definition will be the basic steps in work process management(10). Present investigations indicate that guidelines integration and clinical practice are the biggest current challenge (18, 19, 53). Integration is still central for health information systems. Information systems such HIS with a central database like the EHR will be a significant influence on the coordination and integration tasks and workflows. Because all the information about individual en-

compasses examinations, interpretations, procedures, data management, diseases and injuries and is an important factor for increasing quality treatment, improving decision making and reducing medical errors(5). So can be concluded the purpose use of IT in the clinical process reduce workload and improve the decision-making. It is believed that the workflow technology is useful in many aspects of health care benefits, Such as electronic health records health that formed the cornerstone of future generations' health information and will help many of the patterns and workflow. This system with the effective communication of information between health care expert support of care integration and services provided(15, 43).

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The correlation of modified sharp score with localized and generalized osteoporosis and clinical and functional condition in patients with rheumatoid arthritis

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Abstract

Objective: The aim of this study is to investigate the relationship of the modified Sharp score (MSS) with localized and generalized osteoporosis in terms of the clinical and functional status in patients with rheumatoid arthritis (RA).

Materials and Methods: 81 patients with RA were enrolled in the study. Patients were evaluated with the DAS 28 and Health Assessment Questionnaire (HAQ) . Routine laboratory examinations and the ESR, CRP, RF and Anti-CCP tests were performed. Bilateral hand and foot radiographies were evaluated. The bone mineral density (BMD) were measured through the dual-emission X-ray absorptiometry (DXA).

Results: The MSS was observed to decrease with the BMD. There was a significant correlation between the MSS and the BMD of the hands, lumbar spine and femur. Although the correlation between the MSS and the duration of the complaints was not significant, the correlation between the time since the diagnosis and the MSS was significant.

Conclusion: The correlation between the MSS and hand BMD is relatively stronger than the correlation with the lumbar vertebra and femoral BMD. Besides, the existence of a significant negative correlation between the MSS and the hand BMD may show that the hand BMD measurement is a reliable measure for evaluating the level of RA.

Key words: Modified Sharp Score, hand bone mineral density, rheumatoid arthritis, DAS 28, HAQ.

Introduction

RA is a chronic inflammatory, autoimmune and systemic disease which may involve the cartilage and bones in a number of small, medium or large diarthrodial joints. Although the etiology of the condition is unclear yet, the general belief is that infections, genetic factors, immune system disorders, stress, sex and environmental factors play a significant role on the development of the disease. Since the prevalence of RA in the general population is 0.8%, it can be assumed that there are approximately 500.000 RA patients in Turkey(1,4) .

Generalized and local osteoporosis is an extra-articular complication which has been associated with uncontrolled and long-lasting RA. In the previous literature about established RA, the duration of disease, high disease activity, joint damage, decreased functional capacity and treatment with corticosteroids have been defined as causes of osteoporosis or low BMD values. Periarticular and generalized osteoporosis and local bone erosions are thought to share the same pathogenesis. This hypothesis is supported by the fact that the T-cells, which are activated in RA, release the "receptor activator of nuclear factor kappa B ligand (RANKL)" that leads to the destruction of the bones through the activation of the osteoclasts (6).

Radiological imaging is a frequently used method in the diagnosis and grading of RA and for evaluating the response to the treatment. There are several studies suggesting an association between osteoporosis and the radiological damage. Several scoring systems have been developed in order to follow up the severity and progress of the damage (16,17). The most commonly used method is the MSS developed by Van der Heijde (8). The major difference between the MSS and the normal Sharp score is that the subluxation and luxation of the joints are used to evaluate the joint spacing in MSS.

In this study, our aim is to calculate the radiological damage in the hands and feet of the patients with RA using the MSS and to present the relationship between the score and generalized osteoporosis, local osteoporosis at hand and the clinical and functional status of the patients.

Materials and Methods

For the purposes of this study, 81 RA patients between the ages of 18-75, who were followed up by the Physical Therapy and Rehabilitation Department of the Bezmialem Vakif University, Faculty of Medicine were enrolled. Patients with systemic diseases except for hypertension and those with bone pathologies including fractures of the extremities were excluded from the study.

The demographic characteristics of the patients such as age, sex, weight and height were recorded and their body mass indices were calculated. The onset of the disease, the time since the diagnosis and the administered medications were investigated. Patients were inquired about their alcohol consumption, smoking status and physical exercise habits.

While the disease activity in the patients was evaluated through the Disease Activity Score (DAS) 28, the functional status was determined using the HAQ. The ESR, CRP, RF and anti-CCP values were controlled in addition to the routine laboratory tests. Bilateral hand and foot x-rays were made, and bone and joint stiffness was evaluated by an experienced radiologist using the MSS. The BMD values of the left femur, lumbar spine and both hands were measured using the DXA.

The normality of the data was determined with the help of the Kolmogorov-Smirnov test. The distribution of the age, weight, height, body mass index, DAS 28 score and the femoral, lumbar spine and hand BMD values were found to be normal. The data concerning the duration of RA, duration of complaints, the HAQ score and MSS were shown to be outside the assumption of normality. The results were expressed as standard deviation and percentage. The mean values for the males and females were compared using the Mann Whitney U-test.

Three correlation analyses were performed. Primarily, the correlation between the time since the diagnosis of RA, duration of the complaints and the BMD values (right hand, left hand, lumbar region and femoral neck) was analyzed. Secondly, the correlation between the time since the RA diagnosis, duration of the complaints and the MSS was investigated. Thirdly, the BMD measurements were compared with the MSS. All the correlation analyses were carried out with the help of Spearman's test. Statistical significance of the correlation was based on a correlation coefficient $R \geq 0.25$ and a value of $p < 0.05$. The statistical analysis was performed using the PASW statistical software.

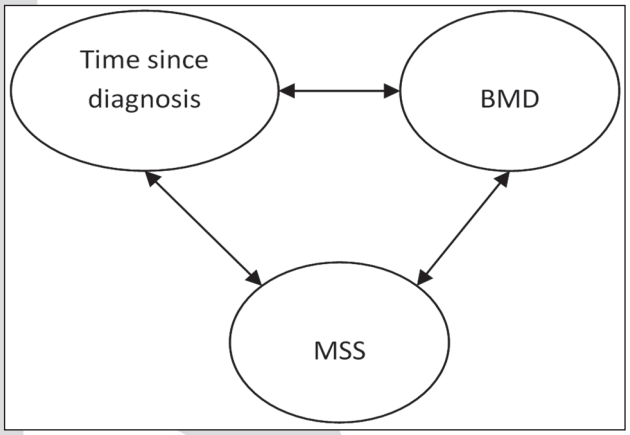

Figure 1. Correlation Analysis

Results

The demographic, anthropometric and disease-related characteristics of the patients are presented in Tables 1-A and 1-B. The clinical characteristics of the patients with RA are summarised in Table 2; and the BMD values of the femoral and lumbar regions in males and females with RA are presented in Table 3.

Table 1A. Demographic, clinical and laboratory characteristics of patients

Year	50.8 ± 9.9
Females / Males	67 / 14
Body weight (kg)	73.4 ± 14.0
Height (cm)	156.3 ± 7.6
BMD (kg/m^2)	30.1 ± 5.7
Duration of disease (years)	10.0 ± 7.1
Time since diagnosis (years)	6.9 ± 6.3
RF Titre	106.7 ± 205.7
Anti CCP	695.3 ± 4599.2
CRP	0.17 ± 0.37
ESR	31.0 ± 18.5
DAS 28	3.9 ± 1.2
HAQ point	0.6 ± 0.6
Sharp point	16.3 ± 31.3

Table 1B. Demographic, clinical and laboratory characteristics of the male and female patients

	Male (n = 14)	Female (n = 67)	P
Duration of complaints (years)	8.4 ± 6.6	10.3 ± 7.3	0.344
Duration of RA (years)	6.6 ± 6.2	7.0 ± 6.3	0.870
RF titre	74.3 ± 142.1	113.5 ± 216.8	0.494
Anti CCP	292.5 ± 346.9	779.7 ± 5059.2	0.016
CRP	0.14 ± 0.15	0.17 ± 0.41	0.975
ESR	30.6 ± 24.8	31.1 ± 17.1	0.565
DAS 28	3.3 ± 1.7	4.1 ± 1.1	0.078
HAQ score	0.4 ± 0.7	0.6 ± 0.6	0.085
Sharp score	28.7 ± 54.1	13.7 ± 23.9	0.214

Table 2. Clinical characteristics of patients with RA

	Male (n = 14)	Female (n = 67)	P
Age	51.4 ±10.8	50.7 ±9.8	0.569
Height	167.2 ±7.7	154.0 ±5.3	0.000
Body weight (kg)	73.7 ± 14.6	73.3 ± 13.9	0.955
BMI (kg/m^2)	26.2 ±4.5	30.9 ±5.6	0.005

Table 3. BMD value of the femoral and lumbar regions in male and female patients with RA

BMD	Total (n=81)	Male (n = 14)	Female (n = 67)	P
Left femoral neck (g/cm^2)	0.900 ± 0.139	0.905 ± 0.160	0.899 ± 0.135	0.822
Lumbar region (g/cm^2)	1.014 ± 0.166	1.017 ± 0.155	1.013 ± 0.169	0.881

Among the patients, 55 (67.9%) were on treatment with MTX, 18 (22.2%) were on SLZ, 2 (2.5%) were on chloroquine, 13 (16%) were on lenflunomide, and 50 (61.7%) patients were on treatment with steroids. The mean BMD values of the phalanges in male and female patients with RA are presented in Tables 4, 5 and 6.

Table 4. The mean BMD values of the phalanges in patients with RA

	Right phalanges	Left phalanges
1. phalanx	0.290 ± 0.055	0.279 ± 0.057
2. phalanx	0.328 ± 0.067	0.321 ± 0.071
3. phalanx	0.241 ± 0.047	0.229 ± 0.050
4. phalanx	0.315 ± 0.060	0.311 ± 0.062
5. phalanx	0.269 ± 0.058	0.258 ± 0.055
6. phalanx	0.335 ± 0.069	0.333 ± 0.071
7. phalanx	0.240 ± 0.050	0.229 ± 0.053
8. phalanx	0.295 ± 0.061	0.287 ± 0.068
9. phalanx	0.200 ± 0.051	0.192 ± 0.051
10. phalanx	0.242 ± 0.077	0.227 ± 0.054

Table 5. The mean BMD values of the right phalanges in male and female patients with RA

	Male (n = 14)	Female (n = 67)	P
1. phalanx	0.324 ± 0.051	0.283 ± 0.053	0.008
2. phalanx	0.362 ± 0.065	0.321 ± 0.065	0.066
3. phalanx	0.251 ± 0.049	0.239 ± 0.047	0.399
4. phalanx	0.330 ± 0.074	0.312 ± 0.056	0.435
5. phalanx	0.282 ± 0.064	0.266 ± 0.057	0.207
6. phalanx	0.349 ± 0.082	0.332 ± 0.066	0.435
7. phalanx	0.252 ± 0.048	0.237 ± 0.051	0.240
8. phalanx	0.310 ± 0.069	0.292 ± 0.059	0.417
9. phalanx	0.209 ± 0.054	0.198 ± 0.051	0.424
10. phalanx	0.251 ± 0.050	0.240 ± 0.082	0.339

The results of the correlation analysis are summarized in Table 7. Initially, the correlation of the time since the diagnosis and the duration of complaints with the BMD was evaluated, and the maximum correlation values for the hand BMD were reported. Although the hand BMD was found to be negatively correlated with the time since the diag-

nosis (-0.366 for the right hand and -0.337 for the left hand), no correlation was observed between the lumbar and femoral BMD values and the time since the diagnosis. On the other hand, the duration of the complaints was found to have a significant negative correlation with all the BMD values. The correlation coefficients between the BMD values and the duration of complaints were found to be -0.305 for the femoral BMD; -0.263 for the lumbar BMD; and -0.385 and -0.342 for the right and left hand BMD, respectively. Therefore, the correlation between the hand BMD and the time since the diagnosis, or the duration of complaints was observed to be relatively stronger than the correlation between the lumbar or femoral BMD, and the time since the diagnosis or duration of complaints.

Table 6. The mean BMD value of left phalanges in male and female patients with RA

	Male (n = 14)	Female (n = 67)	P
1. phalanx	0.310 ± 0.052	0.273 ± 0.056	0.026
2. phalanx	0.368 ± 0.062	0.312 ± 0.069	0.008
3. phalanx	0.242 ± 0.043	0.226 ± 0.051	0.230
4. phalanx	0.337 ± 0.056	0.306 ± 0.062	0.103
5. phalanx	0.280 ± 0.054	0.254 ± 0.055	0.100
6. phalanx	0.357 ± 0.076	0.328 ± 0.069	0.233
7. phalanx	0.243 ± 0.049	0.226 ± 0.053	0.269
8. phalanx	0.301 ± 0.068	0.284 ± 0.068	0.549
9. phalanx	0.202 ± 0.055	0.189 ± 0.050	0.454
10.phalanx	0.246 ± 0.047	0.223 ± 0.055	0.212

Secondly, the correlation between the MSS and the BMD values was evaluated. The MSS was ob-

Table 7. The correlation between MSS, BMD, duration of the complaints and the time since the diagnosis

	Time since diagnosis		Duration of complaints		MSS		Year		HAQ score	
	R	P	R	P	R	P	R	P	R	P
Age	0.018	0.873	-0.064	0.569	0.332	0.002	-	-	0.236	0.034
Time since diagnosis	-	-	0.835	0.000	0.282	0.011	0.018	0.873	-0.052	0.648
Duration of complaints	0.835	0.000	-	-	0.217	0.051	-0.064	0.569	-0.061	0.587
HAQ point	-0.052	0.648	-0.061	0.587	-0.138	0.220	0.236	0.034	-	-
MSS	0.282	0.011	0.217	0.051	-	-	0.332	0.002	-0.138	0.220
Left Femoral neck BMD	-0.249	0.025	-0.305	0.006	-0.369	0.001	-0.315	0.004	-0.036	0.748
Lumbar BMD	-0.224	0.044	-0.263	0.017	-0.317	0.004	-0.215	0.054	0.001	0.991
R-1	-0.279	0.012	-0.272	0.014	-0.307	0.005	-0.215	0.054	-0.034	0.761
R-2	-0.362	0.001	-0.374	0.001	-0.334	0.002	-0.184	0.100	-0.065	0.565
R-3	-0.254	0.022	-0.305	0.006	-0.248	0.026	-0.108	0.338	0.055	0.629
R-4	-0.340	0.002	-0.347	0.001	-0.349	0.001	-0.169	0.132	0.085	0.451
R-5	-0.212	0.057	-0.259	0.020	-0.293	0.008	-0.146	0.192	-0.006	0.959
R-6	-0.366	0.001	-0.385	0.000	-0.466	0.000	-0.288	0.009	0.023	0.837
R-7	-0.194	0.082	-0.214	0.055	-0.359	0.001	-0.215	0.054	0.013	0.906
R-8	-0.316	0.004	-0.323	0.003	-0.421	0.000	-0.257	0.021	0.045	0.688
R-9	-0.248	0.026	-0.236	0.034	-0.405	0.000	-0.178	0.112	0.032	0.775
R-10	-0.287	0.009	-0.283	0.010	-0.385	0.000	-0.257	0.020	0.012	0.918
L-1	-0.172	0.124	-0.135	0.229	-0.309	0.005	-0.258	0.020	-0.063	0.573
L-2	-0.239	0.031	-0.211	0.058	-0.362	0.001	-0.283	0.010	-0.106	0.344
L-3	-0.268	0.015	-0.277	0.012	-0.375	0.001	-0.227	0.041	0.020	0.859
L-4	-0.244	0.028	-0.260	0.019	-0.406	0.000	-0.298	0.007	-0.022	0.843
L-5	-0.264	0.017	-0.275	0.013	-0.448	0.000	-0.258	0.020	0.002	0.986
L-6	-0.272	0.014	-0.287	0.009	-0.456	0.000	-0.315	0.004	-0.022	0.845
L-7	-0.270	0.015	-0.275	0.013	-0.392	0.000	-0.283	0.011	-0.032	0.774
L-8	-0.337	0.002	-0.342	0.006	-0.492	0.000	-0.330	0.003	-0.005	0.966
L-9	-0.243	0.029	-0.269	0.015	-0.434	0.000	-0.281	0.011	-0.085	0.452
L-10	-0.274	0.013	-0.261	0.018	-0.486	0.000	-0.332	0.002	-0.047	0.675

served to decrease in parallel to the BMD. A significant correlation was observed between the MSS and the hand BMD value (-0.466 for the right hand and -0.492 for the left hand). This correlation was found to be stronger than the correlation between the MSS and the lumbar BMD (-0.317) and the correlation between the MSS and femoral BMD (-0.369). Finally, the MSS was observed to increase with the duration of complaints and the time since the diagnosis. While the correlation between the duration of the complaints and the MSS was found to be insignificant, the correlation between the time since the diagnosis and the MSS was observed to be significant.

Discussion

RA is a systemic disease leading to bilateral joint involvement. It is usually observed from the 4th decade of life onwards and affects especially females. The onset and course of the disease are highly variable and although it can go into remission for a long time, it sometimes progresses with exacerbations. Disease activity is evaluated through the ESR and CRP tests. Since elevated values of the rheumatoid factor (RF) and anti CCP have a negative prognostic value, these two tests are valuable as well (1, 2, 4, 7).

The mean age of the patients was found as 50.8 ± 9.9 and the female/male ratio was 4.8/1. These values are in compliance with the related literature. The CRP values of the patients were within normal limits and the ESR values were slightly higher than normal (0.17 ± 0.37 and 31.0 ± 18.5, respectively). This was associated with the regular long-term follow-up of the patients and their appropriately planned treatment. The anti-CCP and RF values were positive in 54.3% and 45.7% of the patients, respectively.

In RA, local periarticular osteoporosis is observed in the hand and foot joints due to the chronic synovitis. In chronic synovitis, the synovial layer that normally has a thickness of one to three cells becomes hypertrophic (and grows to eight to ten cell thickness) due the proliferation of the basic cell population. The subintimal area of the synovium that is connected to the joint capsule normally has a small number of cells, since the blood vessels are located here. During RA, new blood vessels are generated in the subintimal layer (angiogenesis). Also, massive cellular infiltration by the T and B lymphocytes, macrophages and mast cells occurs (5, 9, 10).

The resulting pannus and hypertrophic synovium lead to a bone invasion at the points where the synovium is normally tightly attached on both sides of the joint. This gives way to the erosion that occurs in the adjacent bones and cartilage (11, 12). The subchondral bone resorption caused by the pannus can be observed through the electron microscope (13). The erosions on one or both sides of joint and the intraarticular narrowing indicating the reduction of the cartilage volume can be viewed radiographically (14).

Plain radiographs of the joints are often used for the diagnosis and monitoring of the disease (15). The three diagnostic features of rheumatoid joint destruction; namely the periarticular osteoporosis, intraarticular narrowing and erosions, can be detected with the help of plain X-rays. Many scoring systems have been developed to objectively assess these changes (16, 17).

For the purposes of this study, we used the MSS. In this scoring system, the maximum erosion score for the hand and wrist is 160, while it is 120 for the foot. For the intraarticular narrowing, the maximum score is 120 for the hand and wrist and 48 for the foot. Hence, the total radiological score corresponds to an interval between 0 and 448. In our study, the average MSS was found as 16.3 ± 31.3. A significant correlation was observed between the time since the diagnosis and the MSS. The average time since the diagnosis was found to be 6.9 ± 6.3 years. It is possible that the relatively shorter time since the diagnosis, and the regular follow up and treatment may have led to the lower MSS score observed in this study.

In clinical practice, radiological assessments are used to determine the progression of RA and bone destruction. However, the erosion cannot be detected in the early phases of the disease. Since the new and aggressive treatment methods are effective in preventing the progression of the erosions (18), more precise and sensitive measurements are needed to assess the benefit of the treatments over the bone and articular structures.

In previous studies, a disease-related BMD loss in the hand bones was demonstrated to occur in the

early phases of the disease. Even at the undifferentiated phase of the RA, a bone loss determined through the DXA has been reported in the hands. It has already been established that the BMD is correlated with the duration and activity of the disease, and a decrease in BMD is observed in parallel to the disease activity and duration (2, 3, 18).

Gursoy *et al*.(23) have demonstrated a relationship between the disease activity and the bone loss, although this relationship was statistically insignificant. Kvien *et al*. (24) have observed that the bone loss is related to the duration of the disease. Sambrook *et al.* (21) reported a correlation between the disease duration and the bone loss at the femoral neck, with a negative correlation between the BMD value and the duration of the disease.

Gough *et al*. (25) have shown that the BMD of the patients with a disease duration under 6 months were higher than the BMD of the patients with longer duration of disease. Laan *et al.* (26) have reported the determinants of bone loss in the early periods of RA as the functional status, disease activity and the disease duration.

In RA, the loss of the BMD in the hands develops at the early stages of the disease. This loss is correlated with the disease activity and is considered as an indication of poor prognosis. In their study conducted on RA patients, Deodhar *et al*. have shown that the hand BMD values of the RA patients were significantly lower than the control group. Also according to their observation, while the BMD loss in patients with RA increased over time, the BMD values in the control group did not change (19). In another study by the same researchers, 50 patients with RA were observed over a period of 5 years and the BMD loss was followed up through the DXA method. According to their results, the decrease in the BMD was 5.5% during the first year; 7.5% during the second year, and 9.9% during the third year. The BMD was stabilized within the last two years (29).

Haugeberg *et al*. (18) reported that although changes in the MSS were observed in only 22% of the patients after 48 weeks, the changes in the hand BMD values occurred at earlier stages and a decrease in the hand BMD was detected in 50% of the patients at the end of the 24th week.

The data collected on the prevalence of osteoporosis in men with RA are still inadequate. In the present study, data obtained from males and females were classified and compared in combination; and no statistically significant difference between males and females was observed in terms of the means of the duration of complaints, time since the diagnosis, RF, Anti CCP, ESR, DAS 28, HAQ score and MSS. Also, except for the first phalanx of the right hand and the second phalanx of the left hand, no statistically significant difference in terms of osteoporosis and the BMD values was found between the means of the lumbar spine, femoral neck and hand BMD.

The age, body mass index (BMI), HAQ and menopause are considered to be the most important parameters in the evaluation of the osteoporosis or BMD of the lumbar spine and femoral neck (18, 20). On the other hand, physical activity evaluated by the Framingham activity index was found to be significantly correlated with BMD in patients with RA. Hence, it is characterized as an independent parameter in assessing the femoral BMD (21). These results are supported by a recent study evaluating the relationship between the BMD and muscle strength. This study showed that women with low femoral BMD values had 20% lower quadriceps strength compared to women with BMD values within normal limits (22).

In our study, no significant relationship was observed between the HAQ and DAS28 scores and the hand, lumbar and femoral BMD values. Di Munno *et al.* (27) reported a statistically significant association between the lumbar and proximal femoral BMD values and the functional status.

Sivas *et al.* reported a relationship between the HAQ scores and the forearm BMD values. However, they did not observe any association between the lumbar and hip BMD values, and the HAQ, ESH, CRP, RF values and disease duration (28).

Conclusion

In our study, a statistically significant negative correlation was found between the duration of the RA and the hand BMD. There was also a significant negative correlation between the MSS and the hand BMD value. Based on the literature data and the results of our study, it can be concluded that the hand BMD measurement is a reliable criterion in order to determine the severity and the course of RA.

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sRANKL, TRADE RANKL and OPG system in periodontal diseases

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Abstract

Introduction: After in 1997 the osteoprotegerin (OPG) was discovered, the mechanism occurring in the bones concerning the osteolysis and the bone formation was explained. The cells - osteoblasts and osteoclasts as well as the three cytokine system: sRANKL, TRADE-RANKL and osteoprotegerin participate in this process. The osteoprotegerin has properties protecting against the bone resorption. It is a „trap receptor" because by binding the sRANKL it inhibits the formation of osteoclasts capable of bone resorption.

The aim of the paper is to evaluate the level of osteoprotegerin (OPG), sRANKL and TRADE-RANKL in the blood serum in a periodontal disease group and in a group of healthy volunteers, and to compare the effect of the progression of the periodontal disease on these parameters.

Material and methods: The clinical population consisted of patients with the periodontal disease treated in a periodontal clinic, diagnosed with chronic periodontitis, whereas the reference population comprised 28 volunteers without any periodontal disease. A detailed periodontal examination in the clinical population was conducted. The laboratory tests for the assay of cytokines were carried out using the immunoenzymatic method (ELISA).

Results: In the periodontal disease group, a statistically significant increase in the level of sRANKL and the sRANKL/OPG ratio was found. The level of osteoprotegerin correlates with the progression of the periodontal disease.

Key words: Osteoprotegerin, sRANKL, periodontal disease.

Introduction

The human skeleton consists in 80% of the compact bone, and in 20% of the spongy bone. The principal mineral bone components are calcium and phosphorus. The organic matrix of a bone constitute the osteoid, the principal component of which is the collagen (90-95%), as well as the proteoglycans, glycoproteins, osteocalcin and phosphoproteins. The bone mass undergoes a constant remodelling. In the growth period, the replacement of components of the skeleton is referred to as modelling. It is a structural process that consists in the formation of a new bone at a place separate from its resorption, which enables the bones to grow in length and thickness, achieve their target shape and counteract the gravitation as well as the pressures and stresses when performing mechanical functions. The alveolar process is subject to a special modelling during the ontogeny. The eruption processes of primary and permanent dentition and the effect of occlusal forces require a very dynamic equilibrium between bone resporption and formation. In the human organism, after the termination of growth and attaining approximately the 30th year of life, the process of internal bone remodelling begins to outweigh the modelling. It does not cause growth and change in shape of a bone, but is responsible for its regenerative properties. A loss of the bone tissue at a rate of 0.5-1%/ per year begins at the age between 35 and 40 years and occurs in both sexes. In the menopause in women this rate increases as many as 10 times, and then stabilizes at the level of 1-3% per annum (1, 2).

The modelling and remodelling are based on the bone resorption and formation processes cyclically following each other, guided by 2 main types of cells with an opposite effect. The osteoblasts are

bone building cells, whereas osteoclasts are responsible for the destruction. If the resorption process is intensified and/or the osteogenesis process is inhibited, a loss of the bone mass occurs. On the other hand, the absence of resorption leads to a pathological increase in the bone tissue mass. Many bone diseases are related to an increase in the activity of osteoclasts, they include: osteoporosis, periodontal diseases, rheumatoid arthritis, multiple myeloma, cancer metastases to the bone (3, 4, 5, 6).

The proper maturating and functioning of osteoclasts is guarded by a pathway the principal participants of which are: the osteoprotegerin (OPG), the Receptor Activator of Nuclear Factor NF-kB (RANK) and the RANK Ligand (RANKL). The osteoprotegerin is a protein belonging to the family of tumour necrosis factor receptors, produced inter alia by blood system cells, lungs, kidneys, bones, hematopoietic and immunity cells. The expression of the OPG gene is increased by: cytokines (TNF-α, Il-1α, Il-18, TNF-β), morphotic bone proteins, 17β-estradiol, mechanical loading; whereas the expression is decreased by: glucocorticosteroids, immunosuppressive drugs, PTH, PGE_2, FGF (7).

The RANK (Receptor Activator of Nuclear Factor NF-kB) is a glycoprotein of the TNF (Tumour Necrosis Factor) receptors family being a RANKL receptor of the principal cytokine stimulating the growth and maturation of osteoclasts. RANK – a membrane protein is generated in the osteoclast precursors and in mature osteoclasts, in fibroblasts, dendritic cells, T and B lymphocytes, endothelial cells, chondrocytes and tumour cells (7). An increased expression of the RANK is influenced by: vitamin D_3, TGF-β, IFN-γ, IL-1, RANKL, whereas IL-4 decreases the number of RANK molecules on the surface of osteoclasts (7).

The RANKL belongs to the family of proteins of the Tumour Necrosis Factor (TNF) and is produced by the osteoblast cell line (by mature osteoblasts and their precursors), and by activated T lymphocytes. Its expression is influenced inter alia by cytokines (IL-1, Il-6, Il-11, TNF-α), glucocorticosteroids, PTH, 1.25 $(OH)_2D_3$. It is an agent activating the entire formation process of mature osteoclasts which acts through the RANK receptor located on the surface of the target cells. The osteoprotegerin has the ability to bind with the RANKL, which prevents binding of the RANKL with the RANK and consequently stops the pathway of maturation of the osteoclasts (4, 6, 8, 9, 10, 12, 13, 14, 15).

Periodontitis develops as a result of an exposure of a host organism susceptible to the action of virulent Gram-negative bacteria and consists in the apical migration of the epithelial attachment and the destruction of the connective tissue and the alveolar bone (1, 2 16, 17, 18, 19). The data from the literature confirm that the RANKL-OPG-RANK system controls the pool of bone destroying cells and participates in the pathomechanism of bone loss in periodontitis (20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30).

Many experiments indicate that the periodontium, which contains the osteoprotegerin, plays an important role in the internal remodelling of the alveolar bone tissue (13, 31, 32, 33). In the conditions of the stimulation with proinflammatory cytokines in periodontopathies, the periodontal cells lose their osteoclastogenesis protection properties, the result of which is the loss of the alveolar bone. The inflammation and destruction in periodontitis are connected with the formation of a pathological granulation tissue which contains inflammatory cells (T and B lymphocytes, plasmatic cells, other cells of the monocyte/macrophage line), generating great quantities of the proinflammatory cytokines (2, 27, 28, 29, 30, 31, 32). Crotti et al. (2) showed an increase in the concentration of the RANKL and a reduction of the OPG in the granulation tissue adjacent to the bone of patients with periodontitis, which may prove that a change in the levels of molecules regulating the diversification of osteoclasts can play an important role in the bone loss occurring in the periodontal diseases. Mogi et al. (18) evaluated the expression of the RANKL and the OPG in the gingival fluid. The concentration of the RANKL was higher, and of the OPG lower in the periodontopathies as compared to the control group. So far, not much data concerning the sRANKL/OPG ratio in the circulating blood serum in periodontal disease patients is available in the literature.

Material and methods

The clinical population consisted of periodontal disease patients treated at a periodontal clinic (29 individuals) aged between 22 and 74 years, 51.4 on average. The patients gave their written

consent to the clinical examination and to taking a blood sample for the biochemical tests.

The reference group consisted of 28 generally healthy individuals, without any periodontal disease, the staff of dentistry departments aged between 28 and 66 years, average age 41.5 years. The approval of the Bioethical Committee to conduct the study was obtained.

Periodontal examination

The periodontal examination comprised:

1. The measurement of the simplified O'Leary Plaque Index (PI) (the sum of all surfaces with dental plaque/ sum of all examined surfaces x 100%).
2. The assessment of the Ainamo and Bay Bleeding Index (BI) : the sum of all surfaces in which bleeding occurred/the sum of all examined surfaces x100%), the bleeding index was measured at six measuring points – mesially, medially and distally for the labial/buccal and lingual/ palatal surface of all teeth.
3. The clinical gingival status was evaluated using the Löe and Silness Gingival Index (GI) by assessing the gingiva with all teeth present in the oral cavity, by examining four gingival surfaces surrounding a tooth, i.e. the buccal, lingual, mesial and distal surfaces.
   The gingival status evaluation criteria in this index are entirely based on clinical qualitative changes in the gingival tissues and are as follows: 0- the absence of gingivitis symptoms, the absence of any pathological change in the colour of the gums, 1- a mild gingivitis with a small change in the colour of the gums, a mild change in the structure of the gingival tissue, the absence of bleeding on probing, 2- a moderate gingivitis: redness, swelling, shiny appearance and hypertrophy of the gums, bleeding on pressure or probing, 3- a serious gingivitis manifesting itself in a considerable redness, swelling, ulceration and the susceptibility to spontaneous bleeding.
4. The average Pocket Depth (PD), a value measured from the bottom of a periodontal pocket to the edge of the free gingiva, given in mm.
5. The average level of the Clinical Attachment Loss (CAL), a value measured from the bottom of a periodontal pocket to the cement-enamel junction, given in mm.

The PD and CAL values were measured at six measuring points. For the evaluation of the periodontal status (items 4 and 5) the PD and CAL percentages with the value equal to or higher than 6 mm were used.

Laboratory methods

The assay method for TRADE RANKL, sRANKL and osteoprotegerin in the serum: The ELISA cytokine assay method was used according to the instruction given by the manufacturer (Biomedica Medizinprodukte GMBH&Co KG, A-1210 Vienna). The colour intensity was spectrophotometrically measured with the wavelength of 490 nm, using the Anthos microplate reader.

Statistical analysis

The results of the clinical and laboratory tests were statistically analysed using the IBM SPSS Statistics 20.0 program. The analysed parameters were described by specifying the arithmetic mean (X), the standard deviation (SD), the minimum and maximum values, and the percentages (%). The test results obtained in both groups were compared using the Mann-Whitney Test. The correlation between the parameters was evaluated using the Spearman's nonparametric correlations. The results of the statistical tests were found significant at the level of $p<0.05$.

Results

Clinical examination results

Chronic periodontitis was diagnosed in all patients qualified for the study. The periodontal status was shown in Table 1. The Plaque Index was between 23.7% and 99.3% (mean 60.24%). The BI was between 9.8 and 94.79 % (mean 66.27%).

The Gingival Index was between 0.49 and 3.0; as mentioned above, this index evaluates the gingiva only (mean 2.15).

In evaluating the PD and the CAL the percentage of teeth where the clinical attachment loss and the periodontal pocket depth was equal to 6 mm or

Table 1. Periodontal status in the clinical population

Group	n	Mean	Stand. deviat.	Minimum	Median	Maximum
PI [%]	29	60.24	21.4	23.7	59	99.3
GI	29	2.15	0.69	0.49	2.25	3.00
BI[%]	29	66.27	21.82	9.80	71.25	94.79
PD, CAL [%]	29	47.78	27.57	3.84	41.66	100.00
Number of teeth	29	22.72	4.95	12.00	23.00	28.00

Table 2. Comparison of mean OPG, TRANCE/ RANKL and sRANK values (pg/1 ml) in the control group and the periodontal disease group (Mann-Whitney Test)

Group		n	Mean	Stand. dev.	Minimum	Median	Maximum	p*
Osteoprotegerin	control	28	11.42	3.89	3.48	11.54	21.62	0.219
	period.dis.	29	12.93	5.42	4.20	12.78	28.44	
	Total	57	12.20	4.76	3.48	12.33	28.44	
TRANCE/ RANKL	control	28	32.28	21.11	15.60	26.77	120.30	0.607
	period.dis.	29	48.85	83.67	14.98	24.03	459.20	
	Total	57	40.85	61.99	14.98	25.27	459.20	
sRANKL	control	28	37.32	44.93	3.20	19.80	195.20	**0.002**
	period.dis.	29	83.39	101.19	0.60	43.20	416.00	
	Total	57	61.15	81.88	0.60	35.30	416.00	
sRANKL/ OPG	control	28	3.18	3.22	0.38	1.88	11.54	**0.007**
	period.dis.	29	6.90	7.52	0.05	3.73	29.86	
	Total	57	5.11	6.10	0.05	3.07	29.86	

exceeded this value was taken into consideration. Such progression of the periodontal destruction affected all subjects. Advanced periodontal diseases were found in the range between 3.84 % and 100 % (mean 47.7%).

Laboratory test results

The level of osteoprotegerin in the group of healthy individuals without any periodontal disease was 11.42 pg/ml, whereas in the group of patients with a periodontal disease this level was 12.93 pg/ml. The differences were not statistically significant. The TRANCE/RANKL level in the control group was 32.28 pg/ml, and in the periodontal disease group 48.85 pg/ml. Also in this case the differences were not statistically significant. However, the sRANKL concentration in the control group was 37.32 pg/ml, whereas in the group of patients with a periodontal disease it was 83.39 pg/ml. These differences were statistically significant ($p<0.02$). Also the sRANKL/ OPG ratio in the group of patients with a periodontal disease was higher than in the control group and these differences were also statistically significant ($p<0.02$) (Table 2). When evaluating the correlation in the group of patients with the periodontal diseases (Table 3) it was found that a positive correlation between the level of osteoprotegerin and the level of TRANCE/RANKL exists. Also a positive correlation between the level of sRANKL and the sRANKL/ OPG ratio was found. In addition, a positive correlation between the clinical parameters of the periodontal disease: amount of dental plaque, gingivitis, periodontal pocket bleeding and depth, and the level of osteoprotegerin was noticed. On the other hand, a negative correlation between the gingivitis and the RANKL/OPG ratio was found.

Table 3 presents also the relationships between individual periodontal parameters. A positive correlation between the presence of dental plaque and the occurrence of gingival bleeding, gingivitis and the presence of deep periodontal pockets was found.

Similarly, an analysis showed a positive correlation of the gingivitis with the occurrence of dental plaque, gingival bleeding and a considerable destruction of the periodontal tissue (pockets <6mm)

On the other hand, the gingival bleeding correlated with the presence of dental plaque, the gingivitis and deep periodontal pockets. Deep periodontal pockets correlated with the presence of dental plaque, gingivitis and gingival bleeding.

Table 3. Spearman's nonparametric correlations in the periodontal disease group

Group		Osteopro-tegerin	TRANCE/ RANKL	sRANKL	sRANKL/ OPG	PI	GI	BI	PD/CAL	Teeth
Osteoprote-gerin	r	1.00	0.40	0.32	-0.34	0.44	0.54	0.42	0.45	-0.26
	p		0.03	0.09	0.063	0.017	0.003	0.022	0.013	0.169
TRANCE/ RANKL	r	0.40	1.00	0.44	0.11	0.16	0.24	0.21	0.10	0.02
	p	0.029			0.564	0.410	0.213	0.266	0.615	0.925
sRANKL	r	0.32	0.44	1.00	0.70	0.22	-0.09	-0.02	0.11	0.09
	p	0.089			0.000	0.255	0.649	0.907	0.587	0.648
sRANKL/ OPG	r	-0.34	0.11	0.70	1.00	-0.03	-0.37	-0.27	-0.09	0.18
	p	0.063	0.56	0.00		0.857	0.048	0.157	0.635	0.347
	p	0.530	0.97	0.44	0.271	0.986	0.097	0.338	0.427	0.344
PI	r	0.44	0.16	0.22	-0.03	1.00	0.60	0.49	0.51	-0.58
	p	0.017	0.41	0.25	0.857		0.001	0.006	0.005	0.001
GI	r	0.54	0.24	-0.09	-0.37	0.60	1.00	0.90	0.46	-0.56
	p	0.003	0.21	0.65	0.048	0.001		0.000	0.011	0.001
BI	r	0.42	0.21	-0.02	-0.27	0.49	0.90	1.00	0.43	-0.49
	p	0.022	0.27	0.91	0.157	0.006	0.000		0.021	0.007
PD/CAL	r	0.45	0.10	0.11	-0.09	0.51	0.46	0.43	1.00	-0.43
	p	0.013	0.62	0.59	0.635	0.005	0.011	0.021		0.020
Number of teeth	r	-0.26	0.02	0.09	0.18	-0.58	-0.56	-0.49	-0.43	1.00
	p	0.169	0.93	0.65	0.347	0.001	0.001	0.007	0.020	

Table 4. Spearman's nonparametric correlations in the control group

Group		Osteoprotegerin	TRANCE/ RANKL	sRANKL	sRANKL/ OPG
Osteoprotegerin	r	1.00	-0.05	0.41	0.07
	p		0.80	**0.03**	0.732
TRANCE/ RANKL	r	-0.05	1.00	0.43	0.44
	p	0.804			**0.021**
sRANKL	r	0.41	0.43	1.00	0.91
	p	**0.030**			**0.000**
sRANKL/ OPG	r	0.07	0.44	0.91	1.00
	p	0.732	**0.02**	**0.00**	

Table 5. Spearman's nonparametric correlations in both groups together (control + periodontal disease groups)

Group		Osteoprotegerin	TRANCE/ RANKL	sRANKL	sRANKL/ OPG
Osteoprotegerin	r	1.00	0.20	0.37	-0.06
	p		0.13	0.00	0.645
TRANCE/ RANKL	r	0.20	1.00	0.36	0.24
	p	0.127			0.068
sRANKL	r	0.37	0.36	1.00	0.87
	p	0.004			0.000
sRANKL/ OPG	r	-0.06	0.24	0.87	1.00
	p	0.645	0.07	0.00	

A negative correlation between the presence of all parameters of a periodontal disease and the number of teeth present in the oral cavity was found.

In the control group (Table 4) a positive correlation between the osteoprotegerin and the sRANKL, and a positive correlation between the osteoprotegerin and the sRANKL/osteoprotegerin ratio occurred. When evaluating all patients (58 individuals), in the comparison a positive correlation between the levels of osteoprotegerin and sRANKL, osteoprotegerin and TRANCE/RANKL as well as sRANKL and sRANKL/OPG ratio (Table 5) was found.

Discussion

The OPG/RANKL/RANK system plays an important role in the bone tissue metabolism. Therefore, numerous research studies on the determination of the level of osteoprotegerin in a state of physiology and pathophysiology have been conducted. In the literature, research studies concerning the level of OPG depending on age and gender can be found.

The research of Fahrleitner-Pammerai at al. (36) proved a positive correlation between the level of OPG and the age of healthy women, and the absence of relationship with the ratios of bone turnover evaluated by means of the biochemical indices: the level of osteocalcin, which is a bone formation marker, and the C-terminal telopeptide of collagen (CTx) representing the process of osteolysis.

The research of Kudlacka et al. (37) conducted on a large group of 1134 individuals aged between 24 and 96 years showed a considerable increase of the level of OPG in the serum of women after 60 and men after 70. The same research proved a negative correlation between the levels of OPG and PTH, both in men and women. The bone metabolism increases in women after the menopause. However, some authors suggest that the bone tissue can be more or less sensitive to the estrogen deficiency (38). Women after the menopause with a similar level of estrogens differ in the bone metabolism rate. Women with osteoporosis are characterised by an increased resorption and a compensatory increase of the OPG as compared to healthy women (39).

Examples of inflammatory pathological conditions in which an increased expression of the RANKL occurs are the rheumatoid arthritis and the inflammatory forms of periodontal diseases. The occurrence of a periodontal disease is determined by the presence of microorganisms forming dental plaque. Two types of dental plaque – supragingival and subgingival - are associated with the tooth surface. Mostly Gram-negative bacteria and spirochaetes occur in the bacterial plaque of patients with chronic periodontitis. The microorganisms and their components as well as the molecules produced by the host in response to the presence of microorganisms can contribute to the destruction of the periodontal tissues.

Among the bacterial factors which potentially may play a role in the direct destruction of the periodontal tissues are the proteolytic enzymes, the lipopolysaccharides (LPS) and the lipoteichoic acid of the Gram-positive bacteria, and the products of bacterial metabolism. Under the influence of these products the inflammation mediators are formed (40). The principal role in the bone resorption processes in periodontitis was attributed to the prostaglandin PGE_2, and to the cytokines-IL-1, IL-6 and TNF-α (4, 41, 42). A view was put forward that different mechanisms of bone resorption in periodontitis could exist. In case of the stimulation of osteoclasts by high LPS doses, the bone resorption by a mechanism in which both the IL-1 and the TNF -α participated was observed, whereas in case of the use of lower LPS doses the bone resorption was independent of these cytokines (43). The conducted study on the level of OPG, TRANCE/RANKL and sRANKL is an attempt to evaluate the level of cytokines in the serum depending on the progression of the periodontal disease. This is a pilot study which should be repeated on a larger material and with a precisely specified age because from the quoted data from the literature arises that the level of osteoprotegerin correlates with the age and the hormonal status. Apart from the presented results of our research on the level of osteoprotegerin and other cytokines, the research of Stawińska, who also presented the levels of OPG in a group of patients with the periodontal disease depending on the diagnosis and the progression of the disease, has been published (44). This author found a decrease in the level of osteoprotegerin in periodontal diseases compared to the reference group without any periodontal disease. On the other hand, an increase of the expression of the RANKL in patients with aggressive periodontitis and chronic periodontitis was found. To sum up, it should be stated the most research studies concerning the RANKL, RANK and OPG were conducted in the periodontal tissues or in the gingival fluid. The research concerning these agents in the serum should be related to the gender, age, weight, and the state of health.

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Expression of Ki-67, E-cadherin and Vimentin markers and their relationship with degree of dysplasia in discriminating high from low grade adenomatous polyps using IHC staining

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Abstract

Background and Goals: Transition phases from an adenoma to a colorectal adenocarcinoma are characterized with specific histological changes known as dysplasia, which is divided to two categories of high grade dysplasia and low grade dysplasia. Expression of proliferation index marker of Ki-67, E-cadherin (cell adhesion marker) and vimentin can be used as a diagnostic approach in discrimination of these cases. In this study, the expression method of the mentioned markers was investigated in adenomatous polyps with various dysplasia rates in order to find significant differences in the studied sample.

Materials and Methods: In this case-control study, 60 polypectomy samples from the pathology department of Rohani Hospital diagnosed as adenomatous polyp (high grade or low grade) were compared with IHC staining for Ki-67, E-cadherin and vimentin markers in dysplastic (case) and normal (control) areas and the relationship between degree of adenomatous polyp dysplasia and the expression method of these markers and their co-expression were surveyed.

Findings: The expression rate of Ki-67 in surface epithelium increased from dysplastic areas to non-dysplastic areas (Pv=0.001), rate of positive vimentin cells in dysplastic areas was evidently more than that in non-dysplastic areas (Pv=0.000) and E-cadherin marker in the cells of dysplastic areas compared to non-dysplastic areas was expressed with a shift from cell membrane to cell cytoplasm (Pv=0.000).

Conclusion: The results of this study demonstrated that co-expression of Ki-67 markers and vimentin with 92% specificity and 36% sensitivity could distinguish high grade dysplasia from low grade dysplasia. Co-expression of vimentin and E-cadherin markers with 41% specificity and 91% sensitivity could distinguish low grade dysplasia from high grade dysplasia.

Key words: Dysplasia, adenomatous polyp, Ki-67, vimentin, E-cadherin.

Introduction

Colorectal cancer is the most prevalent cancer of digestive system which is one of the three main causes of mortality from cancer around the world [1]. Nowadays, due to the improvement of medical science and technology, various new diagnostic methods have emerged on the basis of molecular diagnosis to prove and recognize infra-structural changes of various diseases more than before. Although not much novel, IHC is one of the relatively precise techniques which can recognize and check expression method of this type of specific molecules by aiming at specific proteins of the nucleus, cytoplasm and cell membrane by special antibodies; this directly or indirectly expresses cell behavior during various phases such as transition from normal phase to pre-malignant phase and then to the malignant one.

Transformation of normal epithelial tissue to adenocarcinoma in colorectal region requires transition from different grades of dysplasia and this transition is associated with different genetic mutations, disorders and molecular events at the beginning and progress of epithelial proliferative lesions, each of which can be identified in a specific phase or time and based on different sequences of occurrences in pre-malignant lesions of this organ (adenomatous polyps). The presence or absence or even manner of presence of each one of these events can be considered a criterion of le-

sion severity and reversibility or irreversibility in the malignancy pathway.

In this study, expression method of these three proteins with known function in cell proliferation of Ki-67, intercellular adhesion of epithelial cells and also abnormal proliferation inhibition of cells, E-cadherin, and filament indicator of vimentin mesenchymal cells were investigated using the IHC method and the possibility of their practical use in adenomatous polyp discrimination with different degrees of dysplasia (high grade, low grade) was analyzed by proving the relationship between co-expression method of the mentioned markers with dysplasia grade.

In cellular-molecular examination, during tracking series of factors leading to cancer, mutation of APC gene is one of the most important factors in colorectal adenocarcinoma (Adenomatous Polyposis Coli genes), which plays a key role in famililal polyposis. Homozygote loss of APC gene is observed in 70 to 80% of nonfamilial carcinomas and sporadic adenomas [2, 3]. APC is a class of tumor suppressors, the most important function of which is downregulation of β-catenin protein [2] and prevention from the aggregation of cytoplasmic β-catenin; otherwise, β-catenin comes into the nucleus and makes a complex with TCF, which causes an increase in cellular proliferation with increasing transcription from cyclin D1, C-MYC and other genes [3]. On the other hand, E-cadherin, which is a membrane protein, is connected to β-catenin from its cytoplasmic side and prevents its entrance to cell nucleus and proliferation stimulation; these cells are called contact inhibited cells [4]. Also, E-cadherin causes an intercellular connection among epithelial cells and their adherence prevents from the movement and invasion of the mentioned cells; a phenomena which occurs in EMT (epithelial-Mesenchymal Transition) and metastasis through E-cadherin reduction on the membrane surface [5].

Expression reduction of E -cadherin can be due to mutation in gene (in position 16q) or subsequence mutation in β -catenin gene. E-cadherin reduction may be due to the effect of transcription inhibitors such as SNAIL [5]; this effect is accompanied with the increase in vimentin and fibronectin expression in EMT and metastasis [6].

Vimentin is one of the five kinds of cellular intermediate filaments with 57000 Dalton molecular weight, which is the marker of mesenchymal cells, but may be expressed in some tumors with epithelial or neural origins [7].

Vimentin expression increase along with E-cadherin expression decrease in EMT indicates elasticity increase of malignant epithelial cells and tendency to invade other issues and vessels, which has been particularly seen in colorectal invasive adenocarcinoma [6] so that prediction value of this marker in evaluating recurrence of colorectal tumors has been estimated to be greater than that of lymph node assay [8].

During EMT process, epithelial cells, which normally rest on basal membrane with regular arrangement, obtain ECM materials through a series of biochemical changes of mesenchymal cells phenotype which include migration capacity, invasion, resistance against apoptosis and increase in ECM production. In this reversible transformation, some markers of epithelial cells such as E-cadherin, cytokeratin ,Zo-1 and Lamina are lost.

EMT is divided to three subcategories. The first subcategory occurs at emberiogenesis and leads to the differentiation of different organs. The second subcategory occurs at healing time and is accompanied by inflammation and fibrosis formation; the third subcategory is observed in carcinogenesis and causes the invasion of epithelial cells to other issues and vessels.

Ki-67 is an antigen related to a nuclear non-histone protein which is expressed by cells in S, M, G_1-G_2 proliferative phases. This protein is not expressed in G0 phase. Rate of cellular proliferation which is examined by Ki-67 immunoreactivity has been studied as a prognosis indicator in several malignancies and it has been indicated to be related to tumor grade [9].

Materials and Methods

After going to the archives of the pathology department in Rohani Hospital, paraffin blocks of 60 polypectomy &biopsy samples were extracted from 60 patients with (low grade or high grade) adenomatous polyp diagnosis. After cutting, they were stained by H&E staining and IHC staining for Ki-67, E-cadherin and Vimentin markers. The results obtained from marker staining using IHC method in each of the diagnostic groups were

compared with each other and the relationship between the degree of dysplasia and expression of these markers and their co expression were analyzed by SPSS software and statistical tests

Findings

In this study, 60 large colon polypectomy samples of 28 women and 32 men were studied in terms of polyp structure, tubular (n=29), tubulovillous (n=26) and villous (n=5). The patients' age was between 26 and 86 with the mean of 60.37.

Eleven samples were diagnosed as high grade dysplasia and 49 ones as low grade dysplasia (based on morphology in H&E staining).

Ki-67 and vimentin markers were expressed as nuclear and E-cadherin markers were expressed as membranous and cytoplasmic. The mentioned expressions were considered positive. Adenocarcinoma sample of colon and normal colonic mucosa were considered as positive and negative controls for Ki-67 and invasive ductal and lobular breast carcinoma were positive and negative controls for E-cadherin. Mesenchymal and epithelial of non-dysplastic areas were internal positive and negative controls for vimentin. Expression scoring, according to the past studies, are listed in tables1-3.

Table 1. Ki-67 scoring scale

Score	Ki-67 Stained epithelial cells
1	0-10%
2	11-30%
3	30% >

Table 2. Vimentin score scale

Score	Vimentin Stained epithelial cells
1	0-1%
2	2-5%
3	5% <

Table 3. E-cadherin staining score

Score 0	No staining
Score 1	Mild staining
Score 2	moderate staining
Score 3	sever staining

Ki-67 was expressed less in neck of the crypt, and expressed more in surface epithelia in dysplastic areas compared to non-dysplastic areas (pv=0.001). These values are presented in tables 4 and 5.

Value and occurrence percent of Ki-67 marker in deep, neck and surface areas and its relation with dysplasia grade in dysplastic epithelium

At the same time, there was no statistically significant relationship between Ki-67 and dysplasia grade (Pv=0.114). The numbers of this relation are presented in table 5.

Table 4. Marker Ki-67 expression in different regions crypts

		N.ki67.d		N.ki67.n		N.ki67.s	
		Frequency	Percent	Frequency	Percent	Frequency	Percent
Valid	a	37	61.7	5	8.3	58	96.7
	b	17	28.3	49	81.7	2	3.3
	c	6	10.0	6	10.0		
	Total	60	100.0	60	100.0	60	100.0

		D.ki67.d		D.ki67.n		D.ki67.s	
		Frequency	Percent	Frequency	Percent	Frequency	Percent
Valid	a	35	58.3	2	3.3	4	6.7
	b	24	40.0	40	66.7	45	75.0
	c	1	1.7	18	30.0	11	18.3
	Total	60	100.0	60	100.0	60	100.0

Ki-67 marker expression in different areas of crypts
D.Ki67.d, n, s: Ki-67 marker expression in deep, neck and surface of crypts in dysplastic areas, respectively
N.ki67.d, n, s: Ki-67 marker expression in deep, neck and surface of crypts in non-dysplastic areas, respectively.

Table 5. Incidence rates and the percentage of Ki-67 marker in the area of depth, neck and surface, and its relationship with the degree of dysplasia in the dysplastic epithelium

	High grade N/T - %			Low grade % - N/T		
	Deep	**Neck**	**Surface**	**Deep**	**Neck**	**Surface**
Score 1	9/11 58%	0/11 0%	0/11 0%	26/49 53%	2/49 4%	4/49 8%
Score 2	2/11 18%	7/11 64%	8/11 73%	22/49 44%	33/49 67%	37/49 76%
Score 3	0/11 0%	4/11 36%	3/11 27%	1/49 2%	14/49 29%	8/49 16%

Vimentin marker had increase in dysplastic epithelium compared with non-dysplastic epithelium (p=0.000) while this marker is normally expressed only in mesenchymal (not epithelial) cells. Expression percentage of vimentin marker in dysplastic and non-dysplastic epithelium is presented in table 6.

Table 6. Level vimentin marker expression in dysplastic epithelium and non-dysplastic

	%-Dysplastic N/T	**%-Nondysplastic N/T**
Score 1	27/60-45%	60/60-100%
Score 2	29/60-48%	0/60-0%
Score 3	4/60-7%	0/60-0%

Expression rate of vimentin marker in dysplastic and non-dysplastic epithelium

There was a significant relationship between vimentin marker and dysplasia grade of epithelium (p=0.000), which can be proven by referring to the values in table 7.

Table 7. Relationship between vimentin marker expression with degree of dysplasia

Crosstab

			D.vim			
			a	b	c	Total
H&E	mild	Count	32	16	1	49
		% within H&E	65.3%	32.7%	2.0%	100.0%
	sever	Count	0	7	4	11
		% within H&E	.0%	63.6%	36.4%	100.0%
Total		Count	32	23	5	60
		% within H&E	53.3%	38.3%	8.3%	100.0%

Relation ship of vimentin marker expression with dysplasia grade E-cadherin marker had the highest expression in normal areas in the lateral basal membrane of cells, especially in the surface epithelium between the crypts, and in dysplastic areas, membranous expression decreased and cytoplasmic expression increased in the cells of this area (pv=0.000) (Table 8).

Expression of E-cadherin marker in dysplastic (D) and non-dysplastic (N) surface epithelium

Table 8. Marker E-cadherin expression in dysplastic and non- dysplastic's surface epithelium

		D.C.d.E.C		D.M.s.(E.C)		D.C.s.E.C		D.M.d.(E.C)	
		H&E		H&E		H&E		H&E	
		mild	sever	mild	sever	mild	sever	mild	sever
1	Count	6	0	8	5	10	1	43	3
	% within H&E	12.2%	.0%	16.3%	45.5%	20.4%	9.1%	87.8%	27.3%
2	Count	38	6	40	6	34	4	5	5
	% within H&E	77.6%	54.5%	81.6%	54.5%	69.4%	36.4%	10.2%	45.5%
3	Count	5	5	1	0	5	6	1	3
	% within H&E	10.2%	45.5%	2.0%	.0%	10.2%	54.5%	2.0%	27.3%
	Count	49	11	49	11	49	11	49	11
	% within H&E	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

C=cytoplasmic,D=dysplastic,d=deep,E.C=E-cadherin,M=membranous,N=nondysplastic,s=surface * H&E Crosstabulation

Table 9. E-cadherin_Ki-67, Vimentin_ Ki-67& E-cadherin_Vimentin co-expression in surface epithelium of dysplastic area

		E.K.s			V.K.s			E.Vs		
		1	2	Total	1	2	Total	1	2	Total
mild	Count	42	7	49	45	4	49	20	29	49
	% within H&E	85.7%	14.3%	100.0%	91.8%	8.2%	100.0%	40.8%	59.2%	100.0%
	% within IHC	82.4%	77.8%	81.7%	86.5%	50.0%	81.7%	95.2%	74.4%	81.7%
sever	Count	9	2	11	7	4	11	1	10	11
	% within H&E	81.8%	18.2%	100.0%	63.6%	36.4%	100.0%	9.1%	90.9%	100.0%
	% within IHC	17.6%	22.2%	18.3%	13.5%	50.0%	18.3%	4.8%	25.6%	18.3%
Total	Count	51	9	60	52	8	60	21	39	60
	% within H&E	85.0%	15.0%	100.0%	86.7%	13.3%	100.0%	35.0%	65.0%	100.0%
	% within IHC	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

E.K.s=E-cadherin_Ki-67 co-expression in surface epithelium
V.K.s=Vimentin_ Ki-67 co-expression in surface epithelium
E.V.s=E-cadherin_Vimentin co-expression in surface epithelium
1=low dysplasia,2=high dysplasia

This expression shift of E-cadherin marker from membrane to cytoplasm and in the areas with severe dysplasia was significantly more than areas with minor dysplasia (p=0.001).

Table 10. Characteristics of Specificity and sensitivity of markers ki-67 (k), E-cadherin (E), Vimentin (V) in the detection rate of colorectal dysplasia Displaia

	V.K.s	E.K.s	E.V.s
Specificity	92%	86%	41%
Sensivity	36%	18%	91%

Co-expression specificity and sensitivity of vimentin (V), E-cadherin (E) and Ki-67(K) markers in diagnosing dysplasia grade of colorectal adenoma

Discussion and Conclusions

In this study, expression of vimentin, E-cadherin and Ki-67 markers were evaluated in colorectal adenomatous polyps. Ki-67 which is used as a proliferation marker in pathology is a human non-histone nuclear protein which is used to measure mitotic activity in the growth stage of human tumor [9]. Since any disorder in cellular proliferation can lead to tumor formation, recognition of the involved molecules in this process will be a great help in recognizing tumor formation process. The analysis of details of cellular cycle has revealed that Ki-67 is present in all phases of cellular cycle (G_1, G_2, mitosis); however, it is not present in resting cells (G_0). In this study, expression of this marker was surveyed in surface and proliferative (neck of the crypt) areas and deep crypt and the expression of Ki-67 marker statistically increased from proliferation area towards the surface area in dysplastic areas (p=0.001).

Thus, in dysplastic areas, 73.3% of the samples had minimum Ki-67 expression in surface and neck of the crypt and only in 20% of the samples in neck had more than surface expression while these cases were 10% and 90% in in non-dysplastic areas, respectively.

These findings were obtained in the study by Aihvali et al., and Yingh Ao Su et al. in which tissue microarray method was used and colon crypts were divided to three groups, like our study [10].

It can be concluded that with the increase of dysplastic cells in surface, a greater number of dysplastic epithelial cells remain in this area in proliferation cellular cycle and a few number of cells are transferred to the resting phase, which results in more survival of cells and more number of surface epithelial cells in dysplastic areas and ultimately a dysplastic epithelial multilayer.

In this study, no statistically significant relationship was found between the expression of Ki-67 marker and dysplasia grade of adenomatous polyps (pv=0.114).

No similar studies were found in this field. Also, in a study by P. G. Johnson et al., no significant relationship was found between histologic grades of

colorectal adenocarcinoma and Ki-67 expression; however, they observed more expression of ki-67 in carcinoma samples than adenoma samples [10].

In contrast, Masafumi N et al. did not observe any obvious differences between Ki-67 marker expression in carcinoma group and adenoma group [11].

The above findings probably mean that change of cellular nature in dysplasia transformation from low grade to high grade is related to factors independent from cellular proliferation characteristics.

E-cadherin protein is the adhesion of epithelial cells and plays a key role in tissue structure of epithelial cells. This protein is from the membrane protein group and is coded by CDH gene on 16q22 chromosome and its activity depends on calcium ion. The mentioned molecule sticks to γ or β catenin from inside of cytoplasm and to the E-cadherin of adjacent epithelial cell from outside of the membrane and sticks the adjacent cells to one another like a zipper [12]. Thus, loss of membranous E-cadherin can drive the cells out of the control signals, make them lose differentiation, proliferate and invade [12].

In this study, expression method of E-cadherin marker was statistically analyzed in membranous and cytoplasmic forms in normal and dysplastic areas and in two areas of surface epithelial and deep crypt epithelial. E-cadherin marker expression in surface epithelial and also deep epithelial of crypts from non-dysplastic to dysplastic areas (1) and from low grade dysplasia to high grade dysplasia (2) with the mentioned marker shift changed from membrane to cytoplasmic expression. Table 8 presents intensity of E-cadherin marker expression in dysplastic and non-dysplastic epithelium in two levels of surface epithelial and deep crypts. The mentioned expressions were evaluated in two methods of cytoplasmic (c) and membrane (m) and the numbers mentioned in the table show that the maximum intensity of E-cadherin marker expression in non-dysplastic areas in surface and deep of crypts was membranous and was expressed as average to severe intensity in 75% and 6.7% of cases, respectively, while the maximum intensity in dysplastic areas was cytoplasmic and, in 86.6% and 90%, the intensity of average and high was expressed in surface and deep crypts, respectively.

45.5% of the samples with sever dysplasia had high E-cadherin expression in their cell cytoplasm while this value was 10.2% in the samples with low grade dysplasia (in H&E) in both cases. This change was tangible in both areas of surface epithelial and deep epithelial of crypts. Similar results were obtained in the studies by Xiaodi Chen et al. [6]. Also, R. G .hardy et al. demonstrated reduction in E-cadherin (membranous) marker expression in the formation of adenomatous polyps [13] and Hermiston ML et al. defined functional reduction of E-cadherin in intestine crypts during adenoma formation [14]. Furthermore, El-Hariry and B. Nawrocki and S.G.T Riusolino along with others conducted three separate studies and investigated shift of E-cadherin marker expression from membrane to cytoplasm and concluded that this location shift can be due to E-cadherin or catenin molecule hypophosphorusation [14-16]. They also stated that seemingly molecular cytoplasmic expression of E-cadherin was a reflection of breach of change, substitution or combination of molecule in cell membrane or due to an abnormal accompaniment by cytoplasmic proteins [17].

According to the data in table 5 and statistical calculations, it can be concluded that E-cadherin marker expression in dysplastic areas was mainly cytoplasmic (in comparison with membranous dominant expression in non-dysplastic areas) and this expression significantly (pv=0.001) increased with the increase of dysplasia grade.

Vimentin is an intermediate III filament specific to mesenchymal cells which is not usually observed in epithelial cells.

This molecule forms cellular cytoskeleton and maintains integrity of cell and protects the cell from mechanical stresses [18].

Table 3 presents vimentin expression intensity in dysplastic and non-dysplastic epithelium in the studied samples; a significant expression increase (p=0.000) can be observed in dysplastic compared with non-dysplastic epithelium so that none of the non-dysplastic areas of the study showed average to high expression while vimentin in the epithelium of more than half of the studied samples with dysplastic areas had average to high expression. It can be observed in table 4 that this marker was expressed considerably more in high grade dysplasia areas compared with low grade dysplasia areas.

Thus, in 65%, the samples with low grade dysplasia diagnosis expressed less than 1% vi-

mentin epithelial cells while, in all the high grade dysplasia diagnosis samples, more than 1% and 36.4% had more than 5% of their interlayer epithelial cells with vimentin expression.

CYnagan and Dr HoYamamoto et al. conducted a similar study and reported increased vimentin expression in colorectal cancers and concluded that this marker can be beneficial for predicting recurrence of colorectal cancers [19]. Xiaodi Chen et al. referred to an increase of vimentin marker expression in epithelium adenomas and colorectal adenocarcinomas and introduced it as a sign of EMT [6].

One of the goals of this study was to evaluate the relationship between the studied markers' co-expression and dysplasia grade of adenomas epithelial on the basis of morphology diagnosis (H&E). The co-expression amount of Ki-67 and E-cadherin and vimentin markers was evaluated in pairs (table 9); thus, it can be deducted that there was a significant relationship between the co-expression of Ki-67 and vimentin markers in surface epithelium and dysplasia grade (pv=0.013). Also, these was a significant relationship between vimentin and E-cadherin markers' co-expression and dysplasia in the surface epithelium (pv=0.046). However, no significant relationship was found between the co-expression of Ki-67 and E-cadherin markers (pv =0.744). Specificity and sensitivity levels of the mentioned co-expressions in the diagnosis of dysplasia grade within the confidence limit of 95% are given in table 10. Increase in the Ki-67 marker expression in epithelium at the same time as the dysplasia formation in surface areas meant that cellular non-differentiation status along with high proliferation index, which is normally specific to proliferative neck crypt, extended to the surface epithelium. Decrease of E-cadherin membrane expression along with increased cytoplasmic expression and its grade increase with increased dysplasia grade in surface epithelia indicated cell preparation for losing epithelial characteristics and obtaining elasystemic and invasion characteristics. These characteristics were reflected by increased vimentin marker expression and specific markers of mesenchymal tissues in epithelium, which indicates EMT in a way. This process can be beneficial in achieving the goal of this study (which is using the above-mentioned markers for better discrimination of adenomatous polyps with different dysplasia grades).

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The congenital heart anomalies in Turkish subjects

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Abstract

The congenital anomalies of the heart during the first ten weeks of embryonic development and are still present at birth. In this retrospective study of 3493 autopsy subjects, obtained between 2005 and 2011, have been investigated. The anatomic structures of the aortic and pulmonary valves, the origins of the coronary arteries, the myocardial bridge, the patent foramen ovale and the presence of ventricular septal defects are reported. The abnormalities of valves were identified in 17 of the of 3493 cases. Specifically, the bicuspid aortic valve was observed in 14 cases, the quadricuspid pulmonary valve evident in 2 cases and a pentacuspid pulmonary valve was seen in 1 case. The triple origin of the right coronary artery was identified in 11 cases. The double origin of the coronary artery was observed in 39 cases on the right side and in 4 cases on the left side. The presence of the myocardial bridge was observed in 29 cases, while in 153 cases the patent foramen ovale was detected. Furthermore, in 3 cases there were ventricular septal defects. The presence of such data regarding congenital heart anomalies should prove helpful for the cardiovascular surgery and may facilitate further clinical and anatomical research.

Key words: Valve anomalies, multiple coronary artery origins, myocardial bridge, patent foramen ovale, ventricular septal defect.

Introduction

Embryologically, during the first ten weeks of embryonic development, the congenital anomalies of the heart and blood vessels arise and remain present at birth. Approximately 9 people per 1000 are born with a congenital heart defect. While many defects do not necessitate treatment, certain complex congenital heart defects require medication or surgery (1,2).

Congenital anomalies of the semilunar valves are rare and most often involve the presence of a monocuspid (3), bicuspid (4-6) or quadricuspid (3,6-10) valves of the pulmonary and aortic valves. Apart from the quadricuspid valves found in the setting of the common arterial trunk, quadricuspid semilunar valves are exceedingly uncommon, with the pulmonary valve being affected more frequently than the aortic valve. When the pulmonary valve is involved, its abnormal structural design rarely alters the function of the valve and the anomaly often remains silent (11). Bicuspid and quadricuspid pulmonary valves are usually considered to be minor cardiac defects because of their clinical significance (12).

The coronary arteries are the first branches of the aorta, usually arising from two of the three aortic sinuses of the Valsalva. These have previously been named in numerous ways, according to the orientation of the heart (13). In their normal epicardial course, the coronary arteries are embedded in a layer of fat. Generally, the branches passing into the myocardium commence in perpendicular fashion from the parent artery. During their passage through the epicardium, nonetheless, the parent arteries themselves can dive into the myocardium, reemerging at a subepicardial location more distally (14). Anatomic variations in the origin and course of the coronary arteries were reported as being at 0.3% in a necropsy series (1956), and at 1.6% in a large series of patients undergoing coronary arterial angiography (15).

The myocardial bridge (MCB) is a congenital coronary anomaly, where a segment of the epicardial coronary artery courses intramurally. The angiographic prevalence of the MCB is reported to range from 0.5% to 29.4%. Although it is considered to be

benign, the MCB may cause ischemia, acute coronary syndrome, coronary spasms, life-threatening arrhythmias, exercise induced atrioventricular conduction blocks and sudden cardiac death (16).

The patent foramen ovale (PFO) is a remnant opening from the fetal circulation. It is situated in the interatrial septum between the septum primum and the septum secundum which allows the passing of fetal blood from the right atrium to the left atrium. It is an oblique opening resembling a tunnel with an average width of 4.9mm. It closes within two years after birth, at the ratio of 75% (17,18). The interventricular septum is the anatomic structure that divides the right and left s. The formation of the interventricular septum begins at around the fifth week of embryonic development and involves the sequential fusion of 3 independent septa: the muscular, outlet, and inlet septa. 7–9 A disruption in this process leads to the development of ventricular septal defects (19,20).

Several case studies of heart anomalies are presented in the literature (3,21,22). However, there exists an insufficient number of studies with large sample sizes reported in the literature specific to cardiology and anatomy. The present study indicates the incidence of congenital heart anomalies, such as the anatomic variations of aortic and pulmonary valves, the origins of the coronary arteries, the foramen ovale, the presence of atrial septal defect, the presence of ventricular septal defect and the myocardial bridge in the Turkish population over the course of a five year period.

Materials and methods

In this retrospective study, the archive records of 3493 autopsy cases between 2005 and 2011 were obtained from the Akdeniz University Department of Forensic Medicine and the Council of Forensic Medicine Antalya Group Presidency have been scrutinized. This examination focused on the anatomic structure and variations of the aortic and pulmonary valves, the anomalous origin and course of the coronary arteries, the presence of myocardial bridges, and the atrial and ventricular septal defects.

Results

In the present study, 256 patients (7%) of 3493 patients were observed to have abnormalities. The bicuspid aorta was identified in 14 cases (0.4 %) (Figure 1). 2 of these cases were considered to be rare: the first heart anomaly exhibited a transposition of the major arteries; the aorta originated from the right ventricle, while the pulmonary artery originated from the left. The leaflets combined and formed a bicuspid valve in the aortic valve, and the abnormality of the coronary artery origin was also identified. The mitral valves were thickened, with an opening in oval foramen of about 1.5 cm reported (Figure 2). In the second, two small valves within the anterior semilunar valve were detected (Figure 3).

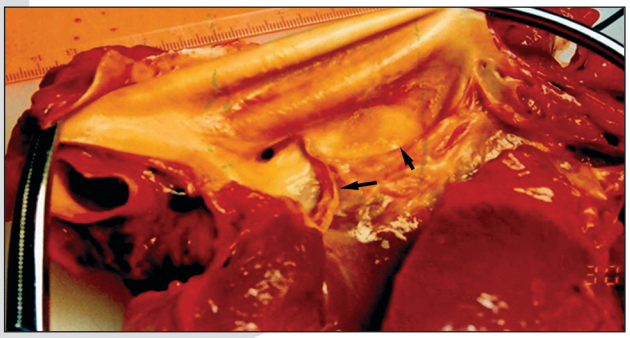

Figure 1. Bicuspid aortic valve

The pulmonic valve with five cusps (pentacuspid) was identified in 1 case (Figure 4), whereas the four cusps (quadricuspid) pulmonic valve was observed in 2 cases (0.05 %) (Figure 5).

In 54 cases (1. 5%) the abnormality of the coronary artery origin was identified. The triple originated right coronary artery was identified in 11 cases (Figure 6), whereas the double originated right coronary artery was observed in 39 cases (Figure 7), while the double originated left coronary artery was found in 4 cases.

The presence of the myocardial bridge was observed in 29 cases (0.83 %) (Figure 8), with all myocardial bridges reported over the anterior interventricular arteries (thickness of 0.5 cm- 1.5 cm).

In 153 cases (4.3 %) the patent foramen ovale was detected with different characteristics of foramen ovale (Figure 9). In 108 cases the dimensions of the opening was 0.2 x 1 cm , in 44 cases as 1.2 x 1.7 cm, and in 1 case (a 4 month old male) with

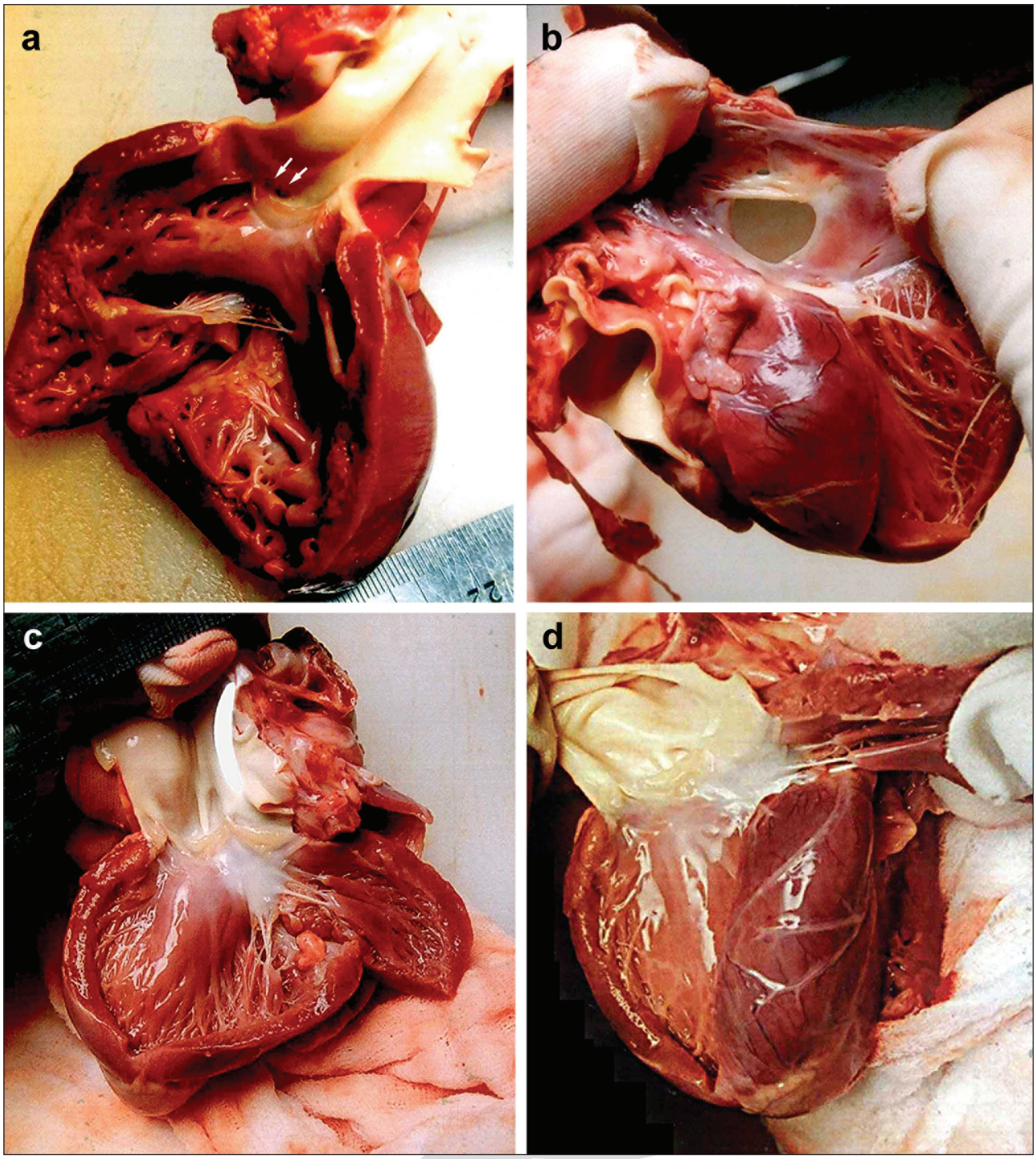

Figure 2. Great artery transposition anomaly of the heart. The right ventricule originated aorta, bicuspid aortic valve and the abnormality of the coronary artery origin (white arrows) (a), atrial septal defect (b), The left ventricule originated pulmonary artery and the thickened mitral valves are shown (c-d).

two separated openings of the foramen ovale (2x3 and 2x2 cm).

Additionally, we observed 3 cases (0.08 %) with a ventricular septal defect (VSD). The mean opening of the VSD was 0.8 x 9 mm. One of these cases was characterized with a bicuspid aorta.

Discussion

Congenital cardiovascular malformations usually occur after an alteration of the embryonic development of a normal structure, or an arrest of development in the early stages of the embryonic or fetal periods (23). Some of these anomalies do not give

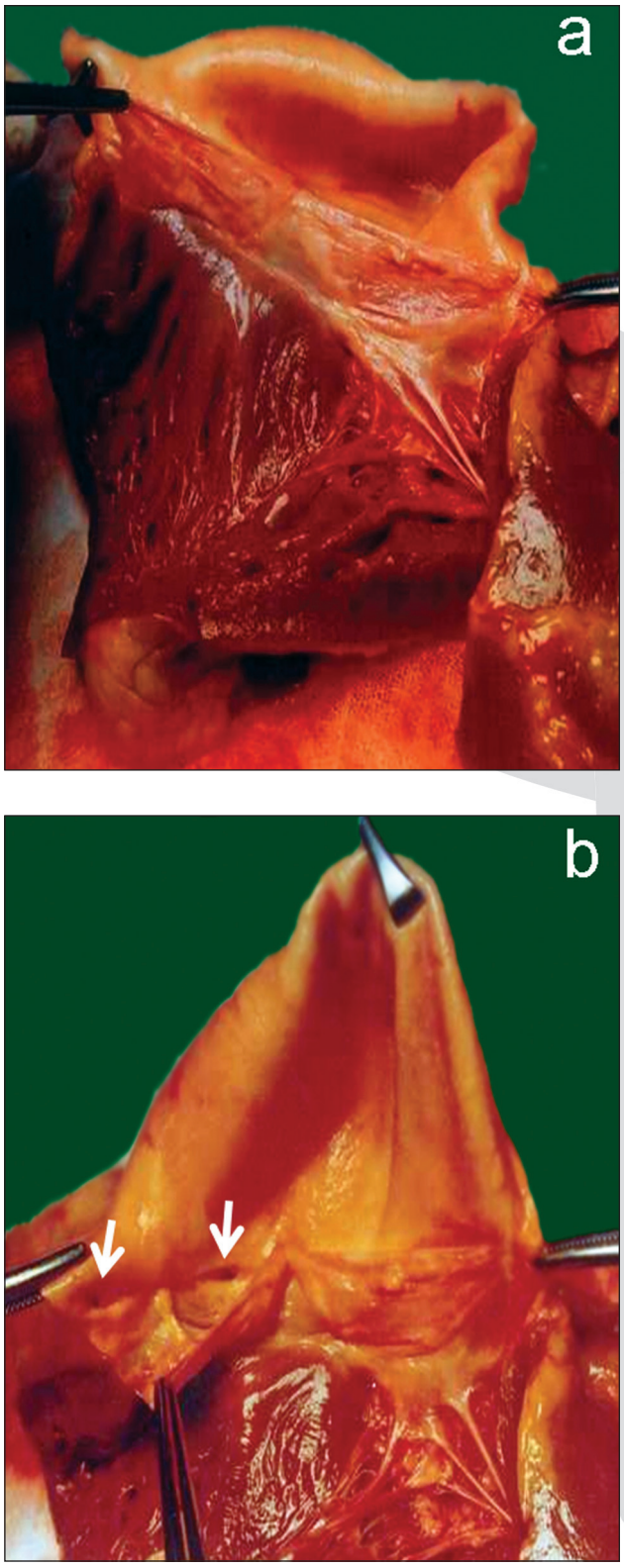

Figure 3. Bicuspid aortic valve (a), two small valves in the anterior semilunar valve (b)

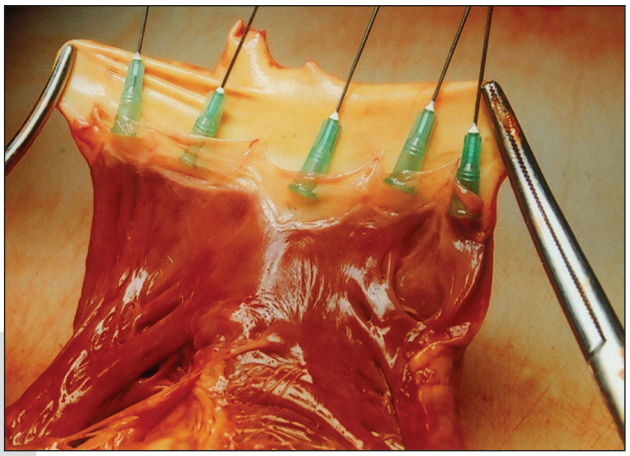

Figure 4. Pulmonary valve with 5 cusps

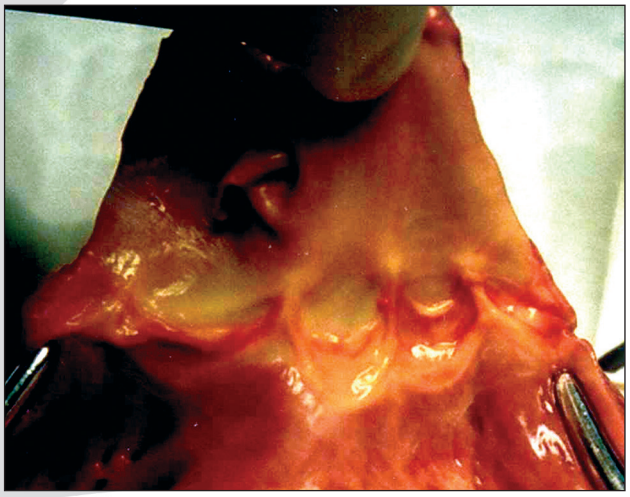

Figure 5. Pulmonary valve with 4 cusps

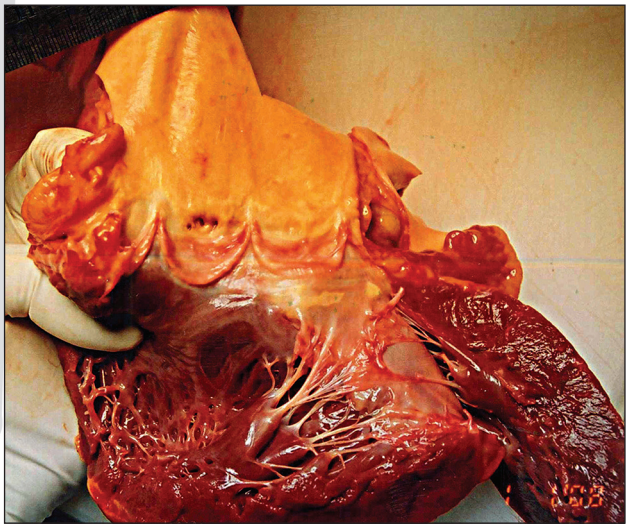

Figure 6. The triple origin of right coronary artery

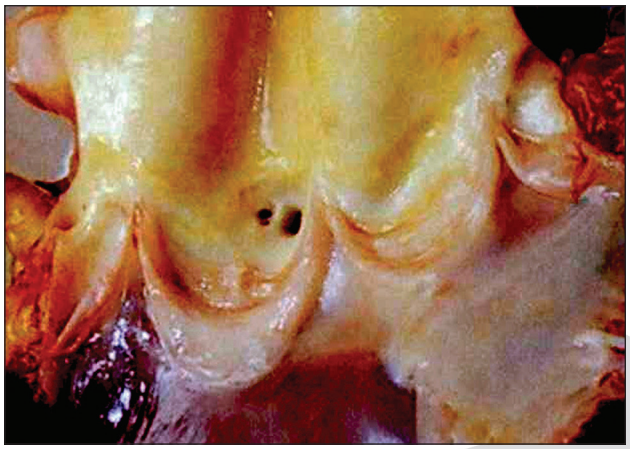

Figure 7. The double origin of right coronary artery

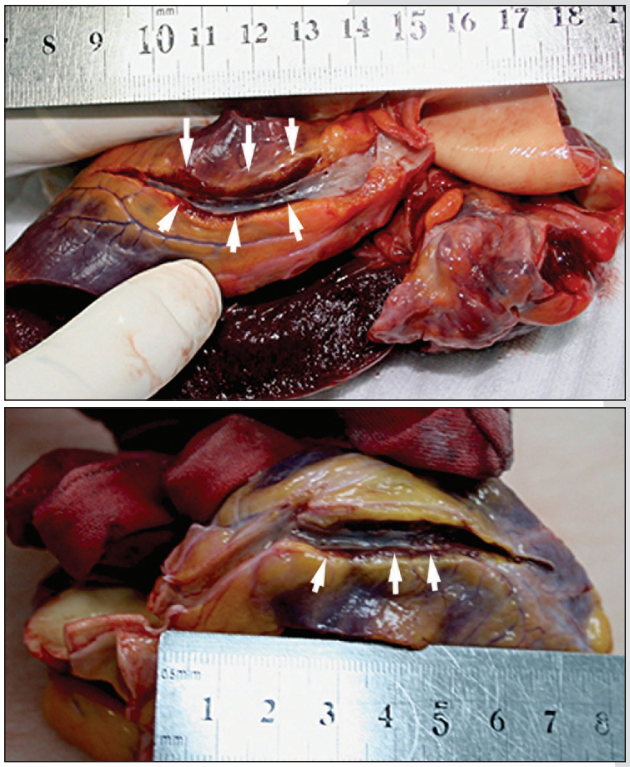

Figure 8. The presence of myocardial bridge over the anterior interventricular arteries (different two cases)

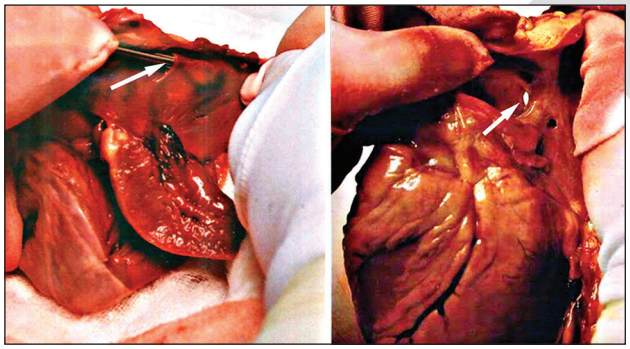

Figure 9. An opening due to the patent foramen ovale

rise to healthy life and result in death at the intrauterine life stage. Because of their early symptoms and indications, these anomalies can be determined at a young age. In a certain section of the patients, it may only be diagnosed during the adult years, or because of the subclinical development, it may not be diagnosed (22). For these reasons, it is difficult to detect congenital cardiovascular anomalies. Through this retrospective study, we have aimed to clarify the incidence of congenital cardiovascular anomalies in the Turkish population.

The congenital anomalies of the aortic and pulmonary valves are rare. The pulmonary valve usually has three leaflets, but in rare cases may have two, four, or more. The lesion is due to a failure in the development of the aorticopulmonary septum, and is almost certainly linked to an abnormal migration of cells into the heart from the neural crest (1,2).

The quadricuspid semilunar valves seem to be far less common and are reported to occur nine times more frequently in pulmonic valves than in aortic valves (3). In his series of 2000 necropsies, Simonds was not able to find even one case of a quadricuspid aortic valve (6). In another series of necropsies and echocardiograms, the quadricuspid aortic valve was reported between the rates of 0.008% and 0.013% (3). Indeed, in the existing literature, the pentacuspid valve is rarer than quadricuspid valve. Cases of a pentacuspid aortic valve diagnosed by transoesophageal echocardiography have been reported by Cemri et al (24), Kamata et al (25), and in our previous autopsy study (26).

A case of a quadricuspid pulmonary valve with an accessory coronary artery was reported by Rivett and Berry (20). Chiu et al (27) reviewed the literature and found one example of a pentacuspid truncal valve from 301 cases. In their report they presented a functional diagram indicating the relationship between the coronary orifice and the truncal valve, according to various truncal rotations. In the present study we identified 10 bicuspid aorta, one pentacuspid pulmonary valve and two quadricuspid pulmonary valves.

While quadricuspid pulmonary valves are infrequently associated with serious clinical complications, the majority of quadricuspid aortic valves are associated with clinically significant dysfunction, most commonly aortic insufficiency (24). In our cases with pulmonary or aortic valve anomalies,

no evidence of heart failure was found clinically or pathologically and we can speculate that the abnormal cusp formations may have been embryologically caused by the possible abnormal proliferations of pulmonary truncus of the common trunk.

The major coronary arteries were identified within the walls of the aortic sinuses before the emergence of coronary arterial orifices, thus suggesting in growth rather than outgrowth of the arterial channels. The origin of the right and left coronary arteries are formed when strands from a peritruncal ring of vascular structures penetrate the aorta at the right and left aortic sinuses of Valsalva (28). The origins of these arteries can vary significantly in relation to the sinutubular junction, and also in their proximity to the zones of apposition between the valvar leaflets, the so-called commissures (29). In this respect, deviations of take-off that are within 1cm of the sinutubular junction in the adult heart are considered normal variations, whereas origins deviating by greater than 1 cm relative to the junction constitute ectopic origin, or high take-off (30). It is exceedingly unusual for a major coronary artery to take origin from the aortic sinus distant from the pulmonary trunk. Based on these origins, therefore, the aortic sinuses of the Valsalva can be named as the right coronary, left coronary and non-coronary sinuses, respectively. In the present study a doubled orificum of the right coronary artery was identified in 6 cases and a doubled orificium of the left coronary artery in 2 cases. Furthermore, in 5 cases a triple orificied coronary artery was observed.

The myocardial bridge (MCB) is a bridging of the heart muscle over one of the major arteries (usually the left anterior descending artery (LAD)) of the heart. Myocardial bridging in the LAD has been detected by angiography, multidetector computed tomography, and by autopsy. It is more frequently detected by MDCT (3.5–58%) than by angiography (0.4–15.8%). MCB frequency is around 50% by autopsy (14, 21). We identified 29 MBs in this series. The incidence of bridging is correlated to the pattern of coronary arterial dominance (15, 31). The functional significance has, however, still to be analyzed, with some evidence pointing to bridging having a protective effect against atherosclerosis, but other findings relating extensive bridging to myocardial ischemia (21, 32). The bridging of arteries other than the anterior interventricular branch, nonetheless, should probably be considered as anomalies rather than normal variations. It is unusual for individuals with myocardial bridges to suffer clinical complications, although on occasion surgical division of the bridge may be indicated (15).

A patent foramen ovale (PFO) is a slit or tunnel-like passage in the interatrial septum formed by failure of postnatal fusion of the septum primum and septum secundum. Persistent PFO occurs in around 20–25% of the adult population, the exact frequency dependent on the method of detection (33). The prevalence of PFO is reported as being between 152% and 353% and the average frequency is about 26%. The prevalence decreases with the progression of age (18). The patent foramen ovale was detected in 153 cases (4.3 %) in our study.

Ventricular septal defects (VSD) are abnormal openings in the septum that allow the shunting of blood between the s. VSDs are the most common congenital abnormality diagnosed in children, with a reported incidence of 53 per 10,000 live births. VSDs occur in 50% of children with congenital heart disease and are an isolated finding in 20% of these cases. In adults, VSDs are the second most common congenital heart condition (after bicuspid aortic valve), with an estimated prevalence of 0.3 per 1000 (34, 35). In the present study we observed 3 cases of VSD, with an incidence of 0.08%. Determination of the incidence of congenital heart anomalies is important in many respects. Knowledge of the relative frequencies of congenital heart diseases associated with congenital anomalies facilitates the individual diagnostic approach. In addition, it can provide great benefits in terms of etiological approaches. Detailed knowledge of the congenital heart anomalies is important for those involved in the provision of cardiac care. Therefore, the data on the incidence of congenital heart anomalies in the Turkish population may facilitate further clinical and anatomical research.

Acknowledgments

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Connexin 32 and Parkinson's disease

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Abstract

Gap junctional intercellular communication (GJIC) plays an important role in brain pathology, but the mechanisms of the changes of gap junctions in Parkinson's disease (PD) are mostly unknown. Astrocytes are involved in PD and thought to be coupled by gap junctions, which consist of connexin (Cx) -26, -32, and -43 gene. In the development of PD, only Cx43 is reported to be enhanced in the astrocyte and Cx26 is stable. However, whether Cx32 is involved in the development of PD remains to be proven. Here we showed that phosphorylated Cx32 (termed P1) was enhanced both in the astrocytes of rat PD model and in cultured astrocytes stimulated with rotenone. Enhancement of P1 levels in cultured astrocytes occurred in parallel with an increase in GJIC, which was accompanied with an increase in Cx32 mRNA levels. In the rat PD model, P1 was selectively enhanced in striatum and hippocampus regions. The P1 levels were lower in the striatum and hippocampus regions than in the cortex, cerebellum, midbrain and thalamus. These findings indicate that up-regulation of P1 in astrocytes also plays a critical role in PD pathology.

Key words: gap junction; connexin 26; astrocyte; Parkinson's disease, dopaminergic, basal ganglia.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease that is characterized by a progressive and loss of dopaminergic (DA) neurons in the substantia nigra and striatum[1-4]. Systemic administration of mitochondrial complex I inhibitor, rotenone, causes selective death of DA neurons and Parkinsonism in rodents, accompanied by behavioral and neurochemical changes, DA degeneration, and the appearance of eosinophilic cytoplasmic inclusions[5, 6]. The reason why DA neurons are particularly vulnerable to rotenone is not fully understood, although their vulnerability seems to be important in the development of Parkinsonism. In addition, accumulating evidence indicates an important role of astrocyte in Parkinsonism[7-9], but the mechanism remains mostly unknown.

It has been reported that several connexins are expressed in neurons and astrocytes where they may function in release of ATP and glutamate[10-13]. It is becoming evident that astrocytes are involved in neurological disorders including PD[7, 8]. Astrocyte gap junctions may be formed of multiple connexins. Presumably, the metabolic and ionic coupling provided by these diverse gap junction types may provide whatever intercellular signaling, which is necessary for brain development and cortica[11]. Hemichannels are large pore ion channels that in the traditional view are formed when half a gap connexin junction opens to the extracellular space. Hemichannels form a novel and unique class of ion channels that likely have diverse physiological and pathophysiological roles in the nervous system[13]. It has also been reported that that gap junctional intercellular communication (GJIC) in astrocytes is indispensable for several glial functions, including neuroprotection[14]. Astrocytes are thought to be coupled by gap junctions, which consist of connexin (Cx) -26, -32, and -43 gene[15]. Cx has recently been observed in ischemia, Alzheimer's disease, Huntington's disease and PD[16-20]. In the development of PD, only Cx32 is reported to be enhanced in the astrocytes and Cx26 is stable[20].

However, whether Cx32 is involved in the development of PD remains unanswered. Therefore, in this study we examined the changes in astrocyte GJIC and Cx32, as well as the phosphorylation status of Cx32, in a rat model of PD induced by chronic exposure to rotenone and in cultured astrocytes stimulated with rotenone. The model has been widely used to investigate the etiology of Parkinsonism[5, 21, 22] and will be useful to study whether Cx32 is involved in the development of PD.

Materials and method

Drugs and chemicals

Rotenone and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rotenone was dissolved in DMSO and stored at −20 °C.

Lewis rats

The animals were acclimated and maintained at 23 °C under a 12-h light/dark cycle (lights on 08: 00–20: 00). Rats were housed in standard laboratory cages and had free access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee. Lewis rats (200–250 g each) were purchased from Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China) and maintained in specific pathogen-free conditions. The rats were randomly divided into a rotenone group (n=9) and a control group (n=9). The rotenone group subcutaneously received rotenone (2.5 mg/kg, diluted in Panacet); the controls only received Panacet.

Primary astrocyte cultures

Primary astrocytes were prepared from whole brains of neonatal Wistar rats (1–2 days of age)[23], which were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). In brief, the brains were digested with 0.05% trypsin–EDTA at 37 °C for 10 min, and then mechanically dissociated by gentle pipetting and passed through a 100 um pore nylon mesh. Cells were plated onto 75 cm^2 plastic flasks and grown in DMEM supplemented with 10% v/v FBS and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO2-containing atmosphere. The medium was changed once each three days. They were harvested when cells reached confluence. Cells were seeded into a secondary culture. The purity of the primary astrocyte cultures was determined by immunocytochemical staining using an antibody against an astrocyte-specific marker (GFAP, dilution 1: 1000; Sigma, St. Louis, MO, USA) or a microglia-specific marker (anti-CD11b, dilution 1: 200; Serotec, Oxford, UK). At 30 d in vitro (DIV), 99% of the primary cultured cells were GFAP positive. No detectable CD11b-positive cells (i.e., microglia) were found[24]. Cultured astrocytes were treated with 0–16 nM rotenone for 2 days.

Fluorescence recovery after photobleaching (FRAP) assay for GJIC

GJIC is measured using FRAP. The quantitative FRAP assay for GJIC was performed as previously reported [20] using laser-scanning confocal microscope (LSCM, Olympus Fluoview FV300, Olympus (China), Ltd., Beijing, China). After bleaching of randomly selected cells with a micro-laser beam, the rate of transfer of 5, 6-carboxyfluoresceindiacetate (Sigma, St. Louis, MO, USA) from adjacent labeled cells back into bleached cells was calculated. Recovery of fluorescence was examined after 0.5 min, and the recovery rate (RR) was calculated as percentage of photo bleached fluorescence per min. The RR was adjusted for the loss of fluorescence measured in unbleached cells, and the results are expressed as the ratio of RR to that of untreated control cells[20].

Extraction of Cx32 RNA and Quantification for Cx32 mRNA

Cells were grown in 6-cm cell culture dishes for at least 48 h. The cells were trypsinized and suspended in DMEM medium containing 10% FCS. Total RNA was isolated from the cells using QIAshredder and RNeasy Mini kits (Qiagen, Inc., Chatsworth, CA, USA). An initial strand of cDNA was synthesized from 500 ng of RNA extracts in a volume of 20 ul using AMV reverse transcriptase XL (TaKaRa, Takara Biotechnology (Dalian) Co., Ltd., Dalian, China) priming with random 9-mers at 42 °C for 10 min. The cDNA strand was stored at 20 °C until use. Expression of Cx32 mRNAs was evaluated by real-time RT PCR. PCR was performed in an ABI PRISM 7900 sequence detector (Applied Biosystems, Foster City, CA, USA) in a final volume of 20 ul. The PCR mixture contained 10 mM Tris–HCl buffer, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTP mixture, 0.5 U of Ampli Taq gold enzyme (Applied Biosystems, Foster City, CA, USA) and 0.2 M primers. The primer and probe sequences for gene amplification were as follows: Cx32, 5'- GCTATGACCAATTCTTCCCC -3' and 5'- GACGTCGCACTTGACCAGCC -3'; GAPDH, 5'-CCCTTCATTGACCTCAACTAC-3'

and 5'-CCACCTTCTTGATGTCATCAT-3'. GAPDH was used as an internal control. The Ampli Taq gold enzyme was activated by heating for 10 min at 95 °C, and all genes were amplified by 50 cycles of heating for 15 s at 95 °C, followed by 1 min at 60 °C.

For the construction of standard curves of positive controls, the total RNA of primary astrocytes was reverse-transcribed into cDNA and serially diluted in water in five or six log steps to afford fourfold serial dilutions of cDNA from about 100 ng to 100 pg. These cDNA serial dilutions were stored at−20 °C. The coefficient of linear regression for each standard curve was calculated, and then when the cycle threshold (CT) value of a sample was substituted into the formula for each standard curve, the relative concentration of Cx32 or GAPDH could be calculated. To normalize for differences in the amount of total RNA added to each reaction mixture, GAPDH was used as an endogenous control. The data represent the average expression of target genes, relative to GAPDH, from three independent cultures.

Immunoblotting

Cells and rat brains were lysed in ice-cold in buffer (50 mmol/l Tris-HCl pH 7.4,150 mmol/l NaCl, 1% [v/v] NP40, 5 mmol/l EDTA, 5% [v/v] glycerol, 10 ug/ml leupeptin, 10 ug/ml aprotonin, 1 mmol/l phenylmethylsulfonyl fluoride, and 1 mmol/l Na_3VO_4) using a polytron. The lysates were then sonicated. The samples were diluted 1: 4 in water, and their protein concentrations were determined using the Bradford method with affinity-purified bovine serum albumin as standard. Ten g samples were dissolved in Laemmli Sample Buffer, separated on 12% acrylamide gel, and transferred to PVDF membranes. Then blots were incubated with anti-Cx32 antibody (Shengshizhongfang BioSci& Tech. LTD. CO., Beijing, China) overnight at 4 °C, followed by PBS with 0.1% Triton X-100 (PBST) washes three times for 15 min each. As an internal control to determine whether equal amounts of protein had been loaded on to the gel, the PVDF membranes were stripped and reprobed with anti-tubulin (T5168, Sigma, St. Louis, MO, USA). Blots were incubated with goat anti-rabbit antibody conjugated horseradish peroxidase or mouse anti-mouse antibody conjugated horseradish peroxidase. Immunoreactive bands were visualized by ECL (GE Healthcare, Shanghai, China) and quantified by densitometry with Image J software 1.45 (NIH, Bethesda, USA) according to software's instruction.

Statistical analysis

The association between Cx32 levels and rotenone treatment in different groups was compared by one-way analysis of variance (ANOVA) followed by the post hoc test of Fisher's protected least significant difference (LSD). We used Spearman's rank correlation coefficient to identify the strength of correlation between Cx32 levels and rotenone treatment. The online software could be available and computes the Spearman Rank Correlation and the two-sided p-value. The ordinary scatterplot between ranks of X & Y was shown at http: //www.wessa.net/rwasp_spearman.wasp/.

Results

Cx32 protein level is up-regulated by Rotenone in astrocytes

Western blotting analysis showed that two forms of Cx32 immunoreactive protein (Mr 31,000–32,000) were observed in all samples: A faster migrating band (non-phosphorylated form, P0, Figure 1) and one slower migrating band (phosphorylated form, P1, Figure 1). P1 is known to localize on the plasma membrane and gap junctions[25]. Densitometric analysis showed that rotenone induced a significant dose- and time-dependent increase of P1 compared with control cells (Figure 1A) over 12–48 h (Figure 1B). Non-phosphorylated form P0 seemed changed slightly (Figure 1).The effect of rotenone on Cx32 mRNA levels was also examined, and Cx32 mRNA levels were found to be modulated by rotenone treatment (Figure 2). The optimized concentration was 25 nm (Figure 2 A). The optimized treating time was 48 h (Figure 2 B).The expression of Cx32 reached the highest level when the astrocytes were treated by 25 nm rotenone for 48 h.

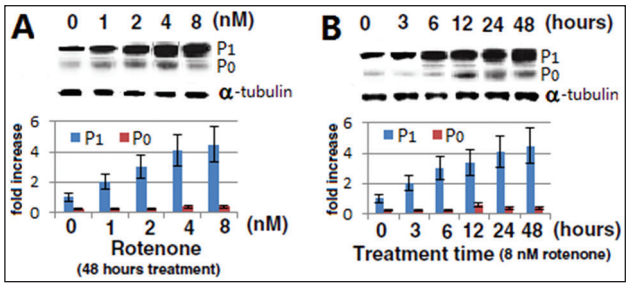

Figure 1. Effects of rotenone on Cx32 levels. Western blot analysis of Cx32 protein expression. Astrocytes were cultured with or without rotenone for 48 h at the indicated concentrations (upper panel) or with 8 nM rotenone for the indicated times (lower panel). Fold increase after culturing with rotenone is shown taking the value of untreated astrocytes as unity.

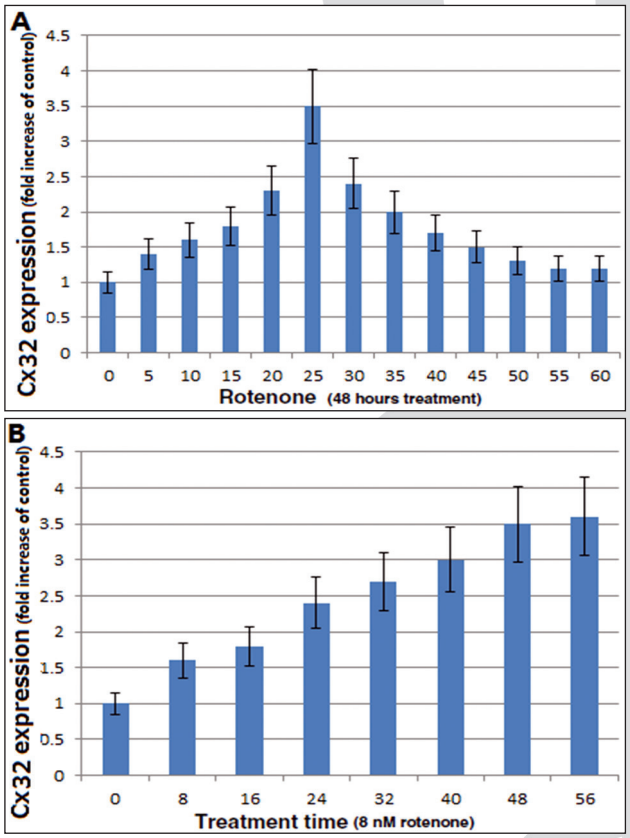

Figure 2. Real-time RT-PCR analysis of Cx32 mRNA expression. Astrocytes were cultured with or without rotenone for 48 h at the indicated concentrations (A) or with 8 nM rotenone for the indicated times (B). The value of untreated astrocytes (control) was taken as unity to calculate the fold increase. Cx32 mRNA levels were normalized by GAPDH mRNA, whose level did not change during culture with rotenone (data not shown). Results are means of at least three experiments. Values are mean ± SE.

GJIC is up-regulated by Cx32

We next examined the effect of rotenone on GJIC in cultured astrocytes. The GJIC was quantitatively assessed in living cells by FRAP assay as previously described[20], in terms of the RR. After photo bleaching, sequential scans detected the recovery of fluorescence in the bleached cells as the dye was transferred to photo bleached cells through GJIC from surrounding nonbleached cells. The RR at 48 h of treatment showed a dose-dependent increase up to 25 nM rotenone, although this was followed by a slight decrease (Figure 3A).

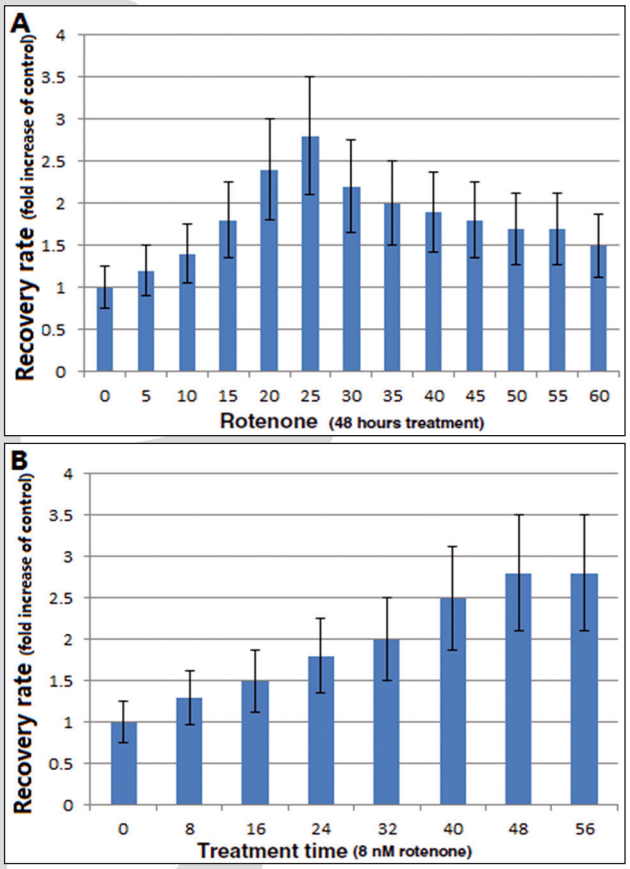

Figure 3. Dose and time course analyses of the effect of rotenone on GJIC in cultured astrocytes. GJIC was assessed by FRAP, in terms of RR (fold increase of control cells). Results are means of at least three experiments. (A) Dose dependence (treatment for 48 h). (B) Time dependence in the case of 8 nM rotenone. Columns show fold increase in RR compared with untreated cells (at 48 h) or compared with cells treated with 8 nM rotenone at 0 h for A or B, respectively. $P < 0.001$ for A and B.

Further, time course analysis showed a time-dependent increase in GJIC after rotenone treatment

(Figure 3B). The amounts of GJIC were consistent with the expression levels of Cx32 (Figure 2 and 3). The results suggest that rotenone treatment of cultured astrocytes generated increased protein levels and a broadened membrane distribution of Cx32, which in turn led to enhancement of GJIC.

Phosphorylated Cx32 levels were enhanced in the rat PD model

To investigate whether Cx32 levels may be altered in Parkinsonism, we examined the Cx32 protein level in rotenone-induced rat PD model. In this model, Cx32 was found in all regions, though at different levels and that the Cx32 protein level was significantly lower in striatum than in other brain regions (Figure 4), though the P1 form of Cx32 was markedly enhanced in striatum of the treated group.

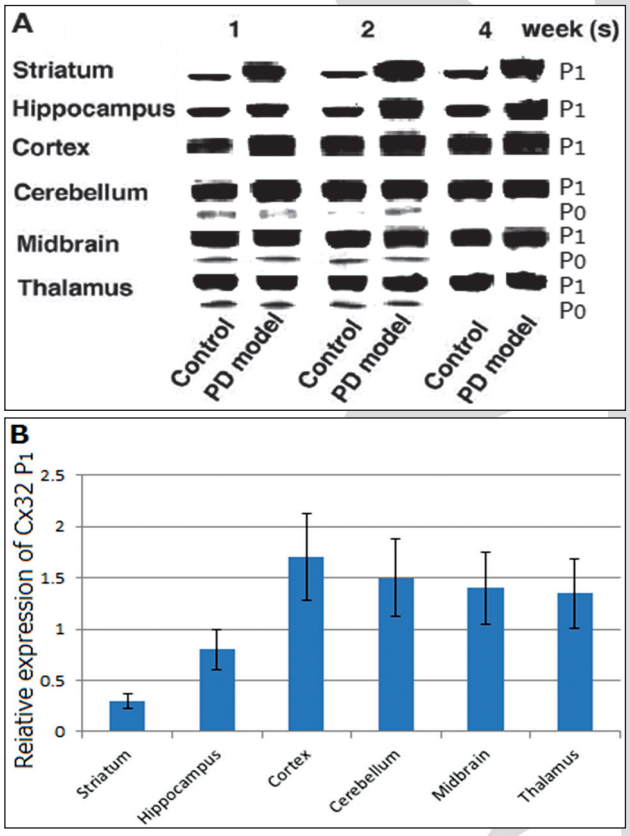

Figure 4. Cx32 levels in the brain. (A) Cx32 levels in the brain of rotenone-treated rats relative to that of Panacet (vehicle)-treated rats (control) at 1, 2, and 4 weeks. Western blotting analysis of Cx32. (B) Cx32 levels were compared between different brain regions by using identical membranes loaded with the homogenates obtained from the different regions. The graph depicts fold increase of total Cx32 expression relative to the control (thalamus). Values are mean ±SE with n=3.

Significant differences of total Cx32 levels were found in striatum of rotenone treated rats at 1, 2, and 4 weeks, as well as in hippocampus of rotenone-treated rats ($P<0.01$). However, no significant changes were observed in other regions (Figure 4A, B).

Cx32 expression levels were modulated by rotenone treatment

The levels of Cx32 in cultured astrocytes were correlated with dosage of rotenone when the treatment concentration was not more than 25 nm ($\rho=0.8$ $P<0.001$). In contrast, the levels of Cx32 in cultured astrocytes were inversely correlated with dosage of rotenone when the treatment concentration was more than 25 nm ($\rho=-0.6$ $P<0.001$) (Figure 5).

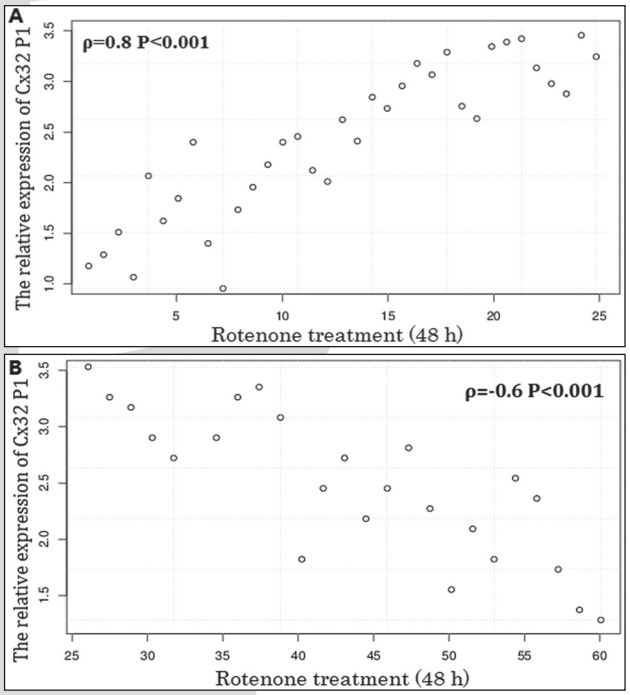

Figure 5. The relationship between the relative expression of Cx32 P1 and the concentrations of rotenone treatment. A, the concentrations of rotenone treatment were not more than 25 nm. B, the concentrations of rotenone treatment were more than 25 nm. Statistical analysis was done by Spearman's rank correlation test. If the value of rho falls between 1 and 0.5, there is a strong positive correlation. If the value falls between -1 and -0.5, there is a strong negative correlation.

All the results suggested that Cx32 levels were modulated by rotenone treatment while rotenone exposure reproduces features of PD[26]. Thus, the levels of Cx32 were closely related with the development of PD.

Discussion

Cx32 electrophoresis studies had found two forms of Cx32 that includes nonphosphorylated Cx32 (P0) and one slower-migrating form, commonly termed P1. Pulse chase analysis had indicated that the Cx32 isoforms progress from P0 to P1 and that the P1 isoform is associated with gap junctional structures[25]. In our study, rotenone treatment induced an increase of Cx32 P1 in astrocytes, and the number of localized foci of total and phosphorylated Cx32 on the plasma membrane was increased. Furthermore, astrocyte GJIC was intensified with rotenone treatment. Therefore, since the increase of P1 of Cx32was proportional to the increase of total Cx32 protein levels, our findings indicate that phosphorylation of P1 was enhanced during the induction of total Cx32 protein by rotenone.

Connexins require an integrated network for protein synthesis, assembly, gating, internalization, degradation and feedback control that are necessary to regulate the biosynthesis, and turnover of gap junction channels. At the most fundamental level, the introduction of sequence-altering, modifications introduces changes in protein conformation, activity, charge, stability and localization. Understanding the sites, patterns and magnitude of protein post-translational modification, including phosphorylation, is absolutely critical. Historically, the examination of connexin phosphorylation has been placed within the context that one or small number of sites of modification strictly corresponds to one molecular function. Connexins undergoing multiple levels of multi-site phosphorylation is the key for understanding of connexin post-translational control[27]. Our in vivo experiment using rotenone-treated rats demonstrated for the first time that P1 of susceptibility of astrocytes to Cx32 induction by rotenone could be of great importance, since astrocytes play direct, active, and critical roles in mediating neuronal survival and function in various neurodegenerative disorders, just as PD [9].

GJIC is involved in cellular growth control and can be restored by Cx32 protein expressions, so Cx32 is closely related with GJIC[28]. The central question is whether the elevation of astrocyte GJIC plays a part in causing the development of PD or whether it is merely a protective response to rotenone. From our results, the amounts of GJIC were closely consistent with the expression levels of Cx32. PD is a neurodegenerative disease that is characterized by a progressive and loss of dopaminergic (DA) neurons in the substantia nigra and striatum[1-4]. The expression levels of Cx32 were enhanced in striatum in PD model (Figure 2 and 3). Thus, GJIC would be also increased in PD model, so the elevation of astrocyte GJIC could cause the development of PD. Our immunohistological analysis suggested that Cx32 could be up-regulated in astrocytes in striatum and hippocampus brain regions. Therefore one possibility is that this difference in the density of astrocytes influences the induction of Cx32 protein by rotenone. Another possibility is that astrocytes in striatum and hippocampus might have different characteristics from those in other areas [29, 30].

This study also has limitations, for instance, induction of Parkinsonism by rotenone will need to be examined further. It will also be necessary to examine astrocytes changes with the use of Cx-specific inhibitors or siRNA in the future.

Author's contribution

YW, ZH and CZ performed all the experiments. XL provided the idea and wrote the paper. All authors read and approved the final manuscript.

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Vitamin D_3 and parathormone–do they affect the periodontal status?

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Abstract

Introduction: The vitamin D_3 (cholecalciferol) is a substance playing an important role in regulating the calcium and phosphorus level and in the bone mineralisation process. It is thus one of the substances playing a key role in regulating the calcium and phosphate management. The perception of the function fulfilled by the vitamin D in the human organism has clearly changed over the last several dozen years. Currently, it is perceived not only as a vitamin, but as a steroid hormone because the receptor for this vitamin in many tissues and organs was discovered. The epidemiological research studies from the second half of the 20th century indicated the participation of the vitamin D in maintaining the proper function of the muscles and nerves, the cardiovascular system, and in preventing cancer and diabetes. The level of vitamin D has a bearing on infections, digestive diseases and autoimmune diseases.

Aim of the paper: Since the role of vitamin D is significant in bone metabolism, we decided to test its level, and the level of the parathormone related to the metabolism of vitamin D_3, in a group of patients with the periodontal disease,.

Material and methods: The examination was conducted in 29 patients with chronic periodontitis and in healthy volunteers (28 individuals) without any periodontal disease. 5 ml of blood were taken from the subjects. The vitamin D and PTH assay was carried out using the electrochemiluminescence method.

Results: No statistically significant differences between the levels of vitamin D3 and parathormone between the individuals from the control group and the periodontal disease patients were found. However, a negative correlation between these parameters in examined patients from both groups was found.

Key words: Periodontal disease, vitamin D, parathormone.

Introduction

The perception of the function fulfilled by the vitamin D in the human organism has changed over the last several dozen years. It is now perceived not as a vitamin, but in view of the discovery of a receptor for the vitamin D in many tissues and organs it is now regarded as a steroid hormone.

In nature occur two compounds which after the metabolic activation exhibit the activity of the vitamin D:

- cholecalciferol- (precursor- 7- dehydrocholesterol) animal vitamin D_3,
- ergocalciferol- (precursor - ergosterol) vitamin D_2, occurring in plant tissues, mainly in yeasts and fungi.

The ergosterol and the vitamin D_2 differ from the 7-dehydrocholesterol and the vitamin D in the presence of a double bond between the C22 and C23 atoms and the presence of a methyl group at the C24 atom, respectively. In the skin, the 7- dehydrocholesterol is subject to a photochemical transformation to cholecalciferol under the influence of the sunlight with the wavelength of 290-315 nm, which is within the range of the UVB radiation. It is estimated that even 90 % of the vitamin D demand may come from the skin synthesis, and what is important no overdose symptoms of vitamin D coming from this source have been observed (3).

The sunlight indeed supplies a sufficient amount of the vitamin D in the equatorial zone, but in the temperate climate zone an insufficient skin exposure to the solar radiation during the winter necessitates the supply of vitamin D with the food. It was assumed that in the United States the

problem of the vitamin D had been eliminated. However, recent research studies have shown a low concentration of the vitamin D in the blood. Potential factors which could have contributed to the increasing deficiency of the vitamin D are: a reduced consumption of foods containing the vitamin D related to the fear of an excessive consumption of fats, a reduced consumption of calcium rich foods, including milk, a frequent use of sunscreen creams, and a reduced exposure to the sunlight in order to reduce the risk of skin cancer and to prevent premature skin ageing caused by the ultraviolet radiation (4).

The vitamin D_3 occurs in few foods: fatty fish (mackerel, salmon, sardines), cod liver oil, chicken eggs, liver (5). The cod liver oil available in pharmacies contains about 1000 IU/5ml. The vitamin D_3 has an influence on the amount of calcium absorbed from the gastrointestinal tract. With an appropriate amount of this vitamin in the organism, the quota of absorbed calcium consumed in the foods is between 30% and 80%, whereas with a low level of vitamin D_3 it is only 10-15% (6). The vitamin D_3 in connection with the vitamin D binding protein (DBP) penetrates through the circulation to the liver where it is hydroxylated to 25 OHD_3.

The next stage of the vitamin D_3 transformations are the reactions occurring in the kidney: 25 OHD_3 is metabolised to 24, 25 $(OH)_2D_3$ or to 1.25 $(OH)_2D_3$ depending on the supply of the organism with the vitamin D_3 and on the concentrations of calcium and phosphorus in the blood (6).

The hydroxylation takes place at the level of liver mitochondria and microsomes. The hydroxylation of the 1α position is principally regulated by the parathormone (PTH), but also by the insulin and the estrogens. This transformation increases with a low level of calcium and phosphorus. The reaction may occur in many other tissues (such as keratinocytes, activated macrophages, prostate, mammary gland, intestinal epithelial and parathyroid gland cells) and affect, through the amount of the active form of the vitamin D_3 being formed, the regulation of proliferation, differentiation and apoptosis of the in situ cells (5).

The activity of the 24-hydroxylase is stimulated by the 1.25 $(OH)_2D_3$, and inhibited by the PTH. In the states of vitamin D_3 deficiency, the 24,25 $(OH)2D_3$ synthesis is clearly reduced; it increases with supplementing the vitamin D_3 reserves in the organism (6).

The products of the vitamin D_3 catabolism are metabolites formed in the target tissues through side chain cleavage, or glucuronic acid conjugation and biliary excretion, or sulphuric acid conjugation and urinary excretion. Another product of the catabolism is the calcitroic acid formed through the 1, 24, 25 $(OH)_3D_3$ synthesis and the oxidative side chain cleavage (6).

The aim of the paper was to evaluate the levels of vitamin D and PTH in periodontal disease patients and to compare such levels in the control group subjects.

Material and methods

The clinical population consisted of periodontal disease patients treated in a periodontal clinic (29 individuals) aged between 22 and 74 years, 51.4 on average. The patients agreed in writing to the clinical examination and to taking a blood sample for the biochemical tests. The approval of the Bioethical Committee to conduct the study was obtained.

The reference population consisted of 28 generally healthy individuals without any periodontal disease, the staff of dentistry departments aged between 28 and 66 years, average age 41.5 years.

Periodontal examination

The periodontal examination comprised:

1. The measurement of the simplified O'Leary Plaque Index (PI) (the sum of all surfaces with dental plaque/ sum of all examined surfaces x 100%).
2. The assessment of the Ainamo and Bay Bleeding Index (BI): the sum of all surfaces in which bleeding occurred/the sum of all examined surfaces x100%), the bleeding index was measured at six measuring points – mesially, medially and distally for the labial/buccal and lingual/palatal surface of all teeth.
3. The clinical gingival status was evaluated using the Löe and Silness Gingival Index (GI) by assessing the gingiva with all teeth present in the oral cavity, by examining four gingival surfaces surrounding a tooth, i.e. the buccal, lingual, mesial and distal surfaces.

The gingival status evaluation criteria in this index are entirely based on clinical qualitative changes in the gingival tissues and are as follows: 0- the absence of gingivitis symptoms, the absence of any pathological change in the colour of the gums, 1- a mild gingivitis with a small change in the colour of the gums, a mild change in the structure of the gingival tissue, the absence of bleeding on probing, 2- a moderate gingivitis: redness, swelling, shiny appearance and hypertrophy of the gums, bleeding on pressure or probing, 3- a serious gingivitis manifesting itself in a considerable redness, swelling, ulceration and the susceptibility to spontaneous bleeding.

1. The average Pocket Depth (PD), a value measured from the bottom of a periodontal pocket to the edge of the free gingiva, given in mm.
2. The average level of the Clinical Attachment Loss (CAL), a value measured from the bottom of a periodontal pocket to the cement-enamel junction, given in mm.

The PD and CAL values were measured at six measuring points.

For the evaluation of the periodontal status (items 4 and 5) the PD and CAL percentages with the value equal to or higher than 6 mm were used.

Laboratory test

A blood sample (5 ml) was taken from the patients. The levels of vitamin D_3(25 -OH) and parathormone were determined at the Department of Laboratory Paediatric Diagnostics of the Medical University of Bialystok by means of the electrochemiluminescence method using streptavidin- and ruthenium compound-coated magnetic particles on the Cobas e411 apparatus of Hitachi.

Statistical analysis

For the comparison of obtained results the IBM SPSS Statistics 20.0 program was used. The analysed parameters were described by specifying the arithmetic mean (x), the standard deviation, the minimum and maximum values, and the percentages (%). For the evaluation of the level of the vitamin D and the parathormone the Mann-Whitney Test and the Spearman's nonparametric correlations were used. The results of the statistical tests were found significant at the level of $p<0.05$.

Results

Clinical periodontal examination

Chronic periodontitis was diagnosed in all patients qualified for the study. The periodontal status is shown in Table 1. The Plaque Index was between 23.7% and 99.3% (mean 60.24%). The Bleeding Index was between 9.8 and 94.79% (mean 66.27%). The Gingival Index was between 0.49 and 3 (mean 2.15). This index evaluates the gingiva only.

In the evaluation of the clinical attachment loss (CAL) and the periodontal pocket depth (PD) the percentage of teeth where the attachment loss and the depth of periodontal pockets was equal to or more than 6 mm was taken into consideration. Such progression of the periodontal destruction affected all subjects. Advanced periodontal diseases were found in the range from 3.84% to 100% (mean 47.78%).

Table 2 presents the level of tested substances in both groups. The mean value of vitamin D in the serum in both groups only slightly exceeded the level of 15ng/ml. In the periodontal disease group, only 7 individuals showed a level of vitamin D exceeding 20 ng/ml, and 5 individuals did not reach the level of 10 ng/ml. In both groups the differences were not statistically significant.

The mean level of the PTH was similar in both examined groups (the mean in the control group was 42.24 pg/ml, and in the periodontal disease group 42.12 pg/ml). No statistically significant differences were found. In the control group, no cor-

Table 1. Periodontal status in the clinical population

Group	n	Mean	Stand. deviat.	Minimum	Median	Maximum
PI [%]	29	60.24	21.4	23.7	59	99.3
GI	29	2.15	0.69	0.49	2.25	3.00
BI[%]	29	66.27	21.82	9.80	71.25	94.79
PD, CAL [%]	29	47.78	27.57	3.84	41.66	100.00
Number of teeth	29	22.72	4.95	12.00	23.00	28.00

Table 2. Comparison of the levels of vitamin D_3 (ng/ml) and PTH (pg/ml) in the control group and in the periodontal disease patients

Group		n	Mean	Stand. dev.	Minimum	Median	Maximum	p*
Vit. D3	Control	28	15.61	8.24	4.00	12.57	38.80	0.797
	Perio.dis.	29	15.34	7.29	0.40	13.60	33.44	
	Total	57	15.47	7.70	0.40	13.32	38.80	
PTH	Control	28	42.24	19.84	17.53	35.56	96.90	0.518
	Perio.dis.	29	42.12	15.62	22.42	37.47	101.90	
	Total	57	42.17	17.62	17.53	37.31	100.90	

**Mann-Whitney Test*

relation between the levels of vitamin D and PTH was found (Table 3). However, a negative correlation between the levels of vitamin D and PTH in the periodontal disease group (Table 4) and in both groups evaluated together (Table 5) was obtained. No correlations among these parameters and the periodontal status and the number of teeth present in the oral cavity were found (Table 6).

Table 3. Spearman's nonparametric correlations between the levels of vitamin D and PTH in the control group

		Vit. D_3	PTH
Vit. D_3	r	1.00	-0.32
	p		0.095
PTH	r	-0.32	1.00
	p	0.095	

Table 4. Spearman's nonparametric correlations between the levels of vitamin D and PTH in the periodontal disease group

		Vit. D3	PTH
Vit. D_3	r	1.00	-0.51
	p		**0.004**
PTH	r	-0.51	1.00
	p	**0.004**	

Table 5. Spearman's nonparametric correlations in both groups together (control + periodontal disease group)

		Vit. D3	PTH
Vit. D_3	r	1.00	-0.43
	p		**0.001**
PTH	r	-0.43	1.00
	p	**0.001**	

Table 6. Spearman's nonparametric correlations between the levels of vitamin D and PTH and the periodontal disease progression

Group		Vit. D3	PTH
PI	r	-0.04	0.18
	p	0.83	0.349
GI	r	0.18	-0.16
	p	0.34	0.407
BI	r	0.02	-0.05
	p	0.91	0.807
PD	r	-0.01	-0.10
	p	0.97	0.592
Number of teeth	r	-0.05	-0.05
	p	0.81	0.804

Discussion

The research of the recent years has emphasised the pleiotropic function of the vitamin D_3 concerning not only the influence on the calcium and phosphate, water and electrolyte, and hormonal management, but also the effects related to the proliferation and the differentiation of cells belonging to the immune system, which is undoubtedly closely connected with the etiopathogenesis of some autoimmune, cancerous and allergic diseases.

The vitamin D_3 is of a great importance in digestive, endocrine, metabolic, nervous system and cardiovascular diseases (7).

A wide range of action of the vitamin D is linked with the common occurrence of the vitamin D_3 receptor in various cells, tissues and organs of the organism (heart, stomach, pancreas, brain, gonads, activated T and B lymphocytes). The vitamin D receptor has a complex structure, and its polymorphism should be also taken into consideration (8, 9, 10).

A vitamin D_3 deficiency below 20 ng/ml was found in 30% of adolescents in and in 70% of

young women in Poland. The main causes of the deficiency were the latitude, the air pollution, a substantial level of cloud cover, the use of UVB protection creams and a reduced supply with the food. The level of 25OHD in the blood should be 20 ng/ml. Such level guarantees correct calcium and phosphate management and a correct state of the bone tissue. The concentration for adults should be higher than 30 ng/ml (5). The disorders of the calcium and phosphate transformation (rickets, osteopenia, osteoporosis) and its complications are generally known. Therefore the role of the vitamin D_3 in the pathogenesis of other diseases is particularly important. As regards the cardiovascular diseases, most observations concern the relationship of a low level of vitamin D_3 with the arterial hypertension and the ischemic heart disease.

The scientists from the Harvard Medical School associate the vitamin D deficiency with an increased risk of heart diseases (11). Individuals with a low level of vitamin D are twice as much exposed to circulation insufficiency, hypertension, ischemic heart disease, circumferential artery diseases or stroke compared to those having the correct level of this vitamin. The arterial hypertension in individuals with a low level of vitamin D is associated with a high renin activity. This leads to a disorder in the functioning of the renin-angiotensin-aldosterone system (3).

The relationship between the coincidence of the occurrence of tumours at particular latitudes and the vitamin D_3 deficiency has for many years been the subject of numerous research studies. The discovery that most tissues have receptors for the vitamin D_3 was a breakthrough in understanding the role of the vitamin D_3 in the mechanism of tumour development. The research showed that the vitamin D_3, by increasing the tissue differentiation and promoting the apoptosis, reduces the formation of metastases and the angiogenesis (12). The presence of different alleles for the VDR gene is responsible for a different risk of tumour formation (13). At present, the vitamin D_3 is considered to reduce the risk of formation of many tumours: bladder, breast, colon, oesophagus, stomach, ovarian, prostate, anal, renal and uterine cancer, and lymphoma (13). The most data obtained so far concerned the relationship between the vitamin D_3 and the colon, breast and prostate tumours (13, 14).

The results of our research showed a low (under 20 ng/ml) level of vitamin D. It seems advisable to administer the vitamin D in order to increase its level in patients with the periodontal diseases. The more so as at present in the prevention of the periodontal diseases the significant role of a proper diet is emphasised. The research on the level of vitamin D was carried out in May and June, i.e. before the maximum sun exposure in the holiday months. The presented research into the levels of vitamin D and PTH in the periodontal diseases should be treated as a pilot study. The problem of the role of the vitamin D_3 and the parathormone in the bone tissue metabolism in the periodontal diseases requires further research.

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Insulin resistance and its relationship with serum Vitamin D concentrations in offspring of patients with type 2 diabetes

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Abstract

Aim: Evidence is accumulating for a role of vitamin D in maintaining normal glucose homeostasis. Offspring of patients with type 2 Diabetes Mellitus (DM) are in increasing risk to develop glucose intolerance. Our objective was to determine the prevalence of insulin resistance in them and its relation to plasma vitamin D concentration.

Material and method: This cross-sectional study conducted on 375 subjects .We randomly selected offspring of patients with type 2 DM referred to two educational hospital diabetes clinics from March to September in 2010.The participants screened with an oral glucose tolerance test (OGTT) and diagnosis of DM or glucose intolerance was made based on American Diabetes Association (ADA) criteria. We also measured Insulin levels, C - reactive protein (CRP), lipid profile and 25-OH-Vitamin D concentrations in the fasting samples. Chi-square test, Bivariate correlation analysis, logistic regression, student-t test and mann-whitney test used for Caucasian and non-Caucasian parametric parameters.

Results: Twelve subjects (3.2%) had DM and 80 persons (21.4%) were in pre diabetes stage. Overweight and obesity were found in 120(33.8%) and 72(20.3%) of the participants respectively. Central obesity found in 145(43%) . Hypertension detected in 60 people (17%). Vitamin D deficiency or Vitamin D insufficiency found in 269 subjects (71.9%). The chance of insulin resistance was significantly increased in subjects with vitamin D deficiency (OR: 4, CI 95%: 2.4-7.4, P: 0.001).

Conclusion: The Findings reveal that vitamin D deficiency is prevalent in offspring of patients with type 2 DM in Iran and associate significantly with insulin resistance. We suggest more studies on the role of vitamin D supplements in this high-risk group to prevent development of glucose intolerance.

Key words: vitamin D status, type 2 diabetes mellitus offspring, insulin resistance, gender, lipid profile, risk factors.

Introduction

Type 2 diabetes (T2DM) is the most common type of diabetes mellitus. According to the World Health Organization, In 2000 at least 171 million people worldwide suffer from diabetes[1], however the incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double.[1] T2DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion.

There are increasing evidences about the effects of vitamin D deficiency on normal glucose homeostasis and occurrence of diabetes mellitus. 25 (OH) D concentration has been reported as a significant and independent predictor of insulin sensitivity and may has a role in the secretion and the action of insulin[2-4], therefore, hypovitaminosis D may be considered as a risk factor for T2DM.

The offspring of patients with T2DM has an estimated risk of 40 percent of developing diabetes. This risk may increases to 60 percent if both parents are affected and they are in increasing risk to develop glucose intolerance. In several studies reviewed by the American Diabetes Association (ADA), 40 to 80 percent of children and adolescents with T2DM had at least one affected parent.[5] Knowing risk factors other than genetic suscepti-

bility in this high-risk group may be important to prevent T2DM in them.

Regarding to reported high prevalence of vitamin D deficiency in general population in Iran[6], the aim of the study was to Compare Vitamin D status as well as prevalence of T2DM in the offspring of T2DM patients.

Materials and methods

Subjects

This cross-sectional study conducted on March to September 2010. We selected our participants in two stages. At first 308 patients with Type2 DM selected by regular systematic sampling from those who referred to two educational hospital diabetes clinics over the past 6 months. A list containing their offspring more than 15 years old was prepared and finally 400 subjects selected randomly from the list based on the table of random numbers and invited for the evaluation. We permitted only one or maximum two subjects from each family to enter in the study. All subjects with recent use of vitamin supplements or medications like corticosteroids or contraceptives and those with liver, renal or inflammatory disorders excluded from the study. All the participants had only one affected parent with T2DM.

Approval obtained from the ethics committee of Zanjan University of Medical Sciences. All participants provided written informed consent before enrolment.

Measures

Body weight was measured to the nearest 0.1 kg with a balanced-beam scale while wearing light clothing, and height was measured with a stadiometer to the nearest 0.5 cm. Body Mass Index(BMI) was calculated based on the weight/ (height)^2 formula. Participants with BMI between 25and 29.9 Kg / m^2 considered as overweight and obesity defined as BMI more than 30 Kg/m^2.Waist circumference between the lowest rib and the iliac crest, at the level of umbilicus, was measured in duplicate to the nearest millimeter using flexible tape. Waist circumference more than 88cm in females and 102cm in males considered central obesity.

Blood pressure was measured with the subject seated using a random zero sphygmomanometer. Systolic (Korotkoff phase I) and diastolic (Korotkoff phase V) blood pressure was measured twice on the left upper arm and the average of the two measurements was used for analysis. Blood pressure more than 130/85 mmHg defined as hypertension.

Venous blood samples collected after at least 12 hours of fasting. The basal levels of insulin and plasma glucose measured and a lipid profile conducted. Insulin levels were measured with sensitivity of 0.25 (μU/ml) via an electrochemiluminescence immunoassay (ECLIA) using commercially available kits (DIAPLUS). The Homeostasis Model Assessment Index (HOMA IR) was used to determine the level of insulin resistance and was calculated according to the following equation: [Insulin (μU/ml)] [FPG (mmol/L)] /22.5. Insulin resistance diagnosed in cases with a HOMA IR of more than 2.1.[6]

We screened the subjects with an oral Glucose Tolerance Test (OGTT) (75gr) and diagnosis of DM or glucose intolerance made based on American Diabetes Association (ADA) criteria. OGTT repeated for those subjects with abnormal results. Plasma glucose concentration measured by the glucose-peroxidase colorimetric method with a sensitivity of 5 mg/dl. Intra- and inter-assay CVs were 1.7% and 1.1% for lower limit and 1.4% and 0.6% for upper limit concentrations, respectively. Based on the ADA criteria, all the subjects with fasting plasma glucose (FPG) between 100 and 126mg/dl or post OGTT glucose levels between140 and 200 mg/dl considered as prediabetes. Those subjects with FPG more than 126mg/dl or post OGTT glucose concentrations more than 200 mg/dl defined as overt diabetes mellitus.

We measured 25 hydroxy vitamin D (25OHD) in the fasting samples with ELISA (DRG, Germany). The sensitivity of the kits was 2nmol/l and the range of measurement was 6.4-250nmol/l. Vitamin D deficiency defined as a serum level of 25OHD of ≤25nmol/l and insufficiency as a serum level between 25 and 75nmol/l. We also measured C - reactive protein (CRP) by a high-sensitivity assay, with the use of the latex particle-enhanced immunoturbidimetric assay with analytical sensitivity of 0.175 mg/dl and upper limit of normal 5 mg/dl. Total cholesterol (TC) and triglycerides (TG) levels assayed with a sensitivity of 5 mg/dl using enzymatic colorimetric tests with cholesterol esterase and cholesterol oxidase, and glycerol phosphate oxidase, respectively. Intra- and inter-

assay coefficients of variation (CV) were 1.6 % and 1.1 % for the lower limit and 0.6 % and 0.9 % for the upper limit concentrations, respectively. High-density lipoprotein cholesterol (HDL-C) measured after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. Triglyceride concentrations more than 150mg/dl, total cholesterol concentrations more than 240mg/dl and HDL-cholesterol less than50mg/dl in females and 40 mg/dl in males defined abnormal in this study.

Statistical analysis

Statistical analysis was conducted using SPSS version 16 .Chi-square test used for non-parametric qualitative parameters while unpaired student-t test and mann-whitney test used for Caucasian and non-Caucasian parametric parameters. Bivariate correlation analysis (calculation of the Pearson coefficient) used to assess the correlation of serum vitamin D levels to each parameter and Odds Ratio was calculated. Independent relationships between serum vitamin D levels and those parameters to which they significantly correlated assessed using multiple linear regression and logistic regression models. Statistical significance was set at $P< 0.05$.

Results

From 400 subjects who invited for the evaluation 375 people including 137 male and 238 female participate in the study. Twenty –five people including 20 male and 5 female without any abnormal past medical history didn't accept to participate in the study. Mean age of the participants was 32.1± 10.8 year (Age range 16-60). Biochemical indices and clinical characteristics of the participants have been shown in table 1.

Findings in this cross-sectional study in the siblings of patients with T2DM showed that 12 subjects (3.2%) were diagnosed to have DM and 80 (21.4%) were in pre diabetes stage.

Furthermore, those participants with DM or prediabetes were older and had higher weight, SBP, diastolic blood pressure (DBP), total cholesterol, triglyceride and LDL-cholesterol concentration than those with normal glucose homeostasis (Table 2).

Vitamin D deficiency or Vitamin D insufficiency found in 269 subjects (71.9%) of the participants. The prevalence was not different significantly between the males and females (67% in males Vs 75% in females, p: 0.07), but was more prevalent in the younger ages (P: 0.003).

Table 1. Biochemical indices and clinical characteristics of offspring of patients with type 2DM (N: 375)

Variable	Mean ± SD		P-Value
	Female (n=238)	Male (n=137)	
Age(Y)	10±31.8	10.3±32.3	0.48
Waist Circumference(Cm)	12.2±90.7	11.5±92.4	0.2
BMI(Kg/m2)	4.8±26.4	3.5±25.5	0.08
Systolic BP(mmHg)	611±128	119±120.1	0.002
Diastolic BP(mmHg)	72 ± 90	27± 90	0.4
FPG(mg/dl)	92.7 ±17.4	93.4±13	0.5
Post GTT glucose(mg/dl)	38.7±108	106 ±38.6	0.5
Cholesterol(mg/dl)	37±172.3	33.2±169.4	0.4
HDL-C(mg/dl)	10.8±38.7	11.9±36.7	0.1
TG(mg/dl)	70.8±142.5	80.7±160.4	0.02
LDL-C(mg/dl)	32±108.5	30.6±103	0.2
Insulin(µIU/ml)	10.3±8.4	8.4±9.1	0.051
Vitamin D(nmol/l)	40.9±53.5	45±64.7	0.01
CRP(mg/dl)	3.1±2	4.1±3.3	0.001
HOMA_IR	2.7±1.7	2.1±1.7	0.13

BP: Blood Pressure, FPG: Fasting Plasma Glucose, GTT: Glucose Tolerance Test, TG: Triglyceride, LDL: Low Density Lipoprotein, CRP: C Reacting Protein, HOMA-IR: Homeostasis Model Assessment Index for Insulin Resistance

Table 2. Clinical and biochemical characteristics of offspring of patients with type2DM based on the results of OGTT in them

Variable	Mean ± SD		P value
	n=92 Diabetes or pre diabetes	n=283 normal	
Age(Y)	35.5±10	30.5±9.9	0.001
Height (Cm)	165±8.8	166±9.4	0.16
Weight (Kg)	76±13	70.5±13	0.001
Waist circumstance (Cm)	96± 11	89.8 ±11	0.001
BMI (Kg/m2)	28±4.6	25.4±4	0.001
Systolic BP(mmHg)	123±12	116±12	0.001
Diastolic BP (mmHg)	76±10	71±9	0.001
FPG(mg/dl)	109.5±23.6	87.6±6	0.001
Post GTT glucose(mg/dl)	144±58	95±17	0.001
Cholesterol(mg/dl)	183±39	167±34	0.001
HDL-C(mg/dl)	34.8±10	38.9±11	0.002
TG(mg/dl)	177±89	140±67	0.001
LDL-C(mg/dl)	114±32	104.6±31	0.001
Insulin(µIU/ml)	12.2±12	7.5±8.2	0.002
Vitamin D(nmol/l)	51±39	60±43	0.07
HOMA-IR	3.2±3.1	1.6±1.7	0.001

BMI: Body Mass Index, BP: Blood Pressure, FPG: Fasting Plasma Glucose, GTT: Glucose Tolerance Test, TG: Triglyceride, LDL: Low Density Lipoprotein, HOMA-IR: Homeostasis Model Assessment Index for Insulin Resistance

Table 3. Logistic regression for association of insulin resistance and Vitamin D deficiency

Dependent Variable	Independent Variable	P-Value	Odds Ratio (CI 95%)
Insulin Resistance	Vitamin D deficiency	0.001	5.8 (2.4-7.5)
	(Age (more than40y	0.001	1.04(1.02-1.9)
	(Gender (female/male	0.06	0.6 (0.2-1.5)
	BMI	0.4	0.97 (0.8-1.1)
	Waist Circumference	0.52	1.02 (0.7- 1.3)
	FPG	0.1	1.2 (0.8-1.5)
	TG	0.07	1.4 (0.9-1.7)
	HDL-C	0.7	1 (0.8-1.2)
	CRP	0.052	1.1 (0.9-1.6)

Vitamin D: ≤ 25 nmol/l Vs >25 nmol/l, Age: ≥40 y Vs <40y, Sex: Female Vs Male, BMI: ≥30Kg/m2 Vs 25-30kg/m2 Vs <25kg/m2 , waist Circumference: ≥88cm Vs <88 cm in female and ≥102cm Vs <102cm in male, FPG: ≥100mg/dl Vs <100 mg/dl ,TG: ≥150 mg/dl Vs <150 mg/dl,HDL-C: ≤50 Vs >50mg/dl in female and ≤40 mg/dl Vs >40 mg/dl in male, CRP: ≥2.5 Vs <2.5.

Table 4. Frequency of Diabetes mellitus and pre diabetes in offspring of patients with type2 DM based on their vitamin D status (n=375)

Serum Vitamin D concentration (nmol/l)	Subjects		P value
	Normal	DM or Glucose intolerant	
25≥	223(79%)	59(65%)	0.05
25≤ (Vit D Deficiency)	60(21%)	33(35%)	0.03

The chance of insulin resistance was significantly increased in subjects with vitamin D deficiency (OR: 4, CI 95%: 2.4-7.4, P: 0.001). After adjusting for other cofounding variables like age, gender, BMI and waist circumference, the chance of insulin resistance remained significantly high in the subjects with vitamin D deficiency. The data revealed that only vitamin D deficiency and age are independent variables affect insulin resistance (Table 3).

Table 4 shows that the prevalence of vitamin D deficiency was significantly higher in participants with hyperglycemia (p=0.003) than those with normal glucose concentrations. (Table 4)

Discussion

In this cross-sectional study, siblings of patients with type 2 diabetes show increased prevalence of type 2 diabetes 24.5% Vs 3.6% of the general population of Iran aged 30 years and over in primary results of the national program for the prevention and control of type 2 diabetes.[7] In most cases, this is accompanied by unfavorable BMI, WC and lipid profile. Results showed that females had higher systolic blood pressure and lower 25OHD concentration. In addition, metabolic syndrome is more prevalent in them. However in one study that had been done in Isfahan results showed women had lower waist circumference, height and weight, waist-to hip ratio, Fasting Plasma Glucose, 30 and 60 minutes plasma glucose, triglyceride and BP and were younger than men. Men had lower BMI, hip circumference, 2-hour plasma glucose, HDL and LDL than women.[8] In another study, sex and age had no effect on 25 (OH) D concentrations.[2]

The siblings of patients with type 2 diabetes who were overweight or obese were at much higher risk of diabetes and IGT than non-obese relatives were. Family history of diabetes is strongly associated with type 2 diabetes in children. The frequency of a history of type 2 diabetes in a first- or second-degree relative has ranged from 74 to 100% [9] and measures of obesity show strong heritability. In another study, the prevalence of insulin resistance among siblings of type 2 DM was 26.67% with the proportion in each family varies between 0-75percent. Central obesity was the most metabolic component commonly found [10]. This suggests that genetic factors besides lifestyle, obesity and dyslipidemia may be among the risk factors for diabetes and IGT.[8]

There are increasing evidences about the relationship between vitamin D metabolism and occurrence of diabetes mellitus. Several investigations suggest that circulating concentrations of vitamin D may be inversely related to the prevalence of diabetes.[11-14] Findings from the present study suggest that vitamin D deficiency is prevalent in offspring of patients with type 2 DM in Iran and associate significantly with insulin resistance. More studies on the role of vitamin D supplements in this high-risk group to prevent development of glucose intolerance is suggested**.**

It should be noted that dietary calcium modulation of intracellular calcium, mediated by suppression of calcitrophic hormones, has previously been demonstrated to attenuate the risk of hypertension, and possibly type II diabetes as well.[3]

In some studies, results did not differ between subjects, with and without, impaired glucose tolerance. In adults without diabetes, correction of vitamin D deficiency is not associated with any effect on blood glucose or insulin concentrations or insulin sensitivity as assessed during an oral glucose tolerance test. These observations do not support an association between glucose/insulin homeostasis and vitamin D, at least in the short term.[15]

In one study has been shown total prevalence of vitamin D deficiency (<25 nmol/L) was found in 70.6% of pregnant women. Prevalence of severe vitamin D deficiency (<12.5) in GDM patients was higher than in normoglycaemic pregnancies. The regression model revealed a strong correlation between the HOMA index and serum levels of vitamin D.[13]

Conclusion

In summary, the findings of this study illustrate the prevalence of diabetes in siblings of patients with type 2 diabetes in Iran. Obesity, hypercholesterolemia and glucose intolerance between different age groups in offspring is more prevalent in older persons (>40 years) and subjects who had insulin resistance were more likely to be older and to have Vitamin D deficiency. They are high-risk group for relationship levels of vitamin D with insulin resistance by the high prevalence of insulin

resistance. The relationship positive between vitamin D with insulin resistance show our attention to the issue of screening in first degree relatives of type 2 diabetes can draw the ground for subsequent studies to investigate the effects of vitamin D supplementation on insulin resistance in this group is dangerous. In addition, primary prevention efforts can be directed to high-risk individuals or to the overall population of siblings of patients. Intervention can take place at an early stage when blood glucose levels are still normal or at the stage of impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) when glucose levels are elevated but not diagnostic of diabetes.

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Lack of difference of gastric mucosal histology between functional dyspepsia subgroups according to Rome III criteria

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Abstract

Background: The aim of this study was to investigate the histopathological changes in gastric mucosa of patients with functional dyspepsia, according to the Rome III criteria.

Methods: Fifty consecutive patients diagnosed as functional dyspepsia by Rome III criteria were included into the study. Histological features of gastric mucosa were evaluated with the updated Sydney System.

Results: Thirteen patients were epigastric pain syndrome alone and, 14 were postprandial discomfort syndrome alone. Twenty three patients had both conditions. There were no differences between groups in the prevalence of Helicobacter pylori infection and the histopathological changes in gastric mucosa. All patients had chronic gastritis, and of these 36 cases were active forms. There were no significant differences in inflammation severity and Helicobacter pylori density in different topographical areas among the subgroups of functional dyspepsia.

Conclusions: Consequently, the characteristics of chronic gastritis of epigastric pain syndrome and/or postprandial discomfort syndrome patients are not distinctive.

Key words: Epigastric pain syndrome, postprandial discomfort syndrome, chronic gastritis, inflammation, functional dyspepsia.

Introduction

Functional dyspepsia is a common disorder of undefined pathophysiology. Visceral hypersensitivity, gastrointestinal motility disorders, Helicobacter pylori (H. pylori) infection and psychosocial factors have been suggested to play a role in the development of functional dyspepsia [1-7].

Although the potential role of H. pylori in functional dyspepsia remains controversial, it does seem that chronic gastritis caused by H. pylori infection might also be implicated in the pathogenesis of dyspepsia. Mucosal inflammation is a common finding among subjects with and without dyspepsia[8]. A number of experimental models of gastritis have demonstrated that the inflammation of the gastric mucosa can affect gastric emptying, accommodation and/or threshold for visceral sensation[9-11]. These conditions may be associated with inflammation induced structural and biochemical changes in gastric tissue, such as changes in the numbers of endocrine cells regulating acid secretion and serum pepsinogen levels [12-13].

The most recent definition of functional dyspepsia based on Rome III criteria has described 2 subgroups: (i) the postprandial distress syndrome (PDS), which referred to mainly food-related dyspeptic symptoms, and (ii) the epigastric pain syndrome (EPS)[14]. This clinical definition is based on symptoms alone and proposed a better association of certain dyspepsia symptoms with the underlying pathophysiological mechanisms in functional dyspepsia[4-15]. However, there have been a few studies that have used the newly defined criteria for evaluating the association of mechanisms with these newly defined subgroups. In a study of patients with upper gastrointestinal symptoms, subtle duodenal eosinophilia was relatively common in some patients with PDS including early satiety[16]. Aro et al found that anxiety is linked to PDS[17]. In two Japanese studies, EPS was present at higher frequency in persons with the G-protein β3 subunit, IL-17F and/or MIF gene polymorphisms[18-19].

Our aim was to investigate the histopathological changes in gastric mucosa of patients with functi-

onal dyspepsia, according to the Rome III criteria, and to evaluate whether there is a difference between the subgroups of EPS and PDS histologically.

Methods

Fifty consecutive patients with functional dyspepsia (41 women and 9 men; age 20 - 55 years, mean age 38,0 ± 9,6 years), according to Rome III criteria [14], participated in this study. All patients presented to the general gastroenterology outpatient clinic because of epigastric symptoms, and all underwent careful history taking and clinical examination, upper gastrointestinal endoscopy, routine biochemistry, and upper abdominal ultrasound. Inclusion criteria were the presence of one or more of bothersome postprandial fullness, early satiation, epigastric pain, and/or epigastric burning for the last 3 months with symptom onset at least 6 months before admission, in the absence of organic, systemic, or metabolic disease. Dyspeptic symptoms had to be present at least 3 days per week, with at least one symptom scored as moderate or severe on the symptom questionnaire. The severity of symptoms were graded according to a four-point Likert scale in which a score of 0 indicated no symptom, a score of 1 mild symptoms missed in daily activities, a score of 2 moderate symptoms annoying but not interfering with daily activities and score of 3 severe symptoms markedly interfering with daily activities.

Exclusion criteria were the presence of esophagitis, peptic ulcer or erosive gastroduodenitis on endoscopy, gallbladder and tract disease, major abdominal surgery, anorexia nervosa, diabetes mellitus, organ failure, and the use of non-steroidal anti-inflammatory drugs, steroids, or drugs affecting gastric acid secretion. The study protocol was approved by the ethics committee of Afyon Kocatepe University, and informed consent of all patients was obtained.

Patients were divided into the 2 subgroups defined in the Rome III criteria: PDS and EPS. The first was diagnosed if patients reported to have bothersome postprandial fullness and/or early satiation. The latter was diagnosed if patients reported epigastric pain, and/or epigastric burning [14]. Overlap between PDS and EPS was allowed according to the definition.

Diagnostic upper gastrointestinal endoscopy were undertaken by 2 experienced endoscopists. In every patient, two biopsy specimens from antrum (2 to 3 cm proximal to the pylorus), two specimens from gastric body (middle of greater curvature and lesser curvature), and one specimen from angulus were obtained for histopathological examination. One experienced pathologists evaluated the biopsy specimens. The biopsy specimens were stained with Hematoxylin&Eosin. H. pylori was histologically detected by May Grunwald-Giemsa staining. Histological parameters of the gastric mucosa were assessed by using the updated Sydney System score definitions [20].

Continuous variables have been expressed as mean ± standard deviation; categorical variables have been presented as counts and/or median (interquartile range). Comparisons of continuous variables were made using one-way ANOVA test. Multiple group comparisons of categorical variables were made by the $\chi 2$ test and Kruskal-Wallis H test. Mann-Whitney U test for two group comparisons were performed. All analyzes were performed with SPSS 10.0 for Windows; $p < 0.05$ was considered statistically significant.

Results

Of 50 patients with functional dyspepsia, according to Rome III criteria, 13 patients were EPS alone and, 14 were PDS alone. Twenty three patients had both conditions. All subgroups of patients were similar with regard to gender, age and body mass index (BMI) (Table).

All 50 patients had histologically active or inactive chronic gastritis. 45 patients (90%) were positive for H. pylori. Neutrophil activity, chronic inflammation severity, and H. pylori density in different topographical areas, according to the Sydney classification, did not differ significantly among the subgroups (Table).

Atrophy was observed in only one patient with EPS and PDS. Two patients with intestinal metaplasia were observed in each subgroups.

Discussion

In this study, we evaluated the histological features of gastric tissue of patients with functional

Table 1. Patient characteristics and histologic findings of subjects with functional dyspepsia according to Rome III criteria

	EPS alone (n = 13)	PDS alone (n = 14)	EPS+PDS (n = 23)	[a]p
Gender (F/M)*	10 / 3	10 / 4	21 / 2	NA
Age (years)†	34,1 ± 7,9	41,3 ± 10,7	38,1 ± 9,5	NA
BMI (kg/m^2)†	24,6 ± 3,6	27,3 ± 4,3	25,9 ± 4,9	NA
H. pylori (*n*)*	12	12	21	NA
Diagnosis (*n*)*				NA
Chronic gastritis	3	5	6	
Active chronic gastritis	10	9	17	
*Antrum histopathology*δ				
Neutrophil activity	1 (2)	1 (2)	1 (1,5)	NA
Chronic inflammation	2 (1)	2 (1)	2 (1)	NA
H pylori density	2 (0)	1,5 (2,25)	2 (1,5)	NA
*Corpus histopathology*δ				
Neutrophil activity	1 (1)	0,5 (1,25)	1 (1)	NA
Chronic inflammation	1 (1)	1 (1)	1 (1)	NA
H pylori density	2 (1,5)	2 (2)	2 (1)	NA
*Angulus histopathology*δ				
Neutrophil activity	1 (1)	1 (1)	1 (2)	NA
Chronic inflammation	1 (1)	2 (1)	2 (1)	NA
H pylori density	2 (1)	1 (2,25)	2 (2)	NA

BMI, body mass index
NA, not applicable
[a]p < 0.05
** χ2 test test was used for comparisons*
† Parameters were expressed as mean (SD) and analyzed by one-way ANOVA test
δ Parameters were expressed as median (interquartile range) and analyzed by Kruskal-Wallis H test

dyspepsia using the the Rome III definition. Our results show no difference between subgroups with PDS and/or EPS.

Whether gastric inflammation are causally linked to functional dyspepsia is controversial. Studies investigating the role of inflammation in gastric mucosa on the basis of dyspeptic symptoms are mainly the work of H. pylori prevalence and eradication. The frequency of H. pylori in patients with functional dyspepsia is similar in the healthy controls and, H. pylori eradication therapy provides symptomatic relief in a small number of patients [15,21,22].

Ongoing symptoms after H pylori eradication therapy may be associated with the persistence of chronic inflammation. Talley et al. have reported that there is a relationship between dyspeptic symptoms and severity of gastritis in H. pylori positive patients with functional dyspepsia[23]. Several studies have shown that gastritis scores are significantly reduced after H. pylori eradication therapy, but chronic inflammation has continues over years[24-26].

In a number of previous studies, the difference of gastric histopathologic features among functional dyspepsia subgroups has been evaluated. Saruc et al. have found that neutrophil activity, H. pylori frequency and density, and cagA-positivity were higher in patients with ulcer-like dyspepsia than in patients with motility-like dyspepsia according to Rome II criteria[27]. Similarly in another study, the frequency and eradication rates of H. pylori were higher in patients with ulcer-like dyspepsia[28]. These observations suggest that there may be similar differences in the newly defined Rome III functional dyspepsia subgroups of EPS and PDS. However, Aro et al. observed that H. pylori infection was not associated with EPS and PDS[17]. Similarly, Futagami et al. found that H. pylori positivity rates did not vary significantly among

EPS and PDS patients and, in addition, there was no significant difference in the degree of gastritis including chronic inflammation and neutrophil activity[29]. In contrast, Arisawa et al reported that the IL-17F genotype was significantly associated with EPS and gastiritis scores in H. pylori positive patients[19]. However, in our results there was no difference in the activity and inflammation scores and H. pylori density of different topographic sites of the gastric mucosa in subgroups of functional dyspepsia. These findings suggest that gastric inflammation alone does not explain the specific dyspepsia symptom pattern and inflammatory features of gastric mucosa in functional dyspepsia subgroups are not distinctive.

In our study, we did observe a significant overlap between subgroups, and approximately half of the study patients had both syndromes. van Kerkhoven et al. also showed that 26 of 60 patients diagnosed with functional dyspepsia according to the Rome III criteria had both EPS and PDS [30]. Therefore, this classification seems to be causing significant limitations in clinical trials.

Our limitations include the number of the study population and the absence of the control group. The number of patients with glandular atrophy and intestinal metaplasia was small. The reasons for this may be small sample size and/or relatively young age of study patients. It has been known that the prevalence of chronic atrophic gastritis increases with age[31]. Due to lack of asymptomatic control group, the evaluation has been carried out in terms of histopatological difference between EPS and PDS.

In conclusion, the pathogenetic factors involved in the formation of EPS and PDS has not been revealed yet. In the present study, in which histomorphological features of gastric mucosa was determined, we did not observe a significant difference between EPS and PDS groups. We observed that EPS and PDS were present concurrently in a significant number of functional dyspepsia patients. The frequency of H pylori infection were similar between groups. As a result, the findings suggest that the features of chronic gastritis are not distinctive among different subgroups of functional dyspepsia by Rome III.

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The effect of nonsurgical periodontal therapy with additional systemic antimicrobials or photodynamic therapy on IL-8 level in GCF of chronic periodontitis patients

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Abstract

The aim of the study was to determine the effect of nonsurgical periodontal therapy with adjunctive use of photodynamic therapy or systemic antimicrobials on IL-8 levels in GCF in chronic periodontitis patients.

Material and methods: 38 patients with chronic periodontitis were included. Subjects were randomly assigned to A group treated with scaling and root planning(SRP) + antibiotic (amoxicillin + metronidazole) or group PDT treated with SRP + photodynamic therapy(PDT). Baseline plaque index(PI), bleeding on probing(BOP), pocket depth(PPD), clinical attachment level(CAL), Sulcus Fluid Flow Rate(SFFR) and IL-8 level were recorded and re-evaluated at 3 and 6 months post treatment.

Results: Both examined therapies caused a statistically significant decrease of all the assessed clinical parameters at the sites of sample collection (except BOP in A group) as well as SFFR. The level of IL-8 per measure point showed a statistically significant decrease in PDT group. In group A the decrease was also noted but there was no statistical significance.

Conclusions: Within the limits of this study it can be concluded that nonsurgical periodontal therapy: SRP with additional antibiotic application and SRP with PDT, are effective methods leading to reduction of clinical parameters and gingival inflammation. Effectiveness of both protocols is comparable however adjunctive PDT may lead to drop of GCF IL-8 levels.

Key words: Interleukin 8, gingival crevicular fluid, scaling and root planing, photodynamic therapy.

Introduction

Chronic periodontitis is bacterial induced inflammatory disease leading to destruction of the tooth supporting tissues. The disease severity depends on the dynamic balance between dental plaque bacteria, genetic factors that condition the innate immunity of the host to bacterial infection, and environmental factors (1, 2). At the moment there is a minor possibility to modify host response to periodontal diseases. That is why effective periodontal therapy is based on reduction or elimination of periodontopathogenes from periodontal pockets (3). Conventional mechanotherapy implied as scaling and root planing (SRP) is very effective in most cases of chronic periodontitis. Mechanical plaque removal especially when it is supported by conscientious oral hygiene and maintenance therapy achieves control of disease. However some patients respond poorly to periodontal therapy despite similar periodontopathogens reduction rate in subgingival bacterial flora as in patients with good outcome of mechanical therapy. It can be explained by reduced host response or extreme virulence of periodonthogenic bacteria colonizing periodontal pockets (4, 5). For these patients the use of an appropriate adjunctive antimicrobial is often beneficial. Different local and systemic antibiotic regimes have been introduced in the treatment of periodon-

titis but in most cases they have limited effects (6). It is known that the amount of antibiotic needed to affect bacteria in biofilm is greater than the amount to inhibit planktonic bacteria. It has been speculated that biofilm-grown cells may be 1000-1500 more resistant to antibiotics than planktonic-grown cells (7). The resistant nature of periodontal bacteria in the biofilm shows the necessity of physical removal of subgingival plaque before systemic antibiotic administration. Selection of an appropriate antibiotic regime comes out of clinician decision. Several reports describe promising results using a combination of metronidazole and amoxicillin. This combination with conjunction with SRP provides a tangible benefit over the SRP alone (8). Unfortunately the increasing risk for developing antibiotic resistance should also be taken under consideration. That is why, photodynamic therapy (PDT), used as an adjunct to conventional periodontal therapy has been proposed as a novel treatment option.

Photodynamic therapy involves three noninvasive ingredients: visible harmless light; a nontoxic photosensitizer; and oxygen. It is based on the rule that the photosensitizer binds to the target cell and can be activated by the light of suitable wavelength. Following these activation, singlet oxygen and other very reactive agents are produced that are extremely toxic for certain cells and bacteria (9). Polysaccharides, present in extracellular matrix of oral biofilm are highly sensitive to singlet oxygen and susceptible to photodamage (10). Microorganisms that are killed by singlet oxygen include viruses, bacteria, protozoa and fungi. Gram positive bacteria are more sensitive to PDT than Gram negative bacteria. It is a result of the differences in the outer membrane structures of both types of bacteria. However it has been demonstrated that photosensitizers such as toluidine-blue O and methylene blue can bind to the outer membrane of Gram negative bacteria and penetrate their cells (9). *In vitro* studies have shown that there is a possibility to efficiently eradicate such pathogens as *P. gingivalis, F. nucleatum, Staphylococcus* sp. by photodynamic treatment, both in aquous suspension and as a biofilm (11). *In vivo* experimental studies have demonstrated that toluidine blue PDT can selectively kill *P. gingivalis* and significantly decrease the level of alveolar bone loss in rats affected by periodontitis (12). PDT can also inactivate virulence factors secreted by microorganisms such as lipopolisaccharides and proteases. *In vitro* studies have proven that the ability of LPS previously treated by photodynamic therapy to activate human peripheral blood mononuclear cells to release pro-inflammatory cytokines (IL-6 and IL-8) was significantly reduced (13). Cytokines in periodontium are synthezised in response to bacteria and their products, inducing and maintaining the inflammatory response. IL-8 is a chemoattractant cytokine produced by a variety of tissue cells and blood cells with a distinct target for recruitment and activation of neutrophils (14). Neutrophils represent the major inflammatory infiltrate in periodontitis. Neutrophil enzymes released after activation can effectively degrade connective tissue. IL-8 is of considerable interest for a better understanding of the mechanisms leading to neutrophil connected tissue destruction (15).

The aim of the present study was to determine the effect of nonsurgical periodontal therapy with adjunctive use of photodynamic therapy or systemic antimicrobials on IL-8 levels in GCF in chronic periodontitis patients.

Material and methods

Patients

Thirty eight patients (29 women and 9 men, mean age 50.53; nonsmokers) with chronic periodontitis, none of whom had undergone any periodontal therapy for at least a year, were included in the study. The exclusion criteria were: severe systemic disease that might affect periodontal therapy, pregnancy and breastfeeding, systemic antibiotic therapy 3 months prior to treatment, allergy to metronidazole and/or penicillin. Inclusion criteria were as follows: moderately advanced chronic periodontitis that had not been treated for at least one year (16), the presence of at least 1 tooth in the quadrant, the overall number of teeth at least 12, and at least 4 sites with PPD>4mm bleeding on probing. All the patients were informed about the study and gave their written consent. The study was approved by the Ethics Committee, Medical University of Białystok (R-I-002/307/2009 and R-I-002/278/2010).

The patients were randomly allocated by randomization table to the A or PDT group. Each group comprised 19 patients. The demographic characteristics of the study groups is presented in Table 1.

Table 1. Demographic characteristics of the study groups

Demographic characteristics	Group 1 (n=19)	Group 2 (n=19)
Age (years mean ± SD)	50.58± 8.11	50,47± 8.63
Female	14	15
Male	5	4

Clinical parameters

Prior to therapy (baseline), as well as 3 and 6 months after it, each patient underwent periodontal examination with a probe PCPUNC 15 (Hu-Friedy Co., Chicago, IL, USA) to determine PPD, GR, CAL, PI and BOP. The examination was performed by one masked and calibrated investigator. At baseline, the clinician chose one periodontal pocket (≥ 4mm deep) to collect GCF for biochemical analyses.

GCF sampling

At baseline as well as 3 and 6 months post therapy, GCF was collected from the periodontal pocket (≥ 4mm depth) chosen on the first visit and sulcus fluid flow rate (SFFR) was determined in relative Periotron-units (PU). The tooth was isolated with cotton rolls, dental plaque was removed gently and the tooth was air dried. GCF was collected using paper strips (Periopaper, Interstate Drug Exchange, Amityville, NY), which were placed in the periodontal pocket at 1-2 mm depth for 30s. The blood-contaminated strips were discarded. The GCF volume absorbed on a paper strip was measured using a calibrated device (Periotron 8000, Oraflow, Plainview, NY). After measurement, the samples were immediately placed in Eppendorf tubes containing 20µl phosphate buffered saline (PBS) and frozen in -20°C.

Periodontal therapy

All the patients received periodontal treatment involving scaling and root planing (SRP) with additional systemic antibiotic therapy (group A) or additional photodynamic therapy (group PDT). Mechanical therapy was performed with the use an ultrasonic device (LM-Instruments, Finland) with slim-line scaler tip (PE-38) and water as a coolant at a single session by one clinician. Local anesthesia was applied when needed.

After SRP, patients from group A were prescribed systemic antibiotic therapy as follows: Amoxicillin 375 mg three times daily and Metronidazole 250 mg three times daily for 7 days (17).

In the PDT group, SRP was followed by PDT done with Helbo System (HELBO® minilaser 2075 F dent, Helbo Photodynamic Systems GmbH & Co KG, Wels, Austria). The system consisted of a hand-held battery-operated diode laser. The laser wavelength was 660 nm, and the power density 60 mW/cm^2. The dye was a commercial solution based on phenothiazine chloride (Helbo Blue Photosentizer®, HELBO® Photodynamic Systems). After SRP, the oral cavity was rinsed with water and the teeth were isolated with cotton rolls. The photosensitizer was carefully applied with a blunt needle at the bottom of the periodontal pocket in a circular motion. It was left there for 3 min, and then rinsed out with water spray. For laser light application, a standard 8.5cm perio tip curved at an angle 60º (HELBO® 3D Pocket Probe) was used. Light was emitted for 10 seconds in each of the six measurement points (mesiovestibular, midvestibular, distovestibular, mesiolingual, midlingual and distolingual). The second PDT session was performed one week later.

GCF IL-8 analyses

The concentration of IL-8 in GCF was determined with the use of commercially available ELISA kit (Human CXCL8/IL8, R&D Systems, Minneapolis, USA) following the producer's instruction. The result was expressed as the amount per measure point (pg/site).

Statistical analysis

The statistical analysis was performed with the use of Statistica 8.0, StatSoft for Windows. Distribution normality was analyzed using the Kolgomorov-Smirnov test with the Lilliefors correction and by the Shapiro-Wilk test. Normal distribution of the quantitative variables was not found. The Friedman's ANOVA test was used to compare three dependent variables (changes in the parameters in time). The changes in qualitative features (BOP point) were checked using the chi-square test of independence. U-Mann-Whitney test was

used for comparison between groups. Statistical differences were considered significant at p<0.05.

Results

38 patients, enrolled to the study, completed the 6 months trial. Healing was uneventful in all patients. Neither pain nor any discomfort was reported by any of the patients following both treatments.

The periodontal therapy has led to statistically significant improvement of most of the evaluated clinical parameters at the sites of sample collection. The only exception was the change in BOP in group A where the reduction did not reach statistical significance. Table 2 shows clinical periodontal parameters (PPD, CAL, PI, BOP) and SFFR at the site of collection for laboratory analysis at baseline, and 3 and 6 months post therapy.

The level of IL-8 per measure point in group PDT decreased statistically significant after treatment (Figure 1). In group A the decrease in time was also noted but there was no statistical significance (Figure 2). Comparison of IL-8 levels between groups did not show any statistical differences. The mean values of total amount of IL-8 at initial, the 3rd month and 6th month in sampling sites (mean±SD) are shown in Table 3.

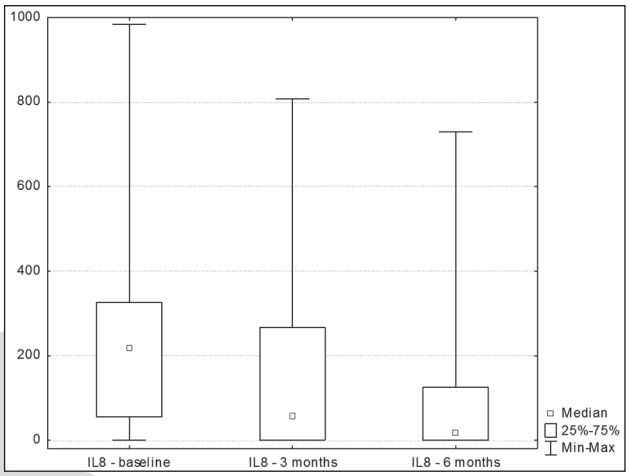

Figure 1. The amount of IL-8 per measure point in group PDT (p<0,05).

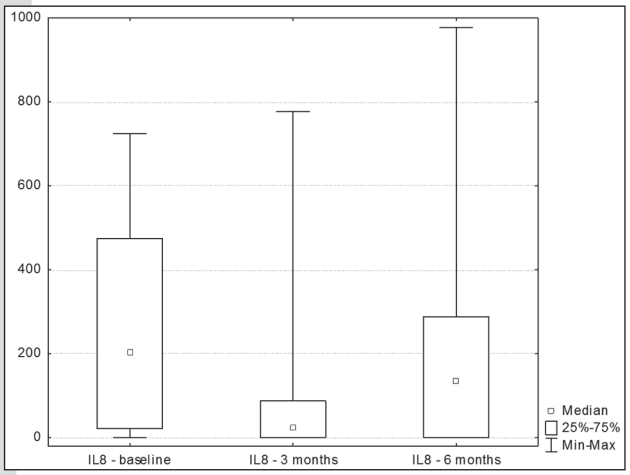

Figure 2. The amount of IL-8 per measure point in group A (non significant).

Table 2. Clinical periodontal data of sampling site

Periodontal parameter	Group	Baseline	3 months	6 months	p
Sampling sites ME (Q1-Q3)					
PPD (mm)	A	7 (6-8)	4 (3-6)	4 (3-5)	p<0.001*
	PDT	6 (5-6)	4 (3-5)	4 (2-5)	p<0.001*
CAL (mm)	A	7 (6-9)	6 (3-7)	5 (4-6)	p<0.001*
	PDT	6 (5-7)	5 (3-6)	4 (2-6)	p<0.001*
PI	A	3 (1-3)	1 (0-1)	0 (0-1)	p<0.001*
	PDT	2 (1-2)	1 (0-1)	0 (0-1)	p<0.001*
SFFR (PU)	A	141 (107-177)	80 (61-125)	88 (52-131)	p<0.001*
	PDT	113 (87-152)	71 (47-100)	83 (55-112)	p=0,2*
BOP(+)	A	12	6	6	p=0.075†
	PDT	15	10	4	p=0.002†

ME- median
Q1- lower quartile
Q3- upper quartile
** the Anova Friedman's test with Kendall's correlation of consistency*
† the Pearson's chi-square test

Table 3. The mean values of amount of IL-8 at initial, the 3rd month and 6th month in sampling sites (mean±SD)

	Parameter	Initial values		3th months values		6th months values	
		A group (n=19)	PDT group (n=19)	A group (n=19)	PDT group (n=19)	A group (n=19)	PDT group (n=19)
Total amount pg/site	IL-8	260,6±251	217,3±227,2*	94,3±187,4	178,2±253,7*	220,4±273	97,8±176,6*

** p<0,05 according to time*
† p<0,05 according to groups

Discussion

The present study was designed to test the applicability of PDT as an alternative adjunctive treatment to general antibiotic administration in chronic periodontitis patients through the evaluation of IL-8 levels in GCF samples.

Usage of amoxicillin and metronidazole as an adjunct to SRP in chronic periodontitis patients has been well established (18, 19, 20). It was clearly demonstrated that general administration of these antibiotics is beneficial not only in patients with specific microbiological profile but in all patients using active drugs. Excellent clinical results were obtained regardless of the presence or absence of six classic periodontal pathogens prior to treatment (21). Unfortunately bacteria strains become resistant, especially through frequent doses of antibiotics (22, 23). That is why precaution is recommended toward using antibiotics basically to limit the development of antibiotic microbial resistance. Additionally there is a concern regarding antibiotic side effects such as gastrointestinal disorders, pseudomembranous enterocollitis and superinfections (24). Therefore, PDT is in interest because of fewer complications and local antibacterial effect (25). Our study revealed that compared to baseline and at 3 and 6 months, both treatments resulted in statistically significant PPD reduction and CAL gain. No statistically significant differences in terms of clinical parameters were found between treatments.

Detection of specific substances in GCF as a measure of host response may develop useful factors for monitoring the disease progression and treatment outcome (26, 27, 28). Additionally GCF sampling is a non invasive method. As it is known not only composition but also volume and flow rate of GCF are of importance. The relationship between the enlarged volume of GCF and increased severity of inflammation has been well documented (29). Both studied groups indicated statistically important decrease in sulcus flowing flow rate what can be explained as a reduction of vascular permeability and inflammation. No statistical differences between examined groups were noted. In our study, all GCF samples were collected over the same period of time and data were presented as the total concentration of IL-8 per measure point. Other authors also suggest higher reliability of results expressed as the amount per measure point rather than concentration (30, 31, 32).

Interleukin-8 has been shown to be important for the initiation and development of inflammation and tissue destruction in periodontal disease. Increased levels of IL-8 in gingival crevicular fluid of patients with periodontal diseases has been reported (33, 34). In terms of periodontal treatment (SRP) on IL-8 levels in GCF different results were obtained. In the study of Tsai et al. (33) the total amount of IL-8 of the periodontitis group was significantly higher than the amount in healthy patients and markedly reduced following periodontal treatment. Similar results were presented by Gamonal et al. (34) where periodontal therapy reduced IL-8 levels. In their research IL-8 was detected in 100% of sites from periodontitis patients and in 75% of sites from healthy individuals. Additionally IL-8 levels were increased in active sites but without significance. Erdemir et al., Jin et al. and Lee et al. also depicted reduction of IL-8 levels after SRP (32, 35, 36). In contrary the study performed by Chung et al. (37) comparing healthy and periodontitis patients prior to treatment showed lower concentration of IL-8 in the patients with periodontitis. SRP resulted in both, an increase or a decrease in total IL-8 and IL-8 concentration in GCF. Several factors could influence this diversity among studies, e.g. patient selection, site selection, classification of periodontal disease,

sampling method, timing of assessments. Our study demonstrated reduction of IL-8 levels in GCF of A group and PDT group, but this drop was significant only in PDT group. In A group we observed a substantial decrease of IL-8 amount after 3 months but in 6 months the amount notable rose. Significant reduction of IL-8 in PDT group can be explained by previous studies which proven inactivating host cytokines by photodynamic therapy (38, 39, 40). The study conducted by de Oliveira et al. (38) examined concentrations of TNF-α and receptor activator of nuclear factor-kappa ligand (RANKL) after periodontal treatment with PDT or SRP. Both therapies led to significant decrease of studied immunological parameters. Study carried by Quadri et al. (39) showed slight decrease of amounts of MMP-8 at the laser side and an increase at the placebo side. Elastase activity, IL-1β concentration showed no statistical differences between the laser and placebo side. Braham et al. (40) found that complete IL-1β or nearly complete TNF-α inactivation occurred when the cytokines were exposed to a 60-second PDT treatment. The authors concluded that this effect along with comensal bacteria recolonisation may aid the host in re-establishing homeostasis and promote tissue healing. It was an in vitro experiment and its effects were not confirmed by clinical study of Giannopoulou et al. (41). Their experiment compared the local biologic effects of PDT, diode soft laser therapy, and conventional deep scaling and root planing by measuring levels of 13 cytokines and nine acute-phase proteins with the use of a bead-based multiplexing analysis system. No significant differences were observed among the three treatment modalities at any time point for any biochemical parameter. The similarity in the total amounts of IL-1β and IL-8 before and after therapy was surprising. However it is hard to discuss above results because the immunological analyses was done by different method.

Conclusions

Within the limits of this study it can be concluded that nonsurgical periodontal therapy: SRP with additional antibiotic application and SRP with PDT, are effective methods leading to reduction of clinical parameters and gingival inflammation. Effectiveness of both protocols is comparable however adjunctive PDT may lead to drop of GCF IL-8 levels.

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Presentation of a model for assessment of medically specialized workforce

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Abstract

Background and Goal: The optimal allocation of recourses is the most important instrument of strategic accomplishment and long- term plans in each organization. In other words, policies and purposes of each organization is reflected in the optimal allocation of resources to activities.

Method: The study method is descriptive- sectional and functional. The study environment is sub-province of Qaemshar and the sample study number includes 75 employed specialists with various specialists in this sub-province in 2010. The data collection instrument is designed due to checklist attendance volume and time- evaluation of services carried out based on one service from the view of self-assertion, time-study and use of experts' ideas in medical affairs. To assess the workforce, software was designed in SQI server environment.

Findings: Based on the obtained results, attendance average to a specialist is 7769 people. The most attendance was 13313 people for neurologist during a year and the least attendance was 4852 people for general surgeons in sub-province of Qaemshahr with the population of 330255 people. Attendance norm to a specialist is 1.76 for 1000 people in this study. The number of the assessed force based on the time- consumption average of a service is 75 people.

Conclusion: Efficiency of the offered model is the ability to assess the workforce with "N" indexes. It has flexibility for workforce allocation with regard to the capacity of attendance number in different parts of the country. So, with using the above- mentioned model, not only the problem of lack of medically specialized workforce will be solved in the district under control of each university, but also this model can announce the number of medically needed workforce to the educational deputy of related ministry for education during next N years scientifically and logically.

Key words: Application, model, estimation, man power, medical.

Introduction

Nowadays, the issue of justice in health and removal of injustice in health department is one of the most important concerns of the health systems in the world and especially in the developing countries. The lack f sufficient human and sufficient human and financial resources along with the growing complexity of health aspects have faced the fair health provision, maintenance and promotion with critical challenges in different countries. That's why it is vital for all people especially policy-makers and managers to notice this paramount issue (1).

According to this viewpoint, the issue of optimal allocation of resources is always one of management subjects in each organization because of limitation of resources and extent of society needs (2).

The optimal allocation of resources is the most important instrument of strategic accomplishment and long- term plans of each organization. In other words, policies and purposes of each organization are reflected in optimal allocation of resources to activities. The rate of achievement to purposes depends on how resources are allocated and controlled (2, 3 and 4). On the other hand, access to health and hygienic services is legitimate right of each citizen. Therefore, creation of proper structures for preparation of health and hygienic facilities in different parts of country is the most important duties of government and especially health, hygiene and medical education ministry. Consequently, level-placement project of health services is codified in the country in order to achieve this goal(5). One of the organizational necessities is achievement of

management duties such as planning the human resources to answer the customer the customer needs with regard to the authorized resources. Otherwise, problem is faced in need- evaluation and guide of the authorized recourses to the intended purposes.

So, planning is the foundation of managing elements so that of planning of human resources can have an effective role in pleasant and good exploitation of human resources with regard to the role and importance of human resources in development of organizations and also in answering and supplying the customer's needs(6). These days, organization role of human resources for optimal and proper allocation of activities along with quick and complete answer to customers is so much that it is believed management can prevent insufficient work and excessive work. For instance, construction of hospitals in one place of the country without attention to the need can waste big amount of capital from the organization and on the other hand, hospital maintenance is an extra and added problem. This was one of the biggest problems in different departments of the above- mentioned ministry (7).

This project has been carried out in order to materialized article 89 of law for the county's development fourth plan and also to determine the attendance number of people to health units and centers. Consequently, the needed workforce is provided based on precise index and different geographic regions and based on a model to supply an element of health justice. This will not be materialized unless a model is designed with awareness from the status quo. Model- making is a regular instrument that can prepare necessary information for decisions in order to achieve goals. Planners can examine some relations and connections among factors systematically with the use of models. Relations that isn't probably easy to comprehend without the use of models. We expect a model to fulfill our demands from planning. Some of these demands include prediction ability, flexibility, function assessment ability, optimal allocation of resources, and compatibility among plans. Thus, it is intended in this study to determine the attendance volume along with time- evaluation of each activity by design of need- evaluation software and human workforce planning according to mathematics. The current study in comparison with division and allocation plan of medically specialized human workforce that has been traditionally used by human workforce planning office of the above- mentioned ministry, has superiority that can be used proportion to the attendance volume in the farthest parts of the country, proportionate to the related attendance index with the same region and time-consumption of each service than human workforce planning. This assessment is functional according to the answer to the need of the same region. Neither the regions that have, nor the region that lack. A comprehensive plan of function software according to activity is provided while this plan is not available in health system right now.

Method

The study method is descriptive, sectional and functional. The study environment is sub- province of Qaemshahr. The number of studied samples are 75 employed specialistts in this place with different specialty in 2010. The data collection intrument is designed due to checklist attendance volume and time- eavlauation of services is carried out based on one service from the view of self-assertion, time-study and use of experts' ideas in medical affairs. To complete checklist attendance volume with seperation of specialists working in this subprovince in 2010, reserachers personally attended specialty clinics, private and governmental hospitals, social welfare organization, health service organizzation, Imam Khomini relief committee and armed forces. To assess the workforce, a software was designed in SQI server environment that can list the attendance according to activity with seperation of specialites.

The applied mathematical model with regard to the attendance volume and time- consumption of a services includes:

A: Assessing physician, s number of a specialty in a base year

$$X_{ij}=R_{ij} \cdot T_s / D \cdot H$$

B: Assessing physician,s number of a specialty in a "n" year

$$X_{ij}= (1+g_r/100)^n \cdot R_{ij} \cdot T_s / D \cdot H \cdot D_f$$

X_{ij}= Assessing physician, s number of a specialty.
R_{ij}= an attendance number of a specialist.
T_s = Service time- consumption (visiting a patient).

D= the number of working days of a physician during a year.
H= the number of population.
N= in "n" year.
D_f= deprivation factor.

Findings

Table 1. The status quo of variables and each specialty

Variables	Descriptive	Status quo
P1	Pediatricians	16
P2	Gynecologists and obstetricians	14
P3	Ophthalmologists	3
P4	Orthopedists	3
P5	ENT specialists	4
P6	General surgeon specialists	8
P7	Urologists	2
P8	Psychologists	1
P9	Dermatologists	2
P10	Gastroenterologists	10
P11	Cardiologists	4
P12	Psychiatrists	2
P13	Physical medicine specialists	1
P14	Infection specialists	4
P15	Neurologist	1
P16	Cosmetic surgery	1
Total		**75**

Discussion and conclusion

Based on the obtained results, attendance average to a specialist is 7769 people. The most attendance was 13313 people for neurologists during a year and the least attendance was 4852 people for general surgeons in sub- province of Qaemshahr with the population of 330255 people. Attendance norm to a specialist is 1.76 for 1000 people in this research. According to this reseach that was carried out by planning office of social welfare organization in 2007, attendance norm of the insured people of social welfare organization to specialists, dentists, paraclinical units and clinical services is as follows:

- Going to general physicians is 2.5 times in a year- going to specialists is 1.5- to dentists is 0.5- going to drugstores is 3 times- going to lab is 0.5 and to radiologist is 0.25(8).
- The current number of working specialists in clinincal ward is 75 people. The most number is 16 people for pediastrians and 14 pople for gynecologist and obstericians while the least number is for cosmetic surgeons, neurologists and physical medicine specialist with 1 person. But what is according to model solution with two indexes of attendance number to a specialist and time consumption of a service is assessed according to four times:

Table 2. Frequency of referes based on each specialty in 2010

Variable	Description	Number of referes	Mean of referes
P1	Pediatricians	113229	7076
P2	Gynecologists and obstetricians	96977	6926
P3	Ophthalmologists	31997	10665
P4	Orthopedists	38624	12874
P5	ENT specialists	35231	8807
P6	General surgeon specialists	38821	4852
P7	Urologists	15929	7964
P8	Psychologists	6075	6075
P9	Dermatologists	19905	9952
P10	Gastroenterologists	82790	8279
P11	Cardiologists	27651	6912
P12	Psychiatrists	12235	6118
P13	Physical medicine specialists	11689	11689
P14	Infection specialists	28675	7169
P15	Neurologist	13313	13313
P16	Cosmetic surgery	8564	8564
Total		**582705**	**7769**

Table 3. Time- evaluation of an activity with separation of specialties according to times of study, self-assertion and ideas of expert group

Variable	Description	Time study	Self- assertion	Ideas of expert group	Mean
P1	Pediatricians	6	15	10	11
P2	Gynecologists and obstetricians	10	17	15	14
P3	Ophthalmologists	9	15	15	13
P4	Orthopedists	12	22	20	18
P5	ENT specialists	14	20	20	18
P6	General surgeon specialists	17	22	22	20
P7	Urologists	13	17	15	15
P8	Psychologists	18	30	25	24
P9	Dermatologists	8	18	20	15
P10	Gastroenterologists	6	15	15	12
P11	Cardiologists	12	20	20	17
P12	Psychiatrists	15	25	25	22
P13	Physical medicine specialists	10	20	25	18
P14	Infection specialists	8	15	15	13
P15	Neurologist	18	20	20	19
P16	Cosmetic surgery	12	18	15	15
Total Mean		**11.75**	**19.31**	**18.56**	**15.6**

Table 4. Assessing the number of specialists according to attendance number and time- consumption of a service with separation of each specilty

Variable	Description	status quo	Assessing based on			Mean
			Time study	time- consumption of self- assertion	time- consumption of ideas of expert group	
P1	Pediatricians	16	7	13	9	10
P2	Gynecologists and obstetricians	14	7	13	11	10
P3	Ophthalmologists	3	2	4	4	3
P4	Orthopedists	3	4	7	6	2
P5	ENT specialists	4	4	5	5	5
P6	General surgeon specialists	8	5	7	7	6
P7	Urologists	2	2	2	2	2
P8	Psychologists	1	1	1	1	1
P9	Dermatologists	2	1	2	2	2
P10	Gastroenterologists	10	4	10	10	8
P11	Cardiologists	4	3	4	4	4
P12	Psychiatrists	2	1	2	2	2
P13	Physical medicine specialists	1	1	2	2	2
P14	Infection specialists	4	2	3	3	3
P15	Neurologist	1	2	2	2	2
P16	Cosmetic surgery	1	1	1	1	1
Total		**75**	**48**	**81**	**71**	**63**

A) Assessing according to time- evaluation of a service with chornometer.
The most prediction was seven people in gynecology, obstetetrics and pediatrcs and the least prediction was one person in neurology, cosmeticts, psychology, dermatology and physical medicine.
B) Assessing according to process owner (physician),s assertion, the most are in gynecology, obstetrics and pediaterics each 13 people and the least are in psychology and cosmetics each of them one person
C) Assessing according to the ideas of expert group. The most prediction includes 11 people in gyneological and obsteric specialty and 10 people for internal specialty. The least prediction is in psychological and cosmetic specialty each of them one person.
D) Asessing according to the average of three time-evaluation methods. The most prediction is in pediatric, gyneological and obsteric specialty each of them 10 people.

The least prediction is in cosmetic and psycological specialty each of them one person. Theodorakis, in an article called injustice in distribution of rural care aid doctors in two iaolated regions of Albania and Greece, concluded that the first care doctors had unfair distribution in both two regions (9). Hiroshi's research results demonstarated that there is improper distribution in mrdical personnel especially doctors gini index = 0.433) (10). Huang concluded that geographical distribution had no progress for all doctors in Tiwan from 1984 to 1998 according to gini coefficient (11). In Kyoko's study, research results showed that most of injustice in distribution of pediatricians in rural areas happened from 1996 to 2004 (12). The efficiency of the offered model is the ability to assess the human workforce with "N" indexes. For example, if the related organizations give allocation of coefficient for deprivation or the underdeveloped with each percentage, the probability of coefficient effect is possible in this model. Or if the related organizations want to assess the workforce number for year "N" with the forms of short- term, middle- term and long-term plans, it can be calculated according to relation B that was mentioned in materials and methods. Another benefit of the offered method is flexibility for workforce allocation with regard to the capacity of attendance number in different parts of the country that in this way it can stop insufficient work and excessive work. At present, workforce allocation is unfortunately done by organizations mostly in traditional and bargaining method rather than mathematically. For using the above- mentioned model, if medical the covered population, not only the problem about lack of medically specialized workforce will be solved in areas under their cover, but also can announce the number of needed medical workforce scientifically and logically to deputy of the related educational ministry for next "N" years.

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Prevalence of osteonecrosis of the jaw in cancer patients who were treated intravenous bisphosphonates

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Abstract

Purpose: The primary aim of the retrospective analysis was to assess the incidence of Bisphosphonate-associated osteonecrosis of the jaw (BONJ) in metastatic cancer patients who were treated zoledronic acid, ibandronic acid and pamidronate.

Patients and Methods: From 15 March 2001 to 10 August 2012, patients with breast, lung, prostat cancer and multiple myeloma receiving intravenous (IV) bisphosphonate therapy in the Department of Oncology, received a dental examination. In all, 272 patients analyzed included age, sex, underlying disease, dental history and examination, bisphosphonates (BP) type, and doses administered.

Results: The mean age was 53,3 (range from 14 to 82), 52.2 % (142) were male and 47.8 % (130) were female. 209 of 272 patients (76.8%) received Zoledronic acid, 60 (22.1 %) received ibandronic acid and 3(1.1 %) received Pamidronat. 112 (41.3 %) were treated for metastatic breast cancer, 102 (37.3%) for metastatic lung cancer, 32 (11.8%) for metastatic prostate cancer and 26 (9.6%) other cancer. The mean receiving time was 12.2 (1-61) months for Zoledronic acid, 15.1 (1-78) months for ibandronic acid, 11 (1-20) months for pamidronate. Osteonecrosis was detected in 6 of 272 (2.2%) patients.

Conclusion: The conclusions of this study validated oral surgery as risk factors for BONJ development. Zoledronic acid and ibandronic acid at the dosages and frequency used in this study seem to exhibit a safer drug profile concerning BONJ complication; however, randomized controlled trials are needed to validate these results. Before initiation of a bisphosphonate, patients should have a comprehensive dental examination. Patients with a challenging dental situation should have dental care attended to before initiation of these drugs. However, pre-therapy dental care reduces this incidence, and non-surgical dental procedures can prevent new cases.

Key words: Bisphonates associated osteonecrosis, jaws, cancer.

Introduction

BPs are a class of drugs that prevent the loss of bone mass, used to treat osteoporosis and similar diseases. Recently three bisphosphonates (zoledronic acid, ibandronic acid, pamidronate) are widely used in the management of metastatic disease to the bone and in the treatment of osteoporosis. Cancer patients with bone metastasis often present with a multitude of complications that include pain, hypercalcemia, spinal cord compression, and pathologic fracture [1]. Bone metastases result in increase of osteoclasts activity by a variety of cytokines produced by tumor cells [2]. BPs play at the cell level by targeting osteoclasts. They inhibit osteoclast recruitment, decrease osteoclast lifetime, and hinder bone surface activity of these cells which will give rise to reduction in bone resorption [3]. When BP absorbed form the gastrointestinal tract around approximately 50% of the dose binds to bone. The half-life of BPs in bone is very long, ranging from 1 to 10 years, depends on the rate of bone turnover. Although these benefits, BONJ is a important complication in a subset of patients taking these drugs [4]. BONJ is a recently reported complication that has drawn the attention of the medical society, partially because

BPs are widely prescribed drugs [5]. Food and Drug Administration (FDA) in the United States raised an alert to the health care professionals on September 24, 2004 regarding the potential risk of performing dental extraction or other oral surgical applications in patients receiving BP therapy. The best part of the reported BONJ were associated with patients when BPs were given intravenously or for longer durations [6]. In addition, some groups of BPs have shown higher tendency to develop BONJ [7]. Reported that BONJ patients were associated with pamidronate and zoledronic acid compared to the other groups of BPs. The incidence, prevalence, and aetiology of the BONJ and risk factors triggering BONJ are mostly secret.. The primary aim of this study was to assess the overall incidence of BONJ in metastatic cancer patients who were treated zoledronic acid, ibandronic acid and pamidronate.

Patient and methods

In this study, all patients with used bisphosphonate were being treated in the period from 15 March 2001 to 10 August 2012 in the Department for Oncology of our region, regardless of the time of first BP administration. Inclusion criteria were metastatic breast cancer, metastatic lung cancer, metastatic prostate cancer, multiple myeloma. On the basis of criteria, 272 patients were retrospective into three arms: zoledronic acid (4 mg, i.v., every 3–4 weeks), ibandronic acid (6 mg, i.v., every 3–4 weeks) and pamidronate (90 mg, i.v., every 3–4 weeks). Using these criteria, 272 patients were identified and included. BONJ was diagnosed if the patient had a BP anamnesis but no radiation anamnesis of the head and neck area in Department of Maxillofacial Surgery. Medication and risk factors for osteonecrosis were recorded from patient histories.

Result

Patients

Completely, 272 patients were included in this study and clinical characteristics of the these patients are shown in Table 1. The majority of patients had breast cancer with bone metastases (n=112) followed by breast cancer patients who had lung cancer (n=102), prostat cancer (n=32) and other (n=26). Of 142 (52,2%)patients were male and 130(47,8%) were female. The average age of the patients at the time of Cancer diagnosis was 53,3 yr . 209 patients had received zoledronic acid 4 mg intravenously (IV) every 3-4 week, 60 patients had taken ibondronate 6 mg intravenously (IV) every 3-4 week, and 3 patients had taken pamidronate 90 mg intravenously (IV) every 3-4 week. All patients had received chemotherapy and all patients (except one) had received corticosteroids. The corticosteroids were applied according to the cover scheme parallel to the chemotherapy. Data about other risk factors associated with osteonecrosis were described in Table 2.

Bisphosphonate therapy and development of osteonecrosis

All of the 272 patients received only one BP. The majority of these patients was administered zoledronic acid (n=209(76,8%)). The mean number of treatment cycles administered to all patients treated with i.v. BPs was 9.5 (range 1–61, median: 8 cycles). The mean duration of treatment was 13.9 months (range 1–61). Osteonecrosis was detected in 6 (2.2%) of 272 patients. Follow up time was 34.5 months for BONJ group. All of these patients' findings were shown in table 2. mean age was 53.4 (range 14-82) and mean treatment duration was 13.9 (1-61) months for patients without BONJ (n=266 98,7%). Follow up time was 27.2 (1-180) months for this group.

Number of treatment cycles and duration of bisphosphonate therapy were higher in diagnosis of BONJ patients. In patients diagnosed with BONJ the mean number of treatment cycles was 20 cycles (range 6–30 cycles) and the mean duration of bisphosphonate therapy was 17.0 (range 5-29 months). However, the mean number of treatment cycles in patients without manifestation of BONJ was 15 cycles (range 2–80 cycles) and the mean duration of BP therapy was 12.9 (range 1-78 months).

Diagnosis and treatment of the 6 patients with ONJ

The site of ONJ development was the mandible in all cases (Table 2). The posterior mandible was the most frequent affected site (n=4). Symptoms of ONJ were alveolitis (n=1), fistula (n=3), abscess formation (n=3) and exposed jawbone (n=6). All patients diagnosed with BONJ after BP therapy had

Table 1. Comparison between all patients with BONJ, and patients with no BONJ

	All patients (n)	Patients with BONJ	Patients without BONJ
Number (percentage)	272 (100%)	6 (2,2%)	266 (97,8%)
Age, years, median (range)	53,3 (14-82)	46.3 (35-65)	53.4 (14-82)
Type of cancer			
breast cancer	112 (41.3 %)	1(16,7%)	110(41,3%)
lung cancer	102 (37,3%)	1(16,7%)	102(38,3%)
prostate cancer	32 (11,8%)	1(16,7%)	31(11,7%)
other cancer	26 (9,6%)	3(50,0%)	23(8,7%)
Type of bisphosphonate			
Zoledronic acid	209(76,8%)	5(83,3%)	204(76,7%)
ibondronat	60(22,1%)	1(16,7%)	59(22,2%)
pamidronate	3(1,1%)	0(0,0%)	3(1,1%)
Used time, months	12.9 (1-78)	17.0 (5-29)	13.9 (1-61)
Follow-up time (month)	25.2 (1-180)	34.5 (30-39)	16.5 (1-180)

Table 2. All patients with BONJ

Age	Sex	BP	Bonj Location	Stage	Description	Radiographic Findings	Predisposing Factors	Corticosteroids	Type of cancer
39	male	zoledronic acid	Mandibula	3	Mandible Pain and paresthesia, non-healing extraction site with multiple fistulas and osteomyelitis	Focal osteolytic region on panoramic x-ray	Tooth extraction 2 yr previously	Yes	Multiple myeloma
53	male	zoledronic acid	Mandibula	3	Intermittent ache, purulence, fistula, and osteomyelitis at previous extraction site	Osteosclerosis on CT	Extraction 4 months previously	Yes	Multiple myeloma
61	male	zoledronic acid	Mandibula	2	Purulence, non-healing extraction site, osteomyelitis, and extensive necrosis	Osteosclerosis on CT	Extraction 6 months previously	Yes	Multiple myeloma
57	male	zoledronic acid	Mandibula	1	4-mm exposed bone <6 wk	Normal panoramic x-ray	Partial denture	Yes	Lung cancer
66	male	zoledronic acid	Mandibula	1	4 mm sequestrum <4 wk at previous extraction site	Osteosclerosis on CT	Partial denture	Yes	Prostate cancer
45	male	ibandronic acid	Mandibula	1	Pain with focal recurrent bony spicules for 2months followed by spontaneous loss of adjacent tooth	Osteosclerosis on CT and intense focal uptake on bone scintigraphy	Periodontal abscess	Yes	Breast cancer

received chemotherapy including corticosteroids for metastatic disease. None of patients were treated with radiotherapy. Three of six patients had at least one recent dental extraction before developing BONJ. An ill fitting prosthesis may have triggered BONJ in 2 patients. None reported other trauma to head or neck. Only limited data were available about the risk factors smoking, drinking, diabetes, anemia and dental health. Figures 1-2 shows a patient (ID-No: 3) with BONJ of the mandible.

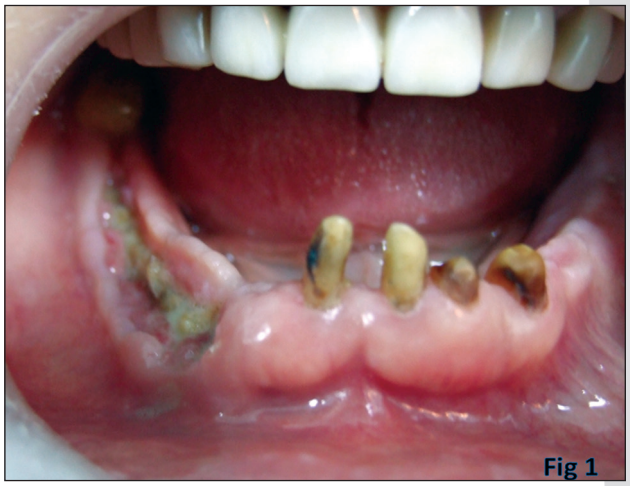

Figure 1. Patient (ID-No: 3) with BONJ of the mandible

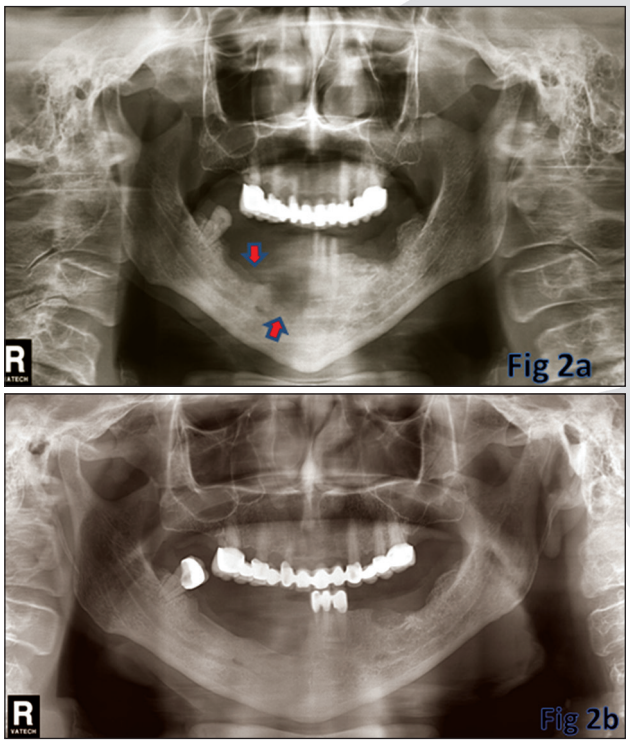

Figure 2. BONJ (Patient ID-No: 3) radiological view of the mandible (a. Pre-treatment b. Post-treatment)

After diagnosis of BONJ BP therapy was stopped in 5 of 6 patients. Three patients received surgical intervention by sequestrotomy and the other three patients were managed conservatively. The management of these patients consisted of periodontal therapy, antibiotics and mouth rinses with chlor hexidine digluconate. Follow-up was possible in all patients (mean time of follow-up: 25,5 months, range 6–45 months). Surgery resulted in improvement in 2 patients. BONJ improved partly after conservative management in 2 of 3 patients.

Discussion

Incidence of BONJ

Osteonecrosis (ON) is a consequence of bone malnutrition and a well-known side effect of high-dose radiation therapy. It is also known to occur after chemotherapy [8]. In 2003 first cases of BONJ were described in cancer patients after long-term BP treatment [9]. Although causality seems obvious, the mechanism for BONJ development remains to be defined. The first attempt to give an incidence of BONJ in cancer patients was made by Durie et al. in 2005 [10]. However, these numbers are possibly not reliable as they are based on patients' answers in a survey. Until now there is a lack of reliable data about BONJ incidences in BPs due to mostly case reports. We therefore retrospectively analyzed data of patients treated with i.v. BPs at the Department of Oncology in our region during 15 March 2001 to 5 November 2012.

Table 3 shows the data from other studies published to date in comparison to our study. There are described incidences from 2.5% for breast cancer [11] and 18.6% for pCA [12]. The only incidences described for pCA so far are 6.5% (3 of 46 patients) [13], 2.9% (3 of 104 patients) [14], and 18.6%(8 of 43 patients) [12].

Six out of 226 patients treated with i.v. BPs developed BONJ which results in an incidence of 2.2%. However, all of them were treated for bone metastases in breast, lung, prostate cancer and multiple myeloma. Published incidences of BONJ in breast cancer patients range from 0.1 to 18,6 % (Table 3). Differences are most probably due to varying methods of data assessment/analysis, the small number of patients studied and the limitations of voluntary case reporting.

In our opinion, prevalence of BONJ in patients receiving BPs may be underestimated because of the retrospective design of most of the other studies and the missing dental examination [15]. It is the most important point that all patients had a dental examination in this study. As early stages of BONJ often are asymptomatic to the patient [5], the rate of detection of exposed or necrotic bone might be higher in this study than in a general medical examination only.

BONJ is generally occurred after the use of i.v. BPs. However, there are also reports about occurrence of BONJ after oral bisphosphonate therapy [16]. In these rare cases, patients with BONJ were treated with zoledronic acid or risedronate for osteoporosis or Paget's disease. All of our BONJ patients had received zoledronic acid. A comparison of BONJ risk in the different subgroups of bisphosphonates is difficult: patients at high risk for BONJ are long-term treated cancer patients. They mostly received different BPs and often were recently switched to aminobisphosphonates. Therefore, to evaluate effects of one single substance is hardly possible, especially in a group of drugs with halflife of several years. It is hypothesized that zoledronic acid due to its superior in vitro potency also has a higher potential for BONJ development. However, data to confirm this observation are still pending. Although the methods of action are not yet completely understood, it is hypothesized that BONJ is related to a defect in jaw bone physiologic remodeling or wound healing. The strong inhibition of osteoclast function precipitated by BP therapy can lead to inhibition of normal bone turnover. Because BPs are preferentially deposited in bone with high turnover rates, it is possible that the levels of BP within the jaw are selectively elevated. To date, there has been no reported case of bisphosphonate-associated complications within bones outside the craniofacial skeleton [17].

Dental procedures are speculated to be a major risk factor for BONJ development [18].This is in line with our findings. BPs accumulate in skeletal sites of high bone turnover. Therefore, maxilla and mandibula are preferential sites for BP accumulation. The inhibition of osteoclasts affects bone wound healing and makes the bone susceptible to osteomyelitis and necrosis [19].

Management of BONJ

Until now, no definitive standard treatment could be established. In our patient's collective three of six patients received surgical treatment. The other patients were treated with antibiotics and mouth washings. Follow-up was possible in all patients. Only five of them reported significant improvement of symptoms which reflects the severeness of this complication.

Current guidelines recommend that treatment of BONJ should be performed by a qualified dental specialist. Management of pain and infection are the most important issues. Surgical treatment should be conservative or delayed [20]. An unsolved issue is the question about discontinuing BPs. Some experts recommend the interruption of BP therapy in their patients after BONJ occurrence [13]. However, since the half-life of BPs in human bone can reach more than 10 years [21] it has been generally questioned whether interruption of BPs will be of any benefit [22]. Based on the report of the American Society for Bone and Mineral Resarch (ASBMR) it is recommended to take the indication for BPs into account. In case of aggressive skeletal metastatic disease it might be better to continue treatment [9]. Since BONJ is a severe complication, all efforts should be undertaken for prevention. Therefore, based on the recommendations of the ASBMR and other cancer societies patients should be informed about the benefit and side effects of bisphosphonates including the risk of BONJ, the signs and symptoms and the risk factors for developing BONJ. Patients should be encouraged to improve oral hygiene and to have regular dental visits at every 6 to 12 months-intervals [9]. A dental evaluation is recommended before starting therapy. Invasive dental procedures should be avoided in patients receiving bisphosphonates. As recently shown by Ripamonti et al. the implementation of such a preventive dental program was able to reduce the incidence of BONJ by 76% in a prospective trial [23]. Despite emerging data, prospective studies are still necessary to gain more information about risk factors, appropriate BONJ treatment and possible differences in bisphosphonates subgroups to minimize the occurrence of this painful side effect.

Conclusions

The reason for the relatively high incidence might be the study design with the oral examination by a dentist. In studies with such small numbers, undetected or non reported cases of bisphosphonate-associated osteonecrosis have a huge influence on the outcome. All patients with BONJ had had a previous dental surgical procedure or suffered from denture pressure sores. Therefore, close co-operation among the medical doctors applying BPs and the oral and maxillofacial surgeons or the highly skilled dentists in this field seems to be reasonable.

One reason for the higher values in our study might be that the similar effect was reported by the Boonyapakorn study [24] in multiple myeloma patients. In this study, dental examination of the patients at risk was also under- taken and revealed a surprisingly high prevalence of BONJ. In a retrospective study with such small numbers of patients, an undetected or nonreported osteonecrosis can have a huge influence on the prevalence or incidence of a disease. A further reason for the high number of patients with BONJ in this study might be that several factors associated with the development for osteonecrosis [28] were present in this patient collective (cortico-steroids, chemotherapy), including additional factors such as a tooth extraction or a dental pressure sore.

This relationship has been described in previous reports [29] and underlines the importance of oral hygiene in preventing dental surgical procedures, thereby also reducing the need for dentures. Prevention results in both a lower incidence of BONJ and lower infection rates of any noninfected, already necrotic, exposed bone [9]. ON as a side-effect of docetaxel has not been described in literature yet, but it is described in patients taking corticosteroids [30]. The appearance of several risk factors might increase the risk of developing BONJ.

Table 3. All published studies with incidences of BP-ONJ compared to results of this study (last line)

Study, yr	Disease	Patients (n)	BONJ (n)	Incidence (n)
Bamias et al, 2005 [13]	Multiple myeloma Breast cancer pCA Other	111 70 46 25	11 2 3 1	9.9 2.9 6.5 4.0
Boonyapakorn et al, 2008 [24]	Multiple myeloma	58	10	17.2
Dimopoulos et al, 2006 [25]	Multiple myeloma	202	15	7.4
Durie et al, 2005 [10]	Multiple myeloma Breast cancer	904 299	62/116 13/36	6.9/12.8 4.3/12.0
Garcia Saenz et al, 2007 [14]	pCA	104	3	2.9
Wang et al, 2007 [11]	Multiple myeloma Breast Cancer	292 81	11 2	3.8 2.5
Zervas et al, 2006 [26]	Multiple myeloma	254	28	11.0
Walter et al, 2007 [12]	pCA	43	8	18,6
Hong et al, 2010 [27]	Osteoporosis	12,752 /9,882	24	0.05/0.07
Lo et al, 2010 [17]	Multiple myeloma Breast cancer pCA Other	8572	9	0.1

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Experiences with renal transplantation in patients on peritoneal dialysis

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Abstract

Aim: Peritoenal dialysis (PD) is the method of choice in patients with end stage renal disease (ESRD) preparing for kidney transplantation (Tx). Peritoneal dialysis provides several advantages for PD patients, including lower incidence of hepatitis B and C infection, reduced sensitization by blood transfusions, good preservation of bladder function, reduced incidence of delayed transplant function, lower rates of recurrent glomerulonephritis. This report presents the results of PD and Tx recorded at our Centre during the past several years.

Our results: In the period from 01 January 1998 to 01 April 2012, seventeen patients from our Centre underwent transplant surgery. Out of the total number of patients, 12 (70.59%) underwent cadaveric Tx, and five (29.41%) received a related donor transplant. Majority of patients were females 13 (76.47%), and only four (23.53%) patients were males. The average age at the moment of transplantation was 39.75 and 37.23 in male and female patients, respectively. The most frequent underlying causes of ESRD among our patients were chronic glomerulonephritis (12), diabetes (3), vesicoureteral reflux (1) and systemic lupus erythematosus (1). The average length of time on PD was 4.69 years. At the moment of Tx, the majority of patients (14) revealed preserved residual renal function (diuresis 500-1000 ml). None of our patients was HBsAg and HCV positive. The average pre-transplant urea and creatinine values were 20 mmol/l and 900 umol/l, respectively. Pretransplant hematocrit level was 0.32. The average urea, creatinine and hematocrit levels were analyzed one month post-transplant and at the end of follow-up period, revealing the following values: urea – 8.5 and 7.49 mmol/l, creatinine – 130.8 and 105.5 umol/l, hematocrit – 0.36 and 0.37, respectively. Besides the standard therapy regimen, majority of patients received cyclosporine A (12 patients), whereas five patients received tacrolimus.

The average period of PD catheter use after Tx was 14 days. In all patients, the post transplant period was characterized by immediate and satisfactory transplant function and absence of overt postoperative complications. During the follow-up period, one patient with chronic transplant failure was transferred to HD (10 years after transplantation), whereas one patient developed cardiovascular complications and died 6 years after transplantation.

Conclusion: The results and experiences from our Centre have strongly confirmed the advantages of PD in renal transplantation.

Key words: Peritoneal dialysis, renal transplantation.

Introduction

Management of end stage chronic renal disease (ESRD) encompasses either one of well-known dialysis modalities, i.e. hemodialysis (HD) and peritoneal dialysis (PD) or renal transplantation (Tx). By the end of last century, renal transplantation in PD patients was considered hazardous because of potential risk of peritonitis or higher incidence of acute transplant rejection. The situation has significantly changed over time. The growing experience in renal transplant practice revealed certain advantages of PD over hemodialysis therapy in a view of outcomes in kidney transplantation (1,2).

Peritoneal dialysis and renal transplantation

Appropriate evaluation and selection of patients is of paramount importance for success of renal transplantation, and good kidney transplant candidates are persons who have living related donor, pre-emptive transplant patients and PD patients.

Advantages of PD in RTx include:

- lower incidence of hepatitis B and C infections
- reduced sensitization by blood transfusions,
- better preservation of bladder function,
- reduced incidence of delayed transplant function,
- lower *rates of recurrent glomerulonephritis*

Better and longer preservation of diuresis as well as residual renal function (RRF), which is particularly important, is reported in PD patients as compared to HD patients. Preservation of diuresis positively affects the bladder capacity enabling better formation of ureterovesical anastomosis, thus decreasing the risk of vesicoureteral reflux, urinary fistula and urinary tract infections (3,4)

Maintaining the hydration status in PD patients is essential in transplantation procedures. Peritoneal dialysis (PD) patients are often hypervolemic; however, patients with preserved RRF are categorized as euhydrated . The most recent research on renal transplant reported better hydration status in CAPD patients with preserved RRF, as compared to anuric patients on HD. Such patients, if hypervolemic, often do not need urgent dialysis, as HD patients do, thus reducing the cold ischemia time.

Hyperhydration, reduced sensitization to HLA antigens and shorter cold ischemia time reduce the incidence of delayed transplant function and risk of acute transplant rejection, and enables better transplant function. Moreover, decreased need for repeated immunosuppressive therapy may reduce the incidence of early and late post-transplant infections (5,6).

Some research revealed lower rate of delayed transplant function, acute rejection, infections and recurrence of underlying disease in PD patients; however, an increased rate of vascular stalk thrombosis was observed. According to some reports, the patient and graft survival rates (one-year and five-year survival) were similar in HD and PD patients. The incidence and severity of episodes of acute rejection did not differ between these two patient categories. The effects of particular pretransplant therapy modality on transplant outcomes have still not been clearly defined. Negative primary selection of patients for PD may give an impression of poorer survival rate as compared with the HD patients.

Besides the common post transplant complications, some specific ones may develop in PD patients. Some 15% of PD patients can manifest:

- infectious complications (peritonitis, exit-site infections, tunnel infections)
- sclerosing peritonitis
- dialysate leakage and transitory ascites
- rarely, intestinal perforation (7, 8, 9)

PD is applicable even after unsuccessful renal transplantation. In patients who experienced loss of transplant function and were transferred to dialysis therapy, higher survival rates were recorded in those with preserved RRF as compared to anuric patients. The probability for preservation of RRF is higher in PD patients than in those on hemodialysis. Patients who experienced loss of transplant function reveal more rapid loss of residual renal function than patients starting the peritoneal dialysis. Continuation of immunosuppressive therapy is recommended with an aim of preserving RRF and improving the patient survival rate. The risk of infections or carcinoma is potentially high and may be linked with immunosuppressive therapy regimen, thus careful assessment of potential clinical benefits is essential. PD following unsuccessful transplantation can be associated with twofold risk of high peritoneal transport status. This predisposes to loss of ultrafiltration capacity, loss of proteins into dialysate, hypoalbuminemia, hypertension and increased risk of cardiovascular episodes. Appropriate selection of dialysis modality after unsuccessful transplantation implicates comprehensive evaluation of several clinical parameters. PD is considered good option in majority of patients (10, 11).

Our experiences

In the period from 01 January 1998 to 01 April, 2012 a total of 17 patients underwent renal transplantation at the Clinical Centre of Vojvodina, Clinic for Nephrology and Clinical Immunology. All PD patients selected for kidney transplantation were subjected to standard preparation protocol encompassing appropriate biochemical (immunological, virological and bacteriological) tests and radiological examination. The patients were examined by urologist, cardiologist, pulmonologist, gynaecologist, psychologist and neurologist. Possible focalosis was excluded through an exami-

nation by the dentist and ENT specialist. During the post transplant period, vital and standard biochemical parameters of transplant function were monitored. First transplantation was performed in one patient in 2001. Subsequently, the transplantations have been performed once or twice yearly, and the number has increased to four transplant procedures in 2006 (Chart 1).

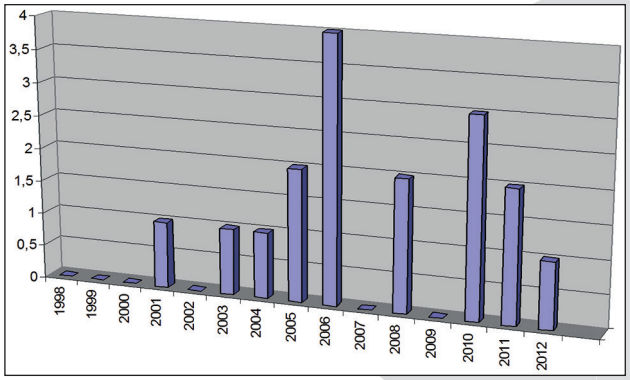


Chart 1. Number of transplant patients by years

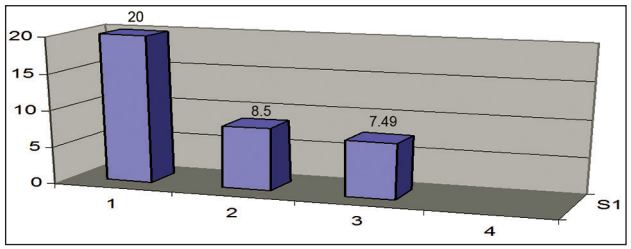
Our of the total number of patients, twelve of them (70.59%) underwent cadaveric Tx, whereas 5 patients (29.41%) underwent related donor Tx. According to sex structure of patient population, the majority of patients were females, i.e. 13 (76.47%) and 4 (23.53%) males.

The average age at the moment of transplantation was 39.75 and 37.23 in male and female patients, respectively.

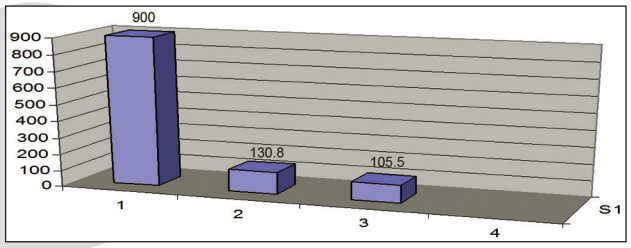
The most prevalent cause of CRD in our patients was chronic glomerulonephritis (12), then diabetes (3) , vesicoureteral reflux (1) and systemic lupus erythematosus (1).

The average length of time on PD until renal Tx was 4.69 years. Majority of patients (14) had preserved residual renal function (dieresis 500-1000 ml), and three patients manifested diuresis below 500 ml. None of the patients was HBsAg and HCV positive.

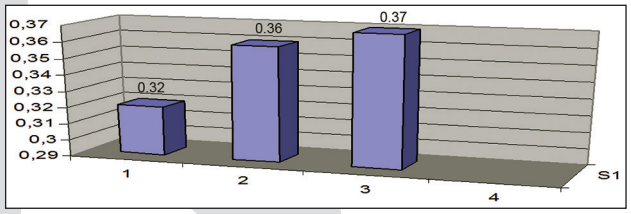
The average pretransplant levels of urea, creatinine and hematocrit were 20 mmol/l, 900 umol/l and 0.32, respectively. The average urea, creatinine and hematocrit levels were analyzed one month post-transplant and at the end of follow-up period, revealing the following values: urea – 8.5 and 7.49 mmol/l, creatinine – 130.8 and 105.5 umol/l, hematocrit – 0.36 and 0.37, respectively. (Chart 2 – a, b, c)



a) urea (mmol/l)



b) creatinine (umol/l)



c) Hct

Chart 2. Laboratory findings before Tx 30 days after Tx last control

Besides the standard therapy regimen, majority of patients received cyclosporine A (12 patients), whereas five patients received tacrolimus.

The average period of PD catheter use after Tx was 14 days. In only one patient, the peritoneal catheter was removed during Tx.

In all patients, the post transplant period was characterized by immediate and satisfactory graft function and absence of overt postoperative complications. During the follow-up period, one patient with chronic transplant failure was transferred to HD (10 years after transplantation), whereas one patient developed cardiovascular complications and died 6 years after transplantation.

Conclusion

The results and experiences from our Centre have strongly confirmed the advantages of PD in renal transplantation.

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Surface of Amygdale complex

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Abstract

Comparison of the surface of amygdale complex at neuropsychiatry disease like posttraumatic stress disorder (PTSD) and temporal epilepsy, as well as control respondents group will contribute to the understanding the role of this part of the limbic system and its connection with the neuropsychiatric pathology. Methods of analysis are Magnet resonance Image (MRI) scan of temporal lobe of cerebrum, measuring of amygdale complex dimensions in the axial projection, with the display of anteroposterior diameters and lateromedial diameters in 10 participants with temporal epilepsy, average age 41,10, years, 10 participants with PTSD, average age 49,10 years, 10 participants from the control group, average age 40,50 years, all participants are male. Calculating of the surface of amygdale complex is done by mathematic formula, obtained by using of amygdale complex dimensions that are measured on MRI scans. There are no significant differences between surface of amygdale complex on the left and the right side within PTSD group and group with the epilepsy. Significance at the level of $p<0,05$ is present inside control group. Significant differences are present on both sides between all respondents groups. The surface of amygdale complex is the biggest within the group of the respondents with the epilepsy: left: 2,549 cm^2 and right: 2,428 cm^2. The smallest one is inside the control group on the left side: 1,259 cm^2.Explanation for the biggest surface of amygdale complex inside the group of the participants with the epilepsy is possible to seek within hyperactive limbic structure, which can contribute, over their abundant projections in prefrontal cortex, to the system disorder. Smaller amygdale complex surface inside PTSD group comparing with group of participants with temporal epilepsy, can be explained by atrophy of the observed structure, probably because of paradoxical neuroendocrine responses with PTSD.

Key words: posttraumatic stress disorder, epilepsy, amygdale complex, MRI

Introduction

Comparison of the surface of amygdale complex at neuropsychiatry disease like PTSD and temporal epilepsy, as well as control respondents group will contribute to the understanding the role of this part of the limbic system and its connection with the neuropsychiatry pathology. Amygdale complex is significantly included into the emotional processes. Nowadays, it is well known that amygdale complex has an important role in numerous processes which include emotional and social processing. The studies of amygdale complex on human beings imply quantitative analyse of its dimensions by using the structural MRI and visualisation of the activities of this structure with different stimuli using Functional Magnet resonance Image (fMRI) and spectroscopy (SPECT). In addition to the studies which examine amygdale of healthy persons, those which compare amygdale of healthy respondents and the patients diagnosed with a variety of neuropsychiatric conditions of very wide range such as among the others: Autism, bipolar disorder, depression, schizophrenia, epilepsy, phobias, PTSD and Alzheimer's disease.

Material and methods

Methods of analysis are MRI scan of temporal lobe of cerebrum, measuring of amygdale complex dimensions in the axial projection, with the display of antero-posterior diameters and lateromedial diameters in 10 participants with temporal epilepsy, average age 41,10, years, 10 participants with PTSD, average age 49,10 years, 10 participants from the control group, average age 40,50 years, all participants are male. MRI scans are made on the device MAGNET IMPACT SIEMENS 1,0 TESLA in T1 and T2 relaxation, in lay-

ers of 5 mm. PD and T_2 dual sequences are used. Head neck spiral and neck spiral itself are used. To analyze the size of amygdale complex and for its comparison Evaluate distance programme was used on the device MRI at the Department of Radiology, Clinical center, University of Sarajevo. All the values are given in centimetres.

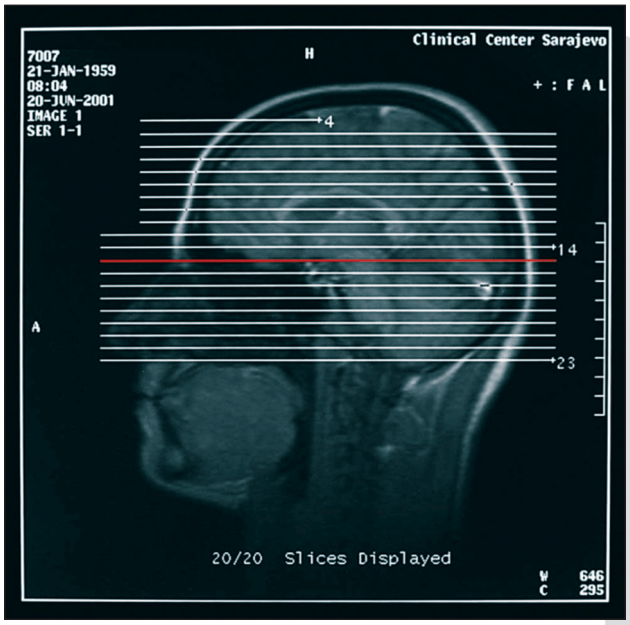

Figure 1. Axial MRI scan – section at the level of amygdale complex

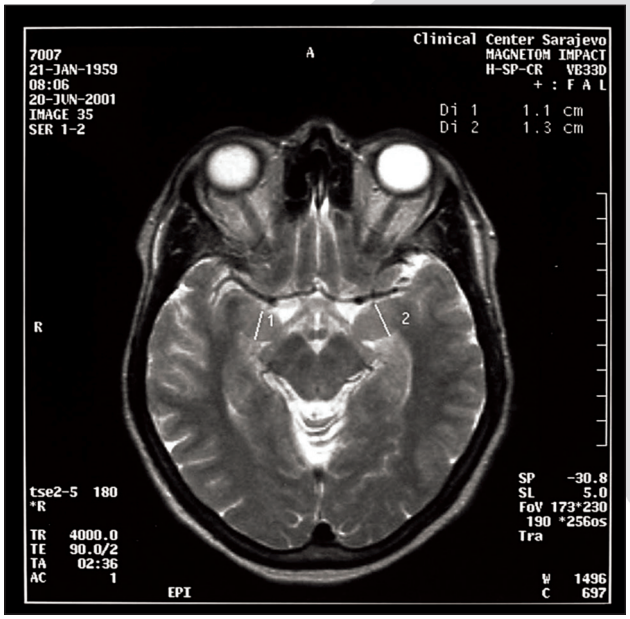

Figure 2. Axial MRI scan - anteroposterior diameter of amygdale complex

Calculating of the surface of amygdale complex is done by means of mathematical formula that is obtained by using the dimensions of amygdale complex that are measured on MRI scans.

$$P = \frac{X1 * X2 * \pi}{4}$$

The mentioned formula is assigned in programme 'Microsoft Office Excel 2010', in which process dimensions were registered in corresponding table cells by the following rule:

Antero-posterior diameter x_1, x_2

Latero-medial diameter y_1, y_2 (in millimeters)

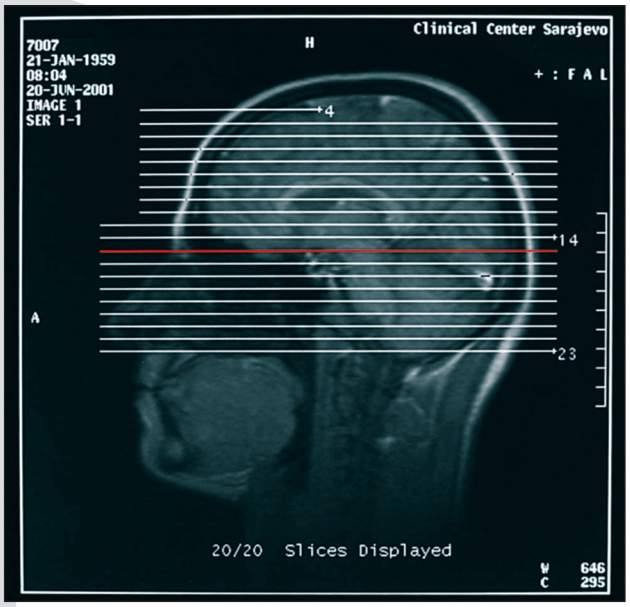

Figure 3. Axial MRI scan – at the level of amygdale complex

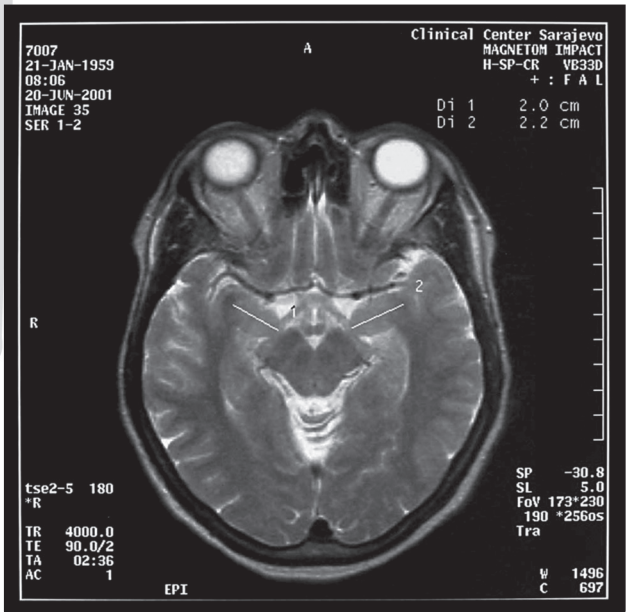

Figure 4. Axial MRI scan lateromedial diameter of amygdale complex

There were compared the surfaces of amygdale complex within each of the respondents group, as well as among three studied group of posttraumatic stress disorder, epilepsy and control group. It was also tested the significance of the differences in average age between the groups of patients.

Methods for statistical analyse used in this study are:

1. Arithmetic mean
2. Standard deviation
3. Standard error
4. Student t – test

Results

From the presented data (Table 1) it is obvious that there are not differences in average surface of amygdale complex between left and right side within PTSD group.

From the presented data (Table 2) it is obvious that there are not differences in average surface of amygdale complex between left and right side within EPI group.

From the presented data (Table 3) it is obvious that there are significant differences in average surface of amygdale complex between left and right side within Control group, at the significant level $p < 0,05$.

Table 1. Average surface of amygdale complex within PTSD group

PTSD group	Surface of amygdale complex		t - test
	Left	Right	
Participant's number n	10	10	t = 0,588 not significant
Arithmetic mean - x in cm²	1,869	1,999	
Standard deviation –SD	0,526	0,462	
Standard error – SDx	0,166	0,146	

Table 2. Average surface of amygdale complex within EPI group

EPI group	Surface of amygdale complex		t - test
	Left	Right	
Participant's number – n	10	10	t = 0,651 not significant
Arithmetic mean - x in cm²	2,549	2,428	
Standard deviation –SD	0,583	0,565	
Standard error – SDx	0,184	0,179	

Table 3. Average surface of amygdale complex within CONTROL group

Control group	Surface of amygdale complex		t - test
	Left	Right	
Participant's number – n	10	10	t = 2,176 significant p < 0,05
Arithmetic mean - x in cm²	1,259	1,999	
Standard deviation –SD	0,419	0,475	
Standard error – SDx	0,133	0,313	

Table 4. The testing results of significant differences between the surfaces of amygdale complex among all three groups

Respondents groups	Left	Right
PTSD / EPI	t = 2,739 significant p < 0,02	t = 1,858 significant p < 0,10
PTSD / Control	t = 2,866 significant p < 0,02	t = 2,905 significant p < 0,02
EPI / Control	t = 5,89 significant p < 0,01	t = 4,00 significant p < 0,01

Table 5. Average age of the respondents

Group of the respondents	Participant's number - n	Average age - x	Standard deviation – SD
PTSD group	10	49,10	5,029
EPI group	10	41,10	8,373
Control group	10	40,50	6,165

Table 6. The testing results of significant differences in average age among all three groups

Respondents groups	t - test
PTSD/EPI	t = 2,590 significant p < 0,01
PTSD/Control	t = 3,418 significant p < 0,01
EPI/Control	t = 0,182 not significant

Significant differences between surfaces of amygdale complex are mutually evident among comparison of all of three studied groups, this finding is relevant for the surfaces of amygdale complex on both, left and right side.

The average age of the respondents shows that it went from 40,50 – 49,10 years, by which possible discriminatory factors of too young or too old respondents are removed. In other words, presence factors of limbic system development or even its involution.

Significant differences within average age of the studied groups exists at the comparison within PTSD group, considering that is the group with the highest average age, which was 49,10 years.

Discussion

The first data about limbic system we can find even in the studies from the second half of nineteenth century. Then series of the studies, with studying of topography and rough morphological characteristics, have been continued and give insight into the structure of the cytoarchitectonical amygdale. Experimental researches on limbic cortex began at the beginning of the twentieth century. It was noticed then, that the temporal lobotomy with the destruction of the amygdale nucleus leads toward meek behavior, while on the other side removal of other parts rinencefalon (cingulate gyrus), piriform cortex and what we call periamygdale complex causes aggressive behavior. Some authors use limbic system as a reference which is related with emotions and tractes that linked them together. Authors differ disinclination (aversion) centres and satisfaction centres (gratification). If aversion centre is simulated, person will experience fear or sadness. On the other side simulation of gratification centre will result with satisfaction. According to the authors, functional inter-relationship between centres of aversion and gratification probably contribute to emotional stability. Hippocamp and amygdale have abundance of aversion centres while nucleus accumbens contains abundance of gratification centre. As a consequence of that, amygdale stimulation can cause fear, while nucleus accumbes stimulation results with feelings of happiness and satisfaction (1). In the study, that was conducted by Tuuli Salmenpera et al, it was proved that volumetric MRI analyze indicated that none of the patients showed significant decrease volume of the significantly reduced volume of the hippocampus, amygdale and peri- or entorhinal cortex during one year observation. So status epilepticus doesn't always lead toward progressive decrease of the volume in the structures of medial temporal lobe of adult patients that are treated without delay in hospital with a pre established protocol for rapid cessation of attack activity.(2) Bower S. et al. found in patients with temporal lobe epilepsy, significantly enlarged amygdale and its asymmetry that corresponds attack lateralization (3). In our study, we found the largest area in the amygdale complex within the group of patients with epilepsy: left: 2.549 cm^2 and right: 2.428 cm^2. Unlike them, surfaces of amygdale complex were significantly lower in the group of patients with PTSD. We can find explanations in paradoxical neuro-endocrine response in patients with a diagnosis of PTSD, which is characterized by elevated concentration of corticotrophin releasing factor (CRF) with normal or even reduced cortisol concentrations in serum. Cortisol, therefore, has the role of anti-stress hormone. It inhibits

with negative feedback brain catecholamines and all neurohormones in the cascade Hypothalamo-hypophysis-adrenal (HHA) axis. All these instances (amygdala, hippocampus, hypothalamus, hypophysis) contain a lot of glucocorticoid receptors and all of them are important targets of cortisol activity. At the moment when external stressor disappears, that means, when the amygdala does not detect this threat any longer, feedback inhibition of HHA axis is allowed, in tandem with the hippocampus, which leads to restitution of hormone levels on baseline values. This specifically means that the organism with the cessation of stressful experiences gradually slows down and its functions then return in normal. Salpolsky Robert M. (4) pointed out in his studies, that exposure to normal levels of glucocorticoids is necessary for the normal functioning of the hippocampus (Diamond et al. 1992) long-term exposure to high levels of glucocorticoids during stress will produce the opposite effect. Mechanism or the way in which glucocorticoids change neurogenesis is still not fully understood, (Sapolsky, 2001). Glucocorticoids lead to atrophy of dendrites, and neurotoxicity compromise the ability of neurons to survive synchronous insult of worsened cascade excessive synaptic glutamate concentration, excessive release of calcium and production of oxidative and cytoskeleton damage, which plays a central role in hippocampal neural damage. In Rachel Yehuda study (5) we find the conclusion that glucocorticoids directly change the micro-architecture, the number and volume of brain cells. In the past twenty years, MRI studies got more significance, in order for better elucidation of structural changes in certain limbic structures, which can be quantified through modern methods of examination (imaging techniques). The results show that people who suffer from PTSD have about 25% lower volume in the hippocampus in comparison of control participants and they have deficits of functions associated with the hippocampus. (6). We exactly use, in our study, structure MRI for the purpose of quantifying surfaces of amygdale complex, regarding its important role in emotional and social processing. Evaluation of patients with temporal lobe epilepsy (ETR) has traditionally focused on hippocampal formation. P. Pereira et al. (7) wanted to explore other possible structural abnormalities and decided to measure the combined volume of the piriform cortex and amygdale complex (PCA). They didn't find asymmetry with control subjects, or influence of age and gender. Patients with right ETR had volume decreased by 18% compared to controls, and 15% compared to those with left ETR, and patients with left ETR 16% and 19%. Woon F. et al. (8) found nine studies by using electronic databases which compare the volume of the amygdale in individuals with PTSD, those exposed to trauma, but who do not have PTSD and non-exposed to trauma. The results showed that there are no significant differences in amygdale volumes in these three groups. In each of these groups the right amygdale was significantly larger than the left one, indicating the preservation of the right amygdale lateralization in exposure to trauma and PTSD. And in our results within the PTSD group, the right amygdale had larger surface: 1.999 cm^2, comparing with the left: 1.869 cm^2. But the significant difference was not found $t = 0.588$.

Conclusions

Explanation for the biggest surface of amygdale complex inside the group of the participants with the epilepsy is possible to seek within hyperactive limbic structure, which can contribute, over their abundant projections in prefrontal complex, to the system disorder. Smaller amygdale complex surfaces inside PTSD group comparing with the group of the participants with temporal epilepsy, cab be explained by atrophy of the observed structure, probably because of paradoxical neuroendocrine responses with PTSD.

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Influence of pre-operative disease course on the orthopedic operation and post-operative quality of life of adolescents with idiopathic scoliosis

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Abstract

Objective: To study the influence of pre-operative disease course on the orthopedic operation and post-operative quality of life of adolescents with idiopathic scoliosis (AIS).

Methods: A total of 200 AIS patients who were treated with posterior correction and all-pedicle internal fixation were divided into two groups according to their pre-operative disease courses (n=100): short course group with a pre-operative course <2 years(S group), long course group with a pre-operative course ≥2 years(L group). Matching design was made in the same gender and Lenke typing, and similar Cobb angles of the major curve between the two groups. Various radiographic measurements and indices like fusion level, intra-operative blood loss and scores of SRS-22 scale were compared between the two groups before and after the operation, and in the follow-up.

Results: The pre-operative Cobb angles of major curve were similar between the two groups, but the flexibility of the major curve in the S group was large than that in the L group ($P = 0.035$). Pre-operative Cobb angles of the minor curve were larger in L group than those in S group ($P = 0.036$). The coronal and sagittal radiographic measurements after the surgery were similar, and there were no statistical significance in differences of the intra-operative blood loss and transfusion between the two groups. The number of fused vertebrae in the L group was more than that in the S group (P=0.026). The parameters in the SRS-22 scale, including function/activity, pain, self-image/appearance, and mental health were not statistically significant between the two groups during follow-up. And the L group had a significantly lower satisfaction rate of treatment compared with the S group (P=0.039).

Conclusions: The flexibility of scoliosis in AIS decreases with the increase of disease course, and the disease course might be a risk factor for the progression of minor curve, which has an influence on the quality of life of AIS patients after operation.

Key words: Adolescent idiopathic scoliosis, disease course, quality of life.

Introduction

Many patients with adolescent idiopathic scoliosis (AIS) go to hospital for the first time only confronting physical deformities (such as razor back deformity, sloping shoulders and waist asymmetry, etc.) due to the insidious onset and indefinite onset age of the disease. In a developing country like China with an imperfect scoliolosis census system, this situation is more widespread. The treatment methods for AIS include surgery, orthosis and follow-up observation. In the AIS patients receiving brace treatment, about 20% to 24% need surgery eventually [1], as well as 13% of the patients under follow-up observation [2]. The risk factors in AIS progression include Cobb angular size, Risser sign and curve type, etc. Its rate of progress is quite different [3,4], which results in great difference in the pre-operative course of the disease. Therefore, the study aims to discuss the influence of pre-operative disease course on the orthopedic operation and post-operative quality of life of AIS patients through the analysis of clinical data of the patients admitted to our hospital between 2010 and 2011.

Materials and Methods

Inclusion criteria and baseline information

The AIS patients who received posterior orthopedic fusion and internal fixation of all-pedicle

screw in our hospital between 2010 and 2011 were selected. The inclusion criteria were as follows: (1) follow-up time was more than 2 years; (2) follow-up data were complete; (3) all patients received treatment for the first time for physical deformity, and the ones who found the disease due to physical examination or by chance (such as chest X-ray film) were excluded.

The patients were divided into two groups according to their pre-operative courses of disease: short course group with a pre-operation course <2 years(S group), long course group with a pre-operation course ≥2 years (L group). In order to control the interference of confounding factors, matching design was made in the same gender, Lenke typing [5], and similar Cobb angles of the major curve (a difference of less than 5 °) between the two groups. There were 100 enrolled cases who met the above criteria in the two groups respectively. There were 89 cases of female and 11 cases of male in each group, with 59 cases of Lenke type 1, accounting for 59%, 2 cases of type 2, 2%, 8 cases of type 3, 8%, 2 cases of type 4, 2%, 24 cases of type 5, 24% and 5 cases of Lenke type 6, 5%. In the S group, the average age was (14.7 ± 1.78) years old (ranging from 11. 0 to 18.1), mean follow-up time (3.4 ± 1.15) years (ranging from 2.0 to 5.5), average disease course (ranging from 7.8 ± 6.96) months (ranging from 0.2 to 20.1), and the mean Risser signs during the surgery (3.1 ± 44)° (ranging from 0° to 5°), and 19 cases or 19% of the patients had received pre-operative brace treatment. In the L group, the average age was (15.3 ± 1.75) years old (ranging from 11.2 to 18), mean follow-up time(3.2±1.25) years (ranging from 2.0-5.5), average disease course (46.5 ± 29.92) months (ranging from 24.0 to 178.5), and the mean Risser signs during the surgery (3.6 ± 1. 20)° (ranging from 0° to 5°) [6], and 48 cases or 48% of the patients had received pre-operative brace treatment. The differences of pre-operative duration and the number of cases receiving pre-operative brace treatment were statistically significant between the two groups (P <0. 0001).

Operative procedures and post-operative treatment

Posterior approach was used in all the patients, and all-pedicle screws were adopted with free hand insertion [7]. The internal fixation devices include Moss-Miami, CDH, and XIA (Johnson & Johnson). Bone graft fusion was performed by mixing materials of autologous bones and (or) Osteoset synthetic bones (Wright Company). The SSEP (somato-sensory evoked potentials) monitoring was conducted during the operation [8], and the awaken test was performed after the completion of the surgery. The patients did off-bed activities 3 days after the operation, and wore protective braces within 3 months after the surgery. All patients did not undergo post-operative pseudarthrosis, rod fracture and revision operation.

Imaging data measurement

Imaging data include pre-operative standing full-spinal anteroposterior and lateral films, left and right curved X-ray films, post-operative and follow-up erecting total-spine X-ray films, all of which were measured by the same orthopedic surgeon. The measurement and analysis of imaging data include the Cobb angles of the major and minor curves before the surgery, 3 days after the surgery and in the follow-up longer than 2 years. The lateral curvature flexibility was measured and calculated through the pre-operative left and right curved X-ray films. The thoracic kyphosis angles (T_5 to T_{12}) and lumbar lordosis angles (L_1 to S_1) were measured by lateral films.

Number of fused vertebrae, intra-operative blood loss and blood transfusion and SRS-22 scale

The number of fused vertebrae, the differential value between lower fused vertebrae and inferior terminal vertebrae, and the intra-operative blood loss and total blood transfusion were compared between the two groups. The patients were asked to fill in the SRS-22 scale in the final follow-up [9] so as to compare the scores of all dimensions in the SRS-22 scale between the two groups.

Statistical analysis

SPSS 16. 0 statistical software was adopted for statistical test on all parameters in the two groups of patients before and after surgery by t-test, with the test level (A) of 0.05.

Results

Coronal imaging measurement results

The results of imaging showed that: the pre-operative flexibility of major curve was larger in the S group [(55.6±18. 65) %] than that in the L group [(48.2±18. 19) %, P = 0. 035]. The immediately post-operative correction rates of the major curve were similar between the two groups [(71. 9 ±9. 92) % in the S group and (70. 0±10. 18) % in the L group, P =0. 198]. It was shown in Table 1 that there was no statistical significance in the difference of pre-operative Cobb angles of the major curve between the two groups, or in the difference of post-operative Cobb angles of the major curve in the last follow-up. The pre-operative Cobb angles of the minor curve in the L group was greater than that in the S group (P = 0. 036). The difference of immediately post-operative Cobb angles of the minor curve was not statistically significant between the two groups, nor the follow-up Cobb angles.

Sagittal imaging measurement results

Table 2 shows that the kyphosis angles of T5 to T12 thoracic vertebras were similar in the pre-operative rear lateral radiographs between the two groups. There was no significant difference in thoracic kyphosis angles in the immediate post-operative and final follow-up. The lumbar lordosis angles (L1 to S1) were similar between the two groups before surgery, immediately after surgery and in the final follow-up, without statistically significant difference.

Number of fused vertebrae, blood loss, blood transfusion and SRS-22 scale scores

Table 3 shows that the difference of the numbers of fused vertebrae was statistically significant (P = 0.026) between the two groups. There was no statistical significance in the difference of differential value between the numbers of lower fused vertebrae and inferior terminal vertebrae (P = 0.096), nor the intra-operative blood loss or blood transfusion between the two groups. Table 4 shows that the differences in scores were not statistically significant between the two groups, including function/activity, pain, self-image/appearance and mental health dimension. The L group had a significantly lower satisfaction rate of treatment compared with the S group (P = 0.039).

Table 1. Comparison of coronal plane changes between two groups

Group	Cobb angle of major curve			Cobb angle of minor curve		
	Preoperative	Postoperative	Follow-up	Preoperative	Postoperative	Follow-up
S	54.0±12.66	15.2±8.56	18.1±7.52	30.2±12.03	14.9±5.57	16.8±5.91
L	54.2±12.98	16.8±8.39	19.9±8.45	34.7±10.76	16.2±6.13	17.5±6.93
P value	0.802	0.335	0.243	0.036*	0.367	0.713

*S: Preoperative disease course< 2 years; L: Preoperative disease course≥2 years, *P < 0. 05*

Table 2. Comparison of sagittal plane change between two groups (n = 100, x±s, α/°)

Group	T_5-T_{12} kyphosis angle			L_1-S_1 lumbar lordosis angle		
	Preoperative	Postoperative	Follow-up	Preoperative	Postoperative	Follow-up
S	26.7±9.55	25.1±7.05	27.1±5.61	58.4±8.61	53.6±7.08	55.2±6.89
L	26.3±9.15	22.6±6.11	25.8±5.34	57.1±10.23	51.8±6.95	54.4±6.03
P value	0.423	0.159	0.512	0.369	0.243	0.401

S: Preoperative disease course< 2 years; L: Preoperative disease course≥2 years

Table 3. Comparison of fusion vertebrae number and intra operation blood loss between two groups (n =100, x±s)

Group	Number of fused vertebrae	<LEV	Blood loss V/ml	Blood transfusion V/ml
S	9.6±1.88	0.56±1.24	985±403	675±319
L	10.4±1.48	0.97±1.23	1065±379	760±289
P value	0.026*	0.096	0.384	0.153

*S: Preoperative disease course< 2 years; L: Preoperative disease course≥2 years; LEV: lower fused vertebrae and inferior terminal vertebrae. *P < 0. 05*

Table 4. Comparison of SRS-22 domains between 2 groups (n = 100, x±s)

Group	Function/activity	Pain	Self-image/appearance	Mental health dimension	Satisfaction rate
S	4.0±0.52	4.4±0.52	4.1±0.43	4.2±0.51	4.0±0.71
L	3.9±0.53	4.3±0.57	3.9±0.59	4.0±0.50	3.8±0.75
P value	0.091	0.283	0.193	0.237	0.039*

*S: Preoperative disease course< 2 years; L: Preoperative disease course≥2 years. *P < 0. 05*

Discussion

As AIS has an occult onset, the majority of AIS patients receive the first diagnosis and treatment due to physical deformities in mainland China where scoliosis screening and physical examination system have not yet perfect. A small number of patients find to have got the disease in medical examination or by chance (e.g. chest X-ray film). In this study, in order to reflect the natural course of scoliosis as much as possible, all enrolled patients were clients for the first diagnosis and treatment due to physical deformities. Giantin, et al. [10] found that the bending flexibility of idiopathic scoliosis was highly correlative with the patient's age (r = -0.6, P <0 01). Lustenberger, et al. [11] also reached a similar conclusion. In this study, although the patients had similar ages and Risser signs when receiving surgery, there was still a certain difference in curve flexibility between the two groups. It indicates that pre-operative duration of disease may also affect the curve flexibility of scoliosis. Although the flexibilities of the two groups were different to some extent, their main curve correction rates were similar, which might be for the reason that both of the groups used the all-pedicle screw system, and the powerful orthopedic force the system provided masked the difference of flexibility between the two groups.

The Cobb angles of the major curve were similar in size between the two groups but the Cobb angles of the minor curve were larger in L group than those in S group. It indicates that the size of Cobb angles of the minor curve is greatly influenced by the duration of disease, which will have a certain effect on the choice of fusion segments in scoliosis orthopaedic surgery. For the patients with Lenke type 1 and 5, fusion is not needed for non-structural minor curve, and selective fusion is only needed in the main curve. But during selective fusion, certain conditions need to be met. Ricart et al. [12] suggested that for patients with main thoracic curve, when the Cobb ratio of thoracic curve to lumbar curve is larger than or equal to 1.2, selective fusion of the thoracic curve can be performed. Ding et al. [13] determined that for the AIS patients with main thoracolumbar/lumbar curve, when the Cobb ratio of thoracolumbar curve to lumbar curve and thoracic curve is greater than or equal to 1.25, selective fusion of thoracolumbar/lumbar curve can be conducted. For patients with a pre-operation course ≥2 years, since the Cobb angles of minor curve increase, the ratio of Cobb angles of the major and minor curves is close to 1. Therefore, selective major curve fusion can not be made in the patients with Lenke type 1 or 5, and thereby fusion segments are increased, which may be one of the reasons that the fusion level in the L group is larger than that in the S group. Poor flexibility of major curve in the L group may be another reason for its high fusion level. Either in pre-operative or post-operative period, the course of disease does not have an influence on the thoracic kyphotic angles and lumbar lordosis angles of AIS patients, nor on the distal fusion level, intraoperative blood loss or total blood transfusion.

Idiopathic scoliosis does an obvious harm to the mental health of young patients [14-15]. Huber and Garcia et al. [16-17] studied the impact of age on the mental health of patients with idiopathic scoliosis, which found that in the patients younger than 15 years old who used the California Psychological Inventory for evaluation after surgery, all of its indicators were within the normal range. On the contrary, the scores of patients older than 16 years old were outside the normal range in the multiple subscales of pre-operative Minnesota Multiphasic Personality Inventory, which were deviated from the normal range after surgery to a higher degree [18,19]. In general, the older the age was, the longer the natural course of scoliosis would be. It seems to suggest that the duration of disease may have an influence on the mental health of patients [20]. However, the conclusion drawn

from the study that "the older the surgical age was, the worse the post-operative psychological outcome would be" is doubtful, because there is a large difference in cognitive maturity among adolescent patients at different ages [21].

In this study, the patients of the two groups had similar ages, however, no significant difference was found in the scores of mental health dimension. There are two possibilities: (1) The duration of disease does not affect a patient's mental health; (2) as there are only five items in the SRS-22 scale in this study for the evaluation of mental health, relatively low sensitivity led by too short scale cannot reflect the true state, and the pre-operative course of disease has no significant effect on the scores of the three dimensions of function/activity, pain and appearance.

Although the improvement in coronal and sagittal deformities was similar between the two groups, there was a significant difference in the scores of satisfaction dimension of the patients, in which patients with a disease course ≥2 years had a lower satisfaction rate of treatment than those with a disease course <2 years. It indicates that patients' expectation on surgery will rise with the increase of disease course, and high expectations often lead to decreased satisfaction on surgical treatment. In addition, pre-operative psychosocial status of AIS patients may affect their satisfaction for surgical treatment, and social psychosocial dysfunction existing before operation may increase the possibility of dissatisfaction for treatment [22,23]. Although the pre-operative psychosocial state of the patients in the two groups were not assessed in the study, it could be inferred that scoliosis might have a longer influence time on the patients in the L group which were more likely to have social psychosocial dysfunction. Of course, it still needs psychological assessment on pre-operative patients in different courses of disease to obtain the real situation.

The main objective of this study is to identify whether the pre-operative disease duration will exert an influence on the AIS orthopaedic surgery and the post-operative quality of life. However, due to the occult onset of scoliosis, it is extremely difficult to obtain the natural disease course of AIS patients, which can only be reflected during the period from finding the disease to surgical treatment. It is one of the limits in this study. The assessment of health-related quality of life of AIS patients is interfered by a number of objective and subjective factors, such as the personality of patient, family economic condition, and deformity rate of progress, etc., all of which are the interference factors of the study. Another disturbed factor is the difference in the number of pre-operative brace treatment cases between the two groups. Eidlitz-Markus and Lange, et al. [24,25] identified that whether to perform pre-operative brace treatment or not also influences the psychological conditions of AIS patients.

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Functions of Toll-like receptors in mediating inflammatory responses of corneal epithelial cells against Aspergillus fumigatus

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Abstract

Objective: To study the roles of Toll-like receptor 2 (TLR2) and TLR4 in mediating inflammatory responses of corneal epithelial cells against *Aspergillus fumigatus* (AF).

Methods: siRNA sequences targeting human TLR4 and TLR4 were designed to construct plasmids expressing TLR2-or TLR4-siRNA, to transfect corneal epithelial cells (THCE) respectively. TLR2 and TLR4 expression was measured by RT-PCR and Western blot and the suppression effects were evaluated. AF mycelium was used to respectively stimulate THCE with siRNA transfection and untransfected control cells to detect IL-1β and IL-6 levels in supernatant at different time points (1h, 3h, 6h, 12h) by ELISA. Results: After the transfection of THCE with TLR2-or TLR4-siRNA plasmid, the levels of TLR2 and TLR4 mRNAs and protein were significantly inhibited. After the stimulation of AF mycelium, the IL-1β and IL-6 expression in the control group was significantly increased, while that in the TLR2-or TLR4-siRNA treatment group was significantly lower compared with the control group.

Conclusion: THCE can recognize AF and mediate the expression of inflammatory cytokines through the expression of TLR2 and TLR4, which plays an important role in the innate immunity of corneal antifungal infections.

Key words: Toll-like receptor, Aspergillus fumigates, nflammatory cytokine, siRNA.

Introduction

Fungal keratitis is a common and severe disease which causes visual deficiency leading to blindness. In recent years, with the extensive application or even abuse of broad-spectrum antibiotics, corticosteroids and immunosuppressants, the incidence of fungal keratitis is on the rise year by year [1, 2]. Fungal keratitis has become the most intractable problem in infectious keratitis today, as well as one of the corneal infections with the most expensive treatment, which has drawn more and more attention of global ophthalmologists [3]. Some studies have shown that Toll-like receptors (TLRs) play a key role in the inflammatory response of corneal fungal infection. This study aims to explore the role of TLR2 and TLR4 in the inflammatory response of corneal fungal infection by constructing plasmids expressing TLR2-or TLR4-siRNA to inhibit TLR2 and TLR4 expression in THCE, which is of vital significance to exploring new and effective treatment of fungal infection by use of genetic intervention technologies.

Materials and Methods

Materials

AF was purchased from China Center for Type Culture Collection (CCTCC); THCE were offered for free by Prof. Fu-SKn X. Yu of the Wayne State University, U.S.A.; the DMEM medium the GIBICO, U.S.A.; Sabouroud medium, fetal bovine serum (FBS), 0.25% trypsin-EDTA digestive juice the Sigma, U.S.A.; Lipofectamine 2000 Invitrogen, U.S.A.; p-silencer 2.0 U6 vector and T4 DNA ligase the Takara, Dalian; TLR2, TLR4, β-actin antibodies the Santa Cruz, U.S.A.; IL-6 and IL-1 β ELISA kits the R & D Company, U.S.A.

Cell culture

AF was inoculated in 200ml sterilized Sabouroud liquid medium, cultured in shaking table at 37 °C for 24h. Every 10ml the said liquid medium containing AF was inoculated in 500ml Sabouroud medium, cultured in a shaking table at 500rpm,

26 °C for 24h. The culture was centrifuged at 3000rpm to precipitate AF mycelium which was rinsed with PBS for three times, then heated and inactivated at 56 °C for 60 min, and grinded by tissue homogenizer for a fragment of 20 ~ 40 μm long. The concentration of the mycelium suspension was adjusted to 5×10^6/ml mycelium fragment to prepare AF stimulus liquid, which was stored at -80 °C. THCE were cultured in DMEM medium containing 10% FBS for passage culture in 5% CO_2 incubator every 2-3 days.

Preparation of TLR2 and TLR4 siRNA

Specific siRNA sequences were designed respectively according to human TLR2 mRNA and TLR4 mRNA sequences and base sequence was rearranged, which didn't have homology with any gene of human through retrieval of GenBank BLAST, as the sequence for negative control (Table 1). siRNA template was designed according to the constitution of hairpin siRNA, including complementary DNA chains of each sense and antisense target sequences, restrictive endonuclease BamH I and Hind III recognition sequence, synthesized by Shanghai Bioasia Biotech Co, Ltd.

siRNA template oligonucleotides were dissolved with TE to adjust the concentration of 1 μg/μl. Annealing of siRNA template nucleotides: reaction was conducted in a 0.5ml sterile centrifuge tube, added in sequence as follows: 2μl sense siRNA template nucleotide, 2μl antisense siRNA template nucleotide, 25μl 2×DNA Annealing Solution, 50μl total reaction system, mixed gently and centrifuged slightly. The annealed siRNA template strand was stored at -20 °C after 90 °C for 3 min and 37 °C for 1 h.

The connection of template strand with pSilencer 2.0-U6 vector: it was successively added with 1μl 10 × ligase buffer, 1μl vector, 1μl template strand, 1μl T4 DNA ligase. The 10μl total reaction system was mixed gently, centrifuged slightly, and stored at 4 °C for overnight. Then, the following steps were taken: transformation of ligation product, screening and extraction of recombinant plasmid. A260 was measured after confirmation by sequencing, and then plasmids were quantified, and preserved at -20°C.

THCE transfection by TLR2 and TLR4 siRNA plasmids: THCE were cultured routinely to 80% -90% fusion. TLR2 and TLR4 siRNA plasmids were transfected to THCE by Lipofectamine 2000 transfection reagent. The operation was performed in accordance with instructions. Transfection control plasmid was set up in each group. The cells continued to be cultured in DMEM containing 10% FBS after transfection.

TLR2 and TLR4 expression suppression level detection: the cells of the interference and control groups were collected 72 hours after transfection. Total RNA and total protein of cells were extracted according to the 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 by 1% agarose gel electrophoresis, RT-PCR augmentation was conducted according to the instructions of reverse transcription kit. (The primer is the same as in table 2,

Table 1. TLR2 and TLR4 siRNA, and the control siRNA sequence

Template	Sequence
TLR2 siRNA template	AATCGGGAGGCTGCATATTCC
TLR4 siRNA template	GGTCAAGCTGGTTTAGAAG
Control siRNA template	AAGATGGGCATCAAGGTGAAC

Table 2. Real time PCR primer and product lengths

Gene	Primer sequence	Product length (bp)
TLR2	F: CTGCAAGCTGCGGAAGATAAT R: AGGACTTTATCGCAGCTCTCAGA	127
TLR4	F: GATTGCTCAGACCTGGCAGTT R: TGTCCTCCCACTCCAGGTAAGT	142
GAPDH	F: GAAGGTGAAGGTCGGAGTC R: GAAGATGGTGATGGGATTTC	152

synthesized by Shanghai Bioasia Biotech Co, Ltd.) Then, the following steps were taken: BCA protein kit quantification, conventional electrophoresis, transfer and immunoassay (40μg total protein in each group was sampled; TLR2, TLR4 and β-actin antibodies were diluted as per 1:500 and secondary antibodies labeled with horse radish peroxidase as per 1:2000), 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.

Cell culture and stimulation: normal THCE in the logarithmic growth phase and THCE for TLR2 and TLR4 siRNA plasmids transfection were selected. When the cells grew to 80% fusion, the AF stimulus solutions (10^6/ml) were replaced respectively, 5 parallel holes for each group. The cell supernatants were collected at 1h, 3h, 6h and 12h after stimulation, centrifuged at 3,000rpm for 10min to remove particles and polymers. The contents of IL-6 and IL-1β in supernatant were measured by ELISA. The coordinate points of each standard sample were connected by a smooth line, with standard concentration as the abscissa and standard absorbance (A)) value as the vertical axis. The concentrations of IL-6 and IL-1β of samples were calculated by the A value. The experiment was repeated three times to take the mean.

Results

Inhibited expressions of TLR2 and TLR4

As shown in Fig. 1, 2, the expression of TLR2 and TLR4 was significantly inhibited after THCE transfection by TLR2 or TLR4 siRNA. After TLR2 siRNA transfection, the suppression efficiency of TLR2 mRNA level was 54.7%, and that of protein level up to about 47.3%. After TLR4 siRNA transfection, the suppression efficiency of TLR4 mRNA level was 48.5%, and that of protein level up to about 57.9%. There was no significant difference in the TLR2 and TLR4 expressions among the control siRNA transfection group, empty vector group and blank control group. It is thus clear that specific TLR2-or TLR4-siRNA can effectively inhibit the expression of corneal epithelial TLR2 or TLR4.

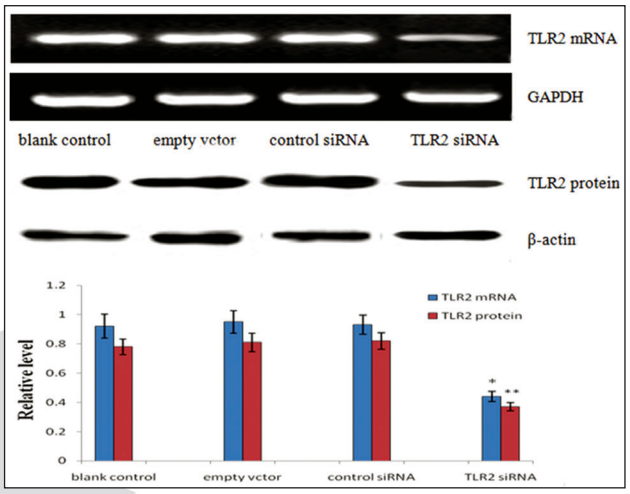

*Figure 1. Inhibited expressions of TLR2, *, ** P<0.05*

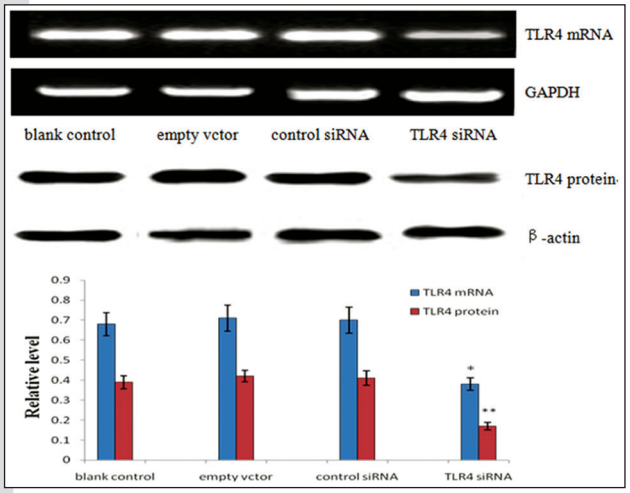

*Figure 2. Inhibited expression of TLR4, *, ** P<0.05*

Blocked inflammatory cytokine expression induced by AF

It was also confirmed that targeting TLR2 or TLR4 siRNA could significantly inhibit the expression of THCE TLR2 or TLR4, which was stimulated by AF mycelium respectively, and the changes of cytokine expression was detected by ELISA. The results were shown in Fig. 3, AF could stimulate the IL-6 and IL-1β expression in THCE was significantly increased, but that was significantly decreased by the AF stimulation after the treatment with TLR2-or TLR4-siRNA, which was significantly different compared with the non-interference group (P <0.05).

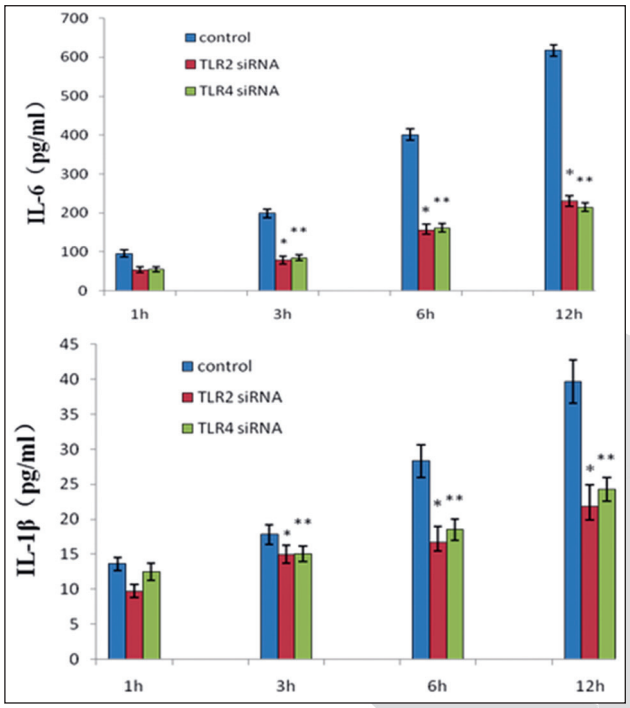

*Figure 3. Blocked expressions of IL-6 and IL-1β, *, ** P<0.05*

Discussion

The pathogenesis of corneal fungal infection has not been fully clear yet. The current research mainly focuses on two aspects: pathogen virulence and host immune mechanism. The innate immune recognition of fungus is fundamental to successful defense and clearance of fungal infection. Recent studies indicate that this defense mechanism is to activate effective immune defense response through the recognition of specific pathogens by TLRs, so as to achieve the purpose of resistance and clearance of pathogens [4]. TLRs are a group of transmembrane receptor family with leucine-rich repeat sequence. There have been a total of 13 TLRs from TLRl to TLR13 found in dendritic cells, monocytes, epithelial cells and vascular endothelial cells of mammals and human so far, which activate downstream signal transduction chain via myeloid differentiation factor 88 (MyD88)-dependent pathway or MyD88-independent pathway, to finally activate nuclear transcription factor κB (NF-κB), interferon regulatory factor (IRF) -3 and other transcription factors, to induce host cells for the expression of inflammatory cytokines and inflammatory cell chemotaxis and infiltration, so as to induce inflammatory response and activate the innate immunity [5-7].

Toll-like receptors were first discovered in drosophila, invoved in its embryo development and defense response. They were also found in the surface of mammalian cells in 1997. A total of 13 members have been found in the mammalian and human TLRs family. Structural studies have shown that TLRs are type I transmembrane protein which is composed of three parts: extracellular domain, cytoplasmic domain and transmembrane domain. TLRs extracellular domain has Leucine-rich repeats (LRR), containing a plurality of ligand binding regions, which participate in the identification of pathogenic microorganisms or its products, and its gene sequence is variable, which is related to the specificity of host response to infection. A conserved sequence exists in TLRs cytoplasmic domain, with high homology to the conserved sequence in the intracellular region of Interleukin-1 receptor (IL-1R), which is called TLR/IL-R homologous region (TIR). It, composed of about 200 amino acid residues, is a key part for the interaction of TLRs with its downstream protein kinase [8]. TLR can identify specific pathogen-associated molecular structure (PAMP), i.e. ligand, through its extracellular region. TLRs have different phenotypes, some expressed in the cell surface and some within cells. TLR phenotypes are related to the characteristics of their ligands, for example, TLR1, 2, 4, 5 and 6 function to recognize the cell wall components of bacteria and other microorganisms, with the expression in cell membrane, while TLR3, 7, 8 and 9 the nucleotide composition of bacteria or virus, with the expression within cell [9].

TLR4, expressed on the surface of human THCE, can react to the stimulation of LPS extracted from Pseudomonas aeruginosa so as to produce inflammatory cytokines and chemokines [10, 11], which is conducive to quickly starting innate immunity and raising inflammatory cells to the infection focus when Grand-negative bacterial infection occurs, in order to effectively remove pathogens. Similarly, it is reported that TLR2 expressed in THCE may trigger an immune response to the PGN of Staphylococcus aureus, secrete inflammatory cytokines, chemokines and antimicrobial peptides, which is of great significance in the pathogenesis of Grand-positive bacterial keratitis [12]. However, Ueta et al. [13] found that TLR2

and TLR4 expression in human THCE is located within the cell, which can not react to LPS and PGN, neither inducing NF-κB transcription, nor mediating the production of inflammatory cytokines. Therefore, it is inferred that an immune stationary state may exist in THCE in order to avoid unnecessary inflammatory response. As THCE are exposed to the commensal flora in the conjunctival sac, there may be a unique down-regulation mechanism so as to avoid adverse TLR activation.

Studies have shown that TLRs of corneal epithelial expression can mediate host's recognition to a variety of pathogens such as Staphylococcus aureus [14, 15], Pseudomonas aeruginosa [16, 17], Wolbachia bacteria [18], etc. and induce inflammatory response, but whether the corneal TLRs mediate the identification of fungi is rarely reported. This study found that the expression of inflammatory cytokines IL-1β and IL-6 was significantly increased when THCE are stimulated by AF, however, AF-induced IL-1β and IL -6 levels were significantly reduced after TLR2 or TLR4 expression in THCE was inhibited by use of RNA interference technologies. This suggests that TLR2 and TLR4 are major receptors of THCE in AF recognition, mediating the inflammatory response of THCE to AF. To further explore the fungal–epithelial interaction and then the activation mechanism of induced stroma cells will help us carry out in-depth studies on the pathogenesis of corneal fungal infection and lead to a new approach to explore new strategy of effectively enhancing the host defense and preventing the consequences of destructive inflammation.

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Pretreatment with melatonin supresses the gentamicin induced renal histological changes

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Abstract

Gentamicin-induced nephrotoxicity is an important cause of renal failure. Because melatonin has potent antioxidative properties, the aim of this study was to determine the potential protective effect of melatonin pretreatment in gentamicin induced nephrotoxicity in rats. Male Wistar albino rats, weighing 200–300 g were divided into 4 groups: vehicle; gentamicin 80mg/kg intraperitoneally for 8 days; melatonin 10 mg/kg administered 3 days prior and concurrently with gentamicin for 8 days; melatonin 10 mg/kg intraperitoneally for 11 days. The histological evaluation of gentamicin-induced structural alterations was performed in order to determine potential beneficial effects of melatonin coadministration with gentamicin. Gentamicin was observed to cause a severe nephrotoxicity which was evidenced by severe tubular necrosis and tubular epithelial loss resulting in tubular cast formation. The glomeruli showed lobularity and dilatation. The interstitium was edematous and infilitrated with mononuclear cells. However, these alterations were considerably reduced with pretreatment and simultaneous administration of melatonin. In conclusion, our results indicate that pretreatment with melatonin attenuates gentamicin associated renal histological changes.

Key words: Melatonin, pretreatment, gentamicin, renal histology.

Introduction

Gentamicin (GM), an aminoglyoside antibiotic, is very effective in the treatment of life-threatening infections caused by Gram negative bacteria (1,2). In many cases, it has been the only effective agent against bacterial strains resistant to other antibiotics (3). Unfortunately, a major complication of therapeutic doses of GM is nephrotoxicity that limits its use (3,4,5). Although the mechanism of the genesis of gentamicin nephrotoxicity is still not completely clear, it is assumed that the reactive oxygen species (ROS) are one of the main causative agents (6,7). The activity of antioxidant enzymes i.e. superoxide dismutase, catalase and glutathion peroxidase is also reduced by gentamicin (8,9,10). Cellular injury and necrosis caused by ROS are the result of DNA, lipid and protein damage (10,11).

Melatonin (N-acetyl-5-methoxytryptamine) is mainly synthesized by the pineal gland (12). It has been shown that it is involved in numerous physiological events including the regulation of circadian rhythm (13), reproduction (14), but it has also antioxidant properties (15).

Because of the increased oxidative stress in the treatment with gentamicin, it seemed reasonable that increasing intrarenal levels of melatonin would decrease oxidant-induced kidney damage. Therefore, the aim of this study was to investigate whether melatonin prevents the renal changes caused by gentamicin using histological examination.

Material and Methods

Thirty two male Wistar rats, weighing 200–300g, were maintained in standardized laboratory conditions with a temperature of 23 ± 2°C, and a 12-hour light-dark cycle. Both standard rat chow and water were provided *ad libitum*. After an acclimation period of 7 days, the rats were randomly divided into four groups, each consisting of 8 animals. Rats of the first group served as controls and received intraperitoneal injection of vehicle (5% ethanol in Ringer solution) during 11 days. Rats of the second group received intraperitoneally gentamicin (80 mg/kg) during 8 days (G). Animals of the third group were intraperitoneally injected with gentamicin (80 mg/kg) during 8 days and

melatonin (10 mg/kg) 3 days before and 8 days concomitantly with gentamicin (GM). Rats of the fourth group received only melatonin (10 mg/kg) during 11 days (M).

The animals were sacrificed under ether anesthesia 24 hours after the last injection. Left kidneys were quickly removed, immersed in 10% formalin, dehydrated and embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (H&E) and periodic acid - Schiff (PAS) staining.

The histological analysis included qualitative and semiquantitative analysis at the level of light microscopy. A minimum of 8 fields for each kidney section were examined and assigned for severity of changes. To evaluate the level of damages, indexes such as tubular degeneration and necrosis, mononuclear cell infiltration, and hyaline casts were scored numerically. The evaluation criteria were as follows: 0 for no detectable lesion (-), 1 for mild changes (+), 2 for moderate changes (++), and 3 for severe changes (+++).

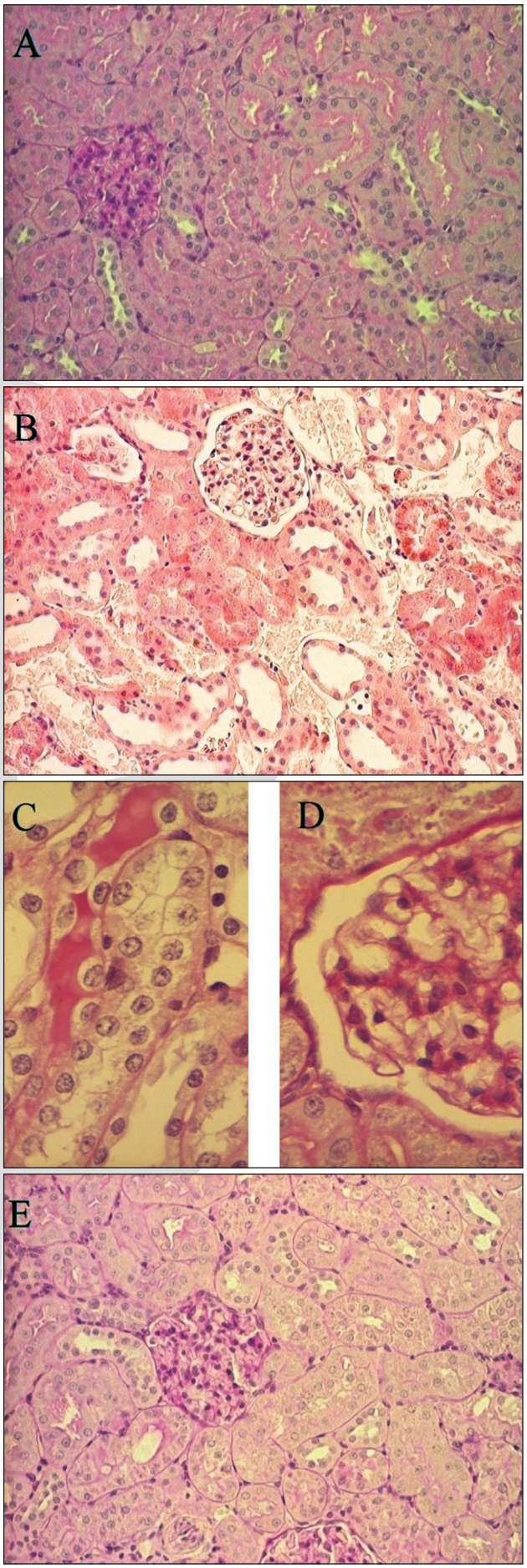
The study was carried out at the Institute of Histology and Embryology of the Faculty of Medicine in Sarajevo. All experiments were done with the approval from the Local Ethics Committee.

Results

Lightmicroscopic examination of kidneys from control and melatonin treated rats showed normal structural characteristics (Figures 1A and 1F).

Table 1. The effect of melatonin administration on renal morphological changes

Histological changes	Control	M	G	GM
Tubular degeneration and necrosis	—	—	++/+++	+/++
Hyaline casts in tubular lumen	—	—	+	—
Interstitial edema	—	—	++	+
Interstitial inflammation	—	—	++	+
Urinary space dilatation	—	—	++	—
Glomerular hyperemia	—	—	++	—



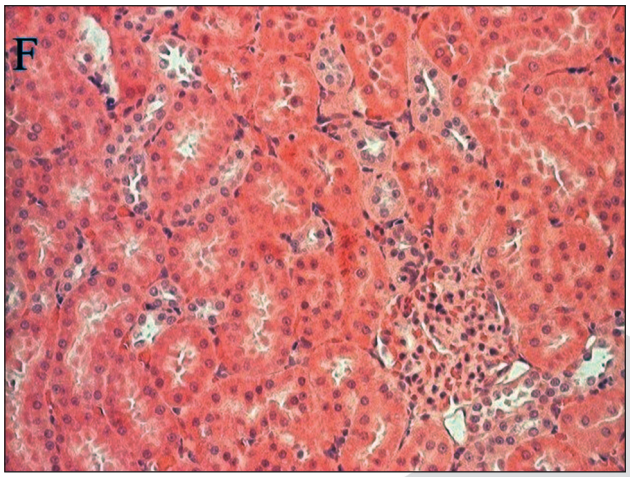


Figure 1. Histological changes in renal tissues in response to gentamicin and gentamicin+melatonin:
A Control (PAS, x 100);
B gentamicin treatment alone (HE, x 100),
C gentamicin treatment alone (PAS, x 400), hyaline cast,
D gentamicin treatment alone (PAS, x 400), dilated urinary space,
E gentamicin+melatonin treatment (PAS, x 100).
F melatonin treatment alone (HE, x 100),

In the proximal tubules of kidneys from the animals treated with gentamicin, diffuse and severe cell necrosis and *granulovacuolar degeneration* were observed (Figure 1B). In addition, degenerated and desquamated epithelial cells were found in the lumens of these tubules. Hyaline casts could be observed in some renal tubules (Figure 1C). Severe mononuclear inflammatory cell infiltrate and interstitial edema were observed (Tab. 1). The analysis also revealed changes of the glomeruli structure represented by increased *lobulation* of the *glomerular tufts*, dilatation and congestion of the glomerular capillaries and diffuse and irregular thickening of the glomerular basement membrane. Dilatation of urinary spaces and thickening of the basal membrane of the parietal layer of the Bowman's capsule were also observed in the renal sections of this group (Figure 1D and Table 1).

The analysis of kidneys from rats treated with gentamicin and melatonin revealed significant improvement in the microstructure of glomeruli and renal tubules compared to the gentamicin treated group (Figure 1E).

Discussion

Gentamicin induced nephrotoxicity is an important clinical cause of acute renal failure (16), but because of its relatively low cost and its rapid action it is still an important antibiotic in the treatment of Gram negative bacterial infections (1,2,17). The genesis of gentamicin nephrotoxicity is still not completely elucidated, but it is supposed that a connection between mechanisms of tubular and glomerular changes involving oxidative stress is the main factor for its development (18,19). The present work demonstrates that rats injected with gentamicin in a dose of 80 mg/kg during eight days display a pronounced damage in renal microstructure. Significant histological changes of the proximal tubules in form of severe necrosis and granulovacuolar degeneration, followed by an extensive mononuclear infiltrate could be observed. Gentamicin toxicity also included the glomeruli that were hyperemic, the glomerular capillaries were dilated and the glomerular basement membrane was thickened. These changes are mostly in accordance with the changes already described by other authors. (19,20,21,22). The dilatation of urinary spaces and thickening of the basement membrane of the parietal layer of Bowman's capsule were also noticed. It has been found that the basement membrane of the glomerulus is essential for a normal functioning of the glomerular filtration barrier (23). Dilatation of the Bowman's spaces could be the result of changes in the epithelium of proximal tubules and the glomerular basement membrane. This alters the functional properties of proximal tubules in the sense of their decrease and results in a glomerular filtration rate decrease and the accumulation of urine in the urinary space (24). Because irreversible renal cell injury and necrosis can be prevented by scavenging of free oxygen radicals (25) in order to develop therapies to minimize oxidative damage, several substances with a possible protective action have been investigated. Administration of different antioxidants, i.e. vitamin E (26), vitamin C (27), etc. reduces the nephrotoxic symptoms produced by gentamicin, but the complete protection could not be reached. Melatonin is a potent antioxidant, a highly efficient free-radical scavenger (28). In conditions of high oxidative stress *in vivo*, melatonin has shown

to be more potent than vitamins C and E in sense of minimizing oxidative damage (29). Therefore, we have decided to test the protective potential of melatonin regarding the gentamicin induced histological changes. Our results show that melatonin pretreatment (10 mg/kg) had beneficial properties on the rat renal microstructural changes; the tubular changes were less pronounced and we could not observe any alterations of the renal corpuscules. Several studies have shown the beneficial effect of melatonin on oxidative induced renal damage in different conditions such as ischemia-reperfusion injury (30), cisplatin (31), heavy metals induced damage (32,33), etc. The results of the research of Nava et al (32) have shown that melatonin reduces the toxic effect of mercuric chloride (2.5 mg/kg sc.) in the sense of reducing oxidative stress and acute renal failure. They have also demonstrated that histological changes in the group treated with melatonin in the form of pretreatment were significantly less pronounced than in the group that did not receive melatonin and that melatonin given at the same time as the toxic substance had no protective impact on the development of micromorphological changes. In a study of Kilic et al (34), melatonin (10 mg/kg) has shown to be a potent agent against the structural alteration of proximal tubules, which confirms our results in the sense of melatonin being an effective agent in the reduction of oxidative damage. Melatonin has reduced tubular necrosis and interstitial inflammation caused by cisplatin. Also Yousef et al (35) have shown that melatonin (10 mg/kg) has renoprotective effects on colistin induced renal changes in rats. Melatonin injected alone did not show any significant effect on the structure of rat kidneys.

Conclusion

Gentamicin induced severe tubular changes and glomerular changes. Melatonin pretreatment prevented gentamicin-induced renal microstructural alterations. Exogenous melatonin given alone did not cause any histological changes. This implicates the potential use of melatonin as a therapeutic agent against acute kidney failure induced by gentamicin.

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Silibin inhibits the epidermal growth factor-induced epithelial mesenchymal transition in A549 and H1650 cells

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Abstract

Context: Non-small-cell lung carcinoma (NSCLC), which accounts for approximately 85% of all lung cancers, performs aggressively, proliferates rapidly, spreads to distant sites early, is sensitive to chemotherapy and radiation extremely and is associated with distinct paraneoplastic syndromes. NSCLC requires the potentiation of epidermal growth factor receptor (EGFR) inhibitors to improve their therapeutic index. Silibinin plus the EGFR tyrosine kinase inhibitor gefitinib synergetically inhibited the proliferation, migration and invasion of NSCLC cells. The reversion of epithelial-to-mesenchymal transition (EMT) by silibinin also results from the cooperation.

Objective: Although the anticancer mechanisms of individual silibinin and the antineoplastic activities of silibinin plus several clinically approved antitumor drugs have been documented, the treatment efficacy of silibinin combined with gefitinib in NSCLC has never been evaluated.

Materials and methods:

Results: In the epithelial A549/H1650 cells expressing EGFR which had undergone EMT and expressed extremely high levels of EGFR, silibinin down-regulated the expression and PI3K/AKT signaling of EGFR, and reverted the mesenchymal phenotype by inducing E-cadherin and down-regulating vimentin as well as N-cadherin.

Discussion and conclusion: This study suggests that the silibinin/gefitinib combination is a feasible therapeutic strategy that guides further clinical evaluation on NSCLC.

Key words: silibinin; gefitinib; non-small cell lung cancer; EGFR; epithelial-to-mesenchymal transition

Introduction

Lung cancer is one of the most common malignancies leading to cancer death all over the world (*1*). Despite the advances in the molecular mechanisms of lung cancer development, prevention and treatment, non-small-cell lung carcinoma (NSCLC) still kills more than 1012,000 patients worldwide annually (*2*). The poor prognosis of NSCLC prevents the treatment of local and regional metastasis in most patients, and leads to an unsatisfactory responsiveness to conventional systemic therapy in recurrent/advanced diseases (*3*). A previous study showed that the over-expression rate of epidermal growth factor receptor (EGFR) in NSCLC is 40-80% and plays a key role in tumorigenesis (*4*). In addition, EGFR-driven cell signaling contributes to disease progression and cancer malignancy, which provide an effective therapeutic target for developing agents resisting to NSCLC (*5-7*).

Gefitinib, an EGFR tyrosine kinase inhibitor (TKI), was the first selective small molecular agent that effectively blocked EGFR phosphorylation and downstream signaling and enabled NSCLC treatment (*8*). However, it has been recently reported the pharmacogenomics of EGFR limited the clinical applications of gefitinib, i.e. the sensitivity of NSCLC to gefitinib is highly correlated with epithelial-to-mesenchymal transition (EMT) (*9*). The trans-differentiation of epithelial cells into mesenchymal cells, known as EMT, is an indispensable process during embryonic development and associated with the development of invasive cancer. EMF may function in establishing the resistance to EGFR TKI in several tumors, especially in NSCLC (*10*). Besides, the mesenchymal-enriched subtype is a distinct type of NSCLC with a defined recurrence-free survival prognosis (*11, 12*).

The EMT-involving mechanisms are associated with the master developmental and oncogenic pathways regulating NSCLC growth, angiogenesis and metastasis, as well as the reprogrammation of specific gene repertoires ascribed to epithelial and mesenchymal cells (*9, 13, 14*). The EMT in NSCLC is stimulated within the tumor microenvironment owing to various reasons. The growth factors that bind to tyrosine-kinase receptors (TKR), such as fibroblast growth factor (FGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) (*10*), all facilitate EMT exogenously. Phosphatidylinositol 3-kinase/ protein kinase B (PI3K/ Akt) cascade is subsequently activated by EGFR among the essential intracellular signal transduction cascades (*15, 16*). The downstream EGFR signaling activates the PI3K/Akt pathway, which is an important intracellular mediator involved in proliferation, migration/invasion and angiogenesis (*17*). Akt activity is relevant to NSCLC progression and poor clinical outcome. The viable non-small cell lung cancer therapy inhibited Akt in the mice with NSCLC (*18*). Therefore, suppressing PI3K/ Akt pathway hypothetically serves as a novel therapeutic intervention in advanced NSCLC. Thus, new therapies combining TKI agents and/or EMT signaling inhibitor potentially circumvent the chemotherapeutic resistance of NSCLC featured by transient or sustained EMT signatures (*19-22*).

Silibinin, which is a flavanoid obtained from milk thistle, is able to down-regulate EGFR and PI3K/Akt activity that inhibits the growth of cancer cells (*23-25*). Moreover, silibinin also suppressed the growth of A549/H1650 by reducing EGFR genes expression (*26, 27*). The down-regulation of EGFR signaling by silibinin in NSCLC owing to the modulated EGFR protein levels suggests that silibinin inhibits the intrinsic EGFR tyrosine kinase activity and suppresses ligand-induced EGFR activation (*23, 25, 28*).

Although the anticancer mechanisms of individual silibinin and the antineoplastic activities of silibinin plus several clinically approved antitumor drugs have been documented, the treatment efficacy of silibinin combined with gefitinib in NSCLC has never been evaluated. Thereby motivated, we herein analyzed the antitumor effects of silibinin plus EGFR TKI gefitinib on a panel of NSCLC cell lines. We demonstrated that they synergetically inhibited proliferation, migration and invasion as well as mesenchymal markers and phenotype in A549/ H1650. Besides, silibinin enhanced the antitumor effects of gefitinib in NSCLC cancer cell lines by inhibiting EGFR-mediated EMT at least partially inhibiting the expression of PI3K/AKT signaling.

Materials and methods

Reagents

Gefitinib (ZD1839, Iressa TM) was provided by AstraZeneca Pharmaceuticals (Macclesfield, UK). Silibinin, which was purchased from Xi'an Helin Biological Engineering Co., Ltd. (Xi'an, Shaanxi, China), was dissolved in dimethylsulfoxide (DMSO) and then added to the media at indicated concentrations to limit the DMSO concentration below 0.1%. The antibodies against N-cadherin, E-cadherin, vimentin and GAPDH were purchased from Bioworld Technology (St. Louis Park, MN, USA), and the antibodies to PI3K/AKT were purchased from Signalway Antibody (Pearland, TX, USA). The EMT sample kit was purchased from Cell Signaling Technology (Danvers, MA, USA).

Cell culture

Human A549 and H1650 NSCLC cells (American Type Culture Collection, Manassas, VA, USA) were grown in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were cultivated in a humidified incubator (37˚C, 5% CO_2) and detached with 0.25% trypsin-0.02% EDTA (Gibco). All other cell culture reagents were obtained From Gibco.

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay

The impacts of silibinin plus gefitinib on A549/ H1650 cell proliferation and viability were evaluated using an MTS assay with the CellTiter 96 ® AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI, USA). Briefly, exponentially growing cells were trypsinized and seeded in 96-well plates ($5x10^3$/well) in complete medium. After being incubated for 24 h, the cells were incubated in the presence or in the absence of silibinin/gefitinib at indicated concentrations

for 24 h. Subsequently, 20 ml of the solution was added to each well. After incubating the plate for 3 h at 37˚C, the optical density (OD) was measured at 490 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Scratch assay or wound healing assay

The wound healing assay was performed to detect A549/H1650 migration. Briefly, A549/H1650 were grown to full confluence in six-well plates and starved overnight. The cell monolayers were wounded with 100 mL of sterile pipette tip, and washed with starvation medium to remove the detached cells. The cells were left either untreated or treated with indicated doses of silibinin/gefitinib in full medium and kept in a CO_2 incubator for 24 h. Thereafter the medium was replaced with phosphate-buffered saline (PBS) buffer, the wound gap was observed and the cells were photographed using an Olympus BX41 microscope fitted with a digital camera.

Cell migration assay

A549/H1650 motility was tested in a Transwell Boyden Chamber using a polycarbonate filter (8 μm pores) coated with 0.1% w/v gelatin in the upper chamber. Briefly, tumor cells (2×10^6) were added to the upper chamber in the absence or in the presence of silibinin, gefitinib or silibinin plus gefitinib at indicated concentrations, while the lower chamber was filled with 600 μl of DMEM with 1% v/v FBS. After being incubated for 6 h, the cells on the top of the filter were wiped with a cotton swab. The filter was fixed by 5% glutaraldehyde at 4℃ and stained with 0.1% crystal violet stain solution, and the cells beneath the surface of the filter, which penetrated the pore of the filter, were fixed onto a glass slide. The cells in five randomly selected microscopic fields (×400) of the lower slide were counted. The experiments were performed in triplicate independently.

Western blot analysis

A549/H1650 were washed with cold PBS buffer and lysed in 10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM EDTA, 1% Nonidet P-40 and 10 μg/ml phenylmethylsulfonyl fluoride. The cell extracts were then transferred to microcentrifuge tubes, mixed and left on ice for 10 min. They were then centrifuged at 12,000 x g for 5 min at 4˚C after 1 freeze/thaw cycle. The protein concentrations in the supernatants were determined using a BCA™ protein assay kit (Pierce, Rockford, IL, USA). The protein samples (50 μg/lane) were resolved by SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The blots were incubated with primary antibodies diluted with Tris-buffered saline-Tween-20 containing 2.5% dried milk overnight. After successive washes with Tris-buffered saline-Tween-20, the membranes were incubated with the corresponding secondary antibodies conjugated to horseradish peroxidase. A SuperSignal kit (Pierce) was used to visualize the immunoreactive bands according to the manufacturer's instructions.

Statistical analysis

The results were analyzed by two-tailed student t-test SD using SPSS 11.0 (Aspire Software International, Leesburg, VA) to calculate the P values. The difference was considered significant if $P < 0.05$.

Results

Effect of silibinin plus gefitinib on proliferation

We first evaluated the anti-proliferative effects of vorinostat on two NSCLC cell lines (A549 and H1650) expressing epithelial and mesenchymal markers (Figure 1A) and EGFR protein levels differently (Figure 1A). Specifically, the considerable expressions of E-cadherin which is related to an epithelial phenotype (*10, 29*) were observed in A549/H1650, and the lowly expressed N-cadherin marking the mesenchymal phenotype was detected in both A549 and H1650. Moreover, A549/H1650 expressed a higher level of vimentin, another epithelial marker. A549 and H1650 both over-expressed EGFR, which are closely epithelial-related.

We examined the anti-proliferative effects of silibinin plus gefitinib on human NSCLC (H1650 and A549) using the MTT assay which measures the mitochondrial dehydrogenase activity within the cell and thereby indicates the cellular proliferation status. Figure 1B shows that the growth of A549 is inhibited moderately by 1 μM gefitinib or 100 μM silibinin, respectively. Silibinin (40 μM) could inhibited the H1650 growth. Combining silibinin with gefitinib inhibited cell growth significantly more pronouncedly than the single-agent treatment did ($P < 0.05$) (Figure 1A).

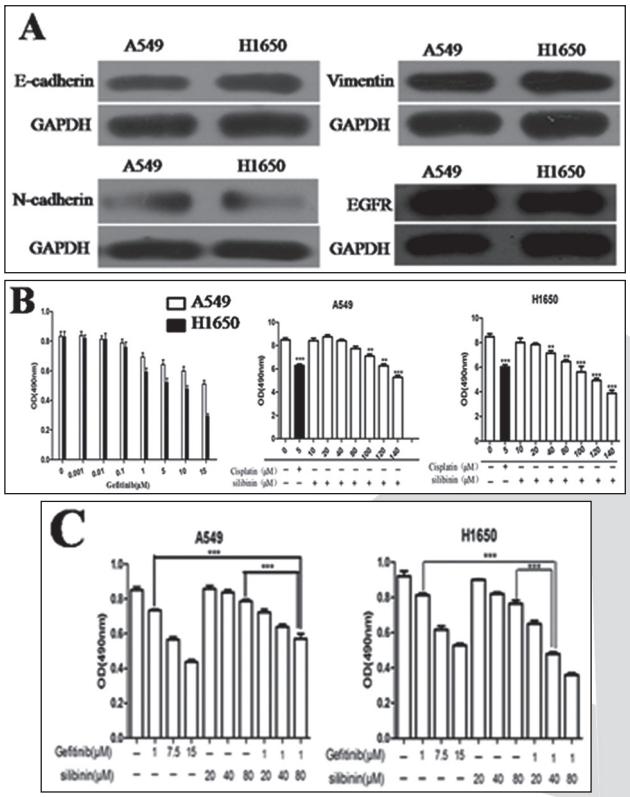

Figure 1. Effect of silibinin plus gefitinib on the proliferation of tumor cells. Anti-proliferative effects of silibinin plus gefitinib were examined in H1650 and A549 cell lines. Tumor cells (1500/well) were seeded into 96-well plates and subsequently exposed to silibinin plus gefitinib for 48 h. The number of viable cells in each well was estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay described in "Materials and Methods". The results were expressed as the percentage of cell growth relative to controls. Each point represents the mean SD of five determinations. Similar results were obtained in replicate experiments. The data points are connected by lines owing to the ease of trend visualization rather than the linkage by chronology or dose.

Effect of silibinin plus gefitinib on A549/H1650 mobility, migration and invasion

Cell migration dominates invasion by allowing primary tumors to metastasize. To examine the migratory behaviors of A549/H1650 under identical conditions, cells were cultivated in Transwell chambers for 24 h in an incubator to assess their migration capability. Figure 2B exhibits that the cell migration capacity of A549/H1650 was significantly lower than that of the control group. Cell mobility measures the metastatic potential of cancer cells. In the wound healing assay, the confluent monolayers of cells were scratched to wound and cultured with various concentrations of silibinin or/and gefitinib for 36 h. Elevating the concentration of silibinin plus gefitinib decreased the wound healing cell mobility of A549/H1650 dose-dependently (Figure 2A). The combination of silibinin with gefitinib inhibited A549/H1650 mobility and migration more effectively than the single-agent treatment did.

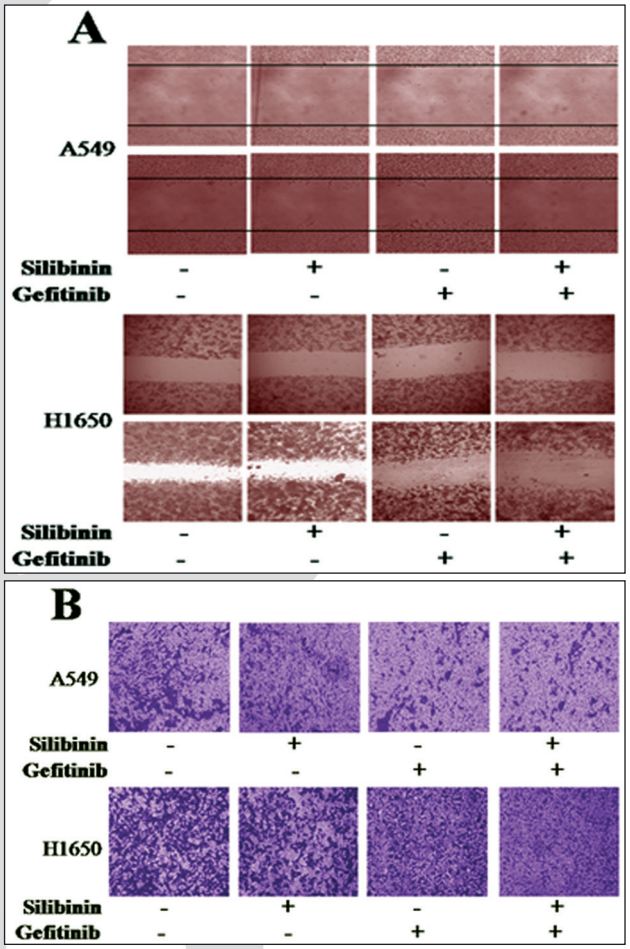

Figure 2. Effect of silibinin plus gefitinib on A549/H1650 cell motility in vitro. A549/H1650 monolayer cells were scraped and treated with silibinin or/and gefitinib, and those in the denuded zone were photographed after indication (0–24 h). The black lines indicate the wound edge. (A) indicates the wound edge changes treated in identical conditions for 24 h. The ability of tumor migration plays a key role in metastasis. We thus evaluated the effect of silibinin plus gefitinib on A549/H1650 migration. The results showed that silibinin plus gefitinib inhibited A549/H1650 mobility and migration more effectively than the single-agent treatment did. (Figure 2B).

Effect of silibinin plus gefitinib on EGFR signaling

To further characterize the downstream EGFR signaling that might correlate with the observed growth inhibition, we examined the influences of anti-EGFR agents on the expressions of several key regulators involved in the EGFR signaling pathway. The Western blot analysis in Figure 3 displays that the single-agent treatment with EGFR inhibitors lowers the EGFR expressions in A549 and H1650. Thereafter the dual anti-EGFR agents further reduced the expression level of PI3K/AKT. The levels of N-cadherin, E-cadherin and vimentin, and downstream regulators of EGFR signaling were dramatically down-regulated upon the dual anti-EGFR inhibitor treatment. These results suggest that silibinin plus gefitinib functioned better than the individual agent did in the inhibition of EGFR signaling.

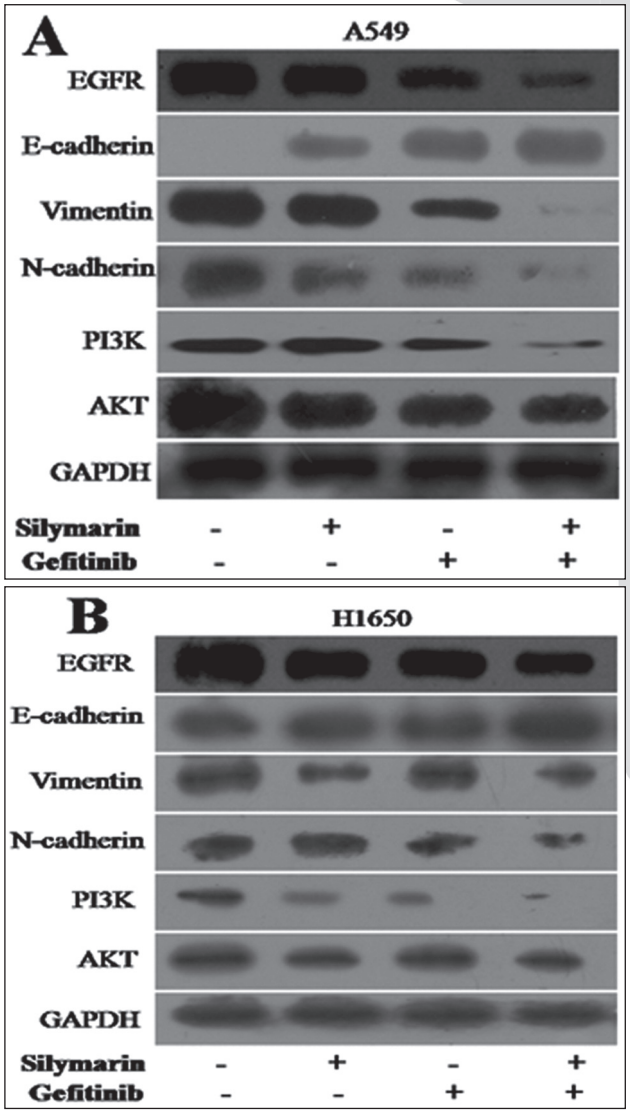

Figure 3. Inhibition of EGFR expression and EMT reversion by silibinin in A549 and H1650 cells. A549/H1650 were treated with the vehicle (0.1% DMSO) or identical conditions for 24 h, and EMT-related proteins and PI3K/AKT were then analyzed by western blot with the indicated antibodies. GAPDH was used as the loading control.

Discussion

Although EGFR is a well-established anticancer target, major cancer patients do not respond to EGFR inhibitors, implying the intrinsic resistance. Besides, most responding patients will eventually progress, implying the acquired resistance (*5, 17, 18*). One of the most extensively accessed mechanisms contributing to the resistance to EGFR inhibitors is the oncogenic shift that triggers EGFR-activated EMT (*9, 21*). In this study, silibinin cooperated with the EGFR TKI gefitinib in the anti-proliferative and inhibits mobility of NSCLC cells, which may originate from the different silibinin-involved EGFR modulations. We also demonstrated silibinin down-regulated the expression and signaling of EGFR in A549/H1650 cancer cells with a prevalent epithelial phenotype.

We showed that silibinin may boost the antitumor effects of gefitinib on NSCLC cells. Both A549 and H1650 cells underwent EMT, as demonstrated by the loss of E-cadherin, the induction of vimentin expression and the expression of extremely high EGFR levels. EMT has been widely correlated with tumor aggressiveness, elevating metastatic potential, and therapeutic resistance to EGFR TKIs in several cancers (*10, 12, 18, 20, 22*). We verified silibinin reversed the mesenchymal phenotype in both A549 and H1650 cell lines by inducing E-cadherin expression and down-regulating the expressions of vimentin and EGFR. In consistent with our data, it has been recently reported that silibinin may reverse the EMT by inducing E-cadherin expression in NSCLC cells.

Thus, we assume that the synergy between silibinin and gefitinib was multi-factorial. In the epithelial NSCLC cells expressing EGFR, silibinin functions by down-regulating the receptor expression and signaling, thereby strengthening the antitumor activity of gefitinib via efficiently and differently blocking the EGFR signaling pathways such as the

PI3K/AKT pathway. On the other hand, silibinin not only enhances the antitumor activity of gefitinib by down-regulating EGFR in the mesenchymal A549 and H1650 cells expressing high levels of EGFR, but also functions through the reversion of EMT. Furthermore, silibinin and gefitinib cooperated in the inhibition of migration and invasion in both mesenchymal A549 and H1650 cells, confirming that the sophisticated antitumor interaction between these two agents is, at least partially, related to the EMT reversion. The interaction pattern in Figure 4 schematizes the predominantly enhanced antitumor effects of the combined treatment the on both A549 and H1650 cells via the down-regulation of EGFR activation and the PI3K/AKT downstream signaling pathway.

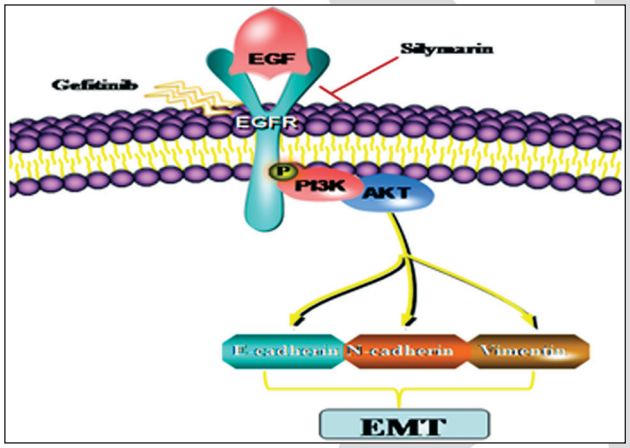

Figure 4. Possible molecular mechanism by which silibinin and gefitinib synergetically regulate EMT. Silibinin enhances the antitumor effect of gefitinib on NSCLC through inhibiting EGFR expression and reversing EMT

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Comparison between traditional open surgery and complete video-assisted thoracoscopic surgery during the treatment of non-small cell lung cancer

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Abstract

Objective: To compare the differences in infection rate between traditional open surgery and complete video-assisted thoracoscopic surgery (VATS) during the treatment of non-small cell lung cancer (NSCLC) and investigate relevant factors.

Methods: 111 NSCLC patients who received the treatment of thoracic surgery in our hospital between January 2011 and June 2012 were selected, of which 56 were applied with complete VATS (experimental group), and 55 underwent traditional open surgery (control group).

Results: In the control group, there were 9 cases of complications, with the incidence of postoperative complications of 16.36% and 4 cases of lung infection, with the infection rate of 7.27%. In the experimental group, there were 2 cases of shoulder movement disorder and 1 case of lung infection. The total incidence of postoperative complications was 5.36%. The incidence of infection was 1.79%. The difference in the incidence of postoperative infections between the two groups was statistically significant (P<0. 01). Thirty days after operation, VEGF in two groups significantly decreased compared with that before treatment and 5 days after operation (P<0. 05) and decreased more significantly in the experimental group (P<0. 05); five days after operation, IL-6 increased to a higher level (P<0. 05) in both groups, and significantly decreased 30 days after operation. Compared with the control group, the level in the experimental group decreased more sharply (P<0. 05) and the blood loss in the experimental group was less than that in the control group (P<0. 05); and hospitalization duration of the experimental group was shorter than that of the control group (P<0. 05). The operative time, postoperative drainage days and drainage volume were not statistically different between the two groups; there was no significant difference in VEGF between the two groups before operation and 5 days after operation.

Conclusion: The difference of immunity after surgery leads to different incidences of infection in the two groups. Compared with traditional open surgery, the complete VATS has less surgical trauma and lower infection rate and can improve the prognosis of patients. It is a safe and reliable operation and worthy of wide application in clinic.

Key words: Complete video-assisted thoracoscopic surgery, traditional open surgery, non-small cell lung cancer, infection.

Introduction

Non-small cell lung cancer (NSCLC) is the most common lung cancer, accounting for 80% to 85% of the total number of lung cancer. It mainly includes three major types: adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma [1]. Compared with small cell carcinoma, the growth and division of its cancer cells is slower, and the diffusion and transfer relatively late [2]. NSCLC is less sensitive to the traditional radiotherapy and chemotherapy, so it should be treated according to the clinical staging of lung cancer [3]. At the stages I, II, IIIA, surgical excision is applied mainly [4], and for patients with severe lymphatic transfer, it can be combined with chemotherapy or radiotherapy before surgery [5]. In recent years, thoracoscopic NSCLC pulmonary lobectomy has been gradually carried out in China, and for elderly lung cancer patients with hypertension, chronic bronchitis and chronic renal failure, thoracoscopic surgery especially demonstrates its obvious advantages, but pulmonary lobectomy by totally video-assisted thoracoscopy is

rarely applied [6]. In this study, we compared the pulmonary lobectomy by complete VATS and traditional open surgery respectively, so as to study the traumas on the body, differences in postoperative infection and the patients' recovery, and the results are reported as follows.

Materials and Methods

General Information

111 NSCLC patients who received the treatment of thoracic surgery in our hospital between January 2011 and June 2012 were selected, of which 56 were applied with complete VATS (experimental group), and 55 underwent traditional open surgery (control group) over the same period. The patients with incomplete medical records were excluded.

Treatment Methods

Control Group

The traditional lateral position and total intravenous anesthesia by double-lumen endotracheal intubation are adopted. Standard thorax posterior lateral incision was made in the 4th and 5th intercostal spaces, with the length of 20 to 30cm. The conventional surgical method was used to handle incomplete lung fissure and pulmonary arterial and venous branches, and bronchus was mechanically sutured by bronchial occluder. Lymph node dissection: levels 2, 3 A, 4,7,8,9,10 and 11 lymph nodes should be routinely dissected in right lung surgery, and levels 5,6,7,8,9,10 and 11 lymph nodes in the left lung surgery. After the dissection was finished, hemostasis was conducted, lung distension removed, drainage thoracic duct inserted, and then chest subsequently closed [7].

Experimental Group

The position and anesthesia method were the same as above. The incision: the observation hole (1.0 ~ 1.5cm) was located in the 7th or 8th intercostal space at the midaxillary line; the main operation hole (3 ~ 4cm) was located in 4th or 5th intercostal space between the anterior axillary line and midclavicular line; the vice operation hole (1 ~ 2cm) was located in the 7th intercostal space at the posterior axillary line. The thoracoscope was inserted into the observation hole, and thoracoscopic instruments placed in the operation holes to separate adhesion and incomplete lobe fissure in thoracic cavity and isolate the pulmonary vein, bronchus and pulmonary artery in sequence, which were handled by endoscopic cutting stapler. The lung lobes were removed and placed in specimen bag. The principles for lymph node dissection were the same as above. After the dissection was completed, hemostasis was conducted, lung distension removed, drainage thoracic duct inserted, and then chest subsequently closed [8].

Indexes of Observation and Statistical Processing

All data was analyzed by SPSS17.0 software to compare the surgical time, postoperative drainage days, drainage volume, intra-operative blood loss, hospital stay, preoperative and postoperative vascular endothelial growth factor (VEGF) in plasma and IL-6 level between the two groups. The measurement data was expressed by $\bar{x} \pm s$, and comparison between groups t-test, $P < 0.05$ for difference with statistical significance.

Results

Patient Information

The ages of patients were (59.56 ± 3.26) and (60.13 ± 3.67) years old respectively in the control group and experimental group, and diameters of lesions (3.11 ± 0.35) and (3.16 ± 0.29) cm respectively; there were no statistically significant differences in gender, age and the extent of disease between the two groups at the time of admission (Table 1).

Surgery-related indicators

The surgical time, postoperative drainage days and drainage volume were not statistically different, the intra-operative blood loss was less in the experimental group than in the control group ($P < 0.05$), and the hospitalization days of the former was shorter than that of the latter ($P < 0.05$) (Table 2).

VEGF and IL-6 levels

There was no significant difference in VEGF between the two groups before and 5 days after operation. The differences were statistically significant between 30 days after surgery and before treatment and 5 days after surgery ($P < 0.05$ for

Table 1. General information

Item		Control (n=55)	Experimental (n=56)
Age (years old)		59.56±3.26	60.13±3.67
Gender	Male	35 (63.64%)	35 (62.50%)
	Female	20 (36.36%)	21 (37.50%)
Lesion diameter (cm)		3.11±0.35	3.16±0.29
Resection position	Left superior lobe	13 (23.67%)	14 (25.00%)
	Left inferior lobe	15 (27.27%)	15 (26.79%)
	Right superior lobe	11 (20.0%)	10 (17.86%)
	Eight median lobe	7 (12.73%)	7 (12.50%)
	Right inferior lobe	9 (16.36%)	10 (17.86%)
Preoperative staging	I	32 (58.18%)	33 (58.93%)
	II	23 (41.82%)	23 (41.07%)
Postoperative staging	I	31 (56.36%)	30 (53.57%)
	II	16 (29.09%)	17 (30.36%)
	IIIA	8 (14.55%)	9 (16.07%)

Table 2. Surgery-related indicators ($\bar{x}\pm s$)

Item	Control	Experimental	P value
Surgical time (min)	145.36±51.72	139.28±55.15	$P>0.05$
Intra-operative blood loss (ml)	185.72±49.64	151.95±32.57	$P<0.05$
Drainage volume (ml)	741.82±41.74	748.16±36.38	$P>0.05$
Drainage time (d)	4.23±1.75	3.56±1.27	$P>0.05$
Hospitalization time (d)	14.21±2.45	10.15±1.68	$P<0.05$

Table 3. VEGF and IL-6 levels ($\bar{x}\pm s$)

Item		Before	5 days after	30 days after
Control	VEGF	2425.7±648.2	2239.1±367.2	2101.3±156.7
	IL-6	34.5±5.2	76.1±4.4	27.2±3.0
Experimental	VEGF	2379.2±587.6	2203.5±269.1	2000.3±147.3
	IL-6	31.4±8.1	64.3±4.9	18.8±2.3

all). VEGF level decreased more significantly in the experimental group (P <0.05). Five days after operation, IL-6 increased to a higher level (P<0. 05) in both groups, and significantly decreased 30 days after operation. Compared with the control group, the level in the experimental group decreased more sharply (Table 3).

Complications

In the control group, there were 2 cases of lung infection, 2 cases of incision infection, 1 case of atelectasis and 2 cases of arrhythmia and shoulder movement disorder respectively, and the complication rate was 16.36%, with the incidence of postoperative infection of 7.27%. In the experimental group, there were 2 cases of shoulder movement disorder, 1 case of lung infection, and the postoperative complication rate was 5.36%, with the infection rate of 1.79%. The difference in the postoperative infection incidence was statistically significant between the two groups (P <0.01).

Discussion

In this study, the three parameters of operative time, postoperative chest drainage days and chest drainage volume were not statistically different between the two groups, which were similar to the literature report [9]; but the degrees of infection of the two groups were different [10]. Compared with the traditional open surgery, the complete VATS had less blood loss, which reduced the probability of transfusion, with a low level of reduced immunity [11]. The infection rate of the

control group was significantly higher than that of the experimental group.

The differences in postoperative VEGF and IL-6 levels also reflected the different degrees of infection, which might also mirror the prognosis of patients, consistent with what was reported in the literature [12]. VEGF, as an important angiogenic growth factor, can act specifically on vascular endothelial cells to cause proliferation [13]. VEGF may release inflammatory mediators [14], so that the tissue metabolites accumulate [15], and inflammatory exudates gather in tissue, making edema, so as to cause local tissue hypoxia which can lead to increased VEGF expression [16]. In severe cases, the proliferation of tumor cells may be promoted, that is, VEGF level is closely related to the extent of infection, tumor control and recurrence [17]. The above theory can confirm and explain the differences in infection between the two groups in this study.

IL-6 participates in the occurrence and development of various tumors, lung cancer in particular. IL-6, as an important growth regulator factor of lung cancer cells [18], is an indicator for the evaluation of prognosis of patients with lung cancer, and may also reflect trauma and degree of infection [19]. Studies have shown that IL-6 can stimulate the release of reactive oxygen and a variety of enzymes, promote the accumulation and activation of granulocytes in the pulmonary capillaries and mediate post-traumatic inflammatory reaction. In some severe cases, it may also lead to multiple organ and tissue damages. In this study, compared with the control group, the experimental group had a significant IL-6 level reduction 30 days after operation and a better recovery, consistent with the conclusion of the abovementioned literature.

To sum up, complete VATS in the treatment of NSCLC can reduce surgical trauma of patients, accordingly decrease damages on postoperative immunity, reduce the incidence of postoperative infection, alleviate the degree of infection, so as to obtain better prognosis, reduce the burden on patients and improve their quality of life, which is worthy of being widely applied in clinic [20,21].

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The immunohistochemical expression of COX-2 and NM23 in gastric adenocarcinoma: correlation with clinicopathological variables

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Abstract

Aim: This study was done to detect the immunohistochemical axpression of COX-2 and NM23 in gastric adenocarcinoma, and to analyze the correlation with clinicopathologic features.

Methods: In a retrospective immunohistochemical study specimens obtained from 56 gastric cancer patients who had undergone gastrectomy with perigastric lymphadenectomy were analysed, in correlation with clasical clinicopathological parameters of tumors, WHO-, Lauren-, Goseki-, and Ming- classification. COX-2 and NM 23 gene expression was compared in gastric adenocarcinoma with paracancerous normal mucosal tissue, which were also collected as control from the same 56 patinets. A semiquantitative immunostaining evaluation was used, counting the percentage of stained cells. Statistical analysis was performed using Kolmogorov-Smirnov test, and Spearman rank correlation test.

Results: NM23 expression was significantly higher in non-neoplastic mucosa than in adjacent gastric adenocarcinoma tissue (p<0,0001). NM23 protein expression did not correlate with gender (p=0,115), tumor size (p=0,844), tumor grade (p=0,172), lymphovascular invasion (p=0,606), lymph node metastases (p=0,311), Lauren classification (p=0,426), Goseki classification (p=0,458) and Ming classification (p=0,212). The expression of COX-2 in gastric cancer was significantly higher than in normal mucosa (p=0,0001). COX-2 protein expression did not significant correlate with tumor size (p=0,301), tumor grade (p=0,054), and lymph node metastasis (p=0,875). It was significantly correlated with gender (p=0,0001), vascular invasion (p=0,045), Lauren- (p=0,0001), Goseki- (p=0,038) and Ming classification (p=0,041). Regression analysis of the Spearman rank correlation coefficient showed a significant (p=0,0001) inverse correlation between proteins COX-2 and NM23 in both carcinoma and non-neoplastic adjacent mucosa (Ro= - 0,835; Ro= - 0,732; respectively)

Conclusion: Significant negative correlation between expression proteins COX-2 and NM23 in both, carcinoma and in adjacent tumor-free gastric mucosa, suggests that COX-2 and NM23 closelly associated to the tumorigenesis and progress of gastric carcinoma.

Key words: COX-2, NM23, gastric cancers, immunohistochemistry

Introduction

Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in production of prostaglandins which contributes to maintain the pathologic and physiologic function of organism. Overexpression of COX-2 has been reported in variety of inflammatory disseases, in many premalignant lesions and various tumors (1). There is now increasing evidence that a constitutive expression of COX-2 plays a role in development and progression of malignant epithelial tumors. COX-2 gene is involved in cancer progression through promotion of tumor cell proliferation, tumor angiogenesis, and resistance to apoptosis. COX-2 has recently been considered to promote angiogenesis in breast and lung cancer. The role of COX-2 has been shown in early stages of carcinogenesis beginning in premalignant adenomatous lesions and in the course of their progression to colon adenocarcinoma, as well as in esophageal, breast, papillary thyroid, medullary thyroid, prostatic, and pulmonary carcinomas, and most relevant, in pheochromocytoma. The impact of COX-2 on gastric cancer remains unclear.

Metastasis suppressor genes have a role in preventing the extension of tumors. NM 23 protein was originallly identified as a metastasis suppressor protein for which the expression level depen-

ds on the state of cell growth. Decreased expression of NM23 has been associated with increased invasiveness in different cancers, including those of the breast, liver, ovary, bladder, and ren. Evidence for their expression in gastric cancer is rather contradictory, both for protein expression status and prognostic value (2) .

This study aims to test the immunohistochemical expression of COX-2 and NM23 in gastric adenocarcinoma, and to analyze their correlation with clinicopathologic features.

Materials and Methods

Patients Selection

The biopsy specimens from 56 patients (40 men, and 16 women) with invasive gastric adenocarcinoma diagnosed at the Departement of Pathology, School of Medicine University of Sarajevo, Bosnia and Herzegovina, were selected for this study. In a retrospective immunohistochemical study specimens obtained from 56 gastric cancer patients who had undergone gastrectomy with perigastric lymphadenectomy were analysed, in correlation with clasical clinicopathological parameters of tumors, WHO-, Lauren-, Goseki-, and Ming- classification. COX-2 and NM 23 gene expression was compared in gastric adenocarcinoma and tumor-adjacent non-neoplastic gastric mucosa. Paracancerous mucosal tissue from 56 patienst were collected as a control. Gastric adenocarcinoma specimens were reviewed using morphologic and immunohistochemical criteria according to the WHO classification of gastric carcinoma (3) and staged according to TNM AJCC classification (4).

The study included 56 patients were found to have no distant metastasis after staging, and relevant surgery. Patients undergoing neoadjuvant chemotherapy and/or radiotherapy were excluded.

All of the samples were routinely fixed in 10% buffered formalin, embedded in paraffin, and cut into 4-5 μm section. Four blocks from each tumor specimens were submitted for paraffin embedding, each containing as mush as possible tumor tissue with the deep advancing edge and piece of adjacent mucosa. One block of them contains a pieces of morphologically normal distant mucosa, at 10 cm from the tumor.

Immunohistochemical Staining

Immunohistochemical analyses of the expressions of NM23 and COX-2 were performed according to the routine processes. All the issue specimens were fixed in 10% neutral formalin and embedded in paraffin. Briefly, 5-μm sections of tumor tissues and non-neoplastic (peritumoral) gastric mucosa were mounted on poly-D-lysine coated slides. Thin sections were deparaffinized in xylene and rehydrated in a series of ethanol solutions (100%, 90%, and 80%) for 5 minutes each, washed in distilled water and three times in 0.05 mol/L PBS (pH 7.4), immersed in 10 mmol/L citrate buffer (pH 6.0) and put in a microwave for 5 min at 60°C for antigen retrieval. Then they were placed in methanol containing 3% H_2O_2 for 30 min at 4°C to block endogenous peroxidase activity and incubated with rabbit serum for 10 min to block non-specific antibody binding sites. After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with primary antibodies, anti-COX-2 mouse monoclonal antibody (dilution range 1: 80, DAKO, Denmark) and rabbit anti-human NM23 (dilution 1: 50; DAKO, Denmark) with affinity for both H1 and H2 components of the NM23 protein The primary antibodies were applied at a working concentration and incubated for 2 hours at 4°C. The secondary antibody and the avidin-biotin-peroxidase complex (ABC) were applied to slides. 3,3 '- Diaminobenzidine (DAB) was used as a chromogen and sections were counterstained with Mayer's hematoxylin. Negative controls were obtained by replacing the primary antibody by non-immunized rabbit or mouse serum.

Quantification of Immunostaining

Positive results were visible as yellou to brown cytoplasmic staining for the investigated antibodies. The expression of the NM23 was evaluated in a semiquantitative manner (5). The criteria used to assess NM23 expression were based on the number of stained cells, and scores were assigned as follows: a) score 0<10%, score 1=10-25%, score 2=26-50%, score 3=51% or more stained cells. Scores 0 to 2 was considered as negative, and score 3 was considered as positive. Non-neoplastic gastric mucosa was used as the internal positive control. Normal tonsil tissue was used as negative control.

The COX-2 immunohistochemical expression was determinated by immunohistochemical score (IHS). This was calculated by combining the percentage of positive stained cells with staining intensity score (6). The percentage of positive cells was categorized as 0 = negative; 1=1-10% positive cells; 2=11-50% positive cells; 3=51-80% positive cells; 4=≥81% positive cells. The staining intensity was categorized as 0=negative; 1= weak; 2 =moderate; 3 =strong. Raw data were converted to HIS by multiplying the quantity score (o-4) by the staining intensity score (0-3). Theoretically, the IHS can range from 0 to 12. An IHS of 9-12 was considered a strong immunoreactivity; 5-8 =moderate; 1-4=weak; and 0=negative. In statistical analysis, COX-2 scores were placed in positive (1-12) and negative (0) group. For each slice, five regions were avaluated at a magnification of x 100. An average score was determinated to avoid random sampling.

Statistical Analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc, Chicago, USA). The correlation among the expression of NM23, COX-2 and clinicopathologic parameters (gender, age, size, tumor differentiation, lymphovascular invasion, number of lymph nodes involved, Lauren- Goseki-, or Ming classification) were calculated by Student's *t*-test, chi-square correlation test and Spearman's coefficient of correlation as appropriate. The statistical significance level was defined as $p<0.05$.

Results

Caracteristics of 56 patients with gastric cancer and staging results of all tumor specimens are summarized in Table 1. Among 56 patients, mean age was 63,3 years (range 48 to 81 years). In all patients tumors were located in antrum. According to UICC 3 (5,3%) patients had tumor size pT1, 15 (26,7%) pT2, 26 (46,4%) pT3, and 12 (21,4%) pT4. Thirteen tumors (23,2%) were classed as grade I and grade IV, fifteen tumors (26,7%) at grade II and at grade III. Among 56 patients 13 (23,2%) were pN0, 26 (46,4%) were pN1, 9 (16,0%) were pN2, and 8 (14,2%) were pN3. Among these patients 44 (78,5%) had lymphovascular invasion, 34 (60%) had inatestinal and 22 (39,2%) had diffuse type carcinoma (Lauren classification). Thirty-five cases were Goseki 1 (62,5%), five cases was Goseki 2 (8,9%), six cases were Goseki 3 (10,7%), and ten cases were Goseki 4 (17,8%).

Table 1. Clinicopathologic characteristics of 56 castric adenocarcinoma with immunohistochemical expression of NM23 and COX-2 proteins

Parameters		No. (%) of patients	*P* value**
Age (median, range)		63 (48 - 81)	
Tumor size (pT)			
1		3 (5,3)	
2		15 (26,7)	
3		26 (46,4)	
4		12 (21,4)	
Grade:*			
1		13 (23,2)	
2		15 (26,7)	
3		15 (26,7)	
4		13 (23,2)	
Vascular invasion:			
Yes		44 (78,5%)	
No		12 (21,4%)	
Nodal status (pN):			
pN0		13 (23,2)	
pN1		26 (46,4)	
pN2		9 (16,0)	
pN3		8 (14,2)	
Lauren classification:			
Intestinal		34 (60,7)	
Diffuse		22 (39,2)	
Goseki grade:			
1		35 (62,5)	
2		5 (8,9)	
3		6 (10,7)	
4		10 (17,8)	
Ming classification:			
Expansive border		14 (25,0)	
Infiltrative border		7 (12,5)	
Mixed		35 (62,5)	
NM23†:			
Non-neoplastic mucosa	Negative	26 (46,4)	
	Positive	30 (53,5)	0,0001
Tumor	Negative	56 (100,0)	
	Positive	0 (0)	0,0001
COX-2‡:			
Non-neoplastic mucosa	Negative	37 (66,0)	
	Positive	19 (34,0)	0,0001
Tumor	Negative	17 (30,3)	
	Positive	39 (69,6)	0,0001

**According to the WHO classification of gastric cancer (3).*
† NM23 expression: 0=≤10%, 1=11-25%, 2= 26-50%, 3=≥51% of stained cells. Scores 0 - 2 was considered as negative, and score ≥3 was considered as positive.
‡ COX-2 expression: 0 = negative; 1= ≤10%; 2= 11-50%; 3= 51-80; 4= ≥81%. The staining intensity : 0=negative; 1= weak; 2 =moderate; 3 =strong. An overall staining score was determined by the product of these two grades. Total core ≥1 was considered positive for COX-2.
*** Spearman rank correlation test.*

The immunohistochemical expression of NM23 was strictly cytoplasmic. There was no nuclear staining. Positive and negative NM23 staining was identified in non-neoplastic gastric mucosa. Normal gastric epithelial cells were homogeneously stained by monoclonal anti NM23 antibody. This was considered as an intrinsic control aldouth we studied positive and negative control slides.

NM23 expression in the adjancent non-neoplastic gastric mucosas were highly variable. Positive NM23 stain (score 3) (Figure 1.) was observed in 30 (53,3%) and negative stained in 26 (46,4%) cases of paracancerous normal mucosal tissue. No expression of NM23 protein in 56(100%) gastric adenocarcinoma (Figure 2.). The normal gastric mucosa had the significantly higher expression of NM23, than gastric adenocarcinoma (p=0,0001).

Negative COX-2 staining was noted in 66,0% (37/56) non-neoplastic gastric mucosa, and in 30,3% (17/56) tumors tissue. The value was significant higher (p=0,0001) in the non-neoplastic adjacent gastric mucosa than in carcinoma. COX-2 expression showed no significant differences regarding tumor size (p=0,301), tumor grade (p=0,054), lymph node metastasis (p=0,875), but was significantly related to the vascular invasion (p=0,045), Lauren- (p=0,0001), Goseki- (p=0,038) and Ming classification (p=0,041). Regression analysis of the Spearman's rank correlation coefficient showed a significant (p=0,0001) negative correlation between proteins COX-2 and NM23 in both carcinoma and non-neoplastic adjacent mucosa (Ro=- 0,835; Ro=- 0,732; respectively) (Table 4.).

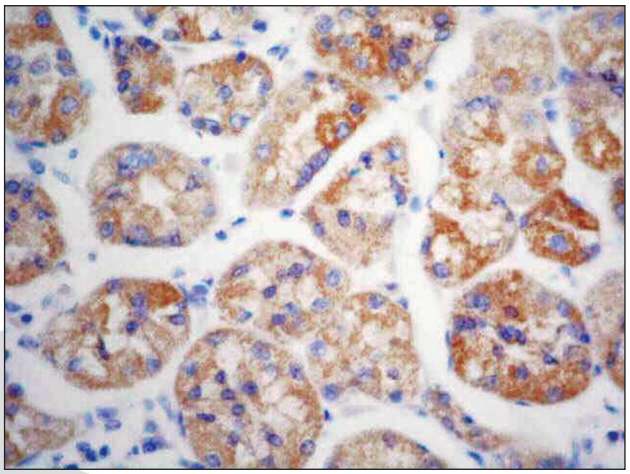

Figure 1. Immunohistochemical expression of NM2 in para-cancerous normal gastric mucosa (x 400)

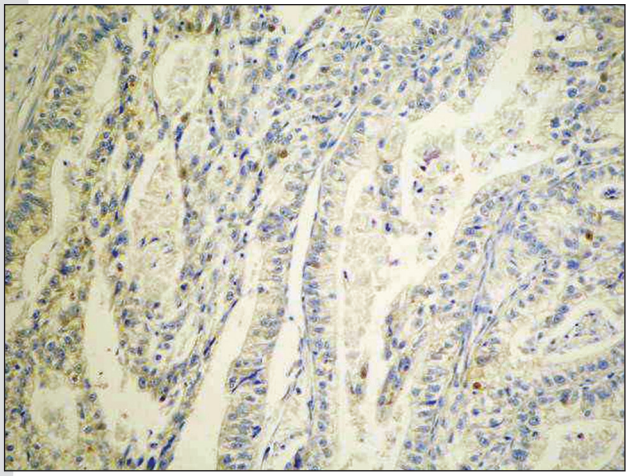

Figure 2. No immunohistochemical expression NM23 in gastric carcinoma (x 400)

NM23 expression was evaluated with respect to patients clinicopathological data (Table 2). NM23 showed no significant differences regarding gender (p=0,115), tumor size (p=0,844), tumor grade (p=0,172), lymphovascular invasion (p=0,606), lymph node metastases (p=0,311), Lauren- (p=0,426), Goseki- (p=0,458) and Ming classification (p=0,212). No significant correlation was found between NM23 expression and analised clinicopathologic factors.

COX-2 expression showed as a yellow or brownish yellou finely granular cytoplasmic staining mostly in cancerous tissue (Figure 3.). COX-2 expression was evaluated with respect to patients clinicopathological data (Table 3).COX-2 expression was noted in 34% (19/56) paracancerous normal mucosal tissue (Figure 4.), and 69,6% (39/56) of

gastric carcinoma. This value was significant higher (p=0,0001) in the carcinoma than in non-neoplastic adjacent gastric mucosa (Table 3.)

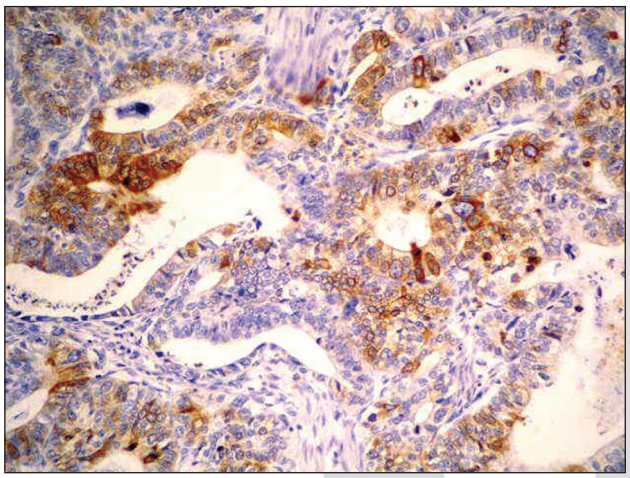

Figure 3. COX-2 immunstaining in tumor cells (x 400)

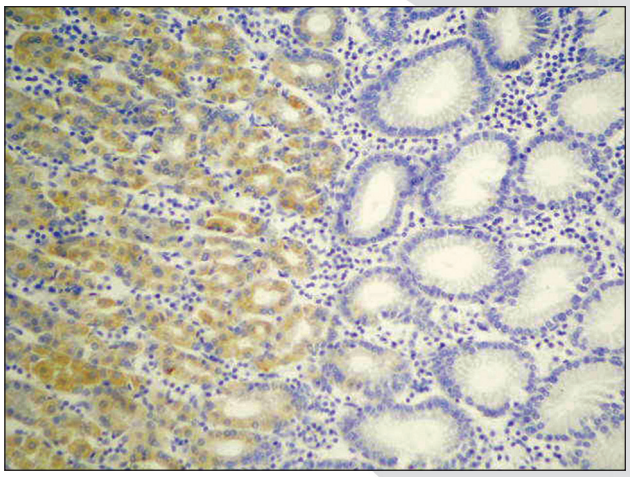

Figure 4. COX-2 immunstaining in paracancerous normalgastric mucosa(x 400)

Discussion

In this study, we investigated the relationship between gastric cancer and immunohistochemical expression of proteins NM23 and COX-2**.** With regard to the prognostic importance of NM23 and COX-2 expression in gastric adenocarcinoma, conflicting results have been reported in the past decade. The hypothesis of this study was based on importance of NM23 and COX-2 expression in predicting a smaller group of patients with biologicallay more aggressive primary tumor at the time of diagnosis.

NM23 protein is a metastasis supressor protein, expressed in all cellular compartement. In vitro correlates of supression include reduced invasion, motility and soft agar colonization, and induction of differentiation. The mehanism by wich NM23 regulates metastasis is not fully understood. NM23 expression has been widely studied in various cancers and with their relation to staging and prognosis. NM23 expression are generally, but not unifomly associated with improved prognosis in various type of carcinomas. Expression of NM23 has been shown to be inversely correlated with the metastatic potential of several human cancer. Reduced expression of NM23 in breast, hepatoecellular and ovarian carcinoma correlates with increassed metastatic potential (7-11), but in esophageal squamous cell, prostate and lung carcinoma, disease progression is associated with increased NM 23 gene expression (12-14)**.** The relatively large number of studies analysed NM23 protein in colorectal carcinoma (5), but a smal number of them analyzed this protein in gastric carcinoma (2, 15).

In the present study expression of NM23 protein was observed in normal gastric mucosa in 53,5% of cases. We observed a similar percentage (46,4%) of cases with negative staining in adjacent non-neoplastic mucosa. There were some differences about expression of NM23 in non-neoplastic mucosae in adjancent gastric cancer beetween different persons. When compared the specimens between the two groups, NM23 expression did not demonstate signficant correlation. Our results do not support findings of Muta's study. Muta (16) analyzed gene and protein expression of NM23, using Northern blot and immunohistochemical tehniques. He noted that expression of NM23 protein in tumor tissue was higher than those in the corresponding normal mucosae. This suggests a linkage of NM23 in the process of the gastric cancer progression. Our results suggest that biological significance of NM23 expression may be quite different in the same organ. Neoplastic gastric tissue showed negative expression of NM23, suggests that absent staining in gastric adenocarcinoma was associated with disease progression, but these mechanism is not understood and remain to be determined conclusively.

In our series, the analysis of NM23 expression revealed a higher tumor grade, higher incidence of metastatic lymphe nodes, higher intestinal type of tumours according to Lauren classification,

Table 2. NM23 tissue status and clinicopathological parameters of 56 patienst with castric adenocarcinoma

Variables	Total (%)	NM23 – No. of case				P value**
		Non-neoplastic mucosa		Tumor		
		positive / negative		positive / negative		
		(score ≥3) / (score 0-2)		(score ≥3) / (score 0-2)		
Sex:						
Male	40(71,4)	22	18	0	40	Ro=0,213
Female	16(28,5)	9	7	0	16	p=0,115
Tumor size (pT):						
1	3 (5,3)	3	0	0	3	
2	15 (26,7)	8	7	0	15	Ro=0,027
3	26 (46,4)	13	13	0	26	p=0,844
4	12 (21,4)	8	4	0	12	
Grade:*						
1	13(23,2)	9	4	0	13	
2	15(26,7)	8	7	0	15	Ro=-0,185
3	15(26,7)	9	6	0	15	p=0,172
4	13(23,2)	4	9	0	13	
Vascular invasion:						
Yes	44 (78,5%)	24	20	0	44	Ro=-0,070
No	12 (21,4%)	6	6	0	12	p=0,606
Nodal status (pN):						
pN0	13(23,2)	8	5	0	13	
pN1	26(46,4)	14	12	0	26	Ro=0,138
pN2	9(16,0)	5	4	0	9	p=0,311
pN3	8(14,2)	4	4	0	8	
Lauren classification:						
Intestinal	34(60,7)	20	15	0	34	Ro=0,108
Diffuse	22(39,2)	9	13	0	22	p=0,426
Goseki grade:						
1	35(62,5)	20	15	0	35	
2	5(8,9)	2	3	0	5	Ro=-0,101
3	6(10,7)	1	5	0	6	p=0,458
4	10(17,8)	4	6	0	10	
Ming classification:						
Expansive border	14(25,0)	6	8	0	14	Ro=0,121
Infiltrative border	7(12,5)	5	2	0	7	p=0,212
Mixed	35(62,5)	18	17	0	35	

**According to the WHO classification of gastric cancer (3).*
*** Spearman rank correlation test according to NM23.*

higher Goseki type 1 tumours and higher nodular/ diffuse type of tumours (Ming classification), and advanced pT categories in patients without protein expression, aldouth this result did not reach statistical significance. In addition we confirmed the higher expression of NM23 in adjacent mucisa when compared to tumor cells of primary gastric adenocarcinoma. This result suggested that loos of NM23 expression in gastric carcinoma tussue may had relation with developement, progression, invasion and metastasis of neoplasm. This finding suggests a potential protective effect of this protein in tumour genesis.

Our results do not support findings of similar studies. There were also some discrepancies amnog previous studies of the same tumours (2,

Table 3. COX-2 tissue status and clinicopathological parameters of 56 patients with castric adenocarcinoma

Variables	Total (%)	COX -2 – No. of case				P value**
		Non-neoplastic mucosa		Tumor		
		positive / negative		positive / negative		
		(score 0) / (score 1-12)		(score 0) / (score 1-12)		
Sex:						
Male	40(71,4)	26	14	11	29	Ro=0,600
Female	16(28,5)	11	5	6	10	p=0,0001
Tumor size (pT):						
1	3 (5,3)	3	0	3	0	
2	15 (26,7)	14	1	6	9	Ro=0,419
3	26 (46,4)	16	10	8	18	p=0,301
4	12 (21,4)	5	7	0	12	
Grade:*						
1	13(23,2)	10	3	4	9	
2	15(26,7)	4	10	0	15	Ro=0,253
3	15(26,7)	11	4	3	12	p=0,054
4	13(23,2)	12	1	6	7	
Vascular invasion:						
Yes	44 (78,5%)	28	16	8	36	Ro=0,316
No	12(21,4)	9	3	4	8	p=0,045
Nodal status (pN):						
pN0	13(23,2)	8	5	5	8	
pN1	26(46,4)	20	6	9	17	Ro=0,06
pN2	9(16,0)	7	2	1	8	p=0,875
pN3	8(14,2)	2	6	2	6	
Lauren classification:						
Intestinal	34(60,7)	22	12	9	25	Ro=0,632
Diffuse	22(39,2)	15	7	11	11	p=0,0001
Goseki grade:						
1	35(62,5)	23	12	13	22	
2	5(8,9)	3	2	1	4	Ro=0,38
3	6(10,7)	5	1	2	4	p=0,038
4	10(17,8)	6	4	1	9	
Ming classification:						
Expanding	14(25,0)	10	4	5	9	Ro=0,37
Infiltrative	7(12,5)	5	2	1	6	p=0,041
Mixed	35(62,5)	19	16	9	26	

**According to the WHO classification of gastric cancer (3).*
*** Spearman rank correlation test according to COX-2.*

15, 17). Lee et al analyzed the relationschip of p53, nm23, PCNA and HER-2 with clinicopathological parameters in gastric cancer and the survival results (18) . He concluded that expression of NM23 and p53 was related with poor prognosis of gastric cancer. Monig et al analyzed clinical significance of NM23 gene expression in gastric cancer (2). Their series did not show a correlation of protein expression in neoplastic gastric tissue in terms of lymphe node and distant metastasis or prognosis in gastric cancer patients. Yeung suggest that NM23 may have a role in gastric carcinoma pathogenesis, but do not show a correlation with metastasis (15). Muller analyzed NM23 expressi-

Table 4. Correlation between NM23 and COX-2 protein expression in gastric adenocarcinoma and tumor adjacent non-neoplastic mucosal tissue of 56 patients

Tissue characters	Total cases	COX-2 expression n (%)	NM-23 expression n (%)	P value*
Non-neoplastic mucosa	56			
Negative		37 (66,0)	26 (46,4)	Ro=-0,732
Positive		19 (34,0)	30 (53,5)	p=0,0001
Tissue cancer	56			
Negative		17 (30,3)	56 (100,0)	Ro=-0,835
Positive		39 (69,6)	0 (0)	p=0,0001

** Spearman rank correlation test.*

on and prognostic impact in 413 gastric carcinoma (13). Expression of NM23 was detected in 84,5% (n=349) of all tumors and demonstrated positive correlation with the intestinal type of tumor, according to the Lauren classification and advanced pT categories, and was also correlated with the presence lymphatic vessel invasion. No correlation be demonstrated between NM23 expresssion and lymphe node involvement. Results of this study showed that expression of the NM23 metastasis supressor gene is correlated with agressive tumor growth and poor prognosis but it is not an indenpendent prognostic marker. Nakamura was found expression of NM23 in 24 out of 31 cases of gastric carcinoma (19). They results suggest that expression of this protein is correlated to tumor progression and / or prolifferation rather than the supression of metastasis. Such a variation may be due to heterogeneity of primary tumour distribution, methods of investigation and scoring systems for pathological variables.

The overexpression of COX-2 has been detected in several types of human cancer (including colorectal, lung, stomach, breast, uterus and pancreas) and is usually associated with poor prognostic outcome. This observation has suggested that COX-2 may play a critical role in the carcinogenesis and progression of tumors. Previous studies show conflicting prognostic significance of COX-2 in gastric carcinoma.

In the present study, COX-2 immunoexpression is significantly higher in tumour tissue than in normal peritumoral gastric mucosa. Our results support findings of similar studies (6, 19). We found that COX-2 expression showed no significant differences regarding tumor size, tumor grade, and lymph node metastasis, but COX-2 protein expression significantly correlate with vascular invasion, Lauren-, Goseki- and Ming classification. COX-2 expression was variabilly noted in our control group which included tissues adjacent to the tumor, which include mucosa with inflammation, dysplastic and metaplastic changes. These changes may be associated with higher expression COX-2 in paracancerous tissues.

COX2 is one of the key isoenzymes in the production of prostaglandins from arachidonic acid, and it takes part in many pathophysiologic processes, such as inflammation (20), immune responses (21, 22), and carcinogenesis (19, 22). COX-2 is normally undetectable in most tissue. It is rapidly induced by growth factors, cytokines, viruses, tumor promoters, and many other stimuli. Some studies indicate that COX2 may play a role in the development of gastric cancer, and its overexpression is associated with nodal metastasis, tumor invasion and differentiation, implicating a poor prognosis (23-27). In Bekdemir's study increased COX-2 expression was detected in tumorous tissue, but no significant relationship was found between histological type of tumor and tumor differentiation degree, with COX-2 expression (28). The study of Saukkonen et al. does not support Bekdemir's findings - they finds significant COX-2 expression in intestinal type tumors (29). Murata et al reported that COX-2 over-expression in gastric cancer was significantly correlated with tumor invasion into lymphatic vessels in the gastric wall and metastasis to the lymph nodes, but not correlated with histopahological grading and size of the tumor (30). Mao et al found that the expression of COX-2 in the gastric cancer tissue was higher than that in normal mucosa (19). They reviewed that the COX-2 protein expression may play an important role in the angiogenesis of gastric cancer, and that it has an obvious relation with the proliferation of gastric

cancer cells and the lymph node metastasis. They suggested that COX-2 could be served as a determinant factor for clinical prognosis and curative effect. Gou et al. was found expression of COX-2 protein in cases of gastric carcinoma, but they did not find a significant association between COX-2 expression and clinicopathological characteristics (6). Lim et al found COX-2 over-expression in gastric cancer tissue as compared to the normal gastric mucosa (31).

Previous studies show conflicting prognostic significance of COX-2 in gastric carcinoma. The difference between studies may be based upon the smaller number of specimens axamined, a blased selection of patienst, different scoring systems and the stage of disease, genetic characteristics of patients, or different antibodies used and their different sensitivity.

In most of the studies COX-2 expression in tumorous tissue was found to be significantly higher than in adjacent non-tumorous tissue. This cleary shows COX-2 influence in tumorous tissue. Recently publiched studies suggest that tumors produce more COX-2 to support their own growth. Mehanisms in which COX-2 is involved are apoptosis, immune suppression, inflammation and angiogenesis (32).

Only a small number studies have examined COX-a and NM23 expression in gastric carcinoma Ji examined 71 cases of gastric cancer, and found low expression (71,8%) of NM23 and overexpression (70,4%) of COX-2 in gastric cancer (33). Their results suggest that abnormal expression of these proteins associate with malignant potential, lymphe node metastasis and clinical stage of gastric carcinoma.We found that owerexpression of COX-2 and no expression of NM23 in gastric carcinoma, was presented as negative correlation. This suggested than COX-2 and NM23 interact in the pathogenesis of gastric cancer.

Conclusion

Significant negative correlation between expression proteins COX-2 and NM23 in both, carcinoma and in adjacent tumor-free gastric mucosa, suggests that expression of COX-2 and depreciation NM23 in gastric adenocarcinoma is a indicator of tumorigenesis and progress of gastric carcinoma It is possible that the NM23 interaction might contribute to metastasis throught induction of COX-2. The detailed biological roles of this marker in gastric carcinogenesis require further investigation.

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Analysis of resistance genotypes and homogeneity in multidrug resistant Acinetobacter baumannii from ICU

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Abstract

Objective: To investigate the phenotypes, genotypes and homogeneities of 18 multidrug resistant Acinetobacter (A.) baumannii from the Intensive Care Unit (ICU).

Methods: The collected bacterial strains from one hospital were identified by Vitek32, and the susceptibility tests were performed by the Kirby-Bauer (K-B) agar diffusion method. PCR were applied to detect various types of β-lactamases, aminoglycoside antimicrobial resistance gene and quinolones gyrA gene. Pulsed field gel electrophoresis (PFGE) was used for homogeneity analysis.

Results: The resistance rate of the bacterial strains was over 88.8% for most of the antibacterial drugs, except 5.6% for imipenem and 22.2% for cefoperazone/sulbactam. 17 of the tested bacterial strains produced AmpC enzyme, 4 produced ESBLs and 4 produced MBL enzyme. The genes coding AmpC, TEM-1, aacA4 and gyrA were found in all of the 18 strains, aacC1 and aadA1 in 17 of them, and OXA-23, PER and aphA1 in one of them, respectively. No SHV, VEB, IM P, VIM and OXA-24 genes were found in any of the bacterial strains. 16 strains appeared high homogeneity for owing completely the same bands in the PFGE, while another two were independent.

Conclusion: In the 18 strains of A. baumannii, one clonal epidemic strain was preferred to cure imipenem for coding with the genes of AmpC, TEM-1, aacA4, aacl, and aadA1 and producing high yield of AmpC enzyme.

Key words: Acinetobacter baumannii, hospital infection, multidrug resistance, drug-resistant gene, molecular epidemiology.

Introduction

Acinetobacter (A.) baumannii, an important pathogen of hospital infection, caused high concern in the clinical and microbiological scholars with its ever-growing infection and drug resistance in recent years. A. baumannii resulted in not only respiratory tract infections, but also sepsis, urinary tract infections, and secondary meningitis. The infection of A. baumannii, also called ICU-acquired infection, was a serious threat to the critically ill, CCU and ICU patients because of the wide distribution and long-term survival (1,2). The development of carbapenem resistant A. baumannii in the mainland and the appearance of "entire resistant" acinetobacter (Pandrug-resistant Acinetobacter baumannii) in both Taiwan and the mainland should be paid high attention on. In this paper, resistance genotypes and homogeneity of multidrug resistant A. baumannii from ICU were reported as follows.

Materials and Methods

Strain sources

18 bacterial strains of the multidrug resistant A. baumannii, 11 from sputum and 7 from wound, were obtained from the ICU patients in a third-grade class-A hospital from January 2011 to July 2011, excluding the ones from the same part of the same patient.

Quality-control strains

Quality-control (QC) strains of Escherichia coli ATCC25922, pseudomonas aeruginosa ATCC 27853, and Enterobacter cloacae 029 M were purchased from the National Center for Clinical LA. baumannii oratories, while the Klebsiella pneumoniae ATCC700603was donated by Inner Mongolian Medical University.

Drug-sensitive slips and powders

The drug-sensitive and drug powder slips were products of the British OXOID. Dry powder clavulanic acid and cloxacillin were from Zhejiang Hisun Pharmaceutical Co., Ltd. and Connaught Pharmaceutical Co., Ltd respectively.

Methods

Isolation, identification and susceptibility testing of the bacterial:

Collected bacterial strains were inoculated on the Vitek-GNI identification card, and identified by the Vitek-32 type automated microbial identification system. The susceptibility tests were adopted the K-B agar diffusion method. Escherichia coli ATCC25922 and pseudomonas aeruginosa ATCC27853 were determined as the quality control strains, which required regular tests according to the American Society for Clinical Laboratory Standards Institute (CLSI) (3).

Detection of ESBLs, AmpC and MBL:

The extended-spectrum β-lactamase enzyme (ESBLs) and AmpC enzyme (4) were examined by the cefoxitin three-dimensional test of the crude enzyme extract, with the Aerobacter cloacae 029M as the positive control of AmpC enzymes and the Klebsiella pneumoniae ATCC700603 as the positive control of ESBLS. In accordance with the literature, metal enzyme (MBL) can be tested by synergy method (5,6).

Detection of resistance gene:

The plasmid and chromosomal DNA were extracted strictly according to the instruction of extraction and purification kit of small mount (3SS pin Plasmid miniprep Kit V 3.1 and 3SS pin Genomic DNA miniprep Kit for Gram Negative Bacterial K713N respectively) from Shanghai Shenergy Gaming Biotechnology Co., Ltd(SGB). The primers, synthesized by SGB were designed by the primer software according to the resistance gene from GenBank, and the relevant literature (7,8) for part of the primers was listed in Table 1.

PCR amplification reaction system:

All of the PCR amplification reaction system was 50 μL, including 5 μL buffer, 1 μL 10 mmol/L 4 × d NTP, 2 μL 50 mmol/L upstream and downstream primers, 0.5 μL pfu DNA polymerase, 2 μL DNA templates, and 37.5 μL multiple distilled sterile water. The Escherichia coli TEM-2 was applied as the TEM-type positive control, while the ATC C700603 pneumoniae was applied as the SHV-type positive control. Different PCR amplification conditions were established according to different primers (9), as shown in Table 2.

Gene sequencing:

Parts of the PCR products were sent to Shanghai Mejorbio Bio-Pham Technology Co., Ltd for the gene sequencing, and the results were in the progress of sequence alignment analysis directly in the GenBank (http://www. ncbi.nlm.nih, gov/BLAST).

Table 1. Primers

Primer	Upstream primer	Downstream primer	Length (bp)
AmpC	5c-GCCTGGTAAGTATTGGAAAG-3c	5c-CCGAAACGGTTAGTTGAGCC-3c	702
SHV	5c-TATTATCTCCCTGTTAGCCAC-3c	5c-GCCTCATTCAGTTCCGTTTCC-3c	479
VIM	5c-CCGTAGAAGAACAGCAAGGC-3c	5c-CCATCGGCAATCTGGTAAAG-3c	589
VRE	5c-AAATGCCAGAATAGGAGTAG-3c	5c-GAAGTCCCTGTTTTATGAGC-3c	611
aacA4	5c-GAAGAAGCACGCCCGACAC-3c	5c-TCGGATCGCTCGCAAGTTG-3c	337
IMP	5c-GCTCACGCAACTGGTCGC-3c	5c-CCGCCTGCTCTAATGTAAG-3c	909
aadA1	5c-TGATTTGCTGGTTACGGTGAC-3c	5c-CGCTATGTTCTCTTGCTTTTG-3c	289
OXA-23	5c-GATGTGTCATAGTATTCGTCG-3c	5c-GTCACGAAAATCAACAACACT-3c	1102
PER	5c-CATTGGTTCGGCTTGAC-3c	5c-CCACTGTAGGCGTTGC-3c	716
aacC1	5c-CCACCTACTCCCAACATCAG-3c	5c-TCACTTCTTCCCGTATGCCC-3c	329
OXA-24	5c-GTACTAATCAAAGTTGTGAA-3c	5c-TGTTTAAGTACAATCCCCTT-3c	1031
aphA1	5c-ATACAGAGACCACCATACAGT-3	5c-GGACAATCAATAATAGCAAT-3c	238
gyrA	5c-AATCTGCCCGTGTCGTTGG-3c	3c-CCACGACGCCCATAGCGG-5c	339
TEM	5c-CAGAAACGCTGGTGAAAG-3c	5c-AACTACGATACGGGAGGG-3c	707

Table 2. PCR amplification reaction

Primer	Initial denaturation	Cycle: 34			Ultimate Extension
		Denaturation	Annealing	Extension	
AmpC	93 ℃ 5 min	93 ℃ 35 s	54 ℃ 45s	72 ℃ 75 s	72 ℃ 7 min
SHV- 1	93 ℃ 5 min	93 ℃ 35 s	54 ℃ 45s	72 ℃ 60 s	72 ℃ 7 min
VIM- 2	93 ℃ 5 min	93 ℃ 35 s	56 ℃ 45s	72 ℃ 60 s	72 ℃ 7 min
VEB- 1	93 ℃ 5 min	93 ℃ 35 s	54 ℃ 45s	72 ℃ 60 s	72 ℃ 7 min
aacA4	93 ℃ 5 min	93 ℃ 35 s	55 ℃ 45s	72 ℃ 30 s	72 ℃ 5 min
IM P	93 ℃ 5 min	93 ℃ 35 s	54 ℃ 45s	72 ℃ 90 s	72 ℃ 7 min
aadA1	93 ℃ 5 min	93 ℃ 35 s	55 ℃ 45s	72 ℃ 30 s	72 ℃ 5 min
OXA-23	93 ℃ 5 min	93 ℃ 35 s	52 ℃ 40s	72 ℃ 75 s	72 ℃ 7 min
PER-1	93 ℃ 5 min	93 ℃ 35 s	54 ℃ 45s	72 ℃ 90 s	72 ℃ 7 min
aacC1	93 ℃ 5 min	93 ℃ 35 s	55 ℃ 45s	72 ℃ 30 s	72 ℃ 5 min
OXA-24	93 ℃ 5 min	93 ℃ 70 s	52 ℃ 60s	72 ℃ 90 s	72 ℃ 10 min
aphA1	93 ℃ 5 min	93 ℃ 35 s	55 ℃ 45s	72 ℃ 30 s	72 ℃ 5 min
gyrA	93 ℃ 5 min	93 ℃ 35 s	55 ℃ 45s	72 ℃ 30 s	72 ℃ 5 min
TEM-1	93 ℃ 5 min	93 ℃ 35 s	56 ℃ 45s	72 ℃ 90 s	72 ℃ 7 min

Table 3. Susceptibility of the strains

Antibacterial agent	Drug resistance	Sensitivity	Medium
Imipenem	1	17	0
Cefoperazone/sulbactam	4	1	13
Ciprofloxacin	16	2	0
Ampicillin/sulbactam	16	0	2
Ofloxacin	16	2	0
Piperacillin	18	0	0
Ticarcillin/clavulanic acid	18	0	0
Cefoperazone	18	0	0
Amikacin	18	0	0
Gentamicin	18	0	0
Aztreonam	18	0	0
Ceftazidime	18	0	0
Piperacillin/tazobactam	18	0	0
Ceftriaxone	18	0	0
Cefoxitin	18	0	0
Cefepime	18	0	0
Cefotaxime	18	0	0
Cefuroxime	18	0	0
Sulfamethoxazole	18	0	0
Amoxicillin/clavulanic acid	18	0	0

Homology analysis:

PFGE was carried out after the digestion of the bacterial strains by restriction endonuclease. According to the method recommended by TENOVER (10), the judgment criteria were as follows: identical; closely related: 2 to 3 different bands; possibly relevant: 4 to 6 different bands; different: no less than 7 different bands.

Data processing:

The susceptibility of the strains was input, analyzed, and counted by the WHONET5.4 software.

Results

Susceptibility

All of the 18 strains were multidrug resistant A. baumannii. 17 strains were susceptible to imipenem while the other one (a029s) was drug resistant. To cefoperazone/sulbactam, one of the strains was sensitive, four were drug resistant, and another 13 were intermediary. To ofloxacin and ciprofloxacin, two of them were sensitive and the other 16 were drug resistant. To ampicillin/sulbactam, two of them were intermediary and the other 16 were drug resistant. All of the strains were resistant to other drugs. The results were shown in Table 3.

Results of ESBLs, AmpC and MBL

Three-dimensional test detected four ESBLs strains with a029s as yield strains; all the strains of high yield AmpC, except a029s, were positive strains including 3 strains producing ESBLs at the same time. The synergy test discovered that obvious MBL positive synergies with CAZ or IPM were observed in four strains. All the results were shown in Table 4.

Table 4. Stain number

Strain No.	ESBL s	AmpC	MBL
a001 s	-	+	+
a007 s	-	+	-
a010 s	-	+	-
a019 s	-	+	-
a026 s	-	+	-
a029 s	+	-	-
a033 s	-	+	-
a039 s	-	+	-
a046 s	-	+	-
a053 s	-	+	-
a066 s	+	+	+
a074 s	-	+	-
a091 s	+	+	-
a103 s	-	+	+
a107 s	-	+	-
a131 s	+	+	+
a139 s	-	+	-
a147 s	-	+	-

Results of drug resistant genes

Genes of TEM-type broad-spectrum, AmpC enzyme, aacA4 aminoglycoside acetyltransferase and gyrA DNA gyrase were detected in all of the 18 strains. PER ultra-broad-spectrum enzyme gene was positive in a029s and negative in the other 17 strains. The OXA-23 carbonpenem gene was positive in the amplified a029s and negative in the other 17 strains. 17 strains were positive in aacC1 and aadA1, while the other one strain was positive in aphA1. None of the SHV type broad-spectrum enzyme or ultra-broad-spectrum enzyme, VEB-type ultra-broad-spectrum enzyme, IM P-type or VIM-type metal enzyme, OXA-24-type carbapenemase genes were detected in all of the 18 strains. All the results were shown in Table 5.

Results of PFGE

According to the analysis of drug resistance gene cluster, 16 strains were identical for the presence of the same bands indicating a high degree of homology, while a029s and a107s strains were independent strains containing different digestion.

Discussion

From the susceptibility testing, imipenem was superior to treat A. baumannii due to its benign bactericidal effect, stability to multiple β-lactamase enzyme, and over 95% sensitive rate. However, because of the facility to induce the generation of other drug resistant bacteria, imipenem should be chosen to use (11).

All of the 18 multidrug resistant A. baumannii generated TEM-1 type ESBLs in this project, however, TEM-type ESBLs A. baumannii had not been found domestic. As a result, TEM-1 type ESBLs was widespread in multidrug resistant A. baumannii for the capacity of extensively hydrolysis of first-generation cephalosporins and the first generation penicillins antibacterials instable to enzyme. Although the enzyme might play a supporting role in the multidrug resistance of A. baumannii, the synergy with other multi-drug resistance mechanisms related to A. baumannii directly. However, SHV-type β-lactamase had not been observed in A. baumannii in this area, which may be related to the lack of this epidemic strain in this area (12).

The three-dimensional test discovered that 4 strains produced ESBLs, amplified strain produced PER-1 type ESBLs, which agreed with the target gene Z21957 in the PCR product bidirectional sequencing comparative analysis. No VEB-type ESBLs was observed in the strains. PER-1 gene

Table 5. Drug resistant genes

Strain No.	acA4	aadA1	aacC1	AmpC	aphA1	gyrA	IMP1	OXA-23	OXA-24	PER-1	SHV1	TEM-1	VEB1	VIM2
a001 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a007 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a010 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a019 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a026 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a029 s	+	-	-	+	-	+	-	+	-	+	-	+	-	-
a033 s	+	+	+	+	+	+	-	-	-	-	-	+	-	-
a039 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a046 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a053 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a066 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a074 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a091 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a103 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a107 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a131 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a139 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-
a147 s	+	+	+	+	-	+	-	-	-	-	-	+	-	-

was first discovered in pseudomonas aeruginosa in France, and PER-1 gene encoding produced ESBLs A. baumannii and pseudomonas aeruginosa were discovered later in Turkey. According to the survey, 45.7% A. baumannii carried this gene and were belonged to different biotypes. PER-1-type ESBLs A. baumannii were widespread in the ICU in different hospitals in Korea, Belgium and Russia (13). The eruption of dissemination of the strains with this gene was reported previously. The major symptom was the complete resistance to the drugs of 3, 4-generation cephalosporins, penicillins, and single-ring antibacterials, the sensitivity to β-lactam inhibitors, cephamycins and carbapenems antibacterials, and significant resistance to aminoglycosides and quinolones. In another three strains of A. baumannii, the three-dimensional test displayed that the genetic testing was negative for ESBLs, which may be caused by other genotypes. Further studies were required (14).

Three-dimensional test revealed that 17 strains of A. baumannii produced AmpC enzyme, and the 18 gene amplified strains produced AmpC enzyme. The unidirectional sequencing comparative analysis of the 4 amplification products presented 100% homology with the target gene AJ009979. The chromosome-mediated high-yield AmpC enzyme, closely related with the C-class cephalosporinases, was important in the multidrug resistant A. baumannii. The inhibition of this enzyme was 40% ~ 50% to sulbactam or tazobactam, and quite weak to clavulanic acid. Like other C-class β-lactamase enzymes, the AmpC enzyme displayed high efficiency in the hydrolysis of cephalosporin, moderate of cefoxitin, and good of penicillins and third-generation cephalosporins with excess enzyme. However, a weak hydrolysis activity appeared in imipenem (15). Recently, more than 27 kinds of plasmid-mediated AmpC enzyme with the size of 7-180kb were emerged throughout the world, which greatly improving the lateral spread of AmpC enzyme. The clinically isolated strains carried the AmpC as well as the β-lactamase gene, resistant to the first to third-generation cephalosporins, cephamycins, aminoglycosides and anti-fake Aeromonas penicillin. However, they were sensitive to the 4th generation cephalosporins, carbapenems, and fluoroquinolones. The genetic testing results revealed that the chromosome-mediated AmpC gene was the major factor for the multidrug-resistance of A. baumannii in ICU of the hospital (16). In a029s, only PCR detected the AmpC gene with multidrug-resistance. Further studies were required because of the various possibilities, such as

the simultaneous load of PER-1 type ESBLs and OXA-23, or the low expression of AmpC, and so on. It was valuable to apply three-dimensional test in the detection of AmpC, presenting that high yield of AmpC could be generated without any inducer and the results were with more than 95% consistency with the genetic tests which could be applied in routine laboratories (17).

Although four A. baumannii exhibited synergy with EDTA from the synergy test, PCR failed to detect the IMP and VIM type metallo-β-lactamase resistance gene and the susceptibility testing revealed susceptivity to imipenem reasons. Advanced research was required for the explanation.

Susceptibility testing revealed that one strain was resistant to imipenem, OXA-23 carbapenemase gene was detected after the gene amplification, and the product was identical to the target gene AY795964.1 by PCR. OXA-23 carbapenemase A. baumannii was reported in Brazil, France and South Korea since the first discovery in the UK. Possessing the characteristic of hydrolyzing lactam antimicrobial, which was closely connected to the multidrug resistant (18), this carbapenemase A. baumannii was significant in the prevention of nosocomial infection in the future although it was not predominant in the main epidemic strains.

Susceptibility results showed that all of the 18 A. baumannii were with aacA4 gene and resistant to gentamicin and amikacin, 17 strains were carrying aacC1, aadA1, and one strain was taking aphA1 gene after the gene amplification. Aminoglycoside modified enzymes were divided into three categories, namely, acetyl transferase enzyme (AAC), adenylyl transferase (ANT) and phosphotransferase (APH). The same as other gram-negative bacilli, A. baumannii, carrying the aacA4, aacC1, aadA1, aphA1, often exhibited multidrug resistance to antimicrobial aminoglycosides. These genes were often transmitted with the gene cassette, showing multidrug resistance (19).

The mutations of DNA Gyrase in A. baumannii usually presented tolerance to quinolones. The mutation of the 83rd point serine lead to high level tolerance to ciprofloxacin and other quinolones, and higher level could be achieved if associated with the mutation of topoisomerase (topoisomerase Ô). By the comparison between 4 amplification products of gyrA and the target gene AF100557, A. baumannii was observed with the drug-resistance to quinolones after the replacement of the serine on the 83rd point by leucine. It was possible that another two strains expressed no resistance to ofloxacin and ciprofloxacin because no gene mutation happened (20).

Multidrug resistant A. baumannii, caused by a single source extensive pollution and broke out easily in a certain amount of time and space, brought difficulty in clinical treatment (21). The infections caused by A. baumannii increased significantly in ICU during the collection of the bacterial strains. AmpC was dominant in the genotyping distribution of multi-drug resistant A. baumannii carrying genotype-similar ESBLs and aminoglycoside modified enzyme. From the PFGE, the digested chromosomal genes of the 16 multidrug resistant A. baumannii were identical, proving the existence of clonal epidemic strains and exhibiting multidrug resistant except to the sensitive or intermediate imipenem and cefoperazone/sulbactam. Multidrug resistant strains carrying OXA-23 type carbapenem and PER-1 type plasmid gene emerged and resistant to imipenem and cefoperazone/sulbactam intermediary and other antibacterial characteristics of the drugs are tolerated. It is important to strengthen the environmental detection and disinfection (22).

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Clinical effects of compound Cordate houttuynia granules on rheumatoid arthritis-interstitial lung disease

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Abstract

Objective: To study the clinical effects of compound Cordate houttuynia granules on rheumatoid arthritis (RA)-interstitial lung disease (ILD).

Methods: 120 patients were selected and randomly divided into a treatment group and a control group equally. The secondary fungal infection patients derived from regular treatment were analyzed to reveal the pathogenic reasons. Both groups were orally administered with formulated Chinese herbal decoctions at dedicated doses, based on which the treatment group were further orally administered with compound Cordate houttuynia granules.

Results: ILD is mainly treated with hormones and immunosuppressive agents, which may jeopardize immune system and induce fungal infection owing to the prolonged treatment duration. In the treatment group, there were 10 markedly effective cases, 39 effective cases and 11 ineffective cases, and the overall effective rate was 81.67%. In the control group, there were 4 markedly effective cases, 27 effective cases and 29 ineffective cases, and the overall effective rate was 51.67%. The overall effective rates of the two groups differed significantly ($P<0.01$). Conclusion: RA-ILD can be effectively and securely treated with compound Cordate houttuynia granules.

Key words: Traditional Chinese medicine treatment, compound Cordate houttuynia granule, rheumatoid arthritis, interstitial lung disease.

Introduction

Rheumatoid arthritis (RA) is a multisystem inflammatory autoimmune disease involving peripheral joints [1]. 30% of RA patients also suffer from pulmonary fibrosis [2]. Currently, interstitial lung disease (ILD) is mainly treated with hormones and immunosuppressive agents, which may jeopardize immune system and induce fungal infection owing to the prolonged treatment duration [3]. According to the traditional Chinese medicine theory quoted in "Suwen·About Paralysis", RA-ILD is ascribed to the lung-energy stagnation syndrome [4], the clinical manifestation and pathological changes of which are in agreement with the phlegm accumulation symptoms. Thus, RA-ILD can be effectively treated with compound Cordate houttuynia granules by preventing the accumulation of phlegm and detoxifying. The compound Cordate houttuynia granules (WS-666(Z-127)-2001, 6 g × 10 bags × 200 boxes/item) consisted of Cordate houttuynia, Radix scutellariae, Radix isatidis, Fructus forsythiae, and Flos lonicerae. In this study, 120 RA-ILD patients enrolled from March 2008 to June 2012 were retrospectively analyzed, aiming to observe the clinical treatment efficacy.

Materials and Methods

Data

120 RA-ILD patients enrolled from March 2008 to June 2012 were selected. The treatment group aging 15-70 years old consisted of 12 males and 48 females. The control group aging 16-72 years old consisted of 13 males and 47 females. 27 out of 120 patients were diagnoses as secondary fungal infection after 3 times of positive examinations.

Diagnosis standards

The patients were diagnosed as RA according to the diagnosis and treatment guidelines of Chinese Medical Association (meet at least 4 standards) [5]: 1) at least 1 h of morning stiffness for more than 6 weeks; 2) arthritis of 3 or more than 3 joints for more than 6 weeks; 3) hand arthritis, and

lumps in wrist, palm, finger or at least one proximal interphalangeal joint for more than 6 weeks; 4) symmetric joint lump for more than 6 weeks; 5) rheumatoid nodule; 6) radiological changes: osteoporosis and joint space stenosis in fingers; 7) positive rheumatoid factor.

Interstitial lung disease diagnosis standards: 1) tussiculation, progressive dyspnea, and Velcro rales over the lung bases; 2) ground-glass, small-nodular, reticular, honeycomb shadows indicated by chest X-ray and/or CT; 3) restrictive ventilatory dysfunction and reduced diffusing capacity indicated by lung function examination; 4) early-stage nonspecific alveolitis and late-stage pulmonary fibrosis. The patients who were in accordance with Standard 1), 2), 3) or 4) were diagnosed [3].

Pathogenic analysis of secondary fungal infection

The secondary fungal infection patients had been administered with hormones or antibiotics and underwent some clinical sign changes, such as more viscous sputum, aggravated symptoms, changed pulmonary auscultation and ineffective antibiotics treatment. Their respiratory tract secretions were subjected to pathogenic analysis.

TCM syndrome differentiation

Arthralgia syndrome was classified into wind, dampness, cold and fever types according to the standard released by the Kunming Conference in April 1988. Blood stasis was diagnosed as the standard released by the 2nd National Blood Circulation Promotion Seminar in November 1986. Qi and blood asthenia, insufficiency of lung yang and insufficiency of lung yin were diagnosed according to the standard released by the National Combined TCM and West Medicine Asthenia and Elderly Disease committee [6].

Treatment efficacy evaluation standard and scoring of imaging and lung functions

Treatment efficacy evaluation

The standard released by the Respiratory Disease Branch of Chinese Medical Association: 1) markedly effective: 1mitigated symptoms and enhanced movement capacity; 2reduced abnormal chest X-ray or HRCT images; 3improved lung functions. 2) Effective: 1alleviated symptoms; slightly reduced abnormal chest X-ray or HRCT images; 3slightly improved lung functions. 3) Ineffective: unchanged symptoms, chest X-ray or HRCT and lung functions [7].

Imaging and lung function scoring

1) Lung CT or HRCT scoring [8]: normal: 1; ground glass: 2; consolidation: 3; ground-glass changes plus traction bronchiectasis: 4; consolidation plus traction bronchiectasis: 5; honeycomb lung: 6. The overall score was obtained by measuring all the abnormal regions in the three lung zones in each lung side. 2) Lung function: diffusion volume of carbonmonoxide (DLCO)≥ 80%: 0; 60% ≤ DLCO<80%: 1; 40% ≤ DLCO<60%: 2; DLCO<40%: 3 [9].

Grouping and treatment

The patients enrolled were randomly divided into a treatment group and a control group. All the herbal tablets were provided by Sinopharm Group Co., Ltd. Both groups were orally administered with the basic herbal formula consisting of Rhizoma et Radix, Radix Angelicae, Radix Saposhnikoviae, Herba Asari, Siegesbeckia orientalis, Sambucus chinensis, Semen Brassicae, Polistes mandarinus, Radix cynanchi paniculati, and Folium et Ramulus. The treatment group were further administered with compound Cordate houttuynia granules (dissolved in boiling water, 1 bag each time, tid). The patients suffering from the arthralgia syndrome resulting from wind, cold and dampness were administered with the basic formula, those with blood stasis were administered with Eupolyphaga seu Steleophaga and Whitmania pigra Whitman, those with Qi asthenia and anaemia were administered with Radix Angelicae and Radix Astragali, those with insufficiency of Yi were administered with Rhizoma Anemarrhenae and Rehmannia glutinosa, those with insufficiency of Yang were administered with Radix Aconiti Lateralis Preparata and Cortex Cinnamomi, those with asthma were administered with Semen Ginkgo and Herba Ephedrae, those with cough were administered with Bulbus Fritillariae Cirrhosae and Folium Eriobotryae, those with expectoration were administered with Trichosanthes kirilowii and Aster tataricus, and those with yellow sputum were administered with Radix scutellariae and

Fructus Gardeniae. All the medicines were boiled routinely to 200 ml (one dose per day, completed by 2 oral administrations). The indexes and scores of both groups were observed after being treated for 3 months. They were followed up 3 times every other 2 months.

Statistical analysis

The data were analyzed by SPSS17.0, and the results expressed as $\bar{x} \pm s$ were subjected to t test and analysis of variance. The grading data were analyzed by Wilcoxon rank sum test. The numeration data were analyzed by χ^2 test. We used $\alpha=0.05$ as the test standard.

Results

Fungal infection reasons and pathogenic analysis

27 out of the 120 cases herein underwent secondary fungal infection, which can be attributed to the old age (over 65 years old), administration of hormones for over 3 months (94 cases), antibiotics polypharmacy (≥ 2 antibiotics types, 83 cases), and the administration history of immunosuppressive agents (44 cases) (Table 1). The reasons all differ significantly. In the sputums of the 27 secondary fungal infection patients, 20 cases of Monilia albican (74.07%), 2 cases of Aspergillus flavus (7.41%), 3 cases of Candida tropicalis and 2 cases of Monilia albican (11.1%) plus other two fungi (7.41%) were detected, respectively. Meanwhile, 13 cases of bacterium (48.15%), including 8 cases of gram negative bacillus, 4 cases of Staphylococcus and 1 case of gram-positive coccus, were also detected.

Table 1. Fungal infection reason analysis

Infection reason		Case No.	Secondary fungal infection	Without fungal infection	P value
Age	≤65	79	11	68	<0.05
	>65	41	16	25	
Hormone administration	≤3 months	94	25	69	<0.05
	>3 months	26	2	24	
Antibiotics	Polypharmacy	83	23	60	<0.05
	No or only one antibiotic	37	4	33	
History of immunosuppressive agents	Yes	44	19	25	<0.01
	No	76	8	68	

Overall treatment efficacy

In the treatment group, there are 10 markedly effective cases, 39 effective cases and 11 ineffective cases, and the overall effective rate is 81.67%. In the control group, there are 4 markedly effective cases, 27 effective cases and 29 ineffective cases, and the overall effective rate is 51.67%. The overall effective rates of the two groups differ significantly (P<0.01) (Table 2).

Table 2. Overall treatment efficacy analysis

Treatment efficacy	Treatment	Control
Markedly effective	10 (16.67%)	4 (6.67%)
Effective	39 (65.00%)	27 (45.00%)
Ineffective	11 (18.33%)	29 (48.33%)
Overall effective rate (%)	81.67*	51.67

*Compared to the control group, $\chi^2=10.555$, *P<0.01*

Symptoms and physical sign scores

The symptoms and the physical sign scores of the treatment group were mitigated and lowered evidently. The two groups differed significantly (P<0.05, Table 3).

Imaging and lung function scoring

The CT or HRCT imaging changes of both groups before and after treatment differed significantly. The results of the two groups after treatment also differed significantly (P<0.01). The lung function scores of both groups before and after treatment differed significantly (P<0.05), and those of the two groups after treatment were similar (P>0.05) (Table 4).

Table 3. Symptoms and physical sign scores

Symptom, physical sign		Treatment	Control
Cough	Markedly effective	18 (30.00%)*	4 (6.67%)
	Effective	36 (60.00%)	39 (65.00%)
	Ineffective	6 (10.00%)	17 (28.33%)
Asthma	Markedly effective	11 (18.33%)*	2 (3.33%)
	Effective	42 (70.00%)	40 (66.67%)
	Ineffective	7 (11.67%)	18 (30.00%)
Burst sound	Markedly effective	10 (16.67%)*	3 (5.00%)
	Effective	42 (70.00%)	38 (63.33%)
	Ineffective	8 (13.33%)	19 (31.67%)

*Compared to the control group, *P<0.05*

Table 4. CT and lung function scores ($\bar{x} \pm s$)

Item	Treatment		Control	
	Before	After	Before	After
CT	42.65±6.07	35.39±5.25#*	42.03±5.36	37.79±6.23#
Lung function score	65.27±9.66	60.17±9.31#	64.97±7.61	60.34±8.52#

*Compared to the results before treatment, #P<0.01; compared to the control group, *P<0.05*

Discussion

RA-ILD, a clinical respiratory system syndrome, is featured in dyspnea, diffusing chest X-ray, restrictive ventilation disorder, diffusing capacity reduction and hypoxemia [10]. Currently, RA-ILD patients were commonly orally administered with hormones or immunosuppressive agents for longer than 1 year, which are prone to jeopardizing the immune system and inducing fungal infection. As a result, the easily neglected infections may even endanger life [11]. The secondary fungal infection rate reached up to 22.50% herein, which is consistent with a previous literature [12]. The lungs are prone to being infected, which is clinically manifested as cough, aggregated sputum, dyspnea, low fever, short breath, chest tightness, and varied respiratory murmur, etc. Fungal infection is often misdiagnosed as aggravated interstitial lung disease [13]. In this study, the patients, who were elderly (over 65 years old) as well as were administered with hormones for over 3 months, antibiotics polypharmacy (≥ 2 antibiotics types) and immunosuppressive agents, were subject to secondary fungal infection. Therefore, the results indicate that the elderly patients, who were of low immune functions, poor nutrition status and heart, lung and kidney diseases, were readily infected by fungi in the presence of long-term hormones, immunosuppressive agents and antibiotics [14].

RA-ILD is ascribed to arthralgia syndrome which includes limb arthralgia and organ arthralgia [15]. Lung-energy stagnation syndrome, which mainly results from the stagnated lung Qi by external factors, is similar to RA-pulmonary fibrosis in the pathological characteristics, clinical signs and disease development. Thus, RA-ILD is ascribed to lung-energy stagnation syndrome in traditional Chinese medicine [16,17], which should be treated by eliminating the pathogenic factors, invigorating blood circulation, reducing phlegm and detoxifying [18].

Compound Cordate houttuynia granules consist of Cordate houttuynia, Radix scutellariae, Radix isatidis, Fructus forsythiae, and Flos lonicerae, etc. [19], targeting to clear fever and toxic materials, resist to bacteria and viruses, and boost the immune function [20,21]. Cordate houttuynia, Radix isatidis, Radix scutellariae, Flos lonicerae and Fructus forsythiae are able to inhibit multiple bacteria. Cordate houttuynia and Radix scutellariae can significantly inhibit the growth of drug-resistant Staphylococci aureus. Besides, Cordate houttuynia and Radix isatidis can resist to Bacillus influenzae. Cordate houttuynia and Radix scutellariae can evidently suppress the growth of Monilia albican and Cryptococcus neoformans. Flos lonicerae and Fructus forsythiae are able to inhibit the Bacillus coli-induced animal peritonitis. Radix isatidis can

apparently inhibit the growth of influenza virus PR3 strain [22]. Radix scutellariae also mitigated the lung injuries of mice and prolonged the survival time by inhibiting the growth of influenza virus PR8 strain. The compound injection containing Fructus forsythiae, Flos lonicerae, Radix isatidis and Coptis chinensis significantly enhanced the phagocytic ability of inflammatory exudate cells, reduced the capillary permeability of rats and mice, and decreased the inflammatory exudate. Cordate houttuynia also boosted the phagocytic capacity of human leukocytes, and elevated the serum properdin levels of rabbits and patients. Moreover, the lysozymes in blood and sputum were also activated [23]. Radix scutellariae specifically raised the cAMP levels of lung and bronchus, inhibited the binding of antigens to IgE, and prevented the labrocytes from releasing histamine. Radix scutellariae extract can reduce the capillary permeability, resist to inflammation and allergic edema, and suppress the passive allergy induced by allogeneic and xenogeneic antibodies [24]. Compound Cordate houttuynia granules do not show obvious toxicity, thought there are few cases of throat dryness, stomach burns, palpitation and hand trembling which do not affect further administration [25]. The overall effective rates of the treatment group and the control group are 81.67% and 51.67%, respectively, revealing that the compound Cordate houttuynia granules are effective and secure.

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Functional hyperprolactinemia in infertile women

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Abstract

Functional hyperprolactinemia (hyperPRL) in infertile women is the presence of abnormally high values of PRL levels in the absence of prolactinomas. The aim of this study was to determine the influence of hyperPRL on gonadotropin and ovarian secretion and endometrial and ovarian morphology.

Patients and methods: Prospective study study included 30 infertile women with functional hyperPRL, and 20 women without hyperPRL. All participants in both groups had tehe following parameters determined: prolactin, FSH, LH, estradiol and progesterone in the early follicular phase and in the middle luteal phase of the menstrual cycle. Morphological characteristics of ovarian and endometrial were observed by ultrasound.

Results: Infertile women with functional hyperPRL had a significantly higher prevalence of galactorrhea (83.3% vs. 3.3%, p <0.001), galactorrhea / amenorrhea (45.0% vs. 3.3%, p <0.001), irregular menstrual cycles (85.0% vs. 10% p <0.001), infertility (66.0% vs. 13.3%, p <0.001) compared to the control group. In the early follicular phase mean PRL (38.4 vs. 10.2, p <0.001), and PRL in the luteal phase (39.1 vs. 9.7, p <0.001) were significantly higher in women with functional hyperPRL compared to the control group. In the early follicular phase the mean LH (4.1 vs. 6.4, p <0.01), FSH (4.2 vs. 5.8, p <0.05) and E2 (87.14 vs. 254.3, p <0.05) were significantly lower than in the control group . Mean values of LH (4.6 vs. 6.1, p <0.05) and FSH (3.6 vs. 6.2, p <0.05) and E2 (84.24 vs. 350.8, p <0.01) were significantly lower, while the value of progesterone (3.6 vs. 25.2, p <0.001) was significantly higher than in the control group. In the middle luteal phase, the mean endometrial thickness (5.7 vs. 8.6, p <0.001), presence of corpus luteum (6.7% vs. 86.7%, p <0.001) and endometrial volume (3.0 vs. 4.9, p <0.01) were significantly lower, while the mean RI (0.72 vs. 0: 42 p <0.00) was significantly higher than the control group.

Conclusion: Results of this study show that infertile women with functional hyperPRL have disturbed gonadal and ovarian function, which results in disruption of menstrual cycles and infertility.

Key words: Functional hyperPRL, hypogonadism, anovulation, infertility

Introduction

Hyperprolactinemia (hyperPRL) is the presence of abnormally high levels of prolactin in the blood. Hyperprolactinemia (hyperPRL) is the most common hypothalamic-pituitary disorder that the clinical endocrinology meets. In addition to the hypothalamic-pituitary disease, hyperPRL can also occur as secondary when using certain medications, chronic diseases such as hypothyroidism, PCOS, chronic liver and kidney disease, stress and neurological disorders. HyperPRL cause endocrine disruption at the level of the hypothalamic-pituitary-gonadal, which results in sexual / reproductive dysfunction and infertility (1).

The most important clinical sign of hyperPRL is galactorrhoea, and is diagnosed in 30-90% of women with hyperPRL.

Prolactin plays a key role in reproductive function and is the most common endocrine disorder of hypothalamic-pituitary axis and occurs in 10% of the population (2).

Primary hypothyroidism is rarely encountered in reproductive females (3). In patients with primary hypothyroidism, there is increased production of thyrotropin (TRH), which can stimulate the secretion of prolactin (1). Hyperprolactinemia can also cause decreased dopamine secretion from the hypothalamus (4). Chronic renal failure in 30% of patients results in increased levels of prolactin (5).

Antipsychotic agents and other drugs often cause functional hyperPRL (especially risperidone, metoclopramide and verapamil, opiates and H2 blockers). Usually in the value of less than 100 ng / ml, but in some patients a level of prolactin can reach and 365 ng / ml (6, 7). Women with polycystic ovary syndrome (PCOS) may have mild hyperprolactinemia in the absence of pituitary lesions. In clinical studies, elevated levels of prolactin were found in the 19% to 50% of women with polycystic ovary syndrome (8, 9).

Subjects and Methods

This prospective study included 30 infertile women with functional hyperPRL, with normal renal and hepatic function. Criteria for inclusion in the study were infertile women with clinical signs of hyperprolactinemia. Infertile women with a tumor and pseudotumor hyperPRL were excluded from the study. The control group consisted of 20 healthy women with normal menstrual cycles.

Study protocol

The primary objective was to determine the level of prolactin, gonadotropins, estrogens and progesterone in the early follicular and luteal phase of the menstrual cycle. The secondary objective was to determine the sonographic features of ovarian and endometrial in the early follicular and luteal phase of menstrual cycle. Patients had these parameters determined: a) anthropometric parameters: FG score, BMI, the percentage distribution of galactorrhoea, iregularanog menstrual cycle, b) hormonal parameters: FSH, LH, estradiol, progesterone, and prolactin in the early follicular phase and high luteal stage d) morphological characteristics of ovarian and endometrial were measured by 4D color Doppler (Voluson E8 Expert, GE Health, USA) in the early follicular and luteal phase of menstrual cycle. Hormon levels of LH, FSH, oestradiol, progesterone and prolactin were determined from blood samples obtained from each subject after overnight fasting in the early phollicular phase (cycle days, 3-5) and mild Lutel phase. The reference values that were used: LH, 5-20 mIU, FSH, 5-20 mIU / ml, and prolactin 2.5-20 ng / ml. Oestradiol, Pg, FSH, LH, prolactin, were determined by using the Wallace Fluroimmunoassay he Delfia Fluorometar. The original Delfia kits (Turku, Finald) for individual hormones were used. Normal levels of prolactin in women is less than 450 mIU / L (20.0 ng / ml and 21.4 ng / mL) (10).

Statistical Methods

Statistical analysis was performed with application of software called SPSS for Windows version 12th Numerical data are presented as mean + /-SD, as median, number%. To test the hypothesis between groups t-test (Mann-Wyitney test and Fisher exact test with a significance level of $p < 0.05$) was used.

Results

Reproductive women with functional hyperPRL had a significantly higher BMI (27.4 vs. 24.6, $p < 0.05$), higher prevalence of galactorrhea (83.3% vs. 3.3%, $p < 0.001$), galactorrhea / amenorrhea (45.0% vs. 3.3%; $p < 0.001$), irregular menstrual cycles (85.0% vs. 10%, $p < 0.001$), infertility (66.0% vs. 13.3%, $p < 0.001$) compared to the control group (Table 1).

Table 1. Anthropometric characteristics of reproductive women with hyperprolactinemia and control group

Parameters	HyperPRL group	Control group	p - value
Total number (№)	40	20	-
Menarche (age)	11.8 ± 1.3	12.4 ± 1.4	p<0.9
BMI (kg/m^2)	27.4 ± 2.1	24.6 ± 2.1	^{a}p<0.05
F-G scor>7 %	9/30 (15%)	2/30 (6.6%)	p<0.6
Galactorrhoea (%)	50/30 (83.3%)	1/30 (3.3 %)	^{c}p<0.001
Irregular MC (%)	51/30 (85.0%)	3/30 (10.0%)	^{c}p<0.001
Infertility (%)	40/30 (66.0%)	4/30 (13.3%)	^{c}p<0.001
Galactorrhea+ amenorrhea (%)	27 /30 (45.0%)	1/30 (3.3%)	^{c}p<0.001

Legend: The results are expressed as mean ± SD, percentage%, statistical significance p<0.05 level of significance, and ^{a}p<0.05, ^{b}P<0.01, ^{c}p<0.001, for the group with hyperprolactinemia Vs. control group, PRL - prolactin. hiperPRL-hyperprolactinemia, MC-menstrual cycle; FG score - Ferriman-Gallwey scores, BMI - body mass index.

In the early follicular phase of the menstrual cycle mean PRL (38.4 vs. 10.2, p <0.001), PRL in the luteal phase (39.1 vs. 9.7, p <0.001) were significantly higher in women with functional hyperPRL compared to the control group. In the early follicular phase of the menstrual cycle mean LH (4.1 vs. 6.4, p <0.01), FSH (4.2 vs. 5.8, p <0.05) and E2 (87.14 vs. 254.3, p <0.05) were significantly lower compared to control group, whereas there was no significant difference in levels of progesterone. Mean values of LH (4.6 vs. 6.1, p <0.05) and FSH (3.6 vs. 6.2, p <0.05) and E2 (84.24 vs. 350.8, p <0.01) were significantly lower, while the value of progesterone (3.6 vs. 25.2, p <0.001) was significantly higher than in the control group (Table 2).

Reproductive women with primary hypothyroidism had in the early follicular phase significantly higher mean values of PRL (32.4 vs.10.2, p <0.001), significantly lower mean LH (4.3 vs. 6.4, p <0.01), FSH (4.7 vs. 5.8; p <0.05) and E2 (95.26 vs. 254.3, p <0.01) compared to the control group, while there was no difference in the levels of progesterone. In the middle luteal phase, the mean PRL (31.4 vs.9.7, p <0.001), progesterone (25.2 vs. 2.9, p <0.001) were significantly higher, while the LH (4.8 vs. 6.1, p <0.05) and FSH (4.1 vs. 6.2, p <0.05) and E2 (89.34 vs. 350.8, p <0.01) were significantly lower in the group of reproductive women with primary hypothyroidism compared to the control group. Mean values were significantly higher than in the control group (Table 3).

Table 2. Serum PRL, LH, FSH, E2 and P in functional hyperprolactinemia and control group in the early follicular and middle luteal phase

Follicular phase	HyperPRL group	Control group	p-value
PRL (ng/ml)	38.6±18.8	10,2±1,8	^{c}p<0.001
LH (U/L)	4,1 ±0,6	6.4±0,2	^{b}p<0.01
FSH (U/L)	4,2±0,7	5,8±0,6	^{a}p<0.05
E2 (pmol/L)	87.14±13,2	254.3±31.9	^{a}p<0.05
P (nmol/l)	1,9 ±0,32	2,3±0,3	NS
Luteal phase			
PRL (ng/ml)	39.1±1.6	9.7±0,8	^{c}p<0.001
LH (U/L)	4,6±0,4	6,1±0,7	^{a}p<0.05
FSH (U/L)	3,6±0,4	6.2±0,6	^{a}p<0.05
E2 (pmol/L)	84.24±18.5	350.8±35.2	^{b}p<0.01
P (nmol/l)	3,6±1.7	25.2±6.4	^{c}p<0.001

Legend: The parameters are expressed as mean ± SD, LH - luteinizing hormone, FSH - follicle stimulating hormone, E2 – estradiol, P-progesteron, PRL – prolactin. Statistical significance was p<0.05 level of significance and ^{a}p<0.05, ^{b}P <0.01, ^{c}P <0.001, for the group with hyperprolactinemia vs. control group.

Table 3. Levels of PRL, LH, FSH, E2 and P in functional hyperprolactinemia (hipotireoidizm) and the control group in the early follicular and middle luteal phase

Follicular phase	Hypothyroidism groupgrou	Control group	p-value
PRL (ng/ml)	32.4±10.6	10,2±1,8	^{c}p<0.001
LH (U/L)	4.3 ±0.9	6.4±0,2	^{b}p<0.01
FSH (U/L)	4.7±0,9	5,8±0,6	^{a}p<0.05
E2 (pmol/L)	95.26±13.8	254.3±31.9	^{b}p<0.01
P (nmol/l)	2.4 ±0.1	2,3±0,3	NS
Luteal phase			
PRL (ng/ml)	31.4±11.8	9.7±0,8	^{c}p<0.001
LH (U/L)	4.8±0,5	6,1±0,7	^{a}p<0.05
FSH (U/L)	4.1±0.7	6.2±0,6	^{a}p<0.05
E2 (pmol/L)	89.34±17.9	350.8±35.2	^{b}p<0.01
P (nmol/l)	2.9±0,6	25.2±6.4	^{c}p<0.001

Legend: The parameters are expressed as mean ± SD, LH - luteinizing hormone, FSH - follicle stimulating hormone, E2-estradiol,P-progesterone, PRL - prolactin. Statistical significance was p <0.05 level of significance ^{a}p <0.05, ^{b}P <0.01, ^{c}P <0.001, for the group with hyperprolactinemia (hypothyroidism) vs. control group.

Reproductive women in the early follicular phase of the menstrual cycle with iatrogenic hyperPRL significantly higher mean PRL (46.4 vs. 10.2, p <0.001), significantly lower mean LH (4.1 vs. 6.4, p <0.01), FSH (3.7 vs. 5.8; p <0.05) and E2 (83.19 vs. 254.3, p <0.01) compared to a healthy control group. Reproductive women in middle luteal phase of the menstrual cycle with iatrogenic hyperPRL had significantly higher mean PRL (48.3 vs. 9.7, p <0.001), significantly lower mean LH (4.2 vs. 6.1, p <0.05) and FSH (3.9 vs. 6.2, p <0.05) and E2 (82.31 vs. 350.8, p <0.01) compared with healthy controls grupu.Values of progesterone in the follicular phase did not differ significantly, while in the middle luteal phase, the mean P (2.5 vs. 25.2; p <0.001) was significantly lower than in the control group of healthy women (Table 4).

In reproductive women with PCOS in the early follicular phase, the mean PRL (29.4 vs. 10.2, p <0.001), LH (9.1 vs. 6.4, p <0.01), E2 (383.18 vs. 254.3, p <0.05) were significantly higher , compared to a healthy control group. Reproductive women in middle luteal phase of the menstrual cycle mean PRL (31.3 vs. 9.7, p <0.001; LH (10.2 vs. 6.1, p <0.05), E2 (326.3 vs. 350.8, p <0.01)Progesteron levels in the follicular phase did not differ significantly, while in the middle luteal phase, the mean P (6.5 vs. 25.2; p <0.001) was significantly lower than in the control group of healthy women (Table 5).

Table 4. Levels of PRL, LH, FSH, E2 and P in functional hyperprolactinemia (iatrogenic) and the control group in the early follicular and middle luteal phase

Follicular phase	Iatrogenic group	Control group	p-value
PRL (ng/ml)	46.4±14.8	10,2±1,8	^{c}p<0.001
LH (U/L)	4.1 ±0.6	6.4±0,2	^{b}p<0.01
FSH (U/L)	3.7±0,5	5,8±0,6	^{a}p<0.05
E2 (pmol/L)	83.19±14.3	254.3±31.9	^{b}p<0.01
P (nmol/l)	2.2 ±0.2	2,3±0,3	NS
Luteal phase			
PRL (ng/ml)	48.3±17.7	9.7±0,8	^{c}p<0.001
LH (U/L)	4.2±0,7	6,1±0,7	^{a}p<0.05
FSH (U/L)	3.9±0.3	6.2±0,6	^{a}p<0.05
E2 (pmol/L)	82.31±15.7	350.8±35.2	^{b}p<0.01
P (ng/ml)	2.5±0.9	25.2±6.4	^{c}p<0.001

Legend: The parameters are expressed as mean ± SD, LH- luteinizing hormone, FSH -follicle stimulating hormone, E2 - estradiol, PRL - prolactin. Statistical significance was p <0.05 level of significance ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, for the group with hyperprolactinemia (hypothyroidism) vs. control group.

Table 5. Levels of PRL, LH, FSH, E2 and P in functional hyperprolactinemia (PCOS) and the control group in the early follicular and middle luteal phase

Follicular phase	PCOS group	Control group	p-value
PRL (ng/ml)	29.4±10.5	10,2±1,8	^{c}p<0.001
LH (U/L)	9.1 ±1.3	6.4±0,2	^{b}p<0.01
FSH (U/L)	5.6±0.6	5,8±0,6	NS
E2 (pmol/L)	383.18±24.2	254.3±31.9	^{a}p<0.05
P (nmol/l)	3.2 ±0.3	2,3±0,3	NS
Luteal phase			
PRL (ng/ml)	31.3±16.8	9.7±0,8	^{c}p<0.001
LH (U/L)	10.2±0.6	6,1±0,7	^{a}p<0.05
FSH (U/L)	5.9±0.4	6.2±0,6	NS
E2 (pmol/L)	326.34±16.7	350.8±35.2	^{a}p<0.05
P (nmol/l)	6.5±1.6	25.2±6.4	^{c}p<0.001

Legend: The parameters are expressed as mean ± SD, LH - luteinizing hormone, FSH - follicle stimulating hormone. E2 - estradiol,P-progesterone, PRL - prolactin. Statistical significance was p<0.05 level of significance ^{a}p <0.05, ^{b}p <0.01, ^{c}p <0.001, for the group with hyperprolactinemia (hypothyroidism) vs. control group.

Table 6. Sonographic features of ovarian and endometrial in the early follicular phase of the menstrual cycle in women with reproductive hyper PRL and the control group.

Parameters	Hiper PRL group	Control group	p-value
Ovarian volume ($cm^{3)}$	4.8±1.1	6.1±0.5	p<0.05
Number of follicles (№)	3.0±0.2	4.9±0.2	p<0.04
RI	0.7±0.02	0.7±0.03	p>0.65
Endometrial thickness (mm)	4.2±0.2	6.0±0.2	p<0.001

Legend: The results are expressed as mean ± SD, with the level of significance p<0.05 for Hyper vs. PRL. control group, Hyper-PRL-hyperprolactinemia, RI - resistance index. SPSS v.17 Paired-Samples T Test.

Table 7. Sonographic features of ovarian and endometrial in middle luteal phase of the menstrual cycle in women with reproductive hyper PRL and the control group

Parameters	Hyper PRL group	Control group	t	95 % CI	p-value
Ovarian volume ($cm^{3)}$	5.2±0.2	6.1±0.2	2.3	0.01 do 0.64	p<0.04
Corpus Luteum (№)	2 (6.7%)	26 (86.7%)	0.08	0.01 do 0.02	p<0.001
RI	0.72±0.03	0.42±0.02	95	0.29 do 0.30	p<0.001
Endometrial thickness (mm)	5.7±0.2	10.8±2.2	-30	-3.14 do -2.7	p<0.001
Ovarian volume (cm^3)	3.0±1.1	4.9±2.2	-18	-5.1 do -3.6	p<0.01

Legend: The results are expressed as mean ± SD, with the level of significance p<0.05 for Hyper vs. PRL. control group, Hyper-PRL Hyperprolactinemia, RI - resistance index. SPSS v.17 Paired-Samples T Test.

In the group of women with functional hyper-PRL mean ovarian volume (4.8 vs. 6.1, p <0.05), endometrial thickness (4.2 vs. 6.0, p <0.001) were significantly lower in the early follicular phase MC comparing to control group (Table 6 (Table 6).

In the middle luteal phase MC mean endometrial thickness (5.7 vs. 8.6, p<0.001), presence of corpus luteum (6.7% vs. 86.7%, p<0.001), ovarian volume (5.2 vs. 6.1 p<0.04), the volume of endometrial (3.0 vs. 4.9, p<0.01) were significantly lower, while the mean RI (0.72 vs. 0: 42 p<0.00) was significantly higher than the control group (Table 7).

Discussion

One of the major causes of infertility is functional hyperprolactinemia. Functional hyperprolactinemia is associated with PCOS, primary hypothyroidism, endometriosis, medication, kidney diseases and stress. The consequences of hyperprolactinemia are hipogonadotropy hypogonadism with menstrual dysfunction, galactorrhoea and infertility (1).

Prolactin inhibits reproductive function suppression of hypothalamic gonadotropin, GnRH, as well as suppression of pituitary gonadotrophic secretion, impairing gonadal steroidogenesis in men or women. The ovaries prolactin block folliculogenesis and leads to hipoestrogenizm and anovulation (11). Affects the shortening of luteal phase menstrual cycle and its inadequate activity. Hyperprolactinemia as possible infertility cause has luteal phase defect - DLF (12).

Results of this study showed that in women with reproductive hyperPRL galactorrhoea (83.3%), irregular menstrual cycle (85%), infertility (66%) and galactorrhea with amenorrhea (45%) are significantly present compared to the control group.

The results can be explained by the fact that hyperPRL has inhibitory effect on GnRH and thus no positive effect on FSH and LH resulting hipogonadotropy hypogonadism. Low estrogen cause irregular menstrual cycles and infertility and galactorrhoea has the result of direct action of PRL on breast. The results of this study are consistent with results of other authors, with a small percentage error of clinical parameters.

Walch and colleagues in their study, which was covered by the age of the patient about 41 years old,were to prove the presence of symptoms hyper-PRL and in 47% loss of libido, galactorrhoea in 2%, while 17% had no clinically visible symptoms (13).

Results of this study indicated that prolactin reaching 365 ng / ml in patients using antipsychotic medication, while the values in patients who were using DA receptor blockers were less than 100 ng / ml. Drugs that commonly cause elevated levels of prolactin are antidepressants, and antipsychotics (risperidone in particular), other dopaminergic

blockers (metoclopramide) and some antihypertensive drugs (6, 7) opiates and H2 blockers.

Hyperprolactinemia is a common and serious side effect of treatment with antipsychotics and one study shows that about 48% to 93% of premenopausal women, and 42% to 47% of men taking antipsychotics have hyperprolactinemia. Elevated concentrations of prolactin can cause sexual dysfunction, amenorrhea, infertility, galactorrhoea and osteoporosis (14).

Results of this study showed that women with reproductive hyperPRL have hipogonadotropy hypogonadism, as FSH, LH and E2 significantly lower ($p < 0.005$) compared to the control group in the early follicular phase. Reproductive women with hyperPRL have chronic anovulation because they have significantly lower luteal progesterone in middle luteal phase ($p < 0.005$) compared to the control group. The results of this study are consistent with results of other authors.

Hajder and his colleagues in their prospective study indicated that hiperprolactinemic women had significantly ($p < 0.01$) higher levels of prolactin, a reduced level of LH and E2 ($p < 0.01$) during the mid-follicular phase in comparison with the control group. In the middle stages of luteal phase hiperprolactinemic women had significantly higher levels of prolactin ($p < 0.01$), decreased LH and FSH compared to the control group (15).

Banu in his study showed higher gonadotropin consumption and better response to stimulation in patients with hipogonadotropy hypogonadism. Implantation rate was higher in patients with hipogonadotropy hypogonadism (36.5% compared to 13%, $p < 0.0001$) and concluded that women with hipogonadotropy hypogonadism undergoing in vitro fertilization were good responders (16).

This research has proven that the values of PRL were significantly higher in hypothyroidism than in the control group, while the values of LH, FSH and E2 decreased in both phases of the menstrual cycle compared to the control group.

In the study of Avasthija and associates incidence of hyperprolactinemia in infertile women with serum prolactin levels> 25 ng / ml was 46%. Serum prolactin levels in hiperprolactinemic infertile women was up to 76.53 ± 55.97 ng / ml (range 48.3 to 200 ng / ml). The incidence of hypothyroidism, hyperprolactinemia was up to 25.5% (17).

Akther and colleagues examined the function of the thyroid hormone and PRL in infertile women and it showed that the prevalence of subclinical hypothyroidism 6.5% of primary infertility and 15% in the secondary, and the prevalence of hyperprolactinemia in 43% of primary and 21% of secondary infertility. Mean levels of prolactin in the primary infertility (495 + /-340nmol / L) was higher than in secondary infertility (340 + / - 310nmol / L), a statistically significant difference ($p < 0.05$). Thus, the incidence of hyperprolactinemia was higher in primary infertility, subclinical hypothyroidism is higher in secondary infertility, and shows no correlation between serum TSH and prolactin levels in the two groups (18). Results of this study show that in women with reproductive hyperPRL which is part of PCOS elevated PRL, LH and E2 in the follicular phase, and P values in the luteal phase were reduced. The results are confirmed by the fact that PCOS leads to increased androgen secretion, the presence of abnormal gonadotropin secretion: LH> 10IU, LH / FSH> 2 and elevated estradiol compared to healthy women.Results are consistant with the results of other authors.

Women with polycystic ovary syndrome (PCOS) may have mild hyperprolactinemia in the absence of pituitary lesions. In clinical studies, were elevated levels of prolactin are found in the 19% to 50% of women with polycystic ovary syndrome (8).

In a multicenter study, which included 200 hiperandrogenic women, there was an elevated serum prolactin. Macroprolactinaemia was observed in 5 patients with serum PRL> 35g / L, which is the diagnosis of PCOS (19). Results of this study showed that in the group of women with hyperPRL mean ovarian volume and the number of antral follicles in the early follicular phase of the MC are somewhat less than in the control group, while the thickness of the endometrium was significantly lower (4.2 vs. 6.0, $p < 0.001$) than in the control group. In the middle luteal phase MC mean endometrial thickness (5.7 vs. 10.8, $p < 0.001$), endometrial volume (3.0 vs. 4.9, $p < 0.05$) and the presence of corpus luteum (2 Vs. 26, $p < 0.001$) were significantly lower than in the control group, while the mean value intraovary RI resistance index (0.72 vs. 0: 42 $p < 0.001$) was significantly higher in women with hyperPRL than in the control group, similar results have been published by other authors. Hajder in their study

showed that in the control group intraovary resistance index during periovulation phase (RI = 0: 53 + -/-0.04), during the mid luteal phase (RI = 0.50 + -/-0.02) during the late luteal phase (RI = 12: 51 + -/-0.04) was significantly lower than the value of resistance index during periovulation phase (RI = 0.70 + -/0.06, p <0.001), mid luteal phase (RI = 0.72 + -/-0.06, p <0.001), and late luteal phase (RI = 0.72 + -/-0.04,, p <0.001) in patients with luteal phase defect (20).

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Clinical effects of laparoscopic cholecystectomy on cholecystolithiasis patients complicated with liver cirrhosis

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Abstract

Objective: To study the feasibility and effect of laparoscopic cholecystectomy on cholecystolithiasis patients complicated with liver cirrhosis.

Methods: The clinical data of 263 cases of cholecystolithiasis patients associated with liver cirrhosis admitted to our hospital from January 2009 to June 2012 were analyzed retrospectively. All the patients were randomly divided into two groups: laparoscopy group (147 cases) and laparotomy group (116 cases). The intraoperative and postoperative indicators were compared between the two groups, and statistical analysis was made.

Results: The operative time, intraoperative blood loss and the average hospital stay of the laparoscopy group were lower than those of the laparotomy group, and the average total cost of hospitalization of the former was higher than that of the latter, with the difference statistically significant ($P < 0.05$); in the laparoscopy group, the changes of total bilirubin, albumin, AST, ALT and GGT before and after surgery were significantly lower than those of the laparotomy group, in which the difference was statistically significant ($P < 0.05$), while there was no significant difference in the changes of direct bilirubin, indirect bilirubin and ALP; the overall incidences of various complications were 8.16% and 13.79% respectively in the two groups, which was not statistically significant ($P > 0.05$).

Conclusion: Laparoscopic cholecystectomy is safe and feasible in the treatment of cholecystolithiasis associated with liver cirrhosis (Child's class A and B liver function), showing distinct advantages.

Key words: Cholecystolithiasis, liver cirrhosis, laparoscopic cholecystectomy.

Introduction

Cholecystolithiasis is a common disease that is frequently encountered in the General Surgery Department. The incidence of cholecystolithiasis in patients with liver cirrhosis is up to 2 to 5 times of that in the normal population [1]. Due to poor liver reserve function, portal hypertension and disturbance of blood coagulation in liver cirrhosis patients, the risk of cholecystectomy is greatly increased, and the mortality of laparotomy was up to 17% to 25% for a time [2], and the cholecystectomy was also listed as a relative contraindication [3,4]. With the continuous development and growing maturity of laparoscopic techniques, indications continue to be broadened, and has been widely used in clinical practice [5]. It has been reported that laparoscopic cholecystectomy is a safe surgical approach [6] for cholecystolithiasis patients with Child's class A and B cirrhosis [7]. 263 cholecystolithiasis patients associated with liver cirrhosis received surgical treatment in our hospital between January 2009 and June 2012, including 147 cases of laparoscopic surgery and 116 cases of laparotomy. The results are reported as follows.

Materials and Method

General information

263 cholecystolithiasis patients associated with liver cirrhosis admitted to our hospital from January 2009 to June 2012 were selected, including 175 males (66.54%) and 88 females (33.46%), with an average age of 53.28 years of old. The cases which did not meet the requirements, with unavailable history and incomplete information were not included in this study.

The patients were treated with laparoscopic cholecystectomy and open cholecystectomy respectively, 147 in the laparoscopy group and 116 in the

Table 1. General information

Item	Laparoscopic cholecystectomy (n=147)	Open cholecystectomy (n=116)	*P* value
Gender			
Male	99	76	0.7289
Female	48	40	
Age			
≤60	105	75	0.1827
>60	42	41	
Child classification			
A	119	89	0.3729
B	28	27	
C	0	0	

laparotomy group. There was no significant difference in age structure, gender ratio and Child classification of liver function between the two groups, as shown in Table 1. The diagnosis of cirrhosis is mainly based on history, laboratory tests, morphological manifestations of liver in preoperative imaging and laparoscopy, in which there were 229 cases of liver cirrhosis after viral hepatitis B and 34 cases of alcoholic cirrhosis. All the patients had no preoperative history of gastrointestinal bleeding, of which 14 cases had undergone splenectomy before.

Preoperative preparation

After admission, the patients were conducted the examinations of blood routine, coagulation function and liver and kidney functions to assess their surgical tolerance. All the patients also underwent B-ultrasonography to learn about whether there were gallbladder shrinking, gallbladder wall thickening, incarcerated gallstones or expansion or calculus of common bile duct, etc., in order to determine the difficulty of surgical procedures, and exclude bile duct diseases at the same time. The degree of hepatic damage was graded in accordance with the Child-pugh grading standards for liver function [8]. No special preoperative preparation was performed generally on Child's class A patients; class B patients were appropriately treated with liver protection, ascites control and reduction of portal venous pressure at first, and received surgery after the liver function improved.

Methods

All the cases were selective operations, in which trachea cannula was used for general anesthesia. For the laparoscopy group, the three-hole or four-hole method was applied to establish pneumoperitoneum gradually; the body position was higher head and lower feet, with an inclination of 30° to the left; in the operation, CO_2 pneumoperitoneum pressure was maintained at 8~ 12 mmHg; Calot triangle was dissected and absorbable clips were used to clip cystic artery and ductus cysticus; antegrade and retrograde techniques were combined to resect cholecyst, burn gallbladder bed and place drainage tube. For the laparotomy group, transrectus incision of the right upper quadrant or kochers incision was adopted to enter the abdomen; Calot triangle was dissected and the cystic artery and ductus cysticus were respectively cut off and ligatured; antegrade and retrograde techniques were combined to resect gall bladder and suture gallbladder bed. Whether to place the drainage tube was decided according to the practical situation. Treatments such as fluid infusion, anti-infection and liver protection were applied after operation.

Statistical analysis

All data were analyzed by SPSS 17.0 and expressed as x±s. Measurement data were subjected to the t test, and numeration data were subjected to the χ^2 test and the Fisher probability test. $P<0.05$ was considered statistically significant.

Results

Clinical data

All the patients receiving cholecystectomy were cured and discharged from hospital, without the incidence of perioperative death, postoperative blee-

ding, bile leakage or bile duct injury. There was significant difference in the operative time, intraoperative blood loss, drainage tube placement, the average cost of hospitalization and the average length of stay between the two groups. But compared with the laparotomy group, the operative time and average hospital stay was shorter, intraoperative blood loss was less, but the cost of hospitalization was higher in the laparoscopy group (Table 2).

Blood biomedical indexes

The patients in both groups were reexamined blood biochemistry three days after the surgery. Compared with the results of the preoperative examination, the majority of patients suffered from varying degrees of hepatic functional impairment. The postoperative changes of liver function indicators of the two groups are shown in Table 3, which can be found that the difference in the changes of total bilirubin, albumin, AST, ALT and GGT in the two groups was statistically significant, and the influence on liver function was less in the laparoscopy group than the laparotomy group.

Postoperative complications

The overall incidences of infection of postoperative incision, ascites, urinary infection, intra-abdominal infection, adhesive intestinal obstruction and other common postoperative complications were 8.16% and 13.79% respectively in the laparoscopy group and laparotomy group. The difference in the postoperative complication rate was not statistically significant between the two groups (Table 4).

Discussion

Laparoscope has been widely used in general surgery since it was introduced into clinical practice. And laparoscopic cholecystectomy has

Table 2. Clinical data (x±s)

Item	Laparoscopic cholecystectomy	Open cholecystectomy	P value
Time (min)	48.35±14.56	54.79±20.17	0.0153
Drainage			<0.0001
Yes	147	96	
No	0	20	
Blood loss (mL)	82.19±21.15	110.53±20.68	<0.0001
Hospitalization time (d)	8.83±3.27	12.15±3.36	<0.0001
Hospitalization expense (¥)	7324.25±208.59	6236.77±240.23	<0.0001

Table 3. Changes of blood biomedical index (x±s)

Item	Laparoscopic cholecystectomy	Open cholecystectomy	*P* value
	Before-After	Before-After	
Total bilirubin	1.24±2.07	2.39±2.01	0.0001
Direct bilirubin	0.49±1.56	0.15±0.91	0.0887
Indirect bilirubin	0.81±2.29	1.25±1.40	0.0874
Albumin	-0.41±1.58	-1.52±1.56	< 0.0001
AST	-0.09±0.97	2.01±1.51	< 0.0001
ALT	1.06±3.44	2.12±2.39	0.0163
GGT	0.16±2.72	0.92±1.25	0.0127
ALP	0.34±1.68	0.73±1.57	0.0830

Table 4. Postoperative complications

Complication	Laparoscopic cholecystectomy	Open cholecystectomy	*P* value
Incision infection	2	5	0.2289
Ascites	4	2	0.4581
Urinary infection	1	3	0.1908
Intra-abdominal infection	2	3	0.4631
Adhesive intestinal obstruction	3	3	0.3036

become a "gold standard" in the treatment of benign gallbladder diseases for its relatively short operative time and hospital stay, less intraoperative blood loss, and fewer postoperative complications [9]. Patients with cholecystolithiasis associated with cirrhosis undergoes a relatively high incidence of postoperative bleeding, liver failure and severe infections and other serious complications for it is often accompanied by portal hypertension, varicose veins and dysfunction of blood coagulation, etc. So the postoperative mortality of traditional laparotomy even reached 17% to 25% for a time [10]. Jiang JK, et al [11] considered that the oppression of increased abdominal pressure caused by the CO_2 pneumoperitoneum in laparoscopic surgery on the liver and portal veins might lead to reduced liver perfusion, resulting in further damage of the liver function, which limits the application of laparoscopic cholecystectomy in patients with liver cirrhosis [12].

With the development of perioperative treatment, improvement of laparoscopic instruments and technological level, and gradual expansion of indications of the laparoscopic cholecystectomy [13], a number of its application in the treatment for patients with cholecystolithiasis associated with liver cirrhosis have been reported, showing an obvious superiority [14].

This study found that laparoscopic cholecystectomy was safe and feasible for patients with Child's class A and B liver function, and there were no perioperative dead cases in both the groups. Laparoscopic surgery had distinct advantages due to its minimally invasive characteristics compared with laparotomy: shorter operative time and hospital stay, less intraoperative blood loss and less impact on liver function [15]. This further proves that the minimally invasive laparoscopic surgery is more suitable for cholecystolithiasis patients associated with liver cirrhosis under certain conditions. Although laparoscopic surgery is safe and feasible, but due to liver cirrosis patients' own particularity and the complexity of surgical procedures, we still need to strictly determine the surgical indications, perform surgery carefully and properly handle after surgery.

For cholecystolithiasis patients associated with liver cirrhosis, the determination of surgical indications mainly depends on the assessment on the reserved function of liver [16]. On the basis of Child-pugh classification of liver function, Child's class A patients generally do not need to be treated specially for their compensatory ability of liver can usually tolerate laparoscopic cholecystectomy; symptomatic treatment can be applied on Child's class B patients, such as liver protection and ascites control, etc. They can select laparoscopic cholecystectomy after their conditions are improved, which is also relatively safe; however, Child's class C patients whose liver function is at the decompensatory stage or with massive ascites, should avoid surgery to the greatest extent [17]. In addition, inflammatory edema at the acute phase may increase the fragility of local tissues so as to cause blood loss, therefore, preoperative inflammation should be under good control [18].

Pneumoperitoneum pressure should be as low as possible in practice. Studies have showed that as pneumoperitoneum may lead to decrease in hepatic blood flow, causing ischemia-reperfusion injury [19], it can reduce the damage to the liver function by use of low pneumoperitoneum pressure and minimizing duration of operation. In addition, shorter operative time can reduce the amount of anesthesia drugs, thereby reducing their toxic effects and the burden on the liver. Cirrhosis and portal hypertension often causes splenomegaly and varicosity of abdominal wall, which may lead to injury of abdominal blood vessels and spleen when applying puncture, so as to cause relevant complications. Therefore, we should be fully aware of the specific conditions of varicosity of abdominal wall and the size of spleen before surgery, in order to avoid damage to the blood vessels of abdominal wall and spleen [20].

Portal hypertension can cause varicose veins in the hepatic hilar region [21], decreased synthesis of blood coagulation factor and disturbances of blood coagulation [22], and be prone to appear hard-to-control bleed loss in surgery. After the laparoscope is led in, the porta hepatis and its surrounding varicose veins should be carefully observed. If there are many thick and intensive varicose veins, laparoscopic surgery will be very difficult, which may also cause serious liver dysfunction due to a long-time pneumoperitoneum and large amounts of blood loss, even if it is barely completed. Under such circumstance, it is wise to choose conversion to laparotomy in a timely manner. If no ob-

vious varicose veins are found, the triangular area may be separated layer by layer, and thick vessels should be occluded. Only electrocautery may cause hemorrhage after operation. In the event of intraoperative blood loss, it should be handled calmly: the bleeding part should be clamped quickly, titanium clips or absorbable clips are prepared, blood is absorbed by aspirator to reveal the position of bleeding, and then clip hemorrhagic focus. Under the circumstance that turbulent blood loss occurs, and it is estimated to be difficult to control, laparoscopy should be converted to laparotomy in a timely manner [23]. Conversion to laparotomy is not a complication, but a measure to prevent the occurrence of serious complications.

Patients with cirrhosis have atrophic right lobe of liver, enlarged left lobe and liver inversion toward the upper right. Porta hepatis is often located high and deep, increasing the difficulty of operation. At this time, an operation hole should be established flexibly according to the practical situation. The gall bladder should be stripped carefully during the operation, thus avoiding gallbladder rupture. Once it is ruptured, bacteria-containing bile and stone will spill, which would increase the probability of postoperative abdominal cavity infection and might cause lethal complications to cirrhosis patients who have poor coagulation function and low resistance and are easy to suffer from coeliac rehaemorrhagia and abdominal abscess after operation [24]. Therefore, the indication for drainage tube placed in the abdominal cavity of such patients should be easing rather than rigid. Drainage tube was placed in all patients in the laparoscopy groups in this study.

As pneumoperitoneum, surgical strike and narcotic drugs may affect the postoperative functions of all systems, the functions of all organs should be detected after the surgery, especially the monitoring of liver function and the examination of whether there was bleeding or bile leakage in the peritoneal cavity drainage tube, which is conducive to an early detection and timely treatment.

In summary, laparoscopic cholecystectomy has the advantages of shorter operative time, less bleeding, less influence on the liver function and fewer surgical complications [25]. As long as we strictly determine surgical indications and perform adequate preoperative preparation, followed by skilled surgery operation and close postoperative observation, laparoscopic cholecystectomy is a safe and effective treatment for cholecystolithiasis patients associated with liver cirrhosis.

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Study on cutting balloon angioplasty for the coronary artery in-stent restenosis for the elderly patients

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Abstract

Aim: To observe the immediate and 6-month effects of cutting balloon angioplasty on the coronary artery in-stent restenosis for elderly patients.

Methods: Sixty-seven patients with in-stent restenosis were randomly divided into cutting balloon (37 cases) and conventional balloon (30 cases) groups. Quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS) were used. Clinical and angiographic follow-up, clinical improvements and QCA outcomes were recorded within 6 months. The study end points included myocardial infarction, coronary artery bypass graft (CABG) and revascularization.

Results: The success rates were 100% in both groups. One patient in cutting balloon group experienced dissection in the distal end after the inflation. The average follow-up time was (6.6±0.4) months. The binary restenosis rates 3 and 6 months after the surgery in cutting balloon group were much lower than those in the conventional balloon group. The diameters of acute gainings in the cutting balloon and conventional groups were (1.71 ±0.51) mm and (1.14 ±0.55) mm. The diameters of late losses in the cutting balloon group were (0.25 ±0.06) mm (3 months later) and (0.37 ±0.07) mm (6 months later), and those in the conventional group were (0.77 ±0.18) mm (3 months later) and (0.88 ±0.17) mm (6 months later).

Conclusions: Cutting balloon angioplasty is reliable, safe and maneuverable, and the binary restenosis is low after the surgery. This is a promising treatment which will be easily accepted by patients.

Keywords: Angioplasty, transluminal, percutaneous coronary, ultrasonography, interventional.

Introduction

Coronary stenting is an effective means to lower the restenosis of conventional balloon angioplasty [1]. According to the statistics, the average into rate of catheter indoor stents is 85% all over the world up to now. The biggest problem brought by a high proportion of stent using is in-stent restenosis (incidence rate is about 20% to 30%) [2]. Therefore, the treatment of in-stent restenosis has been a great subject in the heart disease circle. The conventional balloon angioplasty is feasible and safe, with little complication, but it is very likely to be restenosis again, the reoccurring rate being 30% to 60% [3-4]. Although the other interventional treatment equipments have some effects, such as coronary artery stent for a second time and rotablator, the high proportion of restenosis cannot be lowered clinically.

In recent years, the study of cutting balloon angioplasty for in-stent restenosis shows that it is an effective method [5]. However, there has been no prospective study report about in-stent restenosis for elderly patients. So we have studied the results of coronary angiography and clinical follow-up, using intravascular ultrasound (IVUS), immediately after the treatment and in the 6th month [6].

Materials and Methods

Source of Data

In our hospital, there were 67 cases in which the elderly patients who had coronary artery disease were included, and they were divided into two groups as the cutting balloon and conventional balloon group. There were 37 cases in the cutting balloon group and the average age of patients was (67±6) years old, including 34 males and 48 lesions. Among the stents implanted for the first time, there were 42 tube-stents and 5 winding-stents, the average length of these stents being 15 mm, and the interval between the time of implantation and the time of restenosis was 4 to 17 months. There were 30 cases in the conventional balloon group and the average age of patients was (67±7) years old, including 29 males and 30 le-

Table 1. Clinical data of 69 patients

Group	Cases	Diabetes	Smoking	Hypertension	UAP	OMI	Bypass graft
Cutting Balloon	37	14	7	8	18	7	3
Conventional Balloon	30	14	7	6	15	3	2

sions. Among the stents implanted for the first time, there were 25 tube-stents and 6 winding-stents, the average length of these stents being 14 mm, and the interval between the time of implantation and the time of restenosis was 3 to 15 months. There was no significant difference in the comparison of ages and of risk factors between two groups (Table 1).

The Procedure of Cutting Balloon Angioplasty

After the femoral artery puncture, insert the vagina vasorum, and then projectile injection of heparin was given (5000 to 7500 U). During the operation, add the injection of heparin 5000 U every one hour, so as to maintain activated clotting time (ACT) to be longer than 280 seconds. When the ACT was shorter than 180 seconds, pull out the vagina vasorum. After the surgery, enteric-coated aspirin needed to be taken, 300 mg/d, for a month, and ticlid also be taken, 500 mg/d, for two weeks. The effective length of cutting balloon was 10 mm or 15 mm. In order to minimize the damage to blood vessels, in the beginning the inflating pressure should be 2 to 4 atm, and increase 1 atm every 5 seconds. The maximum of the pressure should be 8 atm and the time of pressure should be 60 seconds. If the imaging result was not satisfactory, the inflation should be done again until satisfaction [7]. The ratio of cutting balloon to the diameter of stent was (1-1.1):1, being 1:1 averagely.

Intravascular Ultrasound (IVUS)

Generally the test should be run before and immediately after the balloon inflation in order to determine the diameter of vessel, patch character and residual stenosis. The standard of successful IVUS is that the cross sectional area of remant patch is smaller than 20% of the patch area and without major complications (including big branches occlusion, distal embolization, spiral dissection and vascular rupture). The acute gaining in diameter is the difference between vessel diameter before inflation and vessel diameter after inflation. Quantitative coronary angiography (QCA) [8] should be undergone before the balloon inflation, after the balloon inflation and at the 6-month after operation, so as to determine minimal lumen diameter (MLD) and referential lumen diameter. The dissection under coronary angiography is classified according to the standard of The U. S. National Heart Association. The standard of successful coronary angiography is that residual stenosis is less than 30%, and without big branches occlusion, serious dissection and vascular rupture. The clinical success criteria are that there should be no major complications, such as death, myocardial infarction, and nor should there be necessity for emergency surgery of coronary artery bypass grafting. The definition of restenosis is that the stenosis of the diameter of morbid vessel is equal or greater than 50% during the period of follow-up.

Follow-Up

The ECG should be recorded immediately and 24 hours after the surgery and blood sample should be taken 24 hours after surgery to determine cardiac enzyme [9]. If the patient experiences ischemic chest pain before hospital discharge, the coronary angiography should be reviewed at any time. The coronary angiography should be undergone 6 months after surgery.

Ridit

All the data should be shown with mean standard deviation. T-test and x^2-test should be run for the ridit.

Results

Comparison of Coronary Artery Angiography Characteristics

There was no significant difference of restenosis sites and their ratio between two groups. But in the cutting balloon group the incidence rate of in-stent restenosis was much higher than that of conventional balloon group (Table 2).

Table 2. Comparison of stenosis after coronary angiography between the two groups

Group	Cases	Target Vessel				Lesions Type			
		Descending Anterior Branch	Circumflex Coronary Artery	Right Coronary	Venous Graft	Local	Diffuse	Body	Ends
Cutting Balloon	37	24(51)	15(32)	6(13)	2(4)*	14(30)	21(42)	7(14)	5(14)*
Conventional Balloon	30	20(66)	8(26)	6(18)	0(0)	12(40)	16(53)	3(7)	0(0)

Characteristics of In-Stent Restenosis in the Cutting Balloon Group

In the cutting balloon group the proportion of diffuse in-stent restenosis (≥10 mm) was 40% and the proportion of local in-stent restenosis (<10 mm) was 25%, the average length of in-stent restenosis was (14±5) mm (3.4～28 mm). In this group the percentage of patients who have venous graft in-stent restenosis and stent-ends restenosis was higher than that of conventional balloon group.

Treating In-Stent Restenosis with Cutting Balloon

The average interval between being put inside the stent and having the restenosis for the patients in the cutting balloon group was (4.1±1.0) months (4～17 months). The maximum of cutting balloon diameter was (3.01±0.24 mm) and the maximum of inflation pressure was 12 atm, the success rate of surgery being 100%. There was only one patient who experienced dissection in the distal end of stent and restriction of forward blood flow, so a new stent was imbedded.

Immediate Results after Surgery and during Hospitalization

There was no patient who was dead, or had myocardial infarction or needed to have the emergency surgery of coronary artery bypass grafting, nor there any patient who experienced angiorrhexis or acute occlusion.

Follow-Up

All 67 patients accepted the clinical follow-up (Table 3). The time of follow-up was (6.4-7.1) months and the average time was (6.6±0.4) months. 64 cases concerning the coronary angiography were followed up at 6-month (including 37 cases in the cutting balloon group and 27 cases in the conventional balloon group). The binary restenosis of cutting balloon group was 14% and 17%, at the 3-month and 6-month, respectively, markedly lower than them of conventional balloon group (37% and 44%, respectively, $P<0.001$). The acute gaining in diameter was (2.08±0.53) mm, and the late loss in diameter was (0.25±0.06) mm and (0.37±0.07) mm, at the 3-month and 6-month, respectively, in the cutting balloon group, markedly lower than them of conventional group [(0.77±0.18) mm and (0.89±0.17) mm, respectively, $P<0.001$].

Table 3. Follow-up results of coronary angiography and intravascular ultrasound

Group	MLD (mm)	DS (%)	CSA (mm²)	DG (mm)
Cutting Balloon Group (37 cases)				
Before Inflation	1.02±0.03	81.0±8.7	2.5±0.6	
Immediately after Inflation	3.13±0.13	11.0±5.3	12.1±6.1	2.08±0.55
6 months after Inflation	2.98±0.32	14.2±9.6	10.2±4.4	
Conventional Balloon Group (27 cases)				
Before Inflation	1.04±0.04	82.1±8.5	2.02±0.5	
Immediately after Inflation	1.81±0.27	16.4±3.6	13.1±8.5	1.76±0.51
6 months after Inflation	1.56±0.13	26.4±17.6	8.64±2.5	

Notes: MLD: Minimum Lumen Diameter, DS: Diameter Stenosis, CSA: Cross Sectional Area, DG: Diameter Gaining: t-test, comparison between immediately inflation and before inflation within two groups, and comparison of 6 months after inflation between two groups, P<0.001.

Discussion

On the surface of the cutting balloon, which was devised by Barath, 3 to 4 metal blades were installed. When the balloon inflated, the blades touched the balloon surface and penetrated into the endangium, causing the continuity of endangium to be discontinued and the decomposition of vascular wall to the inflation pressure to be reduced, thus achieving the maximum of inflation with relatively low inflation pressure. The results of many research centers show that the immediate and long-term effects of cutting balloon angioplasty are better than them of conventional balloon angioplasty. The reascularization rate of primary coronary artery disease at the 6-month is only 10%, markedly lower than that in the conventional group (average rate is 40%). With the extensive application of the stents, in-stent restenosis has become one problem which is more and more difficult to overcome in interventional cardiology. Although the immediate effects of re-dilation and re-stenting of conventional balloon angioplasty are satisfactory, the restenosis rate is still as high as 40% to 50%. The other interventional treatments such as plaque ablation etc. cannot lower the restenosis rate. According to Bauters' report, within 6 months after the implantation of joint stent of conventional balloon angioplasty [10], the restenosis rate was higher than 32%. Our study shows that the restenosis rates were 16% and 17% at the 3-month and 6-month, respectively, in the cutting balloon group, lower than them of conventional balloon group (37% and 44%, respectively).

IVUS is an effective means to study the in-stent restenosis mechanism [11]. Using this method, we studied the structural change of vascular wall before and after the balloon inflation, so as to the reliable basis for the further treatment and the prognosis of restenosis was provided. The main genesis mechanism of in-stent restenosis is neointimal hyperplasia. With the IVUS guidance and the determination of atherosclerotic plaque [12], reliable bases are rendered for reasonably choosing the diameter of cutting balloon. Treating the primary coronary artery disease with cutting balloon angioplasty, the restenosis rate (20%) at the 6-month was much lower than that (35%) of single dilation [13]. Therefore, the technical means we adopted was fractional inflation, which helped the surgeon ascertain the change of vessel after the inflation, in order to reduce the injury to vascular wall as much as possible, and finally there was only one patient who experienced dissection. IVUS showed that after the inflation of cutting balloon the diameter of vessel remarkably increased, without the outer cross-sectional area increasing. This further proved that the damage of cutting balloon inflation to the endangium was much smaller than that of conventional balloon inflation and the intimal hyperplasia of cutting balloon group was lesser than that of conventional balloon group [14].

The distinguishing feature of cutting balloon inflation is that the cutting balloon is easily to be fixed in the stent, not like the conventional balloon which slides easily when inflated [15]. This feature of cutting balloon is the same as its structural characteristic. When inflated, this mobility characteristic of conventional balloon meant that it would bring bigger damage to the normal vascular wall, so the restenosis rate markedly increased during the period of follow-up. When treating the in-stent restenosis with cutting balloon inflation, the position of cutting balloon is very important. Almost one third of the balloon must be placed into the stent to prevent distal dissection.

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An unusual cause of unintentional poisoning: Gliphosate

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Abstract

Glyphosate is a non-selective organophosphate herbicide which has a wide spectrum and systemic effects and used widely in many countries including Turkey. This is resulted in inhibition of aromatic aminoacid, hydroxy phenolic compounds and chlorophyll synthesis and consequently a reduction occcurs in protein synthesis and growing and cell deaths are seen Glyphosate is considered to lead to gastrointestinal irritation, hepatic and renal dysfunction, cardiovascular instability and pulmonary insufficiency also in humans similarly as the result of reduction in protein synthesis and cell deaths by inhibiting some enzyme systems. While it causes death in rats in very low doses, death occurs in humans only in very high doses (330 ± 42 ml).

In this paper, we desired to discuss a group of poisoning cases who were admitted to emergency department with complaints of nausea after noticing that they had mistakenly added glyphosate containing herbicide in the meal instead of oil and who developed thrombocytopenia and elevation in renal function tests.

Key words: Emergency, gliphosate, poisoning.

Introduction

Glyphosate is a non-selective organophosphate herbicide which has a wide spectrum and systemic effects and used widely in many countries including Turkey. Glyphosate which was produced by Monsanto at the beginning of 1970s is commonly used for especailly crabgrass control in agricultural areas.

Glyphosate inhibits 5-enolpyruvyl-shikimic acid-3-phosphate synthase enzyme activity which takes part in shikimic acid metabolic pathway in plants, mycetes and procaryotes. This is resulted in inhibition of aromatic aminoacid, hydroxy phenolic compounds and chlorophyll synthesis and consequently a reduction occcurs in protein synthesis and growing and cell deaths are seen (1).

Glyphosate is considered to lead to gastrointestinal irritation, hepatic and renal dysfunction, cardiovascular instability and pulmonary insufficiency also in humans similarly as the result of reduction in protein synthesis and cell deaths by inhibiting some enzyme systems (2-3). While it causes death in rats in very low doses, death occurs in humans only in very high doses (330 ± 42 ml) (2).

In this paper, we desired to discuss a group of poisoning cases who were admitted to emergency department with complaints of nausea after noticing that they had mistakenly added glyphosate containing herbicide in the meal instead of oil and who developed thrombocytopenia and elevation in renal function tests.

Cases

Seven male patients who work in the same workplace admitted to our emergency department as they noticed that they had added herbicide, glyphosate that they used in the field, into the meal instead of oil. They were conscious and cooperated on admission. They had no complaints except nausea. Vital findings on admision are shown in Table 1. No pathologic findings were found on their physical examination. A vascular access was opened and fluid replacement was initiated. Gastric irrigation was done and activated carbon was given. All patients were monitored in emergency department. No abnormal findings were detected in whole blood count, biochemistry tests and coagulations tests on admission (Tables 2, 3). Blood gases were normal. Their electrocardiograms were normal si-

nus rhythm. A reduction was detected in platelet and hemoglobin values of the first patient on the first day of hospitalization. Peripheral blood smear examination was consistent with the detected platelet values. The patient had no complaints and spontaneous hemorrhages. The patient was discharged with instructions as his platelet and hemoglobin values tended to elevate on the third day of hospitalization. The first patient was invited for control on the seventh day of glyphosate intake. His platelet (243.000/mm³) and hemoglobin values (14.3 g/dl) were within normal ranges. Creatinine values of the second and the third patients were detected to elevate on the second day of hospitalization (1.3 mg/dl and 1.5 mg/dl, respectively). Urinary output of the patients was monitored. No reduction occured in urine volume. The patients were administered IV fluid replacement therapy. Control values were normal on the third day of hospitalization. Remaining four patients were discharged with instructions as they did not have any complaints and normal biochemistry and hemogram values 24 hours after hospitalization (Tables 2, 3). The first three patients were hospitalized for three days and other patients were hospitalized for two days.

Discussion

Glyphosate is a non-selective, wide-spectrum herbicide widely used for plant control in terrestial and aquatic environments and it is an organophosphate compound (4). However it does not exhibit an anticholinesterase-like effect or organophosphate-like effect in central nervous system (5). In many studies, it is stated that the substance responsible for toxicity in humans and mammals is surfactant which is added to facilitate permeating into plant cell. This is considered to achieved by interfering with cytochrome p450 enzyme system and oxidative phosphorilation mechanisms (6). The most commonly used surfactant is polyoxyethylene amine (POEA). Roundup which is the first herbicide containing glyphosate produced by Monsanto in the first years of 1970s is composed of water, glyphosate (41%) (isopropylamine salt) and polyoxyethylene amine (15%) (6).

Diagnosis can be made with medical history, clinical findings and chemical analysis in glyphosate-surfactant herbicide (GlySH) poisonings (2). According to the results of 3 retrospective studies including 246 cases, patients apply with sore throat (41-43%), nausea-vomiting (40%), gastrointestinal mucosal injury (7-43%), hepatic dysfunction

Table 1. Vital signs of patients

Patient	Fever (C°)	Blood Pressure (mm/Hg)	Respiratory rate (/min)	Pulse (/min)
1	36,2	120/80	16	50
2	36,2	110/75	18	59
3	36,4	120/75	15	61
4	36,4	110/70	17	78
5	36,5	110/75	16	75
6	36,2	130/80	17	90
7	36,4	125/75	15	70

Table 2. Complete blood count and coagulation parameters of patients

Patients	Hemoglobin (13,5-18 g/dl)			Haematocrit (42-52 %)			Platelet (150-450x10³/mm³)			PTZ (10-15min)	INR
	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	1st d
1	14.4	12.7	14.1	42.0	37.4	41.2	115	31	80	10.7	0.78
2	14.8	14.0	14.2	42.3	40.7	40.9	179	162	182	11.4	0.83
3	16.4	14.8	14.9	46.6	42.7	42.5	127	118	118	12.7	0.94
4	14.6	14.1	-	41.2	40.4	-	173	163	-	11	0.8
5	14.8	13.5	-	43.7	39.6	-	354	315	-	13.1	0.97
6	15.6	14.4	-	45.4	42.1	-	318	257	-	11.5	0.84
7	12.5	12.6		38.4	39.0		270	266		11.9	0.87

Table 3. Biochemical values of patients

Patient	Sodium (135-150mmol/L)			Potassium (3,5-5,5mmol/L)			Üre a (10-50mg/dl)			Creatinin (0,5-1,2mg/dl)			ALT (7-40 IU/L)			AST (9-45 IU/L)			Amylase (25-125 U/L)		
	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d	1st d	2nd.d	3rd.d
1	136	139	138	3.7	4.1	3.9	34	22	25	1.0	1.0	0.9	21	18	18	33	29	31	90	74	85
2	136	139	137	4.0	4.0	4.0	29	20	17	0.8	1.3	0.8	14	15	15	22	23	20	75	79	67
3	138	141	132	4.0	3.8	4.0	35	23	21	0.8	1.5	0.7	54	46	47	32	30	27	53	48	43
4	139	142	-	3.8	3.8	-	25	16	-	0.9	0.8	-	21	20	-	27	22	-	93	77	-
5	138	142	-	3.9	4.4	-	28	19	-	0.9	0.8	-	27	20	-	26	30	-	84	98	-
6	136	138	-	4.5	4.6	-	31	27	-	0.9	0.9	-	18	13	-	23	16	-	71	60	-
7	134	140	-	4.3	4.2	-	23	16	-	0.8	0.8	-	41	38	-	35	33	-	96	65	-

(19-40%), renal insufficiency (10-14%), abdominal pain (12%), shock (9%), fever (7%), pulmonary edema (5-13%), metabolic acidosis (5-13%) and death (10.5-16.7%) (2). In a study with 131 cases, the most common laboratory findings were leucocytosis (68%), low bicarbonate (48.1%), acidosis (33.8%), hepatic dysfunction (33.6%), hypercapnia (30.9%), hypoxemia (28.4%), renal failure (17.1%) (2).

Our patients had no other complaints than nausea. Thromboytopenia and low hemoglobin were detected in one patient. An elevation was detected in creatinine levels in two patients.

Poisoning may develop as the result of intake by oral, intravenous, inhalation, dermal and conjunctival route. Oral and intravenous intakes are the most toxic ones (7). Skin irritation, photocontact dermatitis and rarely skin burns may be seen in dermal exposures. Clothes should be taken off and skin should be washed with water and soap. Uses as spray form may cause poisionings with inhalation route and disturbances in oral and nasal mucosa, a nasty taste. Conjunctivitis may be seen in ocular exposure however superficial corneal injury may develop if ocular irrigation is delayed (8). GlySH reaches its peak concentration 4-6 hours after oral intake. Its half life is 3-4 hours. Thus gastric lavage should be made and activated carbon reducing surfactant absorption should be administered within 4 hours, preferably within the first hour following oral intake (9). However absence of an erozive injury and perforation in gastrointestinal system should be proven before gastric lavage and activated carbon administeration. Buccal irritation and burns together with symptoms like sore throat, abdominal pain, dysphagia, odinophagia, retrosternal pain, nausea and vomiting may be predictive for this. In a study, corrosive effect of GlySH has been shown to increase with intakes 100 ml or above and determination of corrosive level through endoscopy was recommended (7).

There was oral exposure in all of our patients. The patients had added the glyphosate containing solution into the meal instead of oil mistakenly. Amount of glyphosate was about 35 ml in total for 7 patients. No symptoms or physical examination findings suggestive for corrosive effect were detected in the patients. Gastric lavage and activated carbon that reduces surfactant absorption were administered as corrosive effect was not considered.

GlySH-related poisonings may be categorized in 4 clinical classes. Asymptomatic patients are the ones who have no abnormal complaints, physical examination and laboratory findings and they may be discharged from the hospital after 6-hours of follow up (9). Our 4 patients were asymptomatic. We discharged these patients from the hospital with instructions after 24 hours as they were unproblematic. Mild cases are the ones who are self-limited, who may recover spontaneously and who have stable vital findings. Nausea, vomiting, diarrhea and abdominal pain are the main symptoms. Follow up and supportive treatment will be sufficient for these patients. Remaining three patients were in this group (9,10). Toxic findings begin to appear when 85 ml or above GlySH is ingested (8). These patients exhibit moderate poisioning findings, gastrointestinal bleeding, oral ulceration, epiglottic edema, hypotension responsive to fluid replacement, pulmonary dysfunction not requiring intubation, acid-base disorders, temporal oligouria, temporal renal and hepatic dysfunctions, gastrointestinal symptoms continue for more than 24 hours (9). Patients with severe poisoning findings have hypotension requiring vasopressor treatment, mean arterial blood pressure is <70 mmHg, they have respiratory insufficiency requiring intubation, renal failure requiring dialysis, repeated seizures, their Glasgow coma scale is <10, they are comatous (9,10).

In a study from Sri Lanka, 91.3% cases had minimal symptoms, 5.5% cases showed severe poisoning findings and death occured in 3.2% (10). This death rate is lower than that of other studies (2,11).

Determination of GlySH level with chemical analysis is not enough to know the lethal dose. Because surfactant level which is mainly responsible for toxicity cannot be determined with measurement of blood GlySH level, additionally different amounts of surfactant may be available in different commercial forms (2). However severe poisoning findings and death were seen with 330 ± 42 ml GlySH intake in a study carried out with 131 cases, with 206 ml GlySH intake in the study of Sawada et al., with 269 ± 104 ml GlySH intake in the study of Tanineck et al. (2). Another reason for this difference may be that glyphosate genetics is different between humans (10). High amounts of intake and high plasma GlySH concentration were found to be related with death. Amount of intake was low in our cases as a small amount of GlySH was added into the meal and divided between 7 men. Thus severe poisoning findings were not seen. Low platelet and hemoglobin levels were detected in one patient. Although low leucocyte and eryhtrocyte levels are commonly reported findings in literature, thrombocytopenia is not a common finding. Peripheral blood smear examination of the patient was consistent with laboratory findings. The patient does not have a coagulation disorder in his medical history. Control platelet and hemoglobin values tested one week later were found normal. Thus this is a featured case. Impairment was detected in renal functions of two patients despite low oral intake. These patients responded well to early IV fluid replacement therapy and did not need hemodialysis .

The most important determinant of mortality is pulmonary insufficiency and death rate is high among these patients (2). There are three main mechanisms leading to pulmonary insufficiency. The first mechanism is aspiration pneomonitis. GlySH may lead to frequent vomiting and related aspiration as it is a potent irritant. Aspiration may worsen pulmonary insufficiency in patients who have altered consciousness. While the second reason for respiratory insufficiency is non-cardiogenic pulmonary edema, the third one is GlySH aspiration-related injury of pulmonary endothelium and epithelium, increase in vascular permeability and subsequent respiratory distress syndrome (ARDS) (11). Another reason for death is cardiovascular collapse. This may be caused by cardiogenic shock developing with cardiotoxic effect of GlySH or dehydration-related hypovolemic shock (11). Amount of GlySH intake was low in our patients. Although the patients had nausea, they did not experience vomiting. Glasgow coma scale was 15 and there were no risk factors for aspiration. Mortality in GlySH intake usually develops as the result of aspiration and cardiopulmonary involvement. We consider that severe morbidity and mortality did not develop in our patients due to absence of cardiopulmonary pathologies and aspiration.

GlySH does not have an antidot. Atropine and pralidoxime are not used for treatement although it is an organophosphate. High doses of atropine administered for treatment leads to iatrogenic atropine

intoxication and increases mortality (11). Supportive treatment is applied. Providing airway safety is the first step. The patient should be intubated immediately in case of aspiration risk. Mechanical ventilation support should be provided for patients whose spontaneous respiration is insufficient, who have pulmonary edema or ARDS. An immediate fluid replacement should be initiated via two wide vascular accesses in patients who have severe nausea and vomiting, diarrhea and dehydrated due to corrosive effect in gastrointestinal system. Tretament should be initiated immediately as hypotension and dehydration may impair other organ perfusions and lead to multi-organ insufficiency. Renal replacement therapy may prevent renal insufficieny, acidosis and hyperkalemia. Hemodialysis may be tried if renal insufficiency, acidosis and hyperkalemia are irresponsive to medical therapy. Hemodialysis is effective for GlySH as it is more effective on small molecules. However hemofiltration is more effective on surfactant as surfact is a larger molecule (7,9). Lactic acid free fluids and dialysates should be used if lactic acidosis has developed. Cardiotoxicity should be considered in patients who are irresponsive to fluid therapy and positive inotrpic agents and vasopression treatment should be considered. H2 receptor blockers should be administered for supportive treatment of potential gastrointestinal erosions and ulcerations. Steroids are not used for treatment. Antibiotics should be preserved for possible infections (9). In literature, intravenous fat emulsion (IFE) treatment was tried in a patient who was cardiovascularly supressed and irresponsive to inotropic treatment. The patient has become stable hemodynamically and recovered without sequela. The authors suggest that IFE treatment reduced free serum surfactant level and prevented cardiotoxicity (5). Patients should be transferred to intensive care unit in case of intakes 0.5 ml/kg and above, monitored for pulmonary, cardiovascular and renal functions and supportive treatment should be administered (9).

To conclude; GlySH is an available, commonly used herbicide of which poisoning may be fatal. Clinical findings are closely related with plasma GlySH concentration and vary from asymptomatic findings to fatal manifestations. Securing airway and initiating supportive treatment in the early period would contribute perfusion of all organs and significantly reduce mortality.

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Histopathological characteristics of primary orbital meningioma

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Abstract

Introduction: To understand the biological behaviors of primary orbital meningioma by observing the histopathological characteristics.

Materials and methods: 24 histological samples of primary orbital meningioma patients recruited in Inner Mongolia Baogang Hospital from January to December in 2011 were selected, which were subjected to hematoxylin and eosin (HE) staining as well as immunohistochemistry staining (vimentin, epithelial membrane antigen (EMA), Ki-67, p53).

Results: The orbital meningiomas were mainly endothelial type, transitional type and fibrous type histologically. The expression rates of vimentin and EMA were 91.67% (22/24) and 45.83% (11/24), respectively. However, Ki-67 and p53 were expressed at low levels differently.

Conclusion: The orbital meningiomas were not diverse histologically, and few psammoma bodies were scattered. The expression rate of EMA in intraorbital meningioma was lower than that in intracranial meningioma. The expressions of Ki-67 and 053 in benign, recurrent and malignant meningiomas did not differ significantly.

Key words: Orbital tumor, meningioma.

Introduction

Primary orbital meningioma is one of the common intraorbital tumors, with the age of onset lower than that of intracranial meningioma. Its recurrence rate is as high as 60% [1, 2]. A small number of patients may suffer from the disease bilaterally. The orbital tumor that originates from the optic nerve sheath meninge is called the optic endolemma meningioma, and that occurs in sphenoid or frontal bone wall periosteum is called the sphenoid or frontal meningioma. Most tumors in the optic nerve sheath lead to the loss of visual acuity, and spread into cranial cavity in advanced stage. For the tumor lacking sensitivity to drug treatment and radiation therapy clinically, the best treatment is still surgical resection at present, which often requires the removal of the optic nerve. Therefore orbital meningioma is a highly risky tumor [3]. In this paper, the biological behaviors of primary orbital meningioma were studied by observing the characteristics of histopathology and immunohistochemistry.

Materials and methods

General information

24 histological samples of primary orbital meningioma patients recruited in Inner Mongolia Baogang Hospital from January to December in 2011 were selected.

Reagents and methods

Vimentin, epithelial membrane antigen (EMA), ready-to-use primary antibodies of p53 and Ki-67 were purchased from Shanghai Changjia Biotech Co., Ltd. All samples were fixed by 10% neutral formaldehyde solution, conducted with routine paraffin embedding which were sectioned serially with a thickness of 3μm, stained with HE and then made histopathological classification in accordance with a WHO standard [4]. Each paraffin block was sectioned into five pieces for spare use.

The standard SP method was adopted in immunohistochemical staining according to the reagent instructions. The staining was operated as follows: the paraffin sections were dewaxed to water; incubated with 2.8% hydrogen peroxide at room temperature for 10min and washed with PBS for 5min (three times); blocked with normal goat serum working solution at room temperature for 10min, added primary antibody working solution dropwise, and put in the refrigerator at 4℃ overnight; washed with PBS for 5min (three times); added biotin-labeling secondary antibody working solution dropwise, incubated at 37℃ for 15min, and

washed with PBS for 5min (three times); stained with diaminobenzidine, and then subjected to counterstaining and section sealing. Both the positive and negative controls were set in each experiment.

Results

General information

The clinical data of 24 patients are shown in Table 1, including 9 males and 15 females between 21 and 64 with the average age of 42, in which there were 14 patients with right orbital tumor, 10 with left orbital tumor, 11 with recurrent tumor and 13 with cranio-orbital tumor; 9 cases were found in sphenoid ridge, 7 in optic nerve sheath, 4 in orbital interior, 2 in orbital superior wall, 1 in orbital outer top and 1 in orbital apex. The proportion of male to female was 1: 1.67 (9/15). The first clinic time was 1 month to 15 years after onset with the average course of 4.1 years. Main symptoms included

Table 1. Clinical data of patients

Age	Position	Cranio-orbital	Recurrent	Course /year	Eyesight		Eyeball movement	Exophthalmic extent (mm)	Diagnosis
					Right	Left			
34	Left sphenoid ridge	Y	1st time	14	1.1	0.8	-	4	Endothelial
45	Left optic nerve sheath	N	1st time	11	0.2	0.9	-	2	Endothelial
27	Left orbital interior	N	1st time	136	0.9	0.7	-	6	Endothelial
21	Right optic nerve sheath	Y	1st time	24	1.0	0.9	Insufficient extension	1	Endothelial
56	Left sphenoid ridge	N	1st time	1	1.0	Manual	-	3	Transitional
45	Right sphenoid ridge	Y	1st time	50	0.4	1.1	-	4	Endothelial
39	Right optic nerve sheath, intracranial	N	1st time	36	Light sensing	Light sensing	Limited omni-directionally	7	Endothelial
33	Left sphenoid ridge	N	1st time	45	0.5	0.5	-	6	Transitional
25	Left orbital superior wall	Y	1st time	180	1.0	0.3	-	4	Endothelial
57	Left optic nerve sheath	Y	1st time	8	0.8	0.6	Limited omni-directionally	2	Endothelial
55	Right orbital interior	N	1st time	16	1.2	0.7	-	3	Endothelial
36	Right sphenoid ridge	N	1st time	150	1.5	Anterior ocular index	Insufficient rotation downward	7	Transitional
36	Right sphenoid ridge	N	1st time	50	1.1	1.2	-	9	Transitional
45	Left optic nerve sheath	Y	Recurrent	38	0.9	0.9	-	11	Endothelial
61	Left cranial, orbital apex	Y	Recurrent	18	-	0.5	Fixed outward	3	Endothelial
29	Left sphenoid ridge	Y	Recurrent	66	1.5	1.5	Insufficient rotation upward	13	Endothelial
35	Right orbital outer top	N	Recurrent	84	1.2	1.3	-	7	Endothelial
64	Right optic nerve sheath	N	Recurrent	43	-	0.8	-	2	Transitional
37	Left sphenoid ridge	N	Recurrent	56	0.8	0.2	Limited inward/ outward	4	Secretory
45	Right orbital interior	Y	Recurrent	28	1.1	0.7	-	6	Transitional
49	Left orbital interior	Y	Recurrent	21	0.8	0.1	-	2	Atypical
58	Left sphenoid ridge	Y	Recurrent	72	1.2	0.1	-	4	Atypical
58	Right orbital superior wall, cranial	Y	Recurrent	37	1.1	1.2	-	6	Anaplastic
63	Left optic nerve sheath	Y	Recurrent	9	0.9	-	Limited omni-directionally	9	Anaplastic

protopsis and impaired vision. The exophthalmic extents were 1.0-13.0mm with the average of 5.2mm. 8 patients suffered from different degrees of eye movement disturbance. The results of ocular type-B ultrasonic, CT or MRI examinations show intraorbital space occupying lesion or optic nerve thickening. Observed by naked eye, the tumor was off white with a clear boundary and the optic nerve segment was thickened. All the 11 recurrent cases in this group were the first recurrence after operation (shortest: 9 months, longest: 7 years).

Histopathological characteristics

The 24 cases of meningioma were mainly endothelial type and transitional type histologically. The pathological characteristics include: meningeal endothelial cells and fusiform cells similar to fibroblast were observed under microscope. The former had an unclear boundary, with lightly stained and oval nucleus arranged in a nested structure; the latter were fusiform and arranged in sarciniform, presenting a spiral structure in some regions. A few scattered psammoma bodies were found in individual cases. The secretory meningioma had many pink-dyeing unorganized secretory components scattered among cancer cells. Anaplastic (malignant) meningeoma: obvious cellular atypia in large and irregular shape with rich cytoplasm; nuclear chromatin was thickened and the tumor invaded the optic nerve; focal necrosis occurred in one case. Pathological classification of the 24 samples: 13 endothelial type (recurrent case: 4), 5 transitional type (recurrent case: 2), 2 secretory type and 2 atypical type respectively (recurrent case: 1 secretory type and 2 atypical type) and 2 anaplastic meningioma (both were recurrent cases).

Immunohistochemistry staining

All the 24 samples were subjected to vimentin, EMA, Ki-67 and p53 staining. The positive results of Vimentin and EMA staining are found in cytoplasmic claybank particles (Figure 1a and 1b), and those of Ki-67 and p53 staining are found in nucleus (Figure 1c and 1d). Antibody expression: 22 cases of vimentin positive expression, 11 cases of EMA positive expression, and 4 cases of Ki-67 and p53 positive expression, respectively.

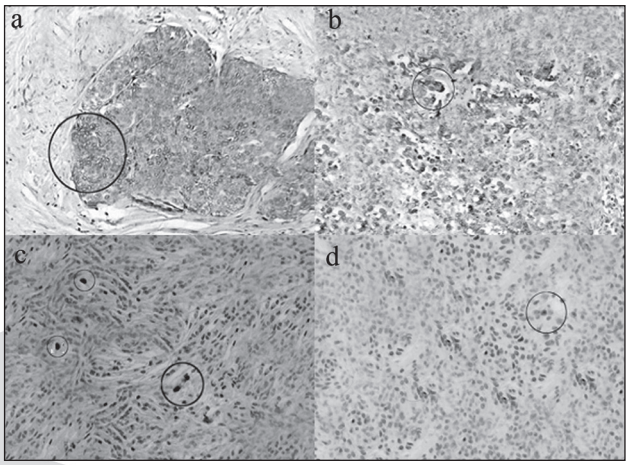

Figure 1. Immunohistochemistry staining

Figure 1a: The tumor was diagnosed as endothelial meningioma pathologically after resection. By immunohistochemical staining light microscope, the vimentin antibody shows diffuse positive expression with a positive part in cytoplasm, which is specifically shown inside the circle; Figure 1b: The tumor was diagnosed as anaplastic (malignant) meningioma pathologically after resection. By immunohistochemical staining light microscope, the EMA antibody shows diffuse and strongly positive (++) expression with a positive part in cytoplasm, which was specifically shown inside the circle; Figure 1c: The tumor was diagnosed as transitional meningioma pathologically after resection. By immunohistochemical staining light microscope, the Ki-67 antibody shows scattered positive expression with a positive part in cytoplasm, which was specifically shown inside the circle; Figure 1d: The tumor was diagnosed as endothelial meningioma pathologically after resection. By immunohistochemical staining light microscope, the p53 antibody showed positive expression (+80%) with a positive part in cytoplasm, which was specifically shown inside the circle.

Discussion

Meningioma, one of the common tumors in the central nervous system, is characteristic in diversified tissue morphologies, various types and different tissue origins [5]. In 1993, meningioma was classified into four major categories by WHO, i.e. benign meningioma, atypical meningioma, papillary meningioma and anaplastic (malignant) meningioma, which were further classified into 11 subtypes [6].

The intracranial meningioma is prone to recurring. It has been reported that the 5-year and 10-year recurrence rates of benign meningiomas are 4% and 8% -16% respectively, and the 5-year recurrence rates of atypical and anaplastic (malignant) meningiomas are 37% and 77%, respectively.

Extracranial meningioma, which is rare, has been reported to occur in nasal cavity and paranasal sinuse [7]. Primary orbital meningioma is one of common intraorbital tumors, accounting for 6% -11% of orbital tumors [8, 9]. Extracranial meningioma may occur according to several different mechanisms: (1) when some blood vessels and nerves cross the skull hole, arachnoid cells appear in the nerve sheath; (2) arachnoid granulations displaced in embryonic development are separated, extruded or taken to other extracranial sites; (3) arachnoid island is shifted under trauma or increased intracranial pressure [10, 11].

Meningioma (either intracranial or extracranial) stems from arachnoid cells, originates from the mesoderm and partly from the neural crest. Arachnoid cells include the outer meningeal nucleus and inner mesh endothelial nuclei fibroblasts, which determines the special immunohistochemical staining of meningioma. Thus the tumor cells express both epithelial markers (e.g. EMA) and mesenchymal markers (e.g. Vimentin), which is rare in other tumors. Primary orbital meningioma is mostly located in the optic nerve, followed by the orbital periosteum (the greater wing of sphenoid and ethmoid in particular), and also some parts without obvious relations with the above mentioned sites, which is considered to stem from ectopic arachnoid cells [12]. Optic nerve sheath consists of dura, arachnoid and pia mater from outside to inside. The meningioma of optic nerve sheath derives from the arachnoid.

The histological morphology of primary orbital meningioma is similar to that of intracranial meningioma. Liu et al. observed the histological features of meningioma in 118 patients, the majority of which was the endothelial type (61%). The transitional type, fibrous type and psammomatous type only accounted for 2% -10%, and there were only three cases of vascular-type meningioma. Orbital meningioma exhibits simple shapes, which may be related with the low incidence [13] and has been demonstrated in this paper. The biological behaviors of orbital meningioma and intracranial meningioma are similar clinically with high recurrence rates. In this study, although the tumor cells were well differentiated in some cases without atypia or nuclear fission, the infiltrative growth of tumor cells could be immersed into the optic nerve or the muscles around.

The immunohistochemical staining results show that the severity expression rates of Ki-67 and p53 were only 16.67% (4/24) despite the meningioma (including malignant one) was incipient or recurrent. The expression of Ki-67 was anaplastic (malignant), 1 case of the endothelial type and 1 case of transitional type (the anaplastic type was a recurrence case). The number of tumor cells with positive expression only reached 13% in each case. One case of endothelial meningioma that was an incipient case showed the highest expression of p53 with the positive expression of tumor cells higher than 75%. In other two cases, the cells with positive expression were no lower than 50% with a endothelial type and a transitional type (the endothelial type was a recurrence case). None of the 24 specimens had positive expressions in both Ki-67 and p53. Five non-recurrent cases with high expression of Ki-67 or p53 were followed up after operation (longest: postoperative one year and two month, shortest: postoperative 3 months), in which no recurrence was found. A previous study has shown that Ki-67 not only served as an objective indicator in evaluating the histological grading of meningioma, but also could be used as one of the indicators to further assess the postoperative recurrence and malignant transformation. Besides, p53 overexpression is an important indicator to predict meningioma recurrence [14]. In this study, Ki-67 and p53 expressions in benign, recurrent and malignant meningiomas did not differ significantly.

EMA, a glycoprotein located in the cell membrane, is a reliable marker of tumor epithelial differentiation. It can be expressed in normal meningocytes and various subtypes of meningioma, and can also express a variety of mesenchymal cell markers (e.g. vimentin). EMA and vimentin are highly express in intracranial meningioma (>80%) [15, 16]. Huang et al. reported that in 60 cases of intracranial meningioma, 59 showed positive in vimentin and 57 in EMA with the positive rates of 98% and 95%, relatively [17]. Chen et al. conducted multiple immu-

nohistochemical studies on 27 cases of intracranial meningioma and 12 cases of extracranial meningioma, the results of which showed that the expressions of vimentin and EMA were 100% [18]. Battistella reported that in 32 cases of extracranial sinus meningioma, the positive expressions of EMA and vimentin were 100%, though the EMA expression was weak or focally positive. The above-mentioned antigen expression of primary orbital meningioma has not been reported abroad [19]. Li et al. reported that the EMA expression rate was 61% in 16 cases of primary orbital meningioma [20]. We observed herein that the expression rates of vimentin and EMA were 91.67% (22/24) and 45.83% (11/24) respectively in 24 cases of meningioma. Most of the EMA expression was weakly positive focally, which was different from that of intracranial meningioma.

Zhang et al. reported that meningioma is a multi-differentiated tumor, which may become mature according to the sequence of meningeal endothelial type, transitional type, fibrous type and other types (e.g. psammomatous type, hemangioma type, microcapsule type, metaplastic type). The easy recurrence of meningioma is mainly associated with endothelial meningioma [21]. In this study, we found 6 cases of endothelial type (an atypical type and an anaplastic type, and the cases were endothelial type in the first surgery), 2 cases of transitional type, and one case of atypical type, secretory type and anaplastic type each, respectively. The results also suggest the high recurrence rate of endothelial meningioma. Further studies are still in need concerning whether the low expression of EMA in orbital meningioma reflects a poor differentiation of tumor in the epithelial direction (meningeal cells) as well as the underlying biological significance.

Moreover, in the pathological diagnosis and differential diagnosis of the orbital meningiomas, researchers should collect more samples and observe slices carefully to find out typical tissue images encountering atypical or rare type of meningioma due to the low expression of EMA.

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Connection between visceral obesity, insulin resistance and C reactive protein

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Abstract

Visceral obesity is associated with hyperinsulinemia, insulin resistance and proinflammatory clinical status.

The objectives of the study will: to examine the relationship between obesity and the emergence of insulin resistance with systemic low-grade inflammation, by determining serum levels of C-reactive protein (CRP) in the sample study population.

Patients and methods of research: The study included 100 obese patients, 30-55 years of age, female gender which, using the anthropometric and metabolic measurements were classified according to BMI> 25 or waist line, marked as "waist circumference" WC> 80 cm. The control group involved 50 people with BMI <25 and waist size WC <80 cm in women. HOMA method was used to calculate insulin resistance for each subject. Homa-index was used to compare groups and correlation analysis, the relation between the results. Correlation was determined between BMI and HOMA-IR. Abdominal obesity was defined as WC> 80 cm in women and> 94 cm in men and obesity (BMI 30 kg/m2), which was used as an indicator of abdominal obesity. The parameters in the study were: age, female gender, BMI (kg/m2), waist size (mm). Also, WC> 80 cm in women, fasting insulin (IU / ml), HOMA IR, CRP mg / l.

Statistical analysis: Statistical analysis was performed in SPSS program, version 15. for Windows.

Results: In this study, which consisted of 100 subjects, female population was included because of gender differences in anthropometric measurements. The average age of respondents was 43.5 years. Respondents were distributed into two groups: 50 patients had a BMI> 25; 50 women had a BMI <25; 50% of the patients had a BMI <25.3%; BMI 25 to 32.1%, BMI> 30t. Based on these data, we can talk about the high prevalence of obesity. Elevated CRP and clinically higher CRP levels were present in 37.6% of respondents. Both overweight (body mass index [BMI], 25 to 29.9 kg / m2) and obese (BMI 30 kg / m2) subjects had had elevated CRP levels than in patients with normal weight (BMI <25 kg / m2), after adjustment for potential side findings, including smoking and mental health status. Waist to hip ratio was positively related to both groups with clinically higher CRP independently of BMI.

Conclusion: On the basis of our study we demonstrated the fact that, with increasing BMI levels of C-reactive protein increase, we found statistical significance statistical difference between the groups. Chronic, subclinical inflammation caused by obesity is a major component in the pathogenesis of insulin resistance and metabolic syndrome. It was found that in obese individuals, adipose tissue becomes infiltrated with macrophages, which may be an important factor in the local production of proinflammatory cytokines.

According to the currently ruling hypothesis, adipokines, probably through CRP, are directly involved in the process of atherogenesis and atherosclerosis contributing to the development of endothelial dysfunction and insulin resistance.

Key words: insulin resistance, visceral obesity, waist circumference, C-reactive protein

Introduction

Obesity has become one of the leading health problems of 21th century. This problem has been influenced by hereditary factors and reduced physical activity as well as excessive consumption of foods rich in concentrated carbohydrates. Obesity, particularly visceral, is a risk factor for cardiovascular disease and type 2 diabetes, hypertension and dyslipidemia. (3.6). Central obesity is associated with hyperinsulinemia, insulin resistance, dyslipidemia and proinflammatory and prothrombotic clinical status. Obesity is conside-

red a chronic inflammatory disease that leads to chronic mass, non-inflammatory diseases such as atherosclerosis, type 2 diabetes, nonalcoholic fatty liver cancer (e.g. prostate cancer) and osteoarthritis. Insulin resistance is a condition in which normal amounts of insulin produce weaker biological response in target cells resulting in compensatory hyperinsulinemia. The biological response to insulin is reduced to 40% in the type-2 diabetes. (11,13). The first step in the action of insulin is activation of insulin receptors. Insulin receptor heterotetramer consists of two alpha and two beta dimers linked with disulfide bond. (5,) Today, it is known that the primary disorder in insulin resistance may be at the level of receptors, the level of postreceptor key cellular enzyme, and mixed receptor-postreceptor defects. CRP concentration in plasma is elevated in obese insulin-resistant individuals. (7,12). Allan Z. Zhao, Ph.D., assistant professor of cell biology and physiology at the University of Pittsburgh School of Medicine, presented the connection between CRP and leptin levels in obese patients. Leptin is a hormone that regulates appetite by signaling CNS, and at the sam time it is a protein that is associated with heart disease. CRP binds to leptin and affects appetite control. Leptin suppresses appetite and reduces weight. (11.6). Leptin is secreted from adipose tissue, so, the more fat is present, the more leptin we have. The name comes from the Greek word *leptos* meaning slim. Leptin in the hypothalamus binds to receptors located on the surface of neurons sending signals that stop the appetite and increase the consumption of calories in the body. Obese people produce more leptin than those who are lean and have normal weight. Obese people are somehow resistant to these effects. Binding of CRP to leptin may be the cause of this happening. Consequently, elevated CRP levels are present in obese patients. CRP is produced in the liver and is typically increased as a result of immune and inflammatory responses, also as a marker for hypertension and heart diseases that occur as complications of obesity. (5,12).

It is known that CRP binds to leptin and prevents its effect on appetite and calories consumption, but how this happens, it is not known.

Materials and subjects

The study included 100 obese patients aged 30-55 years, which were classified by anthropometric and metabolic measurements, according to the BM Index> 25 and waist size marked as "waist circumference" WC> 80 cm in women and> 94 in men.

The control group included 100 patients with BMI <25 and waist WC <80 cm in women and <94 for men. Groups were homogenized by age, gender and level of glycemic control, in order for statistical analysis to be performed methodologically correctly and results interpreted accordingly.

Statistical methods

All research data were entered into a database and analyzed using the statistical program SPSS version 17.0 for Windows-.

Anthropometric measurements

Precision scale was used to measure the weight, the reading was rounded to 200 grams. Height was measured in the standard position, and the measurements rounded to 5 mm. BMI was defined as a weight divided by height (kilograms per square meter). BMI categories (normal weight BMI 18.5-24.9 kg/m2, increased body weight BMI 25-29.9 kg/m2 and obesity BMI 30 kg/m2) was calculated according to the classification of the World Health Organization. WC was measured in the middle, between the lowest rib border and iliac top, in a standing position. The measurement was performed by centimeter tool and rounded to 0.5 cm. Abdominal obesity was defined as WC> 80 cm in women and WC> 94 cm in men, in non-Asian population and obesity as BMI 30 kg/m2, which was used as an indicator of abdominal obesity. (25th)

HOMA

HOMA-method was used to calculate the insulin resistance for each subject.

Homeostasis model assessment formula (HOMA-R). This index is calculated by the following formula:

$$\text{HOMA-R} = \frac{\text{Gb} \times \text{Ib}}{\text{k}}$$

Where **Gb** and **Ib** – are basal glucose and insulin values, **k** is a constant to calculate HOMA - R, so it has the value of 1 or 100% with the meaning of normal basal glucose and insulin. Glucose is displayed in mmol / l; insulin in U / ml, k = 22.5 (Matthews et al. 1985). Homa index was used for the comparison between groups and the correlation analysis, the relation between the results. The correlation between BMI and HOMA-IR was also determined.

This model correlates with estimates using euglycemic clamp method, by which (r = .88).

Test results

The study was conducted on the basis of the usual approach to the subject by means of medcal history, clinical medical examination and laboratory tests. The study was carried out at the Clinic for Endocrinology, Diabetes and Metabolism. The study included 100 patients. The parameters involved in the study were compared in two groups of patients with BMI> and <25 kg/m2.

The average age of the study population with a BMI less than 25 kg/m2 was 44.18 ± 6.32, and the average age with a BMI over 25 kg/m2, was 43.3 ± 6.06. Statistical analysis using the Student t-test showed that there was no statistically significant differences between the two groups in terms of age (t = 1.853, p> 0.05).

The incidence of obesity, the participants divided into two groups BMI> and <25 kg/m2.

Table 1. Age

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25	50	43,36	6,063	,858	35	54
BMI below 25	50	44,18	6,324	,894	36	54
Total	100	43,77	6,177	,618	35	54

t=1,853; p=0,845

Table 2. BMI

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25	50	33,96	5,703	,807	26	46
BMI below 25	50	23,80	1,010	,143	21	25
Total	100	28,88	6,532	,653	21	46

t=153,841; p=0,0001

Table 3. Waist size

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25						
BMI below 25	50	75,44	13,003	1,839	8	88
Total	100	88,03	20,629	2,063	8	140

t=59,113; p=0,0001

Table 4. Hip size

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25						
BMI below 25	50	106,98	13,705	1,938	60	138
Total	100	111,51	12,768	1,277	60	140

t=14,276; p=0,0001

Tabela 5. Hip-ratio (os/ob)

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25						
BMI below 25	50	,8202	,12033	,01702	,63	1,25
Total	100	,8657	,14764	,01476	,61	1,25

t=10,399; p=0,002

The study population included women, because of the gender differences in anthropometric measurements. The mean BMI for the group of patients with normal body weight was 23.8 ± 1.01, and for patients with overweight average, BMI was 33.96 ± 5.7; from the aforementioned points it was clearly presented that there was a significant difference in values. BMI between the groups of patients (t = 153.8, p <.01).

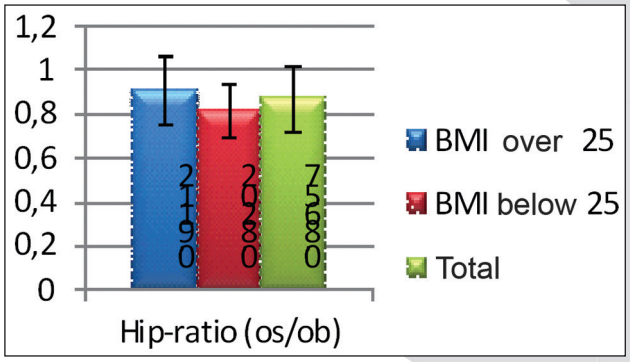

Figure 1. Hip ratio (os / ob)

Value of lower waist and hips was presented by anthropometric measurements, as well as the relationship between the waist and hips. Waist size mean values for a group of patients with BMI> 25 kg/m2 was 100.62 ± 19.1 cm, and for a group of patients with BMI <25 kg/m2 was 75.44 ± 13.0 (Figure 1).

The mean volume of the hips for a group of patients with BMI> 25 kg/m2 amounted to 116.04 ± 9.9, and the mean volume of the hips for a group of patients with BMI <25 kg/m2 was 106.98 ± 13.7 cm (table 4).

Hip ratio was higher in patients with BMI> 25 kg/m2 0.9112 ± 0.16 than for patients with BMI <25 kg/m2 0.8202 ± 0 (Table 5).

Statistical analysis of all three parameters showed that there were statistically significant differences in terms of higher average values in the group of obese patients (p <0.01).

Mean levels of basal insulin level in the group of patients with BMI> 25 kg/m2 was 310.62 ± 129.9 and in the group of patients with BMI <25 kg/m2 was 91.76 ± 19.52 (table and figure 8). Statistical analysis revealed statistically significant differences in basal insulin levels between the groups (t = 138.6, p <.01).

The mean HOMA index (IR) in patients with BMI> 25 kg/m2 was 70.91 ± 31.1 and in the group of patients with BMI <25 kg/m2 was 18.70 ± 6.3. (Table and Figure 9). Statistical analysis revealed statistically significant differences in HOMA index between groups (t = 135.5, p <.01).

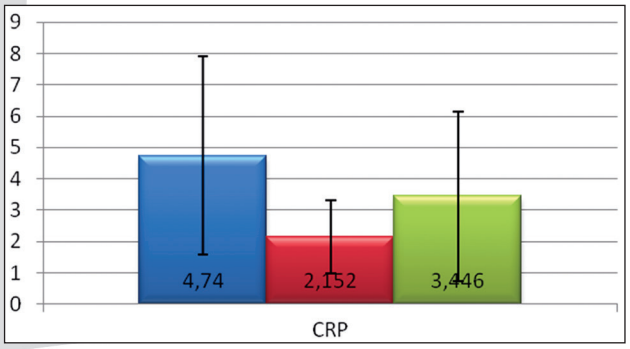

Figure 2. CRP

Mean CRP for patients with BMI> 25 kg / m2 was 4.74 ± 3.15, and in the group of patients with a BMI <25 kg / m2 was 2.15 ± 1.16 (Figure 2). Statistical analysis revealed statistically significant differences in CRP levels between groups (t = 29,574,28, p <.01).

Table 6. Insulin

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25						
BMI below 25	50	91,76	19,523	2,761	60	150
Total	100	201,19	143,678	14,368	60	670

t=138,686; p=0,0001

Table 7. HOMA

	N	Arithmetic mean	Standard deviation	Standard error	Minimum	Maximum
BMI over 25						
BMI below 25	50	18,6970	6,28472	,88879	11,40	38,00
Total	100	44,8042	34,44499	3,44450	11,40	158,35

t=135,486; p=0,0001

Discussion

In this study, which consisted of 100 subjects female population was includd because of the gender differences in anthropometric measurements. The average age of respondents was 43.5 years. Respondents were distributed into two groups: 50 patients had a BMI> 25, 50 women had a BMI <25th 50% . In the group with BMI> 25, 34 respondents had a BMI> 30. Based on these data, we can talk about the high incidence of obesity in our sample.

Waist to hip ratio was positively related to both clinical groups with higher CRP independently of BMI (overweight and obese).

We demonstrated that with increasing BMI, levels of C-reactive protein increase, we also found statistically significant difference between the groups. Subjects with a BMI> 25 had a greater increase in insulin resistance HOMA-IR; there was a significant statistical difference between the groups of subjects with BMI < and > 30 kg/m2. Clinical signs of insulin resistance were elevated fasting insulin> 12 mIU / ml, decreased SHBG, elevated HOMA-IR> 2.16.

Insulin resistance (IR), reduced physiological response of peripheral tissues to insulin action, is one of the main causes of type 2 diabetes, and plays an important role in the pathogenesis of cardiovascular disease. (CVD) (1). Recent studies have shown that the worldwide prevalence of insulin resistance and associated risk factors are markedly increased (2.3). Insulin resistance is probably associated with a chronic inflammatory response that is characterized by an abnormal increase of cytokines and activation of pro-inflammatory signaling pathways (4,5), systemic inflammatory biomarkers. C-reactive protein (CRP) when measured in the blood with high sensitivity of the test is a strong and independent predictor of myocardial infarction, ischemic stroke, type 2 diabetes, hypertension (6.7). Several studies have provided strong evidence of an association between CRP and cardiovascular risk independent of traditional risk factors such as cholesterol, blood pressure, alcohol consumption and smoking. (7.8). Accumulation of experimental and epidemiological data now associate inflammatory processes in impaired glucose metabolism (9). It is also reported that elevated CRP levels may reflect on, not only local inflammation in atherosclerotic lesions, but also on predicting future cardiovascular risks, including insulin resistance (7,10).

Biz Gelayel Luis Revilla, Tania Lopez, Luis Suarez, Michael Williams of the University of Washington: Multidisciplinary Research Training Program, Seattle, WA, USA did a study whose aim was to demonstrate that insulin resistance IR, that is reduced physiological response of peripheral tissues to insulin action, is a major cause of type 2 diabetes. They investigated the relationship of C-reactive protein (CRP8686), markers of systemic inflammation and prevalence in the adult Peruvian population. (6)

Clinical implications

Several studies have shown that women have higher CRP than men (3,4,5). This finding may be due to gender differences in the relationship between CRP and obesity because of CRP levels in women in our study were caused by the amount of total fat. Indeed, studies have failed to show differences in CRP levels between the sexes (34.35). The implications of these interactions are unclear. On the one hand, it is possible that subclinical inflammation, which has been associated with the development of cardiovascular risk factors and adverse cardiovascular events, may disproportionately affect women as they become obese. Such evidence supports the efforts to prevent obesity, which may be particularly valuable for women.

Conclusion

On the basis of our findings in our study, we have shown that increasing BMI increases levels of C-reactive protein; we found a statistically significant difference between the study groups. Chronic, subclinical inflammation caused by obesity is a major component in the pathogenesis of insulin resistance and atherosclerosis. It was found that in obese individuals adipose tissue becomes infiltrated with macrophages, which may be an important factor in the local production of proinflammatory cytokines.

According to the currently ruling hypothesis, adipokines, probably through CRP, are directly involved in the process of atherogenesis and atherosclerosis contributing to the development of endothelial dysfunction and insulin resistance.

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Short term heart rate variability during induction of general anaesthesia with two intravenous anaesthetics

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Abstract

Background: Monitoring of heart rate variability, parameter associated with autonomic nervous system balance, has been advocated as a possibly very important factor of monitoring in the perioperative period.Period of induction of anaesthesia is very important with significant changes of haemodynamics what could be reflected in the changes of the parameter of heart rate variability.

Methods: Seventy patients of ASA I and ASAII status who were scheduled for elective abdominal surgical procedures of moderate stress response, were randomly assigned to group I (thiopentone as induction anaesthetic) and group II (propofol as induction anaesthetic).Monitoring during periinduction period consisted of monitoring mean blood pressure(noninvasively), heart rate, pulseoxymetry and recording of electrocardiogram by holter ECG recorder for further analysis of heart rate variability, in four time segments in periinduction period: Tp,T1,T2,T3. Data were presented as logarithm values of mean values of the total spectrum power values (TP_{lg}), logarithm values of mean values of the power spectrum of low frequencies(LF_{lg}), logarithm values of mean values of the power spectrum of high frequencies (HF_{lg}) and the ratio LF/HF.

Results: Analysis of the values of haemodynamic parameters has shown decrease of mean arterial blood pressure after induction of anaesthesia with intravenous anesthetics thiopental or propofol, with simultaneous changes of heart rate in the four time segments in periinduction period, without statistically significant changes between the groups. Analysis of the values of parameters of heart rate variability measured in the frequency domain has shown changes of logaritmic values of the values of total power spectrum, logaritmic values of the power of low frequency spectrum and logaritmic values of the power of high power spectrum with reduction of the values of total power spectrum, the power of low frequency spectrum and the power of high frequency spectrum, most pronounced just after induction of anaesthesia with slight increase of the values of total power spectrum and individual components of the spectrum of heart rate variability in the later postinduction period, without statistically significant differences between the groups.

Conclusion: According to the obtained results in the groups of patients who underwent induction of general anaesthesia with thiopentone or propofol, there have been reductions of the total spectral power and the power of the spectrum of low frequency components and high frequency components with variations in the relationship between the components in the different periods of periinduction time.

Key words: heart rate variability, intravenous anaesthetics, general anaesthesia

Introduction

There is a strong variability of the oscillations of cardiovascular functions in physiological conditions. Different duration of the consecutive cardiac cycles, denoted as heart rate variability, reflects the feature of the adaptability of cardiovascular system functions in different conditions and under different demands in everyday life. In many experimenatal and clinical trials reduction of this variability of the cardiac cycle length has been shown to be associated with pathological conditions, most notably in the settings of cardiovascular diseases, diabetes, obesity, endocrine, neurological and psychiatric disorders (1,2,3,4). Clinical research in last decades

have shown that measuring of heart rate variability could be very important parameter for assessment of changes in the balance of the autonomic nervous system tone in healthy subjects and a very important predictive factor of morbidity and mortality during the development of the various disease conditions, most prominent after myocardial infarction and in diabetic autonomic neuropathy (4,5,6,7,8). Surgery and general anaesthesia lead to changes in the tone of autonomic nervous system, and in recent period there have been many clinical trials with aim to assess the effects of different anaesthetics on the parameters of the autonomic nervous system tone (9,10,11,12).

The most common way of linear mode of measurig of heart rate variability, adopted as standard measurements enclose measuring of time domain parameters and frequency domain parameters (8). In time-domain analysis different of statistical measures are administered to assess the overall magnitude of fluctuations of the length of consecutive cardiac cycles. In frequency-domain analysis spectral analysis of the signal of electrocardiogram gives information on magnitude of fluctuations in predetermined frequencies. In the complex interaction of parasympathetic and sympathetic activities, resultant neural impulses are directed to sinus node and they are modulated by central and peripheral oscillators. These oscillators produce rhythmic changes in efferent neural discharge, which are manifested as short-and long-term oscillation in heart period (9).

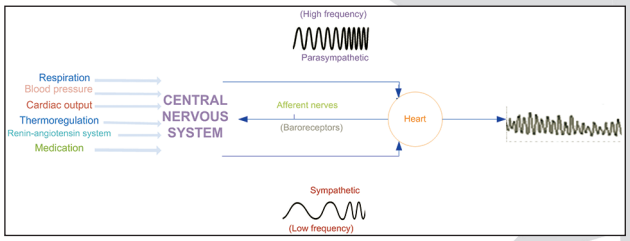

Figure 1. Different frequencies in analysis of heart rate variability, modified from: Schoenauer M. et al. Cardiac autonomic diabetic neuropathy. Diabetes Vasc D Res 2008; 5: 336–44.

The efferent vagal activity is a major contributor to the high frequency (HF) component, as seen in clinical and experimental studies, while low frequency component (LF) is considered by many authors as a marker of sympathetic modulation and by others as a parameter that includes both sympathetic and vagal influences. Consequently, LF/HF ratio is considered to reflect sympathovagal balance.Those numerous factors in control of heart rate variability are depicted in Figure1.

The most common measures of time-domain and frequency domain analysis are enlisted in the Table 1 and Table 2. Parameters calculated from basic measurements represent functions of sympathetic and parasympathetic component of autonomic nervous system (8).

Table 1. Selected Time Domain Measures of HRV (modified from „Heart rate variability: standards of measurement, physiogical interpretation and clinical use". Circulation 1996; 93: 1043-65.)

Statistical Measures		
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording
SDSD	ms	Standard deviation of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer
pNN50	%	NN50 count divided by the total number of all NN intervals

Surgery and anaesthetics that are most commonly administered for induction and maintenanace of anaesthesia can exert different effects on the function of autonomic nervous system, with different levels of reduction of the power of the individual components of the total spectrum and changes in the relationship between those components (10,11,12,13,14). Anaesthetics administered in general anaesthesia show attenuation of excessive sympathetic activity but also supression of parasympathetic reactions (15).

Table 2. Selected Frequency Domain Measures of HRV (from „Heart rate variability: standards of measurement, physiogical interpretation and clinical use". Circulation 1996; 93: 1043-65.)

Variable	Units	Description	Frequency Range
Analysis of Short-term Recordings (5 min)			
5-min total power	ms^2	The variance of NN intervals over the temporal segment	≈≤0.4 Hz
VLF	ms^2	Power in VLF range	≤0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
LF norm	nu	LF power in normalized units LF/(total power-VLF)x100	
HF	ms^2	Power in HF range	0.15-0.4 Hz
HF norm	nu	HF power in normalized units HF/(total power-VLF)x100	
LF/HF		Ratio LF [ms^2]/HF[ms^2]	
Analysis of Entire 24 Hours			
Total power	ms^2	Variance of all NN intervals	≈≤0.4 Hz
ULF	ms^2	Power in the ULF range	≤0.003 Hz
VLF	ms^2	Power in the VLF range	0.003-0.04 Hz
LF	ms^2	Power in the LF range	0.04-0.15 Hz
HF	ms^2	Power in the HF range	0.15-0.4 Hz

Measuring of heart rate variability (HRV) during different anaesthetic procedures can partly elucidate the effects of different anaesthetic agents and drugs on autonomic nervous system.

Numerous clinical studies have investigated the changes of heart rate variability during induction of anaesthesia, with the aim of comparing HRV in unstimulated and stimulation-induced heart rate variability. Galletly DC and coworkers found that after induction of anaesthesia with propofol high frequency heart rate oscillations were replaced by low frequency oscillations, on the contrary maintaining general anaesthesia with volatile anaesthetic isoflurane and nitrous oxide led to partial return of high frequency components but components with lower frequencies were still reduced(16). In the compound trial of Latson TW and coworkers two studies were performed. In the first study etomidate and thiopental sodium were compared as induction agents in 18 ASA I patients with minor surgery. In the second study the effects of sufentanil infusion were analysed in 10 ASA III and IV patients. In the first study greater reduction of heart rate variability was shown with thiopental, but etomidate also showed significant depression of heart rate variability. In the second study correlation was found between mesurement of vagally mediated heart rate variability and changes in parasympathetic tone during opioid induction(12).

Komatsu T et al. investigated the effects of ketamine and midazolam on heart rate variability in two small groups of patients ASA I, and the results showed that both ketamine and midazolam reduced total power and all frequency components, but further analysis revealed expected sympathetic activation with ketamine(17).

Patients and Methods

Protocol of the trial was approved by Institutional Ethic Committee and after obtaining informed consent seventy patients of ASA I and ASA II status, scheduled for elective abdominal surgery were randomly assigned to group I (thiopentone as induction anaesthetic) and group II (propofol as induction anaesthetic). Those patients who had cardiovascular diseases, diabetes mellitus, autonomic nervous dysfunction, rhythm disorders, who had medication that influence heart rhythm and haemodynamics, were not included in the trial. Criteria for exclusion were insufficient recordings and recordings with numerous artifacts. Monitoring of blood pressure, heart rate, peripheral pulseoxymetry, recording of electrocardiogram were started and performed during perioperative period. Patients were given midazolam (Dormicum, F. Hoffman-La Roche Ltd Basel, Switzerland) 7,5 mg forty minutes before induction of anaesthesia, and after preoxygenation for several minutes in the preinduction period opioid analgesic fentanyl $2\mu gkg^{-1}$ (Fentanyl –Janssen-Cilag, Belgium) was administered. Induction of anaesthesia was per-

foremed five minutes later by thiopentone (Thiopental sodium , Rotexmedica GMBH Germany) 4-6 mg kg^{-1} or by propofol (Propofol 1% MCT Fresenius Kabi Austria GmbH) 1,5-3,0 mg kg^{-1}. After induction of anaesthesia and ventilation by face mask for five minutes facilitation of orotracheal intubation was obtained by administration of tracrium (Tracrium , GlaxoSmithKline UK) 0,5 mg $kg^{-1,}$ and balanced anaesthesia was continued.

The recordings of electrocardiogram, taken by Holter ECG recorder (Trillium 3000 Holter System, Forest Medical, USA), were analysed later on by means of Trillium HrvFreq 4.01 ©2006 (ForestMedical,LLC, East Syracuse, USA). Heart rate variability power spectra were determined in four 2-minutes segments, in preinduction period-Tp, in period after administration of fentanyl -T1, after administration of induction agents (thiopental or propofol)- T2 and after orotracheal intubation- T3 period. Power spectral analysis was calculated with application of Hanning window. Data were presented as logarithmic values of mean values of the total spectrum power values (TP_{lg}), logarithmic values of mean values of the power spectrum of low frequencies (LF: 0,04-0,15Hz) (LF_{lg}), logarithmic values of mean values of the power spectrum of high frequencies(HF: 0,15-0,4Hz) (HF_{lg}) and the ratio LF/HF.data were expressed as the means+-SEM. Statistical analysis was performed with χ^2-test for gender, Student t-test for age, Mann-Whitney test for BMI data, and two-way repeated analysis of variance (ANOVA) for parameters of haemodynamics and heart rate variability parameters. P value <0,05 was considered as statistically significant.

Results

Analysis of demografic data have shown that mean age of patients was 47,9±8,9 years, in the range of 29 to 60 years, with no statisticaly significant differences between the groups,while comparison of BMI showed mean values of body mass index of 27,04±2,3 (range 21,9-32 kg/m2), with slightly higher values in group II 27,2±2,4 kg/m2 (range 23,2-32 kg/m2) in regard to group I with mean values of 26,9±2,3 kg/m2 (range 21,9-32 kg/m2), without significant difference between the groups. (Table 3)

Results of our trial have shown variations of haemodynamic variables in both groups, with decrease of the values of mean arterial pressure in both groups after administration of induction agents, thiopental or propofol, and consecutive changes in heart rate, without statistically significant difference between the groups.

Analysis of the changes of the values of power of total spectrum, of low frequency(LF) and high frequency(HF) spectra, demonstrated reductions of the power of all spectral parameters in both groups, with most pronounced decrease after induction, without statistically significant differences between the groups. Analysis of the mean values of LF/HF relationships in the four time segments did not show statistically significant differences between the examined groups.

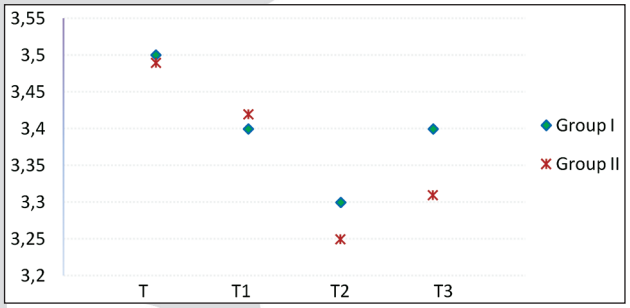

Figure 2. a. Diagram of logarithmic values of mean total spectrum values in the periinduction times

Table 3. Demographic data

	Group I (Thiopentone)	Group II (Propofol)
N	**35**	**35**
Age	48,54 (SE 1,51)	47,23(SE 1,54)
Gender	23 (F) / 12 (M)	21 (F) / 14 (M)
BMI	26,92 (SE 0,39)	27,15 (SE 0,4)
Dose range	4,7 – 5,6 mg kg^{-1}	1,8 – 2,3 mg kg^{-1}

Table 4. Haemodynamic variables and HRV variables in the four time segments

	MAP (mmHg)		HR(f/min)		TPlg		LFlg		HFlg		LF/HF	
	I group (N=35)	II group (N=35)	I group (N=35)	II group (N=35)	I group (N=35)	II group (N=35)	I group (N=35)	II group (N=35)	I group (N=35)	II group (N=35)	I group (N=35)	II group (N=35)
T_p	96,56±9,12	97,42±9,13	76,57±7,3	78,63±7,34	3,49±0,064	3,47±0,056	3,09±0,39	3,08±0,09	2,88±0,153	2,86±0,12	1,73±0,26	1,65±0,15
T1	91,87±7,42	93,76±7,06	73,37±7,1	74,19±6,45	3,43±0,73	3,45±0,078	3,067±0,12	3,05±0,19	2,87±0,132	2,76±0,116	1,66±0,14	1,76±0,32
T2	86,75±6,28	88,3±6,65	82,12±6,1	81,09±5,33	3,36±0,071	3,34±0,069	2,96±0,124	2,92±0,11	2,66±0,098	2,70±0,101	1,86±0,15	1,83±0,31
T3	90,64±3,98	89,32±4,64	82,34±5,5	80,96±3,78	3,41±0,123	3,32±0,148	2,99±0,15	3,02±0,21	2,79±0,213	2,77±0,254	1,66±0,54	1,79±0,63

Values are presented as means±SD, T pre-preinduction time,T1-T3 –time segments in periinduction period, MAP-mean arterial pressure, HR-heart rate,TPlg-logarithmic value of mean total power spectrum, LFlg- logarithmic value of mean LF power spectrum,HFlg- logarithmic value of mean HF power spectrum, I –group with thiopental, II-group with propofol

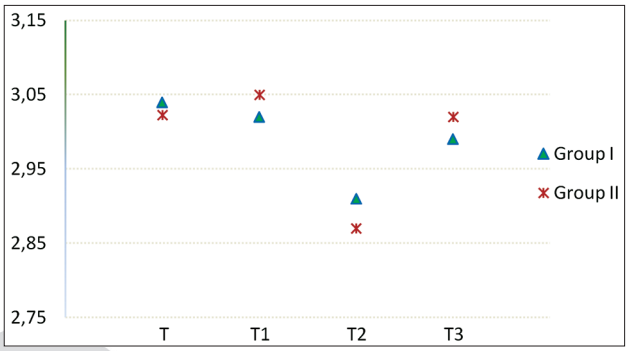

Figure 2. b. Diagram of logarithmic values of mean LF spectrum power values in the periinduction times

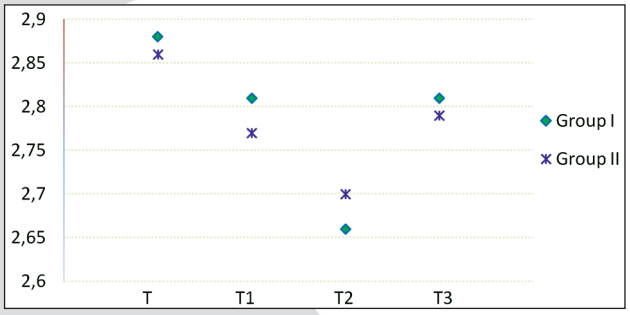

Figure 2. c. Diagram of logarithmic values of mean HF spectrum power values in the periinduction times

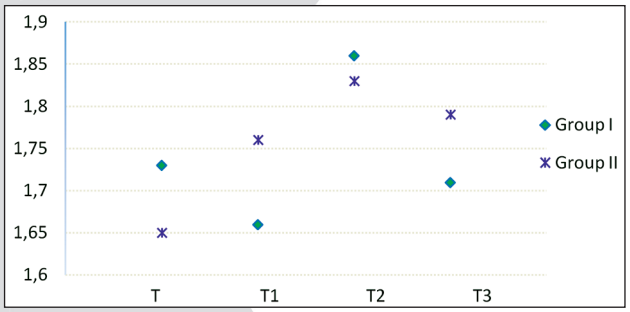

Figure 2. d. Diagram of values of LF/HF relationship in the periinduction times

Discussion

Monitoring of heart rate variability, which has been shown quite important and useful in patients with coronary syndrome and patients with diabetes mellitus, as a parameter of alterations of autonomic nervous system tone and a good predictor of morbidity and complications of the diseases, could be also quite important parameter in the perioperative period. Complexity of the phenomenon of heart rate variability is reflected in different methods of measuring and assessment, different calculations that could be done with basic measurements

and numerous neural and humoral influences that can alter these measurements. Among numerous events in perioperative period the most prominent are stress reaction to surgical procedure and general anaesthesia aimed to attenuate stress response, with maintenance of amnesia and appropriate analagesia during the period of the procedure and in early postprocedural period. These events strongly influence homeostasis in the whole body and especially the balance of autonomic nervous system responsible for fine tuning of physiological reactions and adaptations in the wide range of different environmental situations and demands. Surgical procedure elicit numerous adaptive reactions aimed to restoration of homeostasis, with activation of hypothalamo-pituitary-adrenal axis and the chain of humoral and reflex responses with common denominator of mobilization of glucose from all possible sources and releasing substrates for higher energetic and substrate demands of tissues, organs and systems which function are essential for the adequate stress response(18). The science and practice of anaesthesia has been developing in a major achievement in medicine in last century, enabling safer and more comfortable perioperative period with everdeveloping monitoring systems for different parameters that describe haemodynamic, respiratory, central and peripheral nervous functions and parameters of other homeostatic mechanisms.Changes of the autonomic nervous system(ANS) tone are inevitable during administration of general anaesthesia. It is of great importance to monitor these ANS tone changes and predict possible variations of haemodynamic parameters, what could become more possible by monitoring of heart rate variability.

Numerous clinical trials have been done with induction agents, and most of them have shown that anaesthetic agents attenuate autonomic reflexes what has been reflected in decreasing of the parameters of heart rate variability.

Administration of midazolam in sedation has shown diminished power of spectrum of high frequency and increased power of spectrum of low frequency oscillations what suggested increase of both sympathetic or parasympathetic activity (19). There has been controversy regarding the effects of propofol on the parameters of heart rate variability.

Kanaya et al. in the randomized trial with small group of patients who received propofol and sevoflurane at induction, found that propofol reduced cardiac parasympathetic tone depending on the depth of hypnosis(20).

In the study performed by Deutschman CS at al. induction of anesthesia with propofol was associated with a significant reduction in the values of power of total spectrum, low-frequency spectrum (LF), and high-frequency spectrum (HF), while during the maintenance of anesthesia with propofol as a single anesthetic agent resulted in further reductions in the power of total spectrum and power of low-frequency spectrum, but there was no such effect on the power of high-frequency spectrum (21).

Huang HH. and coworkers who investigated the effects of induction by thiopentone have shown dramatic decrease of the power of total spectrum and individual components of the spectrum but without significant changes in the relationship between the spectral components, what suggested unchanged balance of autonomic nervous system (22).

In the study of Latson TW et al. effects of three induction anaesthetic techniques on heart rate variability were examined (12). These authors demonstrated that induction of anaesthesia both with thiopental and nitrous oxide and etomidate and nitrous oxide was associated with reductions of the powers of total spectrum, low frequency and high frequency spectra, with more pronounced decrease of low frequency spectrum (LF) with thiopental in regard to etomidate(12). In another study Latson TW and O'Flaherty examined effects of propofol and thiopental on induction, in combinations with fentanyl and later different anaesthetic techniques, their results have shown reduction of the power of total spectrum in both groups ,but this effect was less pronounced in the group that had maintenance of the anaesthesia with propofol.

Results of our trial have shown variations of haemodynamic variables in both groups, with decrease of the values of mean arterial pressure in both groups after administration of induction agents, thiopental or propofol, and consecutive changes in heart rate, without statistically significant difference between the groups. Analysis of the changes of the values of power of total spectrum, of low frequency (LF) and high frequency (HF) spectra, de-

monstrated reductions of the power of all spectral parameters in both groups, with most pronounced decrease after induction, without statistically significant differences between the groups. Analysis of the mean values of LF/HF relationships in the four time segments in periinduction period did not show statistically significant differences between the examined groups.

Conclusion

Changes of the parameters of heart rate variability measured in frequency-domain, in periinduction period with two induction agents: thiopental and propofol have shown reductions of the power of all spectral parameters in both groups, with most pronounced decrease of the parameters after induction, without statistically significant differences between the groups. Analysis of the mean values of LF/HF relationships in the four time segments of periinduction period did not show statistically significant differences between the examined groups.

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Posterior fossa decompression with tonsillar resection and duraplasty for Chiari I malformation in 112 adult patients: With a special emphasis on the postoperative complications and clinical outcome

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Abstract

Objective: To evaluate the postoperative complications and clinical outcome in adult patients with Chiari I malformation (CIM) underwent posterior fossa decompression (PFD) with tonsillar resection and wide duraplasty.

Patients and Methods: One hundred and twelve consecutive surgically treated symptomatic CIM patients were retrospectively analyzed. Postoperative complications and factors predicting outcome are discussed, and our results are compared with those of other large series in the literature.

Results: The study included 59 men and 63 women (age range, 19–59 years; mean age at surgery, 40.6 years). Pain, motor and sensory disturbance were the most frequent symptoms. The average duration of preoperative symptoms was 4.1 years, and the follow-up period ranged from 12 to 42 months (median, 27 months). The most common postoperative complications were headache (25.0%), fever (20.5%) as well as pseudomeningocele (18.8%). At the end of follow-up, 64 patients (57.1%) had very good result, 35 (31.3%) had good result, and 13 (11.6%) had poor result. The presence of basilar invagination was associated with poor clinical outcome ($\chi 2$ P=0.038). The extent of cyst on postoperative MR imaging was a predictor of poor clinical outcome ($\chi 2$ P=0.049). Patients with short duration of preoperative symptoms will benefit more from surgical treatment (Regression P=0.021).

Conclusions: PFD and tonsillor resection with duraplasty is an effective treatment of adult CIM, with an 88.4% chance of very good or good result. However, in comparison with other less aggressive approaches, tonsillar resection does not appear to offer a therapeutic advantage but harbors a relatively high incidence of postoperative complications. A mininvasive surgical approach, such as arachnoid-sparing duraplasty or dura-splitting procedure, is advocated.

Key words: Arachnoid-sparing, Chiari I malformation, morbidity, tonsillar resection.

Introduction

Chiari I malformation (CIM), the downward herniation of cerebellar tonsils through the foramen magnum (FM), was firstly described by Chiari more than 120 years ago [1]. Pathologically, the obliteration at the FM will result in reducing compliance of the spinal cerebrospinal fluid (CSF) space and further leading to the formation of syringomyelia [2]. A single operative procedure, posterior fossa decompression (PFD), has been widely accepted as the mainstay of therapeutic modality in the management of CIM. However, additional manipulations, such as tonsillar resection, are still under debate [3, 4]. Many neurosurgeons performed this procedure for several reasons: 1) histologically, the cerebellar tonsils are abnormal secondary to injury and ischemia in a great majority of CIM patients; 2) tonsillar reduction can further enlarge the total volume of the posterior fossa on the basis of PFD; 3) they can also dissect arachnoidal adhesions and explore the patency of the foramen of Magendie. However, tonsillectomy may also associate with a relatively high morbidity [5]. Due to the conflicting and surgeon's preference, it is necessary to evaluate the surgical efficacy and morbidity in CIM patients with or without tonsillectomy.

Patients and Methods

One hundred and twelve consecutive patients (59 males and 63 females) with a mean age of 40 years ranging between 19 and 59 years had surgical treatment for symptomatic CIM over a 3-year period between January 2009 and September 2011. Patients with hydrocephalus, atlanto-axial instability, a history of meningitis, or history of pervious PFD or shut surgery were excluded. The mean interval from the onset of symptoms to surgery was 4 years with a range from 1 month to 30 years. All the patients had preoperative standard craniocervical MR imaging (MRI) that revealed the cerebellar tonsils were at least 5mm below the FM. Clinical and radiological assessments preoperatively mainly considered the duration of symptoms, neurological features, the cyst extension and occupation, the tonsillar herniation evaluation, and other anomalies. After surgery, complications and neurological findings in the latest follow-up were also assessed. Patients were routinely imaged with MRI approximately 3-6 months postoperatively.

118 operations were performed in these 112 symptomatic patients. Once general anesthesia was administered, they were operated in the left lateral position as previously described [4]. A 3~4cm midline incision extending from the inferior portion of the occiput up to the upper cervical spine was used. Suboccipital craniectomy and C1 laminectomy were performed in all cases. Additionally, the C2 laminectomy was performed in selected cases depending on the extent of tonsillar descent. After bone decompression, a thick fibrous band compressing the dura at the level of foramen magnum was usually found and then removed. Under microscopic amplification, dural opening in Y format, and dissection of arachnoidal adhesions between the cerebellar tonsils, medulla and spinal cord were also performed. Elongated cerebellar tonsils were reduced by intrapial aspiration. After tonsillar reduction, exploration of the patency of the foramen of Magendie was usually performed. In nine cases, additional syringostomy was performed. A dural grafting was performed with the use of artificial dura to enlarge the posterior fossa and reconstruct a spacious cistern magna. To prevent CSF leak, the dura and the patch were sealed utilizing fibrin glue. All of the surgeries were conducted by the senior author (S.Q.H) at our department with the same standard surgical technique.

Statistical analysis was performed using chi-square tests to find a correlation between different variables and the outcome according to the Bidzinski Outcome Scale (very good outcome or good outcome, poor outcome) at latest follow-up. The correlation between the duration of symptoms as well as the age onset of CIM and the clinical outcome was also elevated by regression analysis. P values of less than 0.05 were considered significant. Analyses of data were performed using SPSS version 16.0.

Results

The main preoperative clinical manifestations and postoperative status of the 112 patients in this study are detailed in Table 1 and Figure 1. The frequent symptoms were pain (cervicalgia, headache and radiculalgia) and sensory disturbance, which were presented in 82 (73.2%) and 80 (71.4%) patients, respectively. Motor weakness (50.0%), and especially muscular atrophy (14.3%), seems to be less improved or resolved in long-term follow-up than pain (92.7%) and sensory disturbance (73.8%) in our series. 7 patients (6.3%) had scoliosis and all of them were presented in childhood, and only 2 of them underwent corrective surgery showed improvement of this condition whereas the remaining patients were stable. Infrequently, the clinical presentation of an asymptomatic patient in our group was precipitated by minor trauma such as a cerebral concussion.

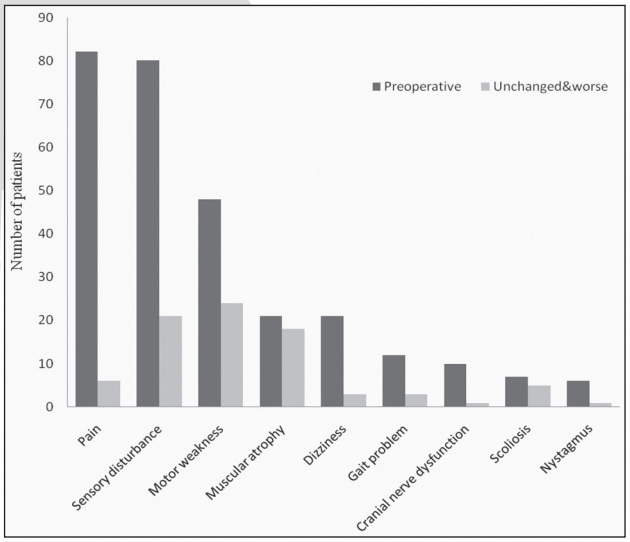

Figure 1. The main preoperative clinical manifestations and postoperative status

Table 1. Preoperative clinical manifestations and their postoperative status in a series of 112 patients with CIM

Clinical manifestations	No. of Patients (%)				
	At presentation	Resolved	Follow-up		Worse
			Improved	Unchanged	
Pain	82(73.2%)	45	31	4	2
Sensory disturbance	80(71.4%)	21	38	11	10
Motor weakness	48(42.9%)	10	14	21	3
Muscular atrophy	21(18.8%)	2	1	17	1
Dizziness	21(18.8%)	12	6	2	1
Gait problem	12(10.7%)	3	6	1	2
Cranial nerve dysfunction	10(8.9%)	2	7	1	-
Scoliosis	7(6.3%)	-	2	5	-
Nystagmus	6(5.4%)	4	1	1	-
Diplopia	2(1.8%)	-	1	1	-
Tinnitus	2(1.8%)	-	-	2	-
Vomiting	1(0.9%)	1	-	-	-
Hiccups	1(0.9%)	1	-	-	-

MRI of the craniocervical junction revealed the presence of a syrinx in 98 patients (87.5%). Degree of tonsillar herniation varied from FM-C1 in 65 (58.0%), C1-C2 in 41 (36.6%), and below C2 in 6 patients (5.4%). Syringomyelia was demonstrated in the cervical spinal cord in 18 patients (16.1%) and a cervico-dorsal extension in 80 cases (71.4%). Degree of maximal axial medullary occupation of cyst more than 75% was presented in 78 patients (69.6%) preoperatively and in 17 (15.2%) postoperatively. Other craniocervical junction anomalies such as occipitalized atlas in 26 patients (23.2%), basilar invagination in 17 cases (15.2%), and Klippel-Feil syndrome in 5 patients (4.5%) were also disclosed.

At a mean follow-up of 26 months (range 12–42 months), there was no surgical mortality in our series. Clinical results were evaluated according to Bidzinski's Outcome Scale [6]. Very good results were achieved in 64 patients (57.1%); good results were achieved in 35 patients (31.3%); and thirteen patients (11.6%) showed poor results. In our group, we have found that the removal of the posterior elements of C1 and C2 in partial cases did not cause instability.

Statistical analysis of the clinical progress, using the Bidzinski Outcome Scale, showed that the preoperative degree of the tonsils' downward displacement no more than C1 ($\chi 2$ P=0.142), the level of maximal axial medullary occupation of cyst less than 75% ($\chi 2$ p = 0.468), and without syrinx ($\chi 2$ P=0.931) were not correlated with better progress. However, there was significant difference in the clinical outcome among patients grouped by the extent of the cyst on postoperative MRI ($\chi 2$ P=0.049). The presence of basilar invagination was associated with poor clinical outcome ($\chi 2$ P=0.038). Longer duration of symptoms prior to surgery was associated with poor results according to the Bidzinski Outcome Scale (Regression P=0.021). It seems that young age at the time of surgery has a better outcome than elderly, while there was no significant difference in our series (Regression P=0.998).

Postoperative complications are detailed in Table 2. Five patients (4.46%) underwent reoperation after the first surgery (range, 1 day to 6 months). Of the three severely affected patients, one underwent extraction of infected artificial dura because of intractable graft-related meningitis and further resolved with the administration of antibiotics for 1 month duration. Another patient, with serious brainstem compression because of acute hydrocephalus and severe cerebellar swelling, has resolved by trepanation and drainage, and PFD with partial cerebellum resection. A third patient, with acute cerebellar hematoma and further deteriorated with acute hydrocephalus underwent evacuation of hematoma and trepanation and drainage.

Table 2. Complications after PDF with tonsillectomy and duraplasty

Complications	No. of patients	Evolution
Increase in pain	3(2.68%)	All patients improved
Increase in limb deficit	4(3.57%)	1 returned to preoperative status
Cranial nerve impairment	5(4.46%)	3 patients returned to normal
Bladder dysfunction	1(0.89%)	Persistent
Ataxia	1(0.89%)	Persistent
Isolated fever	10(8.93%)	No consequence
Bacterial meningitis	6(5.36%)	1 patient had surgery
Aseptic meningitis	7(6.25%)	No consequence
Headache	28(25.0%)	No consequence
Vomiting	7(6.25%)	No consequence
Dizziness	7(6.25%)	3 patients persist
CSF leak	9(8.04%)	3 patients had bacterial meningitis
Wound infection	1(0.89%)	Antibiotic, no consequence
Pseudomeningocele	21(18.8%)	No consequence
Hydrocephalus	4(3.57%)	All patients had surgery
Cerebellar swelling	5(4.46%)	1 patient had surgery
Hiccups	3(2.68%)	All patients improved but 2 persist
Cerebellar hematoma	1(0.89%)	Surgery

With the remaining 2 patients, all of them had V-P shuts because of chronic hydrocephalus, and one patient had a high cervical syringostomy because of the progression of symptoms and exacerbation of syringomyelia despite correct decompression.

Discussion

It is widely believed that the underdevelopment of the mesodermal occipital somite is responsible for the small volume of the posterior fossa and other craniocervical junction anomalies such as occipitalized atlas, and thus for the abnormal CSF flow at the level of FM in patients with CIM [5]. However, the exact pathogenesis of Chiari-related syringomyelia is still unknown, though efforts have been made by Gardner [7], Williams [8], Ball and Dayan [9], Oldfield [10], and Greitz [11]. A new hypothesis, reduced compliance of the spinal CSF compartment which caused by the obliteration of FM will result in the dysfunction of extracellular fluid absorption and further the formation of syringomyelia, offers a better understanding of the clinical or radiological features of the pathogenesis [2]. Recently, computational 3D and magnetic resonance 4D flow analysis confirmed the alterations of CSF hydrodynamics in FM and the reduction of compliance of the spinal CSF spaces [12, 13].

Nowadays, most authors agree that the manifestations unequivocally attributable to CIM should be the indication for decompression surgery. Surgery should be proposed as soon as possible especially in patients with progressing clinical features, because longer duration of symptoms prior to surgery is associated with worse results and sometimes neurological deficits, especially motor weakness and muscular atrophy, are irreversible after structural damage to the spinal cord [5, 14, 15]. Our results demonstrated that the degree of the tonsillar descent, the cyst occupation and extension preoperatively were not correlated statistically with postoperative outcome. These results are in agreement with other reports, implying that we cannot predict the postoperative status of patients only from the MR findings [4, 16]. The interval between onset of symptoms and surgery is an important predictor of outcome, which has been also confirmed by our study [16]. The presence of basilar invagination was associated statistically with postoperative outcome, which may interpreted as the co-occurrence of osseous malformation together further exacerbates the overcrowding of the posterior cranial fossa and the anterior compression may still persist after PFD [17].

Traditionally, surgical treatment of CM-I includes suboccipital craniectomy, cervical lami-

nectomy, lysis of arachnoid adherences, tonsillar reduction, and duraplasty [18]. In light of mininvasive surgery, dura-splitting procedure is favored by more and more neurosurgeons because of well tolerated, less operative and operating room times, without exposure to the risk of CSF-related operative complications, and excellent clinical outcome [19]. Regarding the most appropriate surgical treatment, however, it remains an open subject of debate for lacking of general consensus [1, 3, 4]. Hoffman and Souweidane compared the clinical outcome and CSF-related morbidity between arachnoid-sparing duraplasty and dura-splitting procedures by literature review, and concluded that the two approaches have nearly equal clinical response (duraplasty 82.2% VS dura-splitting 84.1%) and the morbidity is negligible in patients underwent duraplasty [3]. The clinical effect and morbidity between PFD with duraplasty as well as tonsillectomy, however, have not been well-documented in the literature [4, 14, 17, 20, 21].

Comparing the efficacy of tonsillectomy with duraplasty procedures (82.9%) and arachnoid-sparing duraplasty (82.2%) in CIM demonstrates a comparable clinical response with both surgeries (Table 3). Multiple authors reported clinical improvement in greater than 80% of patients in both procedures [3, 4, 5, 16, 19, 21]. Thus, surgical morbidity seems to be the primary difference in the two procedures. The morbidity rates of arachnoid-sparing duraplasty range from 0 to 48% (average 12.4%) [3]. In comparison, the morbidity associated with tonsillectomy is higher ranging from 0 to 42.8% (average 20.4%) [4, 5, 14, 17, 21]. It is well known that arachnoid opening predisposes to CSF-related complications, including CSF leakage, meningitis, pseudomeningocele, and postoperative hydrocephalus. Tonsillectomy with duraplasty have not only CSF-related complications, but also tonsillectomy-related complications, such as cerebellar swelling, cerebellar hemotoma, blood contained CSF-related progressive headache, cranial nerve dysfunction, and transient postoperative apnea [4, 5].

Guyotat et al. reported a complication rate of 0% in eight patients, whereas Navarro et al. reported 42.8% morbidity in 14 patients with tonsillar resection [5, 21]. This range of observed complications with tonsillar manipulations could be the result of the small sample size, which limits the clinical and statistical significance of their results. Though many authors performed tonsillar resection, it difficult to compare their results: 1) the authors analyzed different surgical procedures together and did not report the surgical procedures and clinical results in detail [20]; 2) a unified standard to elevate the clinical outcomes is still lacked [22]. However, all the patients in our series underwent uniformly PFD with tonsillectomy and duraplasty, excepting additional syringostomy was performed in nine patients. Furthermore, a large cohort of 112 patients overcomes the shortcoming of small sample size. At last, all the surgeries were conducted by the senior author (S.Q.H) who has performed the same procedures in more than 70 patients before [4]. Pseudomeningocele (18.8%) and CSF leakage (8.04%) are relatively high in our series. Only utilizing fibrin glue to seal the dural patch maybe the underlying reasons, and tighten three running sutures is advocated [23]. Life-threatening complications, acute hydrocephalus

Table 3. Clinical outcome and morbidity in CIM patients treated with tonsillectomy and duraplasty

Series (ref. no.)	No. of patients	Symptom resolution, % (no.)	Complications, % (no.)
Fischer *et al*., 1995 (21)	18	72.2 (13/18)	11.1 (2/18), 1 aseptic meningitis, 1 tinnitus and mild hearing loss
Guyotat *et al*., 1998 (29)	8	100 (8/8)	0 (0/8)
Arruda *et al*., 2004 (24)	60	91.7 (55/60)	30 (18/60), 14 pseudomeningoceles, 4 meningitis
Navarro *et al*., 2004 (9)	14	60.8 (9/14)	42.8 (6/14), 2 aseptic meningitis, 2 hydrocephalus, 2 pseudomeningoceles, 1 transient postoperative apnea, 1 cerebellar swelling, 1 bacterial meningitis
Ma *et al*., 2012 (6)	76	80.3 (61/76)	13.2 (10/76), 6 progressive headache, 3 bacterial meningitis, 1 CSF leak
Total	176	82.9(146/176)	20.4 (36/176)

and severe cerebellar swelling compressed the brainstem as well as cerebellar hematoma, have also presented in our group [5, 22]. Klekamp et al. reported a series of CIM patients underwent aggressive intrapial manipulation, and one of them died of apnea on the second postoperative day [20]. Cerebellar infraction, mesencephalic dysfunction, and swallowing dysfunction were also noticed, which were most likely the compromise of the small perforating arteries during tonsillectomy and arachnoid dissection.

In comparison with intrapial manipulations, the extra-arachnoidal approach is safer, especially without opening the inner layer of dura [16]. With the help of the microscope and intraoperative ultrasonography, dura-splitting can be achieved with sufficient decompression [16, 19]. Furthermore, superficial infections represent all complications of this procedure with the average complication rate only 6% in literature review [3, 16]. However, a prospective randomized study is still lacked to compare these techniques in a uniform patient population [3, 16, 19]. In comparison with such promising mininvasive procedure, tonsillar management could be kept as a secondary option in recurrent patients and the rare cases in whom CSF circulation at the craniocervical junction is considered to remain insufficient in mininvasive surgery [18, 23].

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Relationship between heart rate and one-back task response time in children with Attention Deficit Hyperactivity Disorder

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Abstract

This study investigated the relationship between heart rate (HR) and one-back task response time in children with Attention Deficit Hyperactivity Disorder (ADHD). Thirty six boys (mean age 11.8±1.5 years) who had been diagnosed as ADHD and were being treated at the time of the study participated. The experiment consisted of three phases performed over a total of 5 min: Rest phase (1 min), Control phase (2 min), and the one-back task phase (2 min). HR was measured during each phase. The HR in the one-back task phase increased significantly compared to the Rest phase. There was a significant negative correlation between the response time of one-back task and magnitude of the HR in the one-back task phase. As HR increased, response time of the one-back task decreased. When sufficient oxygen required for cognitive processing was supplied by increased HR, the speed of information processing increased. The results of the present and a previous study indicate that the administration of high oxygen concentration can positively affect the cognitive performance of children with ADHD.

Key words: Heart rate, response time, one-back task, ADHD children.

Introduction

The administration of highly concentrated oxygen positively affects cognitive processing for healthy young adults (1-10). Highly concentrated oxygen enhances cognitive performance, including memory (5, 7, 8, 10), visuospatial (3, 6), verbal (1), addition (4), and n-back tasks (2). Highly concentrated oxygen induced improvement of cognitive abilities by reducing response time (5-10) and/or increasing the percentage of correct answers (1-8). Numerous studies also have been undertaken to support the effects of highly concentrated oxygen on cognitive performance of healthy young adults. Studies using functional magnetic resonance imaging (fMRI) showed that administration of highly concentrated oxygen increased the amount of brain activation due to the increase of oxygen supply to the brain area that is closely related with cognitive processing, leading to an increase in cognitive performance (11, 12). Cognitive ability has been positively correlated with the absolute values of heart rate (HR) and/or blood oxygen saturation (SpO_2) in healthy young adults (1, 4, 5, 8, 13).

External oxygen administration also positively influences visual matching task performance of children with Attention Deficit Hyperactivity Disorder (ADHD) who have cognitive problems (14). In the study, there was a significant increase in cognitive performance in the presence of hyperoxic air (92% oxygen) compared with the normal air (21% oxygen) condition. When 92% oxygen in the air was supplied, the SpO_2 increased compared to that under the 21% oxygen condition. Thus, increased SpO_2 may have a positive effect on the cognitive ability of ADHD children (14).

Many studies with a variety of cognitive tasks have been performed using various verification methods to define the effect of highly concentrated oxygen on the cognitive ability of normal healthy people (1-13). However, the effect of highly concentrated oxygen administration on the ADHD children has only been reported in a single study (14). Further studies using a variety of verification methods should be done to exactly examine the positive effect of highly concentrated oxygen on patients with cognitive problems, such as ADHD children.

This study tried to obtain a basis to explain the effects of highly concentrated oxygen on the cognitive ability of ADHD children. The relationship between HR and cognitive ability was investigated under a normal air condition in the absence of the supply of highly concentration oxygen. If we have obtained increasing in cognitive ability as increased HR, this result supports the positive effects of highly concentrated oxygen on the cognitive ability of the ADHD children.

Methods

Subjects were 36 boys (mean age, 11.8±1.5 years) diagnosed as ADHD by a psychiatrist at an average age of 9.4±1.8 years and who were under medication at the time of the study. They had no cardiac and respiratory disease and abnormalities. Before any experiments, parental consent was obtained following a detailed and clear explanation of the study objectives and experimental procedures. All experiments were performed under the regulations and with the approval of our Institutional Review Committee.

The experiment consisted of three phases for a total of 5 min (Figure 1): Rest phase (1 min), Control phase (2 min) that had a stabilization period before starting the final phase, and one-back task phase (2 min). The one-back task which relates to working memory task with a low level of difficulty was used as a cognitive task for ADHD children. During one-back task phase, 40 numbers were presented at 3-sec intervals. If the same number was repeated, the subject was asked to press the response button as quickly as possible. Among them the number of correct answers was eight. The one-back tasks were presented using E-prime (Psychology Software Tools, USA). To measure response time and accuracy of one-back tasks using E-prime, response button was set to the space bar of the keyboard. The response time and accuracy rate (number of correct answers/total number of problem × 100) on the one-back test for participants were calculated.

HR was measured in beats per min (bpm) for all phases using a 8600 series pulse oximeter (NONIN Medical, USA) on the subject's left index finger. The mean of HR for each subject were calculated for each phase. Repeated measures ANOVA (PASW ver. 18.0) was used with the three aforementioned phases as independent variables to verify a significant difference in HR according to phases. To investigate the relationship between HR and cognitive ability, Pearson correlations were calculated for the relationship between response time and accuracy of the one-back task and the magnitude of the HR in the one-back task phase.

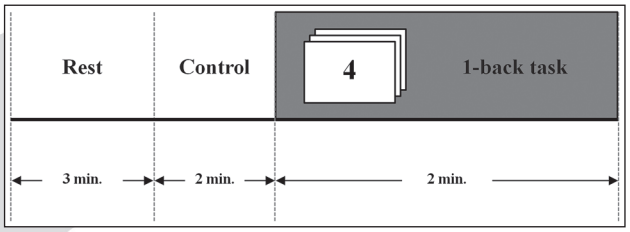

Figure 1. Diagram of the experimental procedure

Results

The mean accuracy rate and response time were 98.13±3.07% and 785.30±259.52 ms, respectively. The mean HR of each phase was 90.92±14.25 bpm in the Rest phase, 92.92±13.61 bpm in the Control phase, and 93.64±15.25 bpm in the one-back phase. There difference in HR in the three phases was significant ($p<.001$). Bonferroni's posteriori tests revealed that the HR in one-back task phase ($p<.01$) and in Control phase ($p<.01$) increased significantly, compared to that in Rest phase (Figure 2). The response time showed a negative correlation with HR ($r=-.516$, $R^2=0.266$, $p=.001$), however, the accuracy rate showed no correlation with HR ($p>.05$) (Figure 3).

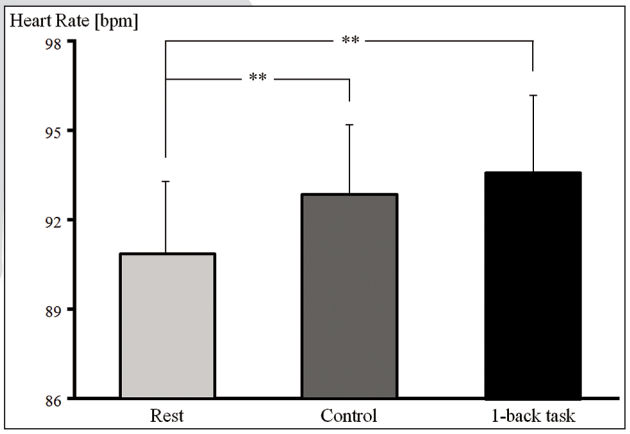

Figure 2. HR in the three phases

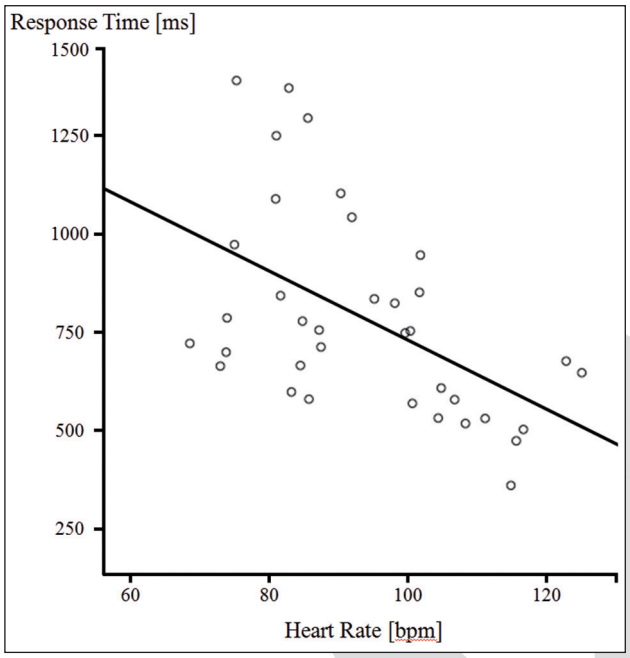

Figure 3. Correlation between response time and HR in the one-back task phase

Discussion

In this study, the correlation between one-back task performance and HR was observed for ADHD children. HR increased in one-back task phase compare to Rest phase. There was a significant negative correlation between response time of one-back task and magnitude of the HR in the one-back task phase.

HR during the cognitive performance phases increases compared with that during the rest phase (1-5). As shown in previous studies, this study also showed that HR increased in the one-back task phase compared to the Rest phase. It is well understood that an increase in fuel (e.g., glucose) supply leads to an upgrade in adenosine triphosphate (ATP) production at times of high demand. Its increased production may enable improvements to be made in information processing during the performance of cognitive tasks. In order to metabolize the fuel, the brain needs more oxygen (8). Therefore, oxygen demand increases during cognitive processing period and this induce an increase in HR (1-5).

Normal young adults with a high HR show better cognitive performance. The response time decreases for the subjects with a high HR during the task phase (5, 8). This study also showed that the response time had a negative correlation with HR, which is consistent with previous studies for normal young adults. This means that increasing HR increases the supply of oxygen for cognitive processing and this induces improved cognitive performance for children with ADHD. This result can explain the positive effects of highly concentrated oxygen on the cognitive performance change of ADHD children. However, since the accuracy rate (98.13±3.07%) was too high, which means the discrimination power was low, there was no correlation between the accuracy rate and HR.

In conclusion, as HR increases, response time of one-back task decreases for ADHD children. When the amount of oxygen required for cognitive processing is supplied by an increased HR, the speed of information processing increases. The results of this study support the previous finding (14) that the administration of high oxygen concentration can positively affect the cognitive performance of ADHD children.

Further studies need to be performed to examine the effect of hyperoxic air on the cognitive processing of the ADHD children using a correlation analysis between SpO_2 and cognitive performance and performing a brain analysis based on fMRI. This evidence will confirm the positive effects of hyperoxic air on cognitive performance of ADHD children, and may well prove beneficial for treating patients with cognitive problems, such as ADHD children.

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Patient satisfaction for nursing care in a University hospital in Turkey

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Abstract

Aim: The aim of the study is to define patient satisfaction for nursing care and related descriptive features.

Methods: The research has been conducted in a university hospital. 281 patients stayed for 3 or more days in the hospital mentioned above and decided to be discharged, which created the subject of the research. After getting the necessary permission from the hospital administration, the data were collected with the help of a questionnaire which included two instruments. The socio-demographic data form included 13 questions and "Scale of Satisfaction for Nursing Care" (SSNC). SSNC was developed by Demir and Eşer in 2004, and validity and reliability analysis were carried out by the researchers. Permission was granted for this instrument to be used in this research. Cronbach Alpha coefficient of the scale was 0.97. The data of the study were evaluated by percent distributions, mean score, analyzes of variance and t test.

Results: Among all participants in the study, the significant age was found as 47.64±20.92. The 50.9% of the patients were female, 71.2% were married. 42.3% of them lived in metropolis. It was determined that 49.5% of the patients stayed for 3-7 days in hospital.

Conclusion: According to the results, mean score of satisfaction was found as 142.20. It is from this data that the patients were satisfied for this population. Mean differences were detected between satisfaction scale point, marital status and having an attendant.

Key words: Patient, nursing services, patient satisfaction, nursing care and quality.

Introduction

Patient satisfaction has become a critical point in the past two decades by which the quality of health care service is evaluated (1,2). When the quality of the health services is in question, patient satisfaction constitutes one of the basic steps (3,4,5). Patient satisfaction is a multidimensional concept which includes the interaction between patient and caregiver, presence of the caregiver, offering and continuity of care, the caregiver's competence and characteristics of communication (6,7).

In studies examining the satisfaction of individuals who receive health care the evaluation of health care offered from patients' viewpoints has revealed the weak and the strong sides of care, and patient satisfaction has become increasingly important as a concept which has a part in the formation of strategies to improve health care (8).

In this direction according to Johansson, Ole´ni and Fridlund (2005) there is a strong correlation between patients' perception of nursing care and their perception of the quality of health care (9).

It is commonly acknowledged that patients' reports of their health and quality of life, and their satisfaction with the quality of care and services, are as important as many clinical health measures (10).

Patients and nurses, as well as their interactions, are situated within the health care system. Therefore, patients perceive themselves, as well as nurses, to be both influenced by, and to have to conform to the limitations of the system. Significant attributes of the system, such as the organisation of care, medical care, the institution facilities and the amenities provided are important determinants of patients' experience. Additionally, these factors have a perceived effect on nurses, as well. The quality of technical aspects of care, although important, may be taken for granted. Conversely, the interpersonal contact with nurses is central to patients' experience; therefore, the latter is a crucial determinant of the overall experience of care. Nurse-patient contacts are often emotionally charged experiences for patients. Humane and close

nurse-patient relations are highly valued by patients and are emotionally moving experiences. On the other hand, failure of interpersonal contact or inappropriate contact is judged sternly. The ensuing satisfaction with care consists of 3 overlapping components: satisfaction with the healthcare system, satisfaction with the interpersonal aspects of nursing care, and satisfaction with the technical aspects of care. The element of satisfaction with the interpersonal aspects of nursing care is paramount and may determine the nature of the overall experience (11).

Especially in patient treatment institutions, nurses are the personnel group that the patients mostly have interaction in treatment process. Nurses maintain the communication between the patients and other health staff, behaves as defender of the patients, give physical and emotional care to the patients, and maintain emotional support to the patients and their families. That's why behavior of the nurses have important effect in patient treatment. In studies done, it was shown that there was a strong relation between patient's satisfaction with nursing care and general patient satisfaction with the hospital services. The most important factor affecting the patient satisfaction in hospital care is the patients' satisfaction with nursing care. It is important to define the patients' satisfaction with the nursing care in order to help inpatients more and enhance the quality of patient care (4, 12).

The aim of the study is to define patient satisfaction for nursing care and related descriptive features.

Materials and Methods

The study was carried out in a university hospital with 2000 bed capacity where 1076 nurses are employed and located in Izmir, the 3rd biggest province of Turkey. 281 patients stayed 3 or more days in above mentioned hospital and decided to be discharged which created the subject of the research. After getting the needed permission from hospital administration, the data which was collected by a questionnaire included two instruments. The socio-demographic data form included 13 questions and "Scale of Satisfaction for Nursing Care" (SSNC). SSNC was developed by Demir and Eşer in 2004, and validity and reliability analysis were carried out by the researchers (12). The scale consists of 34 items and a single dimension, and uses a 5-point scale to indicate the degree of satisfaction. The available lowest point was 34, and the highest point was 170 in the scale. The more the point rose, the more the level of satisfaction rose. Demir and Eşer (2004) determined Cronbach Alfa as 0.96 for the scale. Item analysis was used for the validity of SSNC and item-correlation co-efficiency of 34 items were found between 0.71 and 0.94. The reliability was determined by Cronbach Alfa as (0.97), Split half as (0.94), Spearman Brown as (0.94) in statistical testing. Followed from this data, the scale was valid and reliable for this population.

Ethical Consideration

The data were gathered by the researchers after written official permissions to undertake this study was gained from the ethics committee at School of Nursing of Ege University. Informed consent was obtained from each participant and prior to this study; the patients were informed of the purpose of the research. Permission was granted for this instrument to be used in this research.

Statistical Analysis

The SPSS 15.0 program was used in the evaluation of data analysis. The data of the study was evaluated by percent distributions, mean score, analysis of variance and t test. $p<.05$ was taken as the threshold level for statistical significance

Results

Respondent Profile

Range of patients according to their introductory characteristics was shown in Table 1. It was determined that 50.9% of the patients were female, 49.1% of them were male, the age average was 47.64, 71.2% of them were married, almost half of them have equal income and outcome, half of them have less income than outcome. When the patients' educational statuses were analyzed, it was determined that 39.9% of them were primary school graduates, 33.5% of them were housewives, 19.2% of them were self-employed, and 42.3% of them are living in metropolis at the moment.

Table 1. Distribution of patients according to descriptive features

Descriptive features	Number	%
Age Groups		
18-34	86	30.2
35-54	83	29.5
Over 55	112	39.9
	x= 47.64± 20.92	
Gender		
Female	143	50.9
Male	138	49.1
Educational Status		
Illiterate- Literate	56	19.9
Primary -Secondary School	139	49.5
High School - Faculty	86	30.6
Marital Status		
Married	200	71.2
Single	81	28.8
Occupation		
Clerk	44	15.7
Worker	30	10.7
Self-employed	54	19.2
Retired	50	17.8
Housewife	94	33.5
Student	9	3.2
Level Of Income		
Income is equal to expenditure	130	46.3
Income is less than expenditure	122	43.4
Income is more than	29	10.3
Place Of Residence		
Metropolis	119	42.4
City	32	11.4
Town	83	29.5
Village	47	16.7
Total	**281**	**100**

It was determined that 49.5% of the patients in the scope of the research stayed in the hospital for 3-7 days, 67.6% of them had stayed in a hospital before, 32.6 % of them had stayed in a hospital once, 41.1% of them had stayed in a hospital twice or three times, 78.6% of them had had attendants (Table 2).

Table 2. Range of patients according to staying in hospital and having attendants

Characteristics	Number	%
Period of Staying in Hospital		
3-7 days	139	49.5
8-14 days	80	28.5
15 days and over	62	22.1
Situation of Staying in a Hospital		
Yes	190	67.6
No	91	32.4
Period of Staying in a Hospital Before		
Once	62	32.6
2-3	79	41.1
4 and over	50	26.3
Situation of Having a Hospital Attendant		
Having an Attendant	221	78.6
Having no Attendant	60	21.4
Total	**281**	**100**

The total point mean of the answers given by the patients about their satisfactions with the nursing services was determined as 142.20 (Table 3). It was determined that while there wasn't a meaningful difference between the patients' points in terms of their age, sex, and level of education, there was a difference between the patients' point averages in terms of their marital status, and single patients had significantly high point averages in comparison to married ones. It was determined that there was no difference between satisfaction point averages in terms of staying in hospital before and days of staying in hospital, but there was a statistical difference in terms of having an attendant, and the patients who had attendants, had meaningfully high point averages (Table 4).

Discussion

The total point mean of the answers given by the patients about their satisfaction with the nursing services was determined as 142.20. When it is thought that the lowest available point is 34 and highest available point is 170, this result means that the patients' satisfaction levels are high. It was determined that total point mean of all the items about the satisfaction with nursing applications in the scale change between 3.63 and 4.45. Accordingly, it was determined that the patients were "satisfied" and "very satisfied" with the nur-

Table 3. Total point mean of the scale of satisfaction for nursing care (SSNC)

	Min	Max	Mean	Sd
Patients' Satisfactions with Nursing Care	50	169	142.20	20.50

Table 4. Distribution of Total Point Mean of SSNC According to Some Descriptive Features

Descriptive Features	N	Mean (SD)	F/t	p
Age Groups				
18-34	86	144.22±20.18	F=1.678	p>0.05
35-54	83	141.33±23.50		
Over 55	112	141.50±16.86		
Gender				
Female	143	141.01±26.50	t= 0.94	p>0.05
Male	138	143.44±20.21		
Educational Status				
Illiterate- Literate	56	136.46±21.52	F= 1.843	p>0.05
Primary -Secondary School	139	141.91±20.27		
High School/Faculty	86	142.02±19.31		
Marital Status				
Married	200	141.04±19.92	t=1.486	p<0.05
Single	81	145.04±21.74		
Period of Staying in Hospital				
3-7 days	139	141.21±21.45	F= 1.67	p>0.05
8-14 days	80	140.32±18.59		
15 days and over	62	140.12±14.10		
Condition of Having a Hospital Attendant				
Having an Attendant	221	146.04±21.26	t= 1.685	p<0.05
Having no Attendant	60	141.06±17.60		

sing care. Accordingly with our results, high satisfaction levels were found also in studies that had been done about patients' satisfaction with nursing care (12,13,14,15). Patients expressed overall satisfaction with the nursing care (16).

It can be said that the items having low-point averages are mostly the items about "giving information". Giving information also increases the level of effective service given in according to patient's satisfaction. Patients' and their relatives' information being recorded by the health staff make them understandingly comprehended in their illness cases. Giving information is one of the basic patients' rights at the same time. Enlightening the patients and their relatives about the details of the service will increase the satisfaction and the effect of service (3,4). According to the literature, it is determined that socio-demographical characteristics such as the individual's previous experiences, age, sex, level of education, social status (occupation) have effect on patient satisfaction. In our study, while there were no meaningful differences between the patients' points in terms of their age, sex and level of education. A relationship between educational level and level of satisfaction has not been observed. The results of our study support the results of the study by Skarstein et al. (2002) and Can et al. (2008) (8,17).

Webb, Bower and Gill (1997) found that all demographic variables and patient satisfaction with nursing care revealed no significant relationships (18). Dulgerler, Ertem, Ozer (2012) also found that there was no significant relation between education and total satisfaction. However it was determined that there was a difference between the patients' point averages in terms of the marital status, and single patients had meaningfully high point averages when compared to married ones. It was determined that there was no difference between the satisfaction point averages according to their staying in hospital or days of staying in hospital, according to Dulgerler, Ertem, Ozer (2012). There was signifi-

cant relation between patient whose hospital stay and total satisfaction (19). In our study there was a statistically difference in terms of having an attendant or not, and the patients who had attendants had meaningfully high satisfaction point averages.

In some studies about patient satisfaction, it was determined that old patients were more satisfied in accordance with the young patients (13,20,21,22). Okumuş et al (1993), Özmen (1990), and Çelik (1999) determined that there was no relationship between satisfaction level and patients sexes in the studies they had done (15,23,24). But however, according to Yilmaz (2001) and Can et al. (2008) women were more satisfied than men in terms of the services given in Lookinland and Pool's studies (8,25). At the same time, it was determined that there was a relationship between the patients' sexes and satisfaction levels in the study which was done by Bal et al (2002) in order to determine the patients' satisfaction levels (26).

A lot of studies carried on about this subject showed that the more the education level increased, the more the satisfaction level decreased. But there are studies showing that there is no relationship between these two variables. While it was determined that in the studies done by Özmen (1990), Carr-Hill (1992), and Bengtsson et al (1998), the patients who had high level of education were less satisfied with the services they had, it was determined that in the study done by Yilmaz (2000), the patients who had higher level of education were more satisfied with the services they had (15,21,20,25). Furthermore, Uzun (2003), in her study, stated that there was no relationship between the patients' satisfaction levels and sexes, and besides, there was a meaningful relationship between the education levels and satisfaction levels of the participants (27).

Özbaşaran (1999), in her study that she investigated the satisfaction of inpatients, stated that there was no relationship between the patients' introductory characteristics such as sex, age, level of education, occupation, the clinic staying in, and the period of staying in hospital and the satisfaction with the nursing service (14).

As can be seen by scanning the literature, in the studies done, while it was proposed that some variables can be related to satisfaction, it was argued that some variables can never be related to satisfaction. But there is no parallelism between the results of the studies. These factors may have a determinative role in patient expectations. It is important to succeed in giving the ultimate and people oriented qualified service (28).

Conclusion

As a conclusion, patients are satisfied with nursing services in high levels. It was determined that while there wasn't a meaningful difference between the patients' points in terms of their age, sex, and level of education, there was a difference between the patients' point averages in terms of their marital status, and single patients had meaningfully high point averages in comparison with married ones. It was determined that there was no difference between satisfaction point averages in terms of staying in hospital before and days of staying in hospital, but there was a statistical difference in terms of having attendant, and the patients who had attendants had meaningfully high point averages.

According to the study results, it is suggested that the patients' state of mind effecting their adaptation to treatment should be considered as well as doing the concrete processes about patient care. In in-service training programs that will be prepared as nursing-oriented subjects about nurses' independent functions such as patient training and giving information, and subjects about patient-nurse relationship should be placed. In order to constitute the patient care standards and to enhance the patients' satisfaction with the nursing services, patient satisfaction should be measured periodically and improvement precautions should be taken based on these data.

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Etiology of hirsute in women at reproductive age

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Abstract

The aim of study is evaluation of most frequent causes of hirsute-excessive hair growth on the body in women at reproductive age. Identification of the underlying etiologic detects patients at risk for infertility, diabetes, cardiovascular disease and endometrial carcinoma.

Patients and methods: In a prospective-retrospective, clinical and descriptive type of study hirsute women age between 20 and 40 years without any acute or chronically diseases were included. The study was conducted at Polyclinic and diagnostic department of Clinic for Endocrinology and Diabetes University Clinical Centre of Sarajevo and will include all female patients with hirsute diagnosed and treated in period between 01.01.2005. to 31.12.2011.

Methods: clinical evaluation and laboratory investigation with questionnaire. Statistical analysis: All analysed parameters statistically were performed in Microsoft Excel. In statistical analysis of data were used standard methods of descriptive statistic. Statistic hypothesis were test on level of significance α=0,05, and difference between parameters considered statistically significant is the $p<0,05$.

Results: With PCOS is 48% women, 45% women is with menstrual cycle disorders, 21% is with high level of serum prolactin, 71% is with hyperandrogenism and 54% with obesity. PCOS is the most common cause of hirsute at reproductive age women, in background of the same the most common cause is hyperinsulinemia or insulin resistance. Statistically significant correlation is between hirsute and menstrual cycle disorders and insulin resistance has important role in manifestation of hirsute at reproductive age women.

Conclusion: Hirsute at reproductive age between 20. and 40. years old women with menstrual cycle disorders need to be appropriate evaluated and treatment.

Key words: Aetiology, hirsute, reproductive age, PCOS.

Introduction

Hirsute (*hirsutus, l.*-hairy) is excessive of body hair at women. (1) Hirsute represent excessive of body hair at women on the place where usually body hair don't grow up known as the androgen dependent zones (upper lips, chicks, chins, breasts, chest, median line of stomach, upper part of legs), but that hairs are normally distributed at males like secondary sexual characteristics. Hirsute is clinically manifestation of hyperandrogenism which represent increase of value of some androgens hormones in blood circulation of women. (Table 1.)

Increase of androgens level in circulation is normal at women in period of menopauses between age 45 and 55 and in adolescent period which is called physiology hirsute. (2) At 75 % of women menopauses occur between age 45 and 55 and average at age of 50 years old women. Manifestation of hirsute at reproductive age of women between 20 and 40 years old with menstrual cycle's disorders needs to be adequate evaluated and treated.

Table 1. Causes of hirsute

From ovary	PCOS Tumors Arenoblastoma Hypertecosis
From adrenal glands	Cushing syndrome Tumours Adrenogenital syndrome Carcinoms Congenital adrenal hyperplasia
From hipophysis	Acromegaly Hiperprolactinemia Cushing disease
From thyroid gland	Hipothireosis
From pancreas	Hiperinzulinemia
Use of medicamention and stressful situation	Contraceptive pills, glucocorticoides (ACTH), androgens (testosteron), minoxidil, minociklin, diazoxid, fenitoin, ciclosporine, danazol, synthetic progestagens, valproic acids, psoralen, penicilamine, acetazolamide

Polycystic ovary syndrome (PCOS) which is multiply systemic, reproductive and metabolic disorder is one of the most common causes of hirsute which is characterised by oligo/anovulation, hyperandrogenism, polycystic ovaries, obesity and insulin resistance. (3)

Aim

1. Evaluation of causes for hirsute at women on reproductive age;
2. Prevalence of PCOS, hypertecosis, Cushing's syndrome, congenital adrenal hyperplasia, ovary's tumours, insulin resistance and idiopathic causes at women with hirsute;
3. Correlation between hirsute and menstrual cycle disorders;
4. Correlation between hirsute and insulin resistance;

Subjects and methods

In a prospective-retrospective, clinical and descriptive type of study hirsute women age between 20 and 40 years without any acute or chronically disease will be included. The study will be conducted at Polyclinic and diagnostic department of Clinic for Endocrinology and Diabetes University Clinical Centre of Sarajevo and will include all female patients with hirsute diagnosed and treated in period between 01.01.2005. to 31.12.2011. Mayor criteria for including female patients in study are: women at age from 20 to 40 with hirsute estimated with Ferriman-Galweys score; female patients without any acute or chronically disease. Mayor criteria for not including female patients in study are: female patients with age under 20 and older than 40 years with associated acute and chronically diseases.

Methods

Detailed medical history taking and physical examination of the patients will be carried out in all the cases. 1)

History

Generals points, age of onset, duration and rate of progression of disease, menstrual pattern (oligomenorrhoea, amenorrhoea), other signs of hirsute (acne, alopecia, symptoms of virilisation), medical drugs history. 2)

Physical examination

Age, weight, high, BMI, abdominal circumference, hirsute/Ferriman-Galweys score (FG-score ≥ 8); increased sebum production, oily hair and face, acne, alopecia. 3)

Laboratory's investigations

The levels of serum testosterone, dehydroepiandrosterone sulphate, androstendione, luteinizing hormone, follicle stimulating hormone, LH/FSH rate, prolactin, thyroid stimulating hormone, cholesterol, triglycerides, basal insulin, glucose, HOMA-IR. 4) Ovary echo sound characteristics.

Results

Analysis of body mass index (BMI) at patients with hirsute

Table 2. Analysis of body mass index (BMI) at patients with hirsute

BMI (kg/m²)	Level	N	%	SV	SD
>40	Extreme obesity	4	4	30,4	4,3
35-39,9	Very high obesity	4	4		
30-34,9	Middle obesity	46	46		
25-29,9	Obesity	42	42		
18,5-24,9	Normal weight	4	4		
<18,5	Under normal weight	0	0		

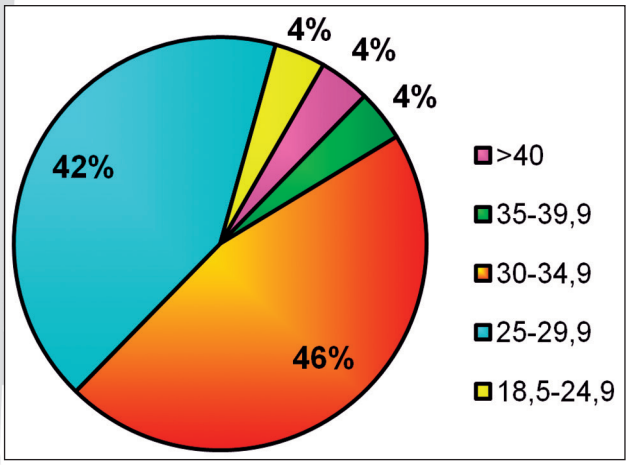

Figure 1. Distribution of patients at reproductive age with hirsute considered body mass index (BMI)

Laboratory investigations

Glucose and insulin blood levels

The average values of glucose and insulin blood levels measured during oral glucose tolerance test (OGTT) are shown in table 3. and 4.

Table 3. Analysis of glucose blood levels during OGTT at women with hirsute at reproductive age

glucose basal (normal 3,1-6,1 mmol/l)	glucose 0' (mmol/l)	glucose 30' (mmol/l)	glucose 60' (mmol/l)	glucose 90' (mmol/l)	glucose 120' (mmol/l)
min	3,2	3,3	3,1	1,7	2,0
max	5,8	11,7	14,2	10,1	7,9
SV	4,6	7,3	6,7	5,6	4,3
SD	0,5	1,5	1,9	1,6	0,8

Table 4. Analysis of insulin blood levels during OGTT at women with hirsute at reproductive age

basal insulin (normal 48-165 pmol/l)	insulin 0' (pmol/l)	insulin 30' (pmol/l)	insulin 60' (pmol/l)	insulin 90' (pmol/l)	insulin 120' (pmol/l)
min	20,8	38,5	10,5	7,7	4,9
max	400,0	3298,0	2961,0	2991,0	1709,0
SV	102,5	558,8	601,8	512,3	142,6
SD	72,7	451,1	467,2	480,3	206,9

In investigation sample of blood levels of glucoses and insulin during OGTT values of basal glucoses were at normal values between 3,1 and 6,1 mmol/l at all patients. Values of basal insulin were increased at 9 (9) patients. At 51 (51%) patients values of insulin were at upper quarter of normal values which lead to insulin resistance. During OGTT after 120 minutes of test higher levels of insulin had 16 (16%) patient with hirsute had higher levels of insulin. In total 76% of patients (graphical 2.) had hyperinsulinemia (basal or reactive).

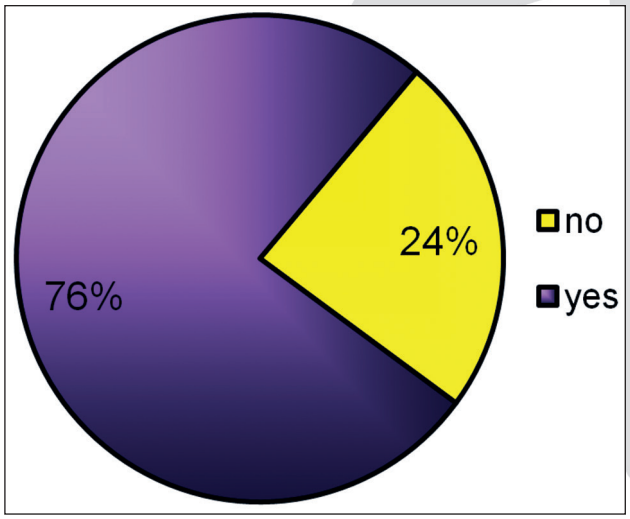

Figure 2. Hyperinsulinemia at women at reproductive age with hirsute

Average values of index of insulin resistance (IR) calculated by HOMA-2 calculator at patients with hirsute at reproductive age is 1,9 ± 1,3. Maximal value is 7 and minimal is 4.

Average values of index of insulin sensitivity (IS%) at patients with hirsute at reproductive age is 80,9 ± 61,1 (table 5.).

Table 5. Values of index of insulin resistance (IR), and insulin sensitivity (IS%) at patients with hirsute calculated by homeostatic model assessment HOMA-2-calculator.

	IR	% B	IS %
SV	1,9	179	80,9
SD	1,3	102,4	61,1

Distribution of patients at reproductive age with hirsute by levels of testosterone, DHEA-S and androstendione

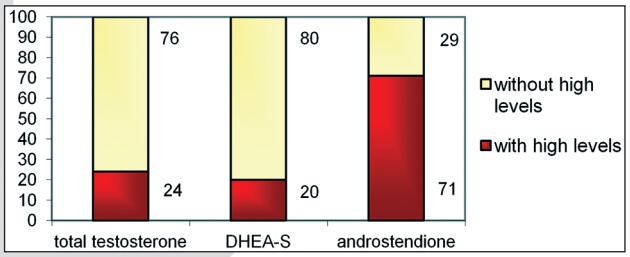

Figure 3. Distribution of patients with hyperandrogenism - high level of androgen hormones in circulation

Analisis of relation LH/FSH

Average values of relation LH/FSH at patients with hirsute hirzutizmom was 1,7+/- 0,9, with higher value 4,5 and smallest 0,3. Total 25% of patients had relation LH/FSH higher than 2 (graphical 4.).

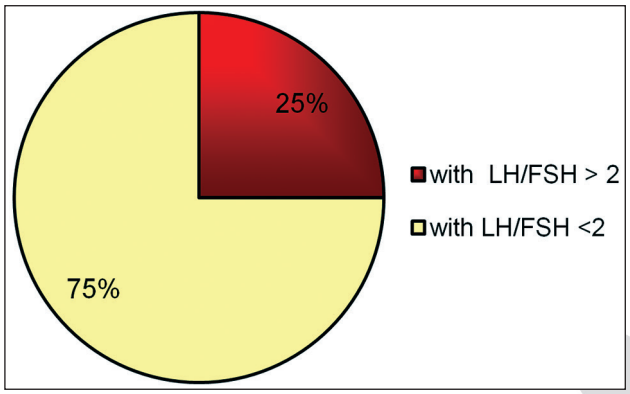

Figure 4. Distribution of patients at reproductive age with hirsute considered values of relation LH/FSH

Analisis of prolactin

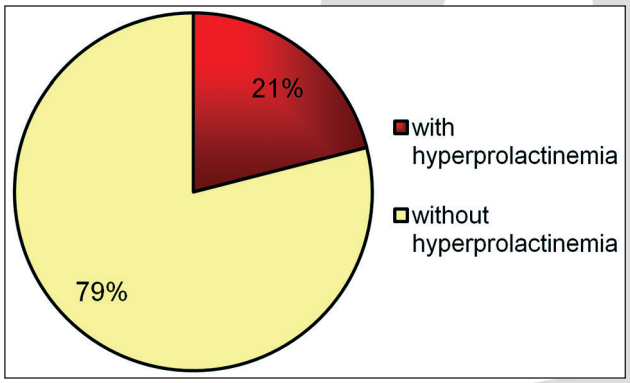

Figure 5. Distribution of hyperprolactinemia at patients with hirsute

Analisis of cortisol

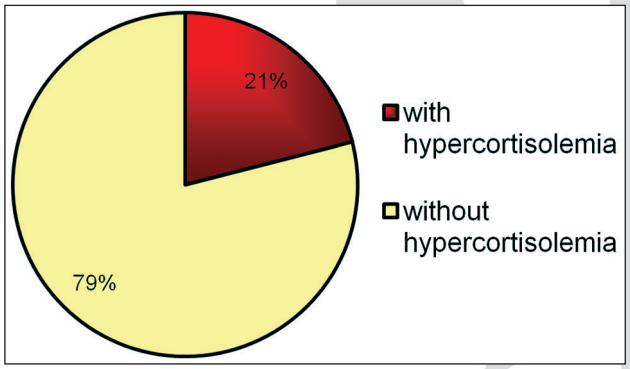

Figure 6. Distribution of hypercortisolemia at patients with hirsute

Analisis of TSH

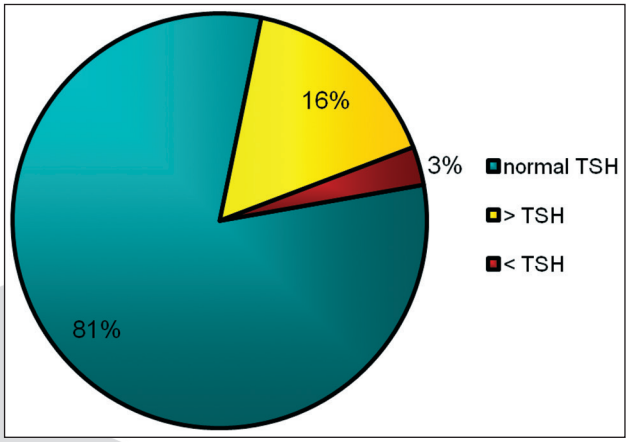

Figure 7. Distribution of patents with hirsute considered TSH level

Analisis of ovary echo sound characteristics

Table 9. Ovary echo sound characteristics of patients with hirsute

Ovary echo sound characteristics	patients	
	N	%
PCOS	48	48
solitary cistis	5	5
endometriosis	1	1
adnexitis	2	2
normal characterics	44	44
total	100	100

Primary infertility and irregular menstrual cycles

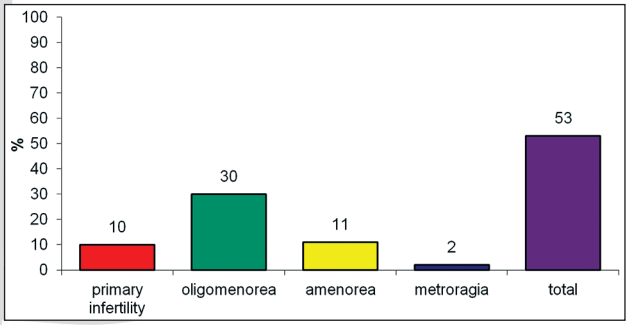

Figure 8. Distribution of primary infertility and irregular menstrual cycles at patients with hirsute

Evaluation of etiology of hirsute

With detailed clinical evaluation, physical exam, analysis of hormones, laboratories investigation and echo sound of ovaries and adrenal glands done at all 100 patients with hirsute at reproductive age we found the causes of hirsute at patients with consider that at one patient in etiology are more than one causes. (Graphical 9.).

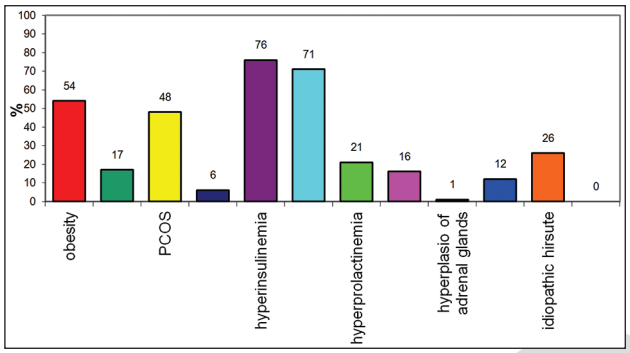

Figure 9. Etiology of hirsute at women at reproductive age

Correlation between hirsute and menstrual cycles disorders and correlation between hirsute and insulin resistance

Statistically significante positive correlation in investigation example of 100 patients is obtained for level of hirsute and menstrual cycles disorders značajna (r=0,64) which consider statistically significant (graphical 10.).

Statistically significante positive correlation in investigation example of 100 patients is obtained for level of hirsute and index of insulin resistence (r=0,73). Index of insulin resistence had statistically significant correlation with level of hirsute (graphical 11.).

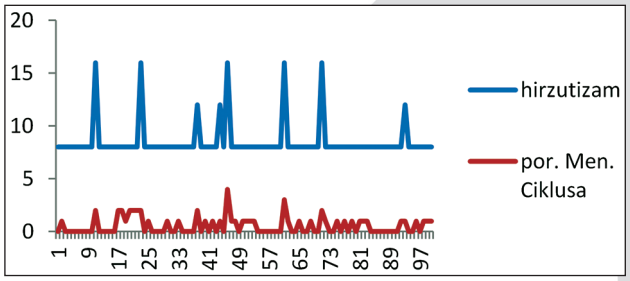

Figura 10. Correlation between level of hirsute and menstrual cycles disorders at women with hirsute at reproductive age (r=0.64)

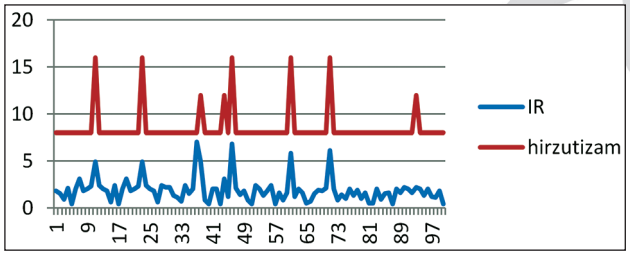

Figure 11. Correlation between level of hirsute and index of insulin resistance (IR) at women with hirsute at reproductive age (r=0.73)

Discussion

Manifestation of hirsute women with high levels of androgens hormones in circulation cause irregular menstrual cycles disorders. Most important androgens hormones in circulation are testosterone, dihidrotestosterone, androstendione, and dehidroepiandrosterone sulphate. In our research 24 (24%) patients had high level of total testosterone in blood circulation. 71 (71%) of patients had high levels of androgens hormones in circulation. In study of others authors most important role in manifestation of hirsute have increase secretion of androstendione (4) which we in our study also shown because 71 (71%) of our patients had increase level of androstendione. Androgens hormone at women can be produced in ovary, adrenal glands, in skin and in body fat. Insulin resistance can be associated with polycystic ovary syndrome at 50% patients. In our research polycystic ovary syndrome had 48% patients with hirsute. In our research micro adenoma had 12% of patients with hirsute. In other studies hirsute can be associated with acromegalia at 10-15 % of patients. Idiopathic hirsute which is characterised by high level of circulation androgens but normal ovulatory cycles is affecting 20% women with hirsute at reproductive age. (5) In our research idiopathic hirsute had 26% of patients. In other study 1, 5% to 2, 5% women with excessive body hair have congenital adrenal hyperplasia and 0, 2% of hirsute women are with tumours' with produce of excessive androgens. (6)

In our research 1 (1%) patient has congenital adrenal hyperplasia, 21 (21%) of patients have hyperprolactinemia, 17 (17%) have high level of cortisol and 16 (16%) patients are with hypothyreosis. Average value of body mass index (BMI) is 30, 4 ± 4, 3 and 54 (54%) of patients is with obesity. Normal waist diameter according to the European standards (80 cm) have 2 (2%) and all other 98 (98%) patients have over sized waist diameter over 80 cm which means that 98% patients have androgen distribution of body fat. 80 to 90% of female patients with overweight and PCOS (polycistyc ovary syndrome have insulin resistance. Body fat is important for energy and thermoregulation but also produce hormones known as lepton. Testosterone, estron, estradiol and cytokines. Women body fat produce 50 % of all testosterone of women with high elevation of producing es-

tradiol and estrone. Adiponectin is hormone synthesised from proteins in body fat cell which are called *adiposities* under control of insulin and insulin like growth factor (IGF-1). In our research is statistically positive correlation between of level of hirsute and index of insulin resistance (r=0,73).

Leptin is protein synthesised also in body fat and its concentration in blood is proportional with quantity of body fat and metabolism speed. High level of leptin has overweight patients. (7)

In our research with menstrual cycle's abnormality are 45 (45%) patients. Results of study conducted in Tehran's at sample of 102 patients, age between 18 to 45 years, shown that 85 patients had PCOS and 51 patients had hirsute with menstrual cycles disorders which confirm that PCOS and idiopathic hirsute are leading endocrinopathy in women at reproductive age. (8, 9)

Results in a hospital study done in Saudian Arabia at sample of 101 patients with hirsute at reproductive age shown that main cause of excessive body hair growth is PCOS. After it is idiopatic hirsute at 11 % patients. (10)

Statistically positive correlation in our research is for level of hirsute and menstrual cycles disorders (r=0,64). In cross-sectional study conducted in Iran which considered two groups of patients, one group wuth idiopatic hirsute and other group with PCOS, results shown also statistically positive correlation between level of hirsute and insuline resistence. (11)

Better understanding of management of hirsute at reproductive age women would help to faster decision of diagnostic procedures and therapy which could prevent infertility, diabetes, cardiovascular disease or endometrial carcinoma. In a study in Kavkaz at 69 women at reproductive age with hirsute and overweight results shown that women have high risk for diabetes (12)

In Europe in use is one questionnaire form done by European consensus and which can be modified for every research but can help during the processing diagnostic works, taking therapy and controls for hirsute women at reproductive age. (13)

Conclusions

PCOS is the most common cause of hirsute at reproductive age women, in background of the same the most common cause is hyperinsulinemia or insulin resistance. Statistically significant correlation is between hirsute and menstrual cycle disorders and insulin resistance has important role in manifestation of hirsute at reproductive age women. Hirsute at women at reproductive age women with menstrual cycle disorders need to be appropriate evaluated and treatment.

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Developing a performance measurement model for Iranian health centers

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Abstract

Background: All over the world there is a pressure to improve the performance and it is not achievable unless we have a performance measurement model. Although a network of primary health facilities has been constructed from Alma-Ata declaration, Iran has lacked a standardized performance measurement model for health centers. The main objective of this study was to develop a performance measurement model for assessing the Iranian health centers.

Methods: After the research review, Delphi process was conducted to select the appropriate performance criteria. AHP method was used to give weights to each criterion.

Results: A seven criteria model was developed for Iranian health centers.

Conclusion: This model can be used as a template for measuring the health centers performance in developing countries.

Key words: Performance, measurement, Iran, Iranian health centers, analytical hierarchy process.

Introduction

Performance measurement is a form of organizational assessment. Although using formal performance measurement is developed in many countries, it is developing with a low speed in health sector [1]. Measuring health performance is important for stakeholders, decision makers and patients [2]. May be one of the most important causes to use performance measurement system is to obtain a general strategic report of organization [3, 4]. Using a unique performance measurement system helps the health organizations to avoid separate and different evaluation process of quality, efficiency and financial performance of organization [5].

In 1966 Donabedian started assessing the quality of health care by three elements: structure, process and outcome [6]. Most performance measurement methods have focused on health systems and hospitals evaluation and just few studies have done for primary health centers [2]. Although after the Alma Ata declaration primary health care has been the main strategy in health systems [7], during the past decade the attention was focused on assessing hospital services performance [8].

There are some challenges in performing a performance measurement system. Outcomes of health services can be achieve in future years so it is hard to measure the outcomes. Inefficacy of reliable data resources about health is another challenging item [9]. There is evidence that health center factors partly explain access to health centers [10-12], quality of services [2, 12], human, fiscal and information resources [8], health status [13], patient satisfaction [14], personnel satisfaction [15] and efficient management [13].

In past several decades it has seen many efforts to develop and improve performance measurement systems in different countries. In these days more emphasis is on primary health center performance. Till now many authors have developed indicators and definitions for health center performance measurement [16]. In Iran the majority of rural and urban population receives primary health services from health centers. Formally each health center offers services to 12500 people. Primary health services are free and are funded mainly by health minister. Health centers have some main activities: organizing health facilities, monitoring, leadership, coordination, decision making, innovation, and community partnership. There are also some specific activities. Public health training, visiting students, women health, children care, vaccination, dental health and environment health are some of specific activities of health centers in Iran.

To our knowledge there has not been previous study regarding the Iranian health center per-

formance with respect to developing the criteria and indicators of performance measurement. The main objective of this study was to develop a performance measurement model for assessing the Iranian health centers.

Methods

This study was conducted in two stages: first, a systematic review of the literature was conducted to identify the list of health center performance criteria measures; second, the Delphi process was used to select the appropriate performance criteria measures for Iranian health centers.

We searched the literature for relevant criteria for health center performance measurement, to be discussed by different countries. The criteria and options to be discussed were derived from the literature review of Australia, Canada, United States, United Kingdom, New Zealand, Germany, Egypt, Ghana, Seri lanka, Afghanistan, China, India and Iran. 24 criteria were found from the literature review as following: health status, accessibility, human resources, medical equipment and physical environment, quality, patient experience, financial performance, research and development, IT and information systems, drug management, productivity, care process, organizational structure, organizational process, health system services, outsourcing, non medical indicators, community characteristics, primary health protections, health cost, organizational sector, logistic services and insurance system. We used the Delphi Procedure to have the experts proof on criteria.

Statistical methods and experimental procedures

To reach consensus a Delphi procedure was used. For the questionnaire content the criteria collected from literature were as the basis for the questions, which the experts were then asked to proofread. We designed an ascending assessment scale from 1 to 5: 1: Extremely disagree, 2: Disagree, 3: Neutral, 4: Agree, 5: Extremely agree. The numbers represented the degree of agreement towards the health center performance criteria by the experts.

To answer the questions of the questioner the experts must have sufficient professional knowledge, experience and wisdom. Therefore, this study selected experts with one of the following qualifications:

(1) A current or previous health center manager with at least 5 years of practical experience;
(2) A PhD degree of health services management and expertise-related experience;
(3) A professional and technical staff and 10 or more years of practical experience.

Based on the qualifications, 18 people were selected as experts for Delphi procedure. Each expert was asked to fill out the questionnaires. They could suggest any item related to health center performance measurement that was not concluded the questioner. The answers were collected and analyzed by SPSS 19. For the first round of questionnaires in the survey, we issued a total of 18 questionnaires, and retrieved 18 questionnaires, for a return rate of 100%. We had this consequence with 2 other rounds. After retrieving each round of Delphi, data were analyzed and another questioner was designed for the next round. The analysis functions included averaging, standard deviation and t-test.

In this study we conducted a 3 round Delphi to select the health center performance criteria. After designing the first model due to the Delphi results, we sent back the model to the experts to confirm it. If two from third of the experts agreed to change the criteria name of the indicators or add or omit the indicators we consider it to develop the main model.

The Analytical Hierarchy Process (AHP) technique was developed by Saaty [17, 18] as a powerful instrument used for multiple criteria decision making purposes [19]. AHP uses pair wise comparisons to identify the priority of alternatives in a multi-criteria decision-making problem [20]. At the top of the hierarchy in this study is the health center performance.

AHP basically enables decision-makers to prioritize the alternatives making a series of tradeoffs. First, we should define the criteria. Second, make a series of pair wise comparisons. Third, estimate relative weights for measurement of overall performance [21].

After revealing the health center performance criteria, 8 experts estimated relative weights by using AHP method. Application of AHP to rank-order the seven criteria required 3 steps. In Step 1, the 18 experts selected 7 main criteria.

In Step 2, seven experts made comparisons among health center performance criteria and discussed why a given indicator would be more or less important than another and the degree of the difference. In order to help the comparison it was created a nine-point scale of importance between two criteria. The suggested numbers to express degree of preference between each two criteria are shown in Table 1. Intermediate values (2, 4, 6 and 8) can be used to represent comparisons between the preferences.

Table 1. Nine point scale and its description

Definition	Intensity of importance
Equally importance	1
Moderately more importance	3
Strongly more importance	5
Very Strongly more importance	7
Extremely more importance	9

In Step 3, researchers calculated the weights for each criterion by K. Goepel Version 9.5.2012 software.

Results

The number of participants that filled in the questionnaires and attended the Delphi procedure is presented in Table 2.

55.5% of participants were female and 44.5% were male. 22.2% were educated in health services management filled and 33.3% were general practitioners.

Selected criteria from analysis of the data collected in the first round of questionnaires were as follows: health status, accessibility, quality improvement and management. The other criteria were incorporated in the second round of questionnaires in the survey. In the second round 2 criteria were selected as: human resource and financial performance. From the last round IT and information system was chosen. Table 3 shows the results of Delphi rounds for selecting health center performance criteria.

16.7% of criteria were statistical significant in first round and 58.3% of criteria were asked in the second questionnaire. In the second and third round the return rate of questionnaires were 100% as the

Table 2. Participant characteristics in Delphi procedure

		General physician		Health services management		Public health	
		N	%	N	%	N	%
Gender	Male	3	50	2	50	5	62.5
	Female	3	50	2	50	3	37.5
	Total	6	100	4	100	8	100
Job experience (years)	Mean	15.7		13.5		12.8	
	Min-Max	5-30		4-30		2-18	

Table 3. Details of Delphi rounds data in selecting criteria

Delphi Rounds	No. of Criteria (%)	No. Accepted (%)	No. of Not Accepted (%)	No. of criteria for next Delphi round (%)
First	(100%) 24	(16.7%) 4	(25%) 6	14 (58.3%)
Second	(100%) 14	(14.3%) 2	(57.1%) 8	4 (28.6%)
Third	(100%) 4	(25%) 1	(75%) 3	0 (0%)

Table 4. Statistical analysis of selected criteria of first model

Round of Delphi	Selected Criteria	Average	Standard Deviation
First	Health status	4.51	0.54
	Accessibility	4.42	0.67
	Quality improvement	4.50	0.38
	Management	4.63	0.57
Second	Human resource	4.70	0.54
	Financial performance	4.67	0.57
Third	IT and information system	4.54	0.70

first round. Finally the first model for Iranian health centers was developed. The statistical analysis of seven selected criteria is shown in Table 4.

After developing the first model, it was sent to experts again. They were asked to proof the model and make some changes if necessary to develop the main model. The experts suggested renaming the management criteria because the management term is a general term and the financial and human resources are the parts of management; so they confirm to change the term to "planning and policy making". The AHP hierarchical structure for this study appears in Figure 1.

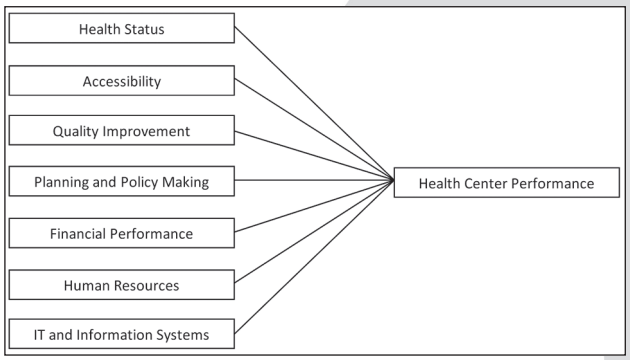

Figure 1. Iranian health center performance measurement model

Base on experts opinions health status got the highest score and IT and information systems got the lowest. The consistency rate was 8.3% and it shows that the data were appropriate. In Figure 2 shows the normalization matrix of 7 experts.

Normalization	1	2	3	4	5	6	7	
	normalized matrix							Criteria Weights
Health stats	0.36	0.40	0.38	0.44	0.25	0.24	0.21	**36%**
Accessibility	0.15	0.17	0.18	0.15	0.18	0.23	0.18	**17%**
Quality improvement	0.23	0.23	0.24	0.27	0.20	0.23	0.19	**23%**
Planning and policy making	0.07	0.09	0.07	0.08	0.18	0.21	0.12	**9%**
Financial performance	0.06	0.04	0.05	0.02	0.04	0.02	0.13	**5%**
Human resource	0.08	0.04	0.05	0.02	0.14	0.05	0.14	**7%**
IT and Information system	0.04	0.02	0.03	0.02	0.01	0.01	0.02	**3%**

Figure 2. The normalization matrix

Discussion

Having a strong primary health system needs an appropriate performance measurement model to cause a positive impact on population health [22]. Developing Iranian health center performance model provides a new insight to the primary health center. Although a network of primary health facilities has been constructed from Alma-Ata declaration, Iran has lacked a standardized performance measurement model for health centers. In Iran health center performance measurement was due to two criteria of health status and human resource. We reported on the development and prioritize of Iranian health center performance model that combines seven criteria. Our model will be used to guide the development of quality, accessibility, to health status, managerial aspects and information system performance of health centers. Our model components also do not follow a consistent pattern over time.

In developing our model although we consulted the experts and professionals in health system, we developed a review on performance models of other countries. We used the actual measures and concepts in the model to measure the health center not the providers or population.

To date the majority of published studies on developing performance models has focused on different criteria [8, 12, 23, 24]. However very little studies have also prioritized the criteria. Our model gives some weights to the criteria based on the experts' opinions. These weights can help the evaluators to measure the performance of health centers exactly.

In all over the world there is a pressure to improve the performance and it is not achievable unless we have a performance measurement model. The results and the usage of them require the participation of health workers and academia to develop the performance measurement model and discuss about the indicators and results [12]. In this research we got feedback from the managers, health workers and academia to develop a model.

Conclusion

This model can be used as a template for measuring the health centers performance in developing countries. We believe that a health center performance model should include not only health status criteria but also the other criteria as we include our model. The indicators can be used to measure each criterion, but due to health systems they remain to be studied.

This model gives the insight to evaluate health centers in a developed way by calculating weights of each criterion. This study is unique because a new methodology was used to perform a health center performance measurement model.

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The effect of a low dose ingestion of glycyrrhetinic acid (liquorice) on blood pressure in normotensive individuals

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Abstract

Purpose: Anecdotal evidence suggests that the consumption of liquorice is associated with an increase in blood pressure. This may be of clinical significance for cardiac patients and those enrolled in exercise-based cardiac rehabilitation programs. If liquorice alters blood pressure, accurate measurement thereof will be identified.

Methods: 51 healthy, normotensive participants consumed 1g of liquorice per 1kg of body weight (75mg - 200mg glycyrrhetinic acid) over a period of 2 hours. Blood pressure was measured before and then at 30-, 60-, 90- and 120 minutes post-consumption.

Results: The results showed no significant changes in blood pressure throughout the duration of the test ($p = 0.3738$).

Conclusion: The consumption of 1g of liquorice per 1kg of body weight does not alter blood pressure over a period of 2 hours.

Key words: Liquorice, glycyrrhetinic acid, blood pressure, hypertension**.**

Introduction

Several epidemiological studies have demonstrated that ingestion of substances containing glycyrrhetinic acid may contribute to hypertension, hypokalemia and muscle weakness (1, 2, 3, 4). It has been proposed that glycyrrhetinic acid elevates blood pressure through a process of producing pseudo-aldosteronism by inactivating 11-beta-hydroxysteroid- dehydrogenase and by binding mineral corticoid receptors in the kidneys (5). Consequently inhibition of 11-bHSD2 causes an increase of cortisol to be restricted in the kidneys therefore causing a hypermineralocorticoidism state in individuals (4).

In a study [6] healthy volunteers given 814mg of glycyrrhetinic acid a day experienced hypokalemia and increased body weight (due to water retention) after 1-2 weeks. Kidney retention of water and sodium lead to increased blood volume, hence increased blood pressure. Chronically elevated blood pressure also known as hypertension - a disease of vascular regulation resulting from malfunction of arterial pressure control mechanisms (central nervous system, rennin – angiotensin – aldosterone system and extracellular fluid volume). It is a relatively common form of cardiovascular disease (7) and is associated with an increased risk of mortality and morbidity. Furthermore (8) states that hypertension is one of the prevalent chronic diseases for which treatment is available. Despite this, awareness, treatment and control of high blood pressure remains insufficient (9).

In the most sensitive individuals, a regular daily intake of less than ~100mg of glycyrrhetinic acid, corresponding to 50g of liquorice sweets, seems to be enough to produce adverse effects (hypokalemic hypertension in the absence of a renal artery stenosis) (10). Most individuals who consume 400mg glycyrrhetinic acid daily experience adverse effects. Provided glycyrrhetinic acid has no other effects at lower doses, a daily intake of 10mg of glycyrrhetinic acid represents a safe daily dose for healthy adults.

It was found (11) that the effects of eating 50 – 200g of liquorice (75 – 540mg glycyrrhetinic acid) is dose-dependent, but the prolongation of the consumption from 2 – 4 weeks does not influence the response, whereas (12) revealed that liquorice – induced hormonal changes were evident after only 1 week of ingesting 100g of liquorice. It has been suggested (11) that incidence of liquorice – induced hypertension is underestimated and could be misunderstood. In addition, there is anecdotal evidence that liquorice consumption may induce spikes in blood

pressure. From a physiological perspective this would not be beneficial (especially to patients with cardiovascular disease). The aim of this study was to investigate the acute or short term effect of ingesting liquorice on blood pressure. In practice these findings may be of clinical importance when advising patients on the short term effects of liquorice, and also for accurate measurement of blood pressure.

Methods and Materials

Participants

Fifty one healthy individuals (Table 1) volunteered for the study and signed an informed consent form. The study was approved by the Faculty of Science and Agriculture's Ethics Committee. All participants were normotensive, with no current illness or chronic diseases. In view of previous research, the study was designed with the goal of eliminating those factors known to influence 11-beta-hydroxysteroid-dehydrogenase such as alcohol, tobacco, and furosemide, as well as factors influencing the Renin-Angiotensin-Aldosterone System (RAAS) such as ACE–inhibitors, AII–inhibitors and diuretics (11-18)

Blood Pressure Measurements

Blood pressure measurements were taken according to the American Heart Association (AHA) guidelines (19) with an A&D Medical blood pressure device, Model UA-767PLUS30 (A&D Instruments, Australia). Participants were required to remain seated for five minutes prior to resting blood pressure being obtained. Participants were asked to refrain from strenuous exercise and alcohol for 24 hours prior to testing, and to refrain from smoking, eating and caffeine ingestion for 3 hours prior to the test. Participants were also asked to answer a short questionnaire about their health and emotional state prior to testing to eliminate any extraneous variables. Blood pressure was measured twice and an average of the two scores was recorded. Thereafter, participants were required to ingest 1g of liquorice for every 1kg of body weight (which ranged from 75mg-200mg glycyrrhetinic acid). This dose was selected as it mimics the approximate amount of liquorice that an individual may ingest at any one time. Blood pressure was taken again at 30-, 60-, 90- and 120 minutes after ingestion.

Statistics

Data are expressed as means, standard deviations and 95% confidence intervals. A repeated measures analysis of variance (ANOVA) was computed to determine if differences existed at multiple time points. Significance was set at $p < 0.05$.

Results

Table 2 indicates that the mean changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from the baseline and after liquorice ingestion did not reach statistical significance ($p = 0.3738$).

Table 1. Participant characteristics

	Males	Females	Combined
Height (m)	1.87	1.65	1.76 ± 0.19
Weight (kg)	88.3	72.4	80.35 ± 8.64
BMI (kg/m^2)	25.2	26.62	25.91± 1.5
Age (years)	26.5	31.1	28.8 ± 9.7

Table 2. Blood pressure changes at 30 – 120 minutes post liquorice consumption

Period	Mean ± SD	Mean ± SD	Max		Min		Mean Diff	95% CI	
	SBP	DBP	SBP	DBP	SBP	DBP		SBP	DBP
Pre	118.92 ± 13.45	70.94 ± 10.32	139	90	92	50		115.1 - 122.7	68.04 - 73.84
30min	115.55 ± 12.18	67.8 ± 8.68	138	87	88	47	3.14	112.1 - 119.0	65.36 - 70.25
60min	113.2 ± 10.39	67.8 ± 9.52	133	89	90	44	4.31	110.3 - 116.1	62.88 - 70.38
90min	113.8 ± 10.74	68.84 ± 9.28	129	90	89	38	-9.67	110.8 - 116.8	57.15 - 104.1
120min	115.78 ± 11.32	69.51 ± 9.33	130	88	89	44	1.43	112.6 - 119.0	66.88 - 72.13

SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; CI = Confidence Interval; SD = Standard Deviation

Discussion

Research has indicated that a significant rise in blood pressure in normotensive and hypertensive individuals (4, 5, 11, 12) follows ingestion of liquorice over a prolonged period of 1–4 weeks. Our results indicate that a moderate consumption of liquorice (1g per 1kg body weight) over a short period of time (2 hours) does not raise blood pressure in normotensive individuals. It is unclear if the same response would be mirrored in patients diagnosed with cardiovascular disease. Epidemiological studies suggest that the rennin – angiotensin – aldosterone system is also affected by gender. Contradictory to common opinion and previous studies (4), (20) it is evident that a significant difference in the blood pressure increases between genders occur, with a greater decrease in aldosterone secretion in males, although females received a higher dose of liquorice per kilogram body weight which suggests that gender may influence blood pressure responses to liquorice ingestion. Results from our study indicates the contrary and agree with common opinion, as no significant difference was found between blood pressure measurements in male and female participants.

In addition it has been suggested (11) that liquorice–induced elevations in blood pressure, encompasses a linear dose-response relationship. The dose in the current study was selected to mimic the real–life setting, since continuous high–dose liquorice comsumption is unlikely for the average person. Therefore the 1 gram per kilogram of body weight consumed by each individual in the study could have attributed to the non-significant change in blood pressure, suggesting that a higher dose of liquorice, such as 2 grams per 1 kilogram of body weight (150mg - 400mg glycyrrhetinic acid), may elicit a more significant response on blood pressure measurements. These findings mirror that of (4) a previous study where no changes in blood pressure were found after ingesting 50 – 200g of liquorice.

Continuous liquorice ingestion for longer periods of time may have an effect on blood pressure. This may explain the non-significant findings in the study, concluding that the active ingredient in liquorice, glycyrrhetinic acid may require to be ingested for longer than 2 hours prior to taking results to ensure better digestion, as well as continuous ingestion to elicit an effect on blood pressure.

In conclusion, this study has shown that in normotensive individuals consuming 1 gram per 1 kilogram of body weight of liquorice over a 2 hour period, blood pressure remains unaltered.

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List of Abbreviations

11-bHSD2; 11-beta-hydroxysteroid- dehydrogenase
AII–inhibitors; Angiotensin II-inhibitors
ACE–inhibitors; Angiotensin Converting Enzyme Inhibitors
AHA; American Heart Association
ANOVA; Analysis of Variance
CI; Confidence Interval
DBP; Diastolic Blood Pressure
RAAS; Renin-Angiotensin-Aldosterone System
SBP; Systolic Blood Pressure

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Association between chronic mental stress and prevalence of injury risk in a sample of elite baseball players at a Korean high school

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Abstract

The purpose of this study was to examine whether chronic mental stress is associated with the prevalence of injury risk in a sample of elite baseball players at a Korean high school. The subjects included 75 elite baseball players aged 15–19 years who visited the Korea Institute of Sports Science, Seoul, Korea in April 2012. Stress hormone levels in saliva, which is a marker of chronic mental stress, were measured and the Functional Movement Screen™ (FMS™), which is marker of prevalence of injury risk, was conducted. The association between stress hormone levels and FMS™ was assessed using logistic regression analyses. Stress hormone levels were classified as low and high for logistic regression analyses. Each FMS™ score was classified into 1 of the following 4 groups: (1) very low prevalence of injury risk, (2) low prevalence of injury risk, (3) high prevalence of injury risk, and (4) very high prevalence of injury risk. The half and quartile cutoff points of mental stress levels and prevalence of injury risk, respectively, were obtained from our data. Odds ratios (ORs) (95% confidence interval [CI]) between mental stress levels and prevalence of injury risk were 0.336 (0.089–1.267, p = 0.107) for a low prevalence of injury risk, 1.576 (0.417–5.950, p = 0.502) for a high prevalence of injury risk, and 0.582 (0.158–2.138, p = 0.415) for a very high prevalence of injury risk as compared to a very low prevalence of injury risk. We conclude that chronic mental stress level was not correlated with prevalence of injury risk in our sample of elite baseball players at a Korean high school.

Key words: Elite baseball player, Functional Movement Screen™, Prevalence of injury risk, Stress hormone levels.

Introduction

Baseball is the most popular sport in Korea, and national attention towards this sport increases each year. At the 2008 Summer Olympics, Korea won the gold medal in the final match against Cuba. In 2010, Korea is currently ranked third in the world after Cuba and the United States (1).

Because baseball is one of the most popular sports in Korea, many children and adolescents begin to play baseball at an early age in an attempt to become elite players. However, although baseball is considered relatively safe compared to many other sports, impact injuries frequently occur from contact with a bat or ball (2). Additionally, elbow and shoulder injuries can occur from overuse and overload (2).

According to Kieling et al. (2011), 10%–20% of children and adolescents worldwide have mental health problems and account for a large portion of the global burden of disease (3). Mental health problems are primarily associated with mental stress in daily life; moreover, because the adolescent period is a phase of rapid psychological change, these individuals experience more stress due to the socioeconomic environment encountered in their daily lives (4-5). Hence, mental stress in adolescents is related to an increased number of injuries and delayed wound healing (6). Levels of stress hormone in saliva, such as salivary amylase, are excellent indices for chronic psychological mental stress (7-11).

Plisky et al. (2006) have used movement examinations involving inclusive movement patterns to predict injury. They hypothesized that conducting exams for concurrently assessing multiple domains of function such as range of motion, balance, and strength would improve the accuracy of identifying elite players at risk of injury using pre-participation assessment (12). Furthermore, the Functional Movement Screen™ (FMS™), which is a marker of prevalence of injury risk, is a comprehensive test that assesses the quality of essential movement patterns by identifying an individual's imbalances, limitations, and neuromuscular impairments (13-14).

We hypothesized that chronic mental stress may affect the prevalence of injury risk. However, to our knowledge, no study has been conducted to assess the relationship between chronic mental stress and prevalence of injury risk in Korea. Therefore, the purpose of this study was to examine whether mental stress is associated with prevalence of injury risk in a sample of elite baseball players at a Korean high school.

Methods

Subjects

The subjects included 75 elite baseball players aged 15–19 years who visited the Korea Institute of Sports Science, Seoul, Korea in April 2012. Stress hormone levels were measured and the FMS™ was conducted. All subjects completed a written consent form before participating in this study, and all study procedures were approved by the Human Care and Use Committee of the Institute of Sports Science. Subject characteristics are shown in Table 1.

Experimental procedures

Measured stress hormone levels

Stress hormone levels were evaluated using a hand-held salivary amylase monitor (CM-2.1; Nipro, Osaka, Japan) between 09:30 and 10:00 a.m. Subjects were prohibited from consuming food/liquids and from smoking for 2 h before the measurement. This analyzer is used to automatically analyze salivary amylase activity using a dry-chemical system over a total testing period of 1 min (15-16). The testing strip tip was soaked in a buffer containing 2-chloro-4-nitrophenyl-4-O-beta-D-galactopyranosylmaltoside, which acts as a substrate, and chromogen; the testing strip was then dried. One unit of activity (U) per mass of enzyme is defined as the production of 1 μmol of the reducing sugar maltose in 1 min (15). The tip of the testing strip was set under the tongue for 30 s to collect the saliva. Next, the testing strip was immediately inserted into the analyzer, and the result was displayed automatically. After measuring stress hormone levels in saliva, levels were classified as low or high for logistic regression analyses. Half cutoff points for mental stress levels were obtained from our data.

The FMS™ includes 7 tests that are scored on a 0–3 ordinal scale. Activities tested included the squat, hurdle step, lunge, shoulder mobility, active straight leg raise, push-up, and rotary stability. A score of 3 indicated that the movement was completed as instructed and that the subject experienced no compensation or pain. A score of 2 indicated that the movement did not induce pain, but the subject experienced some degree of compensation. A score of 1 indicated that the subject was

Table 1. Subject characteristics (Mean ± SD or %)

Variables	Category	High school elite baseball player (N = 75)
Anthropometry	Age (years)	16.60 ± 1.03
	Height (cm)	177.10 ± 5.85
	Weight (kg)	73.01 ± 8.33
	Body mass index (kg/m^2)	23.26 ± 2.26
Position	Infielder	20 (26.7%)
	Outfielder	19 (25.3%)
	Pitcher	29 (38.7%)
	Catcher	7 (9.3%)
Functional Movement Screen™	Prevalence of injury risk (point)	13.72 ± 3.00
Stress hormone levels in saliva (Ku/L)		59.16 ± 41.70

unable to complete the movement as instructed. A score of 0 was assigned if the subject experienced pain during any part of the movement. The reliability of the FMS™ has been demonstrated by Minick et al. (2010) (17), and all details pertaining to score measuring procedures have been reported previously (18-19).

After total scores from the 7 tests were aggregated, scores were classified into 1 of the following 4 groups: (1) very low prevalence of injury risk, (2) low prevalence of injury risk, (3) high prevalence of injury risk, and (4) very high prevalence of injury risk. The quartile cutoff points of the prevalence of injury risk were determined based on the data.

Statistical analysis

All results are summarized as the mean ± standard deviation. Logistic regression analyses were conducted to determine whether stress hormone levels in saliva were related to prevalence of injury risk. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS Ver. 20.0 (SPSS, Chicago, IL, USA).

Results

Logistic regression analyses

The results of logistic regression analyses of the prevalence of injury risk for low and high mental stress levels in elite baseball players at a Korean high school are shown in Table 2. Odds ratios (ORs) (95% confidence interval [CI]) between mental stress levels and prevalence of injury risk were 0.336 (0.089–1.267, $p = 0.107$) for a low prevalence of injury risk, 1.576 (0.417–5.950, $p = 0.502$) for a high prevalence of injury risk, and 0.582 (0.158–2.138, $p = 0.415$) for a very high prevalence of injury risk as compared to a very low prevalence of injury risk.

Discussion

The aim of this study was to investigate the association between mental stress levels and prevalence of injury risk in a sample of elite baseball players at a Korean high school.

Adolescence is a period of building health behaviors, and many adult mental health disorders begin during this period (20). Moreover, because adolescents experience high mental stress due to the socioeconomic environment, clinical management of this stress is required. According to Richardson et al. (2010), while approximately 8.5% of adolescents reported depression and anxiety from mental stress, only 22% of those had been treated or diagnosed by a clinician (21).

Adolescent elite sports players also experience high mental stress due to injuries to the shoulder, elbow, wrist, back, spine, hip, knee, thigh, leg, ankle, and foot (22). Because the subjects involved in this study were both adolescents and elite players, we hypothesized that mental stress may be strongly associated with prevalence of injury risk in adolescent elite baseball players.

However, the results of this study showed that mental stress level is not correlated with prevalence of injury risk. Although adolescent elite baseball players are under significant mental stress compared to adults or non-players, there is no effect on prevalence of injury risk. This may be because adolescence is a phase of physical and psychological growth involving activation of hormones that are different from those activated during adulthood (4, 23). Nevertheless, further studies are necessary to understand the relationship between mental stress levels and prevalence of injury risk.

This study has several limitations. First, this was a retrospective cohort study, which assessed the relationship between mental stress and injury risk prevalence*;* we did not examine the cause and

Table 2. Prevalence of injury risk according to mental stress levels in elite baseball players at a Korean high school

	Group	β	SE	OR	95% CI	p
Stress hormone levels (high/low)	Very low prevalence of injury risk	Ref.				
	Low prevalence of injury risk	-1.092	0.678	0.336	0.089-1.267	0.107
	High prevalence of injury risk	0.455	0.678	1.576	0.417-5.950	0.502
	Very high prevalence of injury risk	-0.542	0.664	0.582	0.158-2.138	0.415

SE, standard error; OR, odds ratios; CI, confidence interval
Tested using logistic regression analysis

effect. Second, the subjects involved in this study may not have been representative of all Korean high school elite baseball players because all participants resided in Seoul.

Conclusion

We conclude that stress hormone level is not correlated with prevalence of injury risk in elite baseball players at a Korean high school.

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An ongoing debate: Papillary thyroid carcinoma in association with Hashimoto's thyroiditis

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Abstract

Background: Malignancy risk in patients with Hashimoto's thyroiditis (HT) involved with nodules is higher than other nodular thyroid diseases. Our aim was to investigate the association between HT and papillary thyroid carcinoma (PTC).

Methods: A total of 927 thyroidectomy operations was performed in our clinic between 2000 and 2012, 237 of them were diagnosed as PTC (25.5 %). Thyroidectomy was applied in 144 HT patients with suspicious nodules (15.5 % of thyroidectomy patients) and 74 of them were diagnosed as having PTC addition to the HT. These patients (HT+PTC) were evaluated as group I (n=74) and patients with only PTC as group II (n=163)

Results: Of the 74 HT patients were with PTC (51.3 % of all suspicious nodules with HT whereas 31.2 % all PTC). There were higher levels of preoperative, free T4, anti-TPO, Anti-Tg (p= 0.02, p= 0.001, and p= 0.004, respectively) in group I when compared to group II.

Conclusion: Patients with HT should be followed up closely because of the risk of nodule formation. In case of such a nodule formation, fine needle aspiration has to be done to exclude possibility of malignancy.

Key Words: Thyroid-stimulating hormone, thyroid cancer, Hashimoto's thyroiditis, cancer, iodine intake.

Introduction

Papillary thyroid carcinoma (PTC) is the most frequently encountered endocrine neoplasia which represents 70 -80 % of all thyroid cancers (1). Hashimoto's thyroiditis (HT) is the most common inflammatory disorder of the thyroid gland; it causes hypothyroidism, and its frequency has increased over the past 50 years (2). The pathogenesis of HT includes a combination of genetic and environmental factors (3). HT affects approximately 5 % of the population, usually diagnosed in the fourth to sixth decade of life, and has a prediction for female sex (female to male ratio: 15/1) (4).

The relationship between HT and PTC was first proposed by Dailey, et al. in 1955 (5). Since this initial report, the causal association of the two diseases has remained controversial, while various authors were reporting no association between HT and PTC (6-9), others reported a variable frequency as high as 58 % (4,10-12). Besides possible ethnic, geographic, and gender differences in the prevalence of both diseases, differences in patient selection for thyroidectomy and histopathologic interpretation of HT also contribute to this variability (13,14). HT and PTC share many morphologic and molecular features, but the clinical implications and importance of this correlation are unclear (15).

The purpose of this retrospective study was to evaluate the relationship between PTC and HT in our clinical experience and to determine the features of the tumors in these HT coexisting PTC patients.

Materials and Methods

Patients

In this retrospective study, we analyzed clinico-histopathologic data of 927 patients who underwent a total or near total thyroidectomy for any thyroid pathology from October 2000 to February 2012 at the Cumhuriyet University Hospital, Sivas, Turkey. Approval of Institutional Ethics Committee of Cumhuriyet University has been received (2012-03/25). All patients were from the region of Sivas. In that period, 927 thyroidectomy surgeries were performed and 334 of them were

due to malignancy. In our research, we evaluated 237 PTC including various subtypes (25.5 % of all thyroid specimens and 70.9 % of malignant cases). Of the 144 patients operated due to suspicious nodules with HT (15 % of all thyroidectomies), 74 of them were with PTC (51.3 % of all suspicious nodules with HT whereas 31.2 % all papillary carcinomas). HT and PTC coexistent patients were evaluated as group 1 (n=74) and PTC without HT patients as group 2 (n=163).

All the clinico-pathological features of the patients including tumor localization, extension, lymph node involvement, distant metastasis, and staging were evaluated.

Group 1 patients (n=74) included pathologic confirmation of PTC and the following criterias: (1) positive for anti–thyroid peroxidase (anti-TPO) antibody, (2) positive for antithyroglobulin antibody (anti-Tg), and (3) pathologic confirmation of HT. In addition, patients with PTC with the following criteria were enrolled as group 2 (n=163): (1) negative for autoantibodies (both anti-TPO antibody and anti-TG antibody), (2) absence of HT pathology.

The serum autoantibody levels were evaluated within three months of surgery. Serum antithyroid antibody levels were measured with anti-TPO Architect Anti-TPO Reagent kit (Abbott, USA) and Architect anti-Tg (Abbott). The thyroid-stimulating hormone (TSH) and Free thyroxine hormone (FT4) level was determined by immunoradiometric assay (7K62 Architect TSH reagent kit, Free T4 reagent kit, Abbott, Ireland). The analytical sensitivities of the assay for anti-TPO antibody, anti- Tg, TSH, and FT4 were ≤ 1.0, ≤1.0, ≤ 0.0025 IU/mL, and ≤ 0.4 ng/dl, respectively. The normal ranges for anti-TPO were <5.61 IU/ml, and anti-Tg were <4.11 IU/ml. The ranges for TSH were 0.35-4.94 μIU/ml and for FT4 were 0.7-1.48 ng/dl.

A total thyroidectomy was the surgical procedure of choice for patients with papillary thyroid carcinoma at our hospital. Clinically overt cervical lymph nodes and /or positive fine needle aspiration biopsy (FNA) result were accepted as the indication of modified neck dissection, and were performed in twenty patients. Presence of atypical cells in FNA was accepted as suspicious malignancy. The American Thyroid Association (ATA) consensus statement also recommends therapeutic central neck dissection in patients with clinically involved nodes and prophylactic central neck dissection in advanced primary tumors (T3 or T4) without evidence of nodal involvement (16). A central lymph node dissection (n= 19) was performed according to the ATA consensus (16).

Preoperative assessment for cervical lymph node metastasis included ultrasonography (USG), computerized tomography (CT) when recommended by the radiologist who performed the USG. Metastatic lymph node was confirmed by FNA cytological study before surgery.

Statistical Analysis

Statistical analyses were performed with SPSS version 14.0 software (SPSS, Chicago, IL). Unless otherwise noted, descriptive statistics are reported as the mean SD. Categorical variables are reported as frequencies and proportions. The independent two-sample t test was used to compare the two independent groups. The chi-square test was performed to analyze categorical data as appropriate. Analysis of covariance was used to compare the mean number of metastatic lymph nodes between the two groups after adjusting for age, sex, tumor size and preoperative TSH.

Results

Patient Characteristics

The study included 39 (16.4 %) men and 198 (83.5 %) women ranged from 19 to 83 (mean age, 48.35 years). The mean tumor size was 1.86 (0.1–9) cm in the patients with papillary thyroid carcinoma. Among the 237 cases in the study, 89 patients (37.5 %) had multicentrity and 88 patients (37.1 %) had multifocality on tissue pathologic study. Extrathyroid extension disease and lymphovascular invasion were identified in 31 patients (13 %) and 45 patients (18.9 %), respectively. Lymph node metastasis and distant metastasis were identified in 20 patients (8.4 %) and 5 patients (2.1 %) respectively. According to the American Joint Committee on Cancer/Union Internationale Contre le Cancer's pathologic tumor-node-metastasis classification (17), there were 148 patients with stage I (62.4 %), 51 with stage II (21.5 %), 23 with stage III (9.7 %), and 15 (6.3 %) with stage IV disease. In terms of subtypes of PTC; the follicular variant was accounted for 41.3

% (n=98), classic papillary thyroid carcinoma 34.1 % (n=81), oxyphilic variant 4.6 % (n=11), solid variant 1.6 % (n=4), warthin variant 1.6 % (n=4), uncertain malignant potential variant 1.6 % (n=4), Tall cell variant 1.6 % (n=1), Hurtle cell variant 1.6 % (n=1) of all cases. Furthermore, 33 (13.9 %) patients had micropapillary carcinoma (Table 1).

Clinical/Pathologic Characteristics

The clinical parameters of the study are exhibited in Table 2; Serum antithyroid antibodies (anti-TPO, anti-Tg) and free T4 levels were only statistically significant between two groups (p =0.004, p=0.001, and p=0.028, respectively). There were no differences in age, gender, TSH levels, tumor

Table 1. Pathologic variants of Patients

Pathological variants of PTC	Group I Hashimato&Papillary n=74	Group II Papillary n=163	P*
Classic	26 (35.1%)	55(33.7%)	0.834
Solid	1 (1.4%)	3 (1.8%)	0.630
Folliculer	28 (37.8%)	70 (42.9%)	0.459
Oxyphilic	4 (5.4%)	7 (4.3%)	0.706
Tall Cell	0 (0.0%)	1 (0.6%)	-
Hurthle	0 (0.0%)	1 (0.6%)	-
Uncertain malign pot.	0 (0.0%)	4 (2.5%)	-
Warthin	3 (4.1%)	1 (0.6%)	0.091
Micropapiller carcinom	12 (%16.2)	21 (12.9%)	0.492

**p<0.05 is accepted as statistically significant*

Table 2. Clinical features of patients

Parameters	Group I Hashimato&Papillary n=74	Group II Papillary n=163	P*
Age	44.09±13.27	50.28±13.54	0.33
Gender, m/f	10/64	29/134	0.26
Free T4 ng/dl	0.99±0.52	0.84±0.62	0.028
TSH, mIU/L	3.75±2.64	4.70±9.13	0.068
Anti-Tg, IU/ml	69.57±91.92	56.39±93.77	0.004
Anti-TPO, IU/ml	78.09±118.25	62.04±97.85	0.001
Tumor Size, cm	1.73±1.93	1.91±1.43	0.39
Multifocality	24 (32.4 %)	64 (39.2 %)	0.22
Multicentrity	28 (37.8%)	61(37.4%)	0.95
Extrathyroid extension	10 (14 %)	21 (12.8 %)	0.22
Lympho vasculer Invasion	9 (12.2%)	36(22.1%)	0.49
Distant metastasis	2 (2.7%)	3 (1.8%)	
LN Disection	10 (13.5%)	10 (6.1%)	0.054
Number of Dissected LN	14.11±8.1	14.92±11.60	0.396
Number of metastased LN	5.5±4.79	5.7±5.79	0.475

**p<0.05 is accepted as statistically significant*

Table 3. Distrubitions of patients according to the TNM stage

TNM stages	Group I Hashimato&Papillary n=74	Group II Papillary n=163	P*
Stage I	43 (58.1%)	105 (64.4%)	0.353
Stage II	19 (25.7%)	32 (19.6%)	0.294
Stage III	5 (6.8%)	18 (11.0%)	0.302
Stage IV	7 (9.5%)	8 (4.9%)	0.182

**p<0.05 is accepted as statistically significant*

size, multifocality, multicentricity, extrathyroid extension, lymphovascular invasion, lymph node metastasis, and distant metastasis, sub-types of papillary carcinoma between the two groups. Additionally, in terms of tumor stage no difference was detected between two groups (Table 3).

Discussion

In literature, chronic inflammation is thought to predispose an individual to neoplastic transformation and autoimmune diseases are associated with several kinds of cancers (18). The most widely studied and best established of these links are colon carcinoma associated with inflammatory bowel diseases (chronic ulcerative colitis and Crohn's disease), esophageal adenocarcinoma associated with reflux esophagitis (Barrett's esophagus), hepatitis predisposing to liver cancer, schistosomiasis causing an increased risk of bladder and colon carcinomas and chronic Helicobacter infection leading to cancer of the stomach (19). HT is also a chronic inflammatory disease; therefore this situation may lead to cancer progression. However, the mechanism behind relationship between inflammation and thyroid cancer remains unclear. It seems as if excessive reactive oxygen species as a consequence of imbalanced intracellular redox systems may have a role in progression of thyroid cancer (20). Dailey et al. (5) reported that the risk of malignancy would be greater in patients with thyroid nodules who had thyroidectomy if they also had HT. Numerous clinical studies have been performed to confirm the relationship between HT and thyroid carcinoma (4,6-12).

It is still uncertain whether thyroid cancer is induced by HT or not. Several possible mechanisms have been proposed to clarify this probability. Both chronic iodine deficiency and chronically high iodine intake have been associated with the development of goiter (i.e., hypertrophy and hyperplasia of the thyroid cells) and attributed to excessive secretion of TSH by the pituitary. In turn, goiter has been associated with thyroid cancer risk; particularly in women (21).

Iodine is required by the thyroid to synthesize thyroid hormones. In animal models iodine may trigger thyroid autoimmunity in genetically susceptible animals (22). In addition, iodine affects thyroglobulin (Tg) antigenicity, thymus development, T and B lymphocyte, macrophage, and dendritic cell development (23). In general, chronic iodine deficiency and residence in an endemic goiter area are associated with an increased risk of follicular histological type of cancer whereas chronically high iodine intake may increase the risk of the more common papillary histological type of thyroid cancer (24). Iodine prophylaxis programs in the United States have observed an increasing incidence of HT. An increase in lymphocytic infiltration in cases of thyroidectomy was observed in the 1960s in comparison with the 1920s before iodine administration (25, 26). Similarly, in the region of Salta in the Argentina, cases of thyroidectomy due to thyroid cancer were investigated histologically before and after iodine prophylaxis over a 31-year period. There was a 17 % increase in lymphocytic infiltration (27). Iodine prophylaxis began in our country in 1968, and in 1998, the government declared to producers the obligation of adding iodine to salt and bread. The daily requirement of iodine is approximately 150μgr, and 1gr salt includes 70μgr iodine. According to the Turkish Hypertension and Renal Disease Society, (SALTurk 2008 Conference), a typical Turkish daily diet includes 18gr salt, and this amount of salt means 1260μgr iodine consumption. Thus, there is an over consumption of iodine in our country. So the high level of iodine consumption in our region may cause an increase in detection of HT and also PTC.

TSH may have a trophic effect on thyroid cancer growth, which is most likely mediated by TSH receptors on tumor cells (18, 28). This is consistent with clinical results showing that TSH suppression is an independent predictor of relapse-free survival from PTC (29). Therefore, the increased prevalence of hypothyroidism (i.e., elevated TSH levels) in HT could be one possible explanation for the association of PTC with HT. In our study, the mean TSH concentration was not significantly higher in the group 1 patients (HT and PTC) compared with group 2 (patients with PTC alone). Although we are not able to explain this difference completely, there are some possible explanations. Most of the patients with HT were using thyroxin due to hypo function of the gland after initial diagnosis. If a suspicious or malignant nodule had been detected (with FNA) during follow-up, pa-

tients would have consulted to general surgery department. Therefore most of the patients were in thyroxin therapy before the operations and this may be the reason of insignificancy TSH results between groups.

In our current study, we demonstrate a 31.2 % incidence of HT with PTC coexistence. Ott and colleagues (10) reported a 38 % incidence of PTC associated with HT in 161 patients with thyroid cancer. Cipolla and colleagues (4) reviewed 89 patients and showed a 27 % incidence of HT-associated PTC. Singh and colleagues (11) reviewed 388 patients and found HT associated with PTC in 15 %. In another study, Tamimi (12) performed a histopathologic analysis of surgically resected thyroid tumors together with the frequency and severity of chronic lymphocytic infiltration of the thyroid among patients with follicular adenoma, follicular carcinoma, and papillary carcinoma. The prevalence of lymphocytic infiltrate, which is indicative of autoimmune thyroiditis, was significantly higher in their patients with PTC (58 %) than in patients with follicular carcinoma (20 %) or follicular adenoma (14 %). HT was detected as associated with different types of thyroid carcinomas in various previous publications (12, 15). Interestingly; in the present study only PTC was detected as associated with HT. We demonstrate similar results to those of Dailey (5), Ott (10), Cipolla (4) and Singh (11).

Conversely, Crile et al. (6) did not diagnose a single case of malignancy during their 3,000 patient-years observation of 373 HT cases. In another prospective study, HT cases (46.4 % of patients had thyroid nodules) were followed for more than 10 years, and the incidence of malignancy was found to be 6.3 % (8). The authors concluded that this rate was not higher compared to the incidence for thyroid cancer among thyroid nodules in the population. Both studies had no control group. Holm and colleagues (7), in another large population-based study, showed only an increased association between HT and lymphoma. In a recent study from Turkey, Anil C et al. (9) reported 164 patients with thyroid nodules associated with HT. There were 551 patients with thyroid nodules without HT. The malignancy rate was found 1 % in HT group and 2.7 % in the control group. They concluded that thyroid nodules in patients with HT had no additional risk of malignancy than in those without HT. The results of these studies were alike with the present study. However, the methodologies of their studies were different and both of them reported their results in FNA base and possible inconsistency between FNA and final pathology were not mentioned in those studies. No micro papillary thyroid cancer was detected in both trials; therefore the ratio of micro-cancers could be unrealistic in those studies. Micro nodules in HT are difficult to identify due to the alterations in the structure of thyroid gland, so it is possible to miss micro papillary carcinoma in those trials.

This inconsistency in results of these studies was attributed to differences in gender, ethnic and geographic characteristics, selection of the patients undergoing thyroidectomy, and histopathological interpretations (13).

The most important limitation of this study is being organized in retrospective manner. Also, initialization of thyroxin therapy in endocrinology department before the nodule formation is another important limitation. However, we found a high level of relationship between HT and PTC in final pathology results in presence of nodules in HT patients.

Additionally, extrathyroidal extension, lymphvascular invasion, lymph node dissection, numbers of nodes, number of positive nodes, and TNM staging results are similar in two groups which may be speculate as HT may have no role in pathologic features of PTC.

Conclusion

Overall, based on the present study and similar studies, we conclude that PTC may develop in cases of HT with thyroid nodules. Patients with HT should be followed up closely because of the risk of nodule formation. In case of such a nodule formation, FNA has to be done in a reasonable time to exclude possibility of malignancy.

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A study of the physiological differences between inline skating and cycling at equal exercise intensities

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Abstract

This study aimed to examine the differences in the amounts of glucose, free fatty acids (FFA), lactate, phosphorus, and total nitric oxide (NO) produced while inline skating or cycling at 2 different intensities (55% heart rate maximum [HR_{max}] and 85% HR_{max}). Ten male college students who regularly exercised more than 5 h per week and did not suffer from hyperextension, cardio dysfunction, or stomach problem were selected for the test group of this study. The test consisted of inline skating and cycling in the field for 30 min at an intensity of 55% HR_{max} or 85% HR_{max}. Blood samples were taken at rest (before exercise) and at the end of exercise to analyze the changes in the concentrations of lactate, phosphorous, glucose, FFA, and total NO. The data were analyzed using technological statistics generated by the SPSS version 12.0 statistical package, and paired-sample t-tests were performed to compare the results for inline skating and cycling at each intensity. We found that inline skating and cycling at 55% HR_{max} produced significantly different amounts of lactate, phosphorus, glucose, FFA and total NO ($p < 0.05$). The amounts of lactate, glucose and FFA produced differed significantly between inline skating and cycling at 85% HR_{max} intensity ($p < 0.05$). We conclude that cycling had a more negative metabolic effect, as assessed by the levels of lactate, phosphorus, glucose, FFA, and total NO production, than inline skating at a similar intensity, which could be due to the greater exercise of particular active muscles in cycling relative to inline skating.

Key words: Inline-skating, cycling, lactate, phosphorus, glucose, FFA, total NO.

Introduction

Despite the increased leisure time and improved quality of life resulting from technological advances, modern-day people still suffer from obesity, diabetes, heart attacks, clogged arteries, high blood pressure, hyperlipidemia, osteoporosis, and other diseases of adulthood due to insufficient physical activity (1-4). In Korea, many people have responded to the rising concern about these health risks by showing more interest in increased physical activity levels and utilization of leisure time for healthy lifestyles (5). Two physical activities that have gained interest among the Korean population are inline skating and cycling (5).

Inline skating has attracted great interest from teenagers but has also increased greatly in popularity among adults. One of the primary reasons for the spotlight on inline skating is due to the high exercise effects obtainable within a short time period. Inline skating is a perfect example of an aerobic exercise that uses the whole body and produces results after just 30 min of exercise. It also has anti-aging and heart conditioning effects in adults, thus providing health benefits and pleasure simultaneously (6).

Cycling provides relief from stress and emotional distress through enjoyment of acceleration and speed (7). In addition, the peddling process is known to aid in skeletal development as well as preventing and combating various adult diseases, which makes it a very appropriate physical activity for ordinary citizens; it also carries a relatively low risk for injury (8).

Physical activity typically requires the body to provide energy to support the muscular activity. Carbohydrates and fat are the main energy sources used during exercise; the amounts used are not

equal at all times but rather depend on the intensity and duration of the exercise. Carbohydrates are used during early exercise and high-intensity exercise, while fat is burned mostly during low-intensity and aerobic exercise (9).

In order for carbohydrates to be used as a source of energy during high-intensity exercises, a glucose molecule from the cytoplasm must undergo glycolysis to produce 2 adenosine tri-phosphate (ATP) and 2 nicotinamide adenine dinucleotide (NADH) molecules. The last step of the process produces 2 molecules of pyruvate as the end product. When enough oxygen is available, substrate-level phosphorylation during the Krebs cycle will produce 2 molecules of ATP, while oxidative phosphorylation will produce 6 NADH and 2 flavin adenine dinucleotide dihydrogen ($FADH_2$) molecules (9). In addition, fatty acids from the triglyceride (TG) stored in the adipocytes along with TGs in the muscles will be released into the blood and free fatty acids (FFA) bound to albumin taken back up into the muscles during moderate intensity exercise (10).

FFA released from fatty acids are a very importance source of energy for long-period endurance activities. Early during exercise, the capillary vessels of the muscle expand and accelerate the use of FFA. Exercise also stimulates lipolysis, and this process gradually increases during exercise and does not stop even after the end of the activity itself (9). In the study of the relationship between fatty acids and athletic performance by Rennie et al. (1976), long-term endurance training increased the oxidative ability to use FFA and suppressed muscular glycogen usage, enabling glycogen sparing and resulting in positive effects on performance (11).

Nitric oxide (NO) plays an important role in hemodynamics and metabolic control, and Kingwell (2000) states that adaptation of the NO system through training can maintain and improve blood vessel and heart health (12). NO, which is produced from L-arginine by the activity of the enzyme Nitric Oxide Synthase (NOS), has a very short half-life and is used to expand the blood vessels of heart disease patients and thus prevent increases in blood pressure (13); it also facilitates energy distribution and the elimination of metabolites, resulting in increased athletic performance. Levels of NO not only correlate with cardiovascular stress levels but are also closely linked to athletic performance (14-15).

Although both inline skating and cycling are popular aerobic exercises in Korea, they differ in their use of muscle activity: inline skating is a full-body exercise, while cycling is considered a target-muscle exercise. Although the levels of muscle activity, metabolic energy, and fatigue substances differ between inline skating and cycling, there is little research examining the differences between these activities. Furthermore, there are to date no studies comparing metabolic energy substrate utilization-related NO production and metabolite accumulation or investigating differences in metabolic parameters. Therefore, this study aimed to investigate the energy use, blood levels of fatigue factors, and NO production associated with inline skating and cycling at equal exercise intensities.

Methods

Subjects

Ten male college students who regularly exercised more than 5 h per week and did not suffer from hyperextension, cardio dysfunction, or stomach problems were selected for the test group of this study. They visited an exercise physiology laboratory at Yonsei University, Seoul, Korea for measurement of physiological variables and NO production during different sporting events performed at defined intensities. In order to investigate the differences in energy substrate utilization and NO production during inline skating and cycling at 2 physical intensity levels (55% heart rate maximum [HR_{max}] and 85% HR_{max}), subjects participated in 4 separate experiments (a. inline skating at 55% HR_{max}, b. inline skating at 85% HR_{max}, c. cycling at 55% HR_{max}, and d. cycling at 85% HR_{max}). The experiments were conducted at Mokdong Inline-Skate Rink, Seoul, Korea between August 1, 2005 and September 30, 2005.

The subjects underwent basic physical tests to measure their height, weight, percent body fat (%Fat), and maximum oxygen intake (VO_{2max}) and then proceeded to intervals of inline skating and cycling at both intensity levels (55% HR_{max} and 85% HR_{max}). The training method (inline skating or cycling) was the independent variable, and the lactate, phosphorous, glucose, FFA, and total NO levels were the dependent variables.

All subjects signed a form indicating written consent to participate in this study. The characteristics of the subjects are shown in Table 1.

Table 1. Characteristics of subjects

Variable	Male (n = 10)
Age, years	23.9 ± 1.1
Height, cm	175.2 ± 2.1
Weight, kg	68.9 ± 2.4
Body fat, %	13.7 ± 3.4
VO_{2max}, mL/kg·min	53.9 ± 2.5

Experimental procedures

(1) Body composition measurements

In order to ensure proper testing and the validity of the experiment, the subjects were instructed to refrain from heavy physical activity, smoking, drinking, any dietary treatment, and any type of drug usage. The basic physical tests were conducted in the exercise physiology laboratory at Yonsei University in Seoul, Korea. In order to ensure the reproducibility of the results, the subjects were asked to get plenty of rest the night before and to consume only water within 6 h of the start of the testing to maintain the balance of the constituents in the blood. In addition, to ensure that the body was in an active state, the experiment was conducted between 1 and 3 PM. The subjects were familiarized with the equipment before the experiments so that they would feel comfortable during the experiments.

The subjects arrived with empty stomachs (except for water). Their height and weight were measured using an automatic anthropometer (Fanics, FE810, Korea), and their body composition and body mass index (BMI) were measured using the Body Composition Analyzer Model 310 (Biodynamics, Inc., USA).

In order to ensure accurate body composition measurements, the subjects were prohibited from consuming food or beverages other than water after dinner on the previous day, all jewelry or accessories (ring, necklace, watches) were removed, and the subjects were asked to lie on the bed with their arms and legs extended 6 inches from the body. Electrodes and cables connected to the Body Composition Analyzer were placed on the top of the right foot and wrist, the back of the right hand, and 3 cm under the joint of the third toe. The subjects kept still during the measurements (16).

(2) Maximum Oxygen Intake measurements

The Maximum Oxygen Intake (VO_{2max}) was measured using the CPX system (Cardio Pulmonary Exercise test system) (MedGraphics, Inc., USA) by breath-by-breath methods. The heart rate was measured using the heart check system of a heart rate sensor (Polar-610, Finland). The heart rate was recorded every 3 min beginning from the relaxed state, in which the heart rate was below 70 beats per minute and the maximum oxygen intake below 3.5 mL/kg·min. The measurement was conducted on a Quinton Q65 Treadmill using the Bruce protocol, starting at a speed of 1.75 mph at a 10% incline with increases of 0.8–0.9 mph in the speed and 2% in the incline every 3 min. The subjects' perceptions of tiredness were carefully observed during exercise using the Borg 20 rate of perceived exertion (RPE) scale, and they were watched for signs of exhaustion.

(3) Exercise Methods

1) Inline skating

The experiment was conducted at Mokdong Inline-Skate rink, Seoul, Korea, to which the subjects were transported in the research team's vehicles. The subjects performed inline skating at an intensity of 55% HR_{max} for 30 min. Two weeks later, the subjects exercised under the same circumstances except at an intensity of 85% HR_{max}.

2) Cycling

Two weeks after the inline skating experiments, the subjects performed cycling at an intensity of 55% HR_{max} for 30 min. Two weeks later, the subjects exercised under the same circumstances except at an intensity of 85% HR_{max}.

(4) Blood sample collection and analysis

Blood samples were collected before and after each of the 4 experiments (inline skating at 55% HR_{max}, inline skating at 85% HR_{max}, cycling at 55% HR_{max}, and cycling at 85% HR_{max}). A total of 8 samples were collected from each subject, and 8 mL of blood was extracted from the antecubital vein using a 21-gauge needle on each occasion. Each blood sample was stored in an EDTA-treated tube at a temperature of 4°C until analysis. A 5-mL plain vacuum tube was used for analysis of the phosphorous, FFA, and glucose, a lactate-spe-

cified tube for the lactate analysis, and an EDTA tube for the NO analysis.

1) Lactate

The blood lactate level was analyzed by the enzymatic method using a spectrophotometer. The blood sample (2 mL) was mixed with a deproteinizing agent (4 mL), and the mixture was centrifuged to separate the supernatant. The blank tube was filled with 100 µL of deproteinizing agent and distilled water (DW) and 2.9 mL of reagent, while the test tube was filled with 100 µL of supernatant and 4 mL of DW. The tubes were incubated in a 37°C water bath for 15 min and then allowed to equilibrate at room temperature for 2 min before their absorbance values at 340 nm were measured.

2) Phosphorous

The blood phosphorous level was analyzed by the ultraviolet (UV) method using an ADVIA 1650 (Japan). A 2-mL aliquot of the blood sample was centrifuged in a 5-mL vacuum tube for 10 min at 3,000 rpm. The serum was extracted and stored at -70°C. Centrifuged serum (0.5 mL) was mixed with 250 µL of sulfuric acid, surfactant reagent, and ammonium molybdate reagent to produce a color reaction, and the absorbance at the dominant wavelength of 340 nm and the optical density at 540 nm were measured.

3) Free Fatty Acid

Blood sample was collected using a vacuum tube containing a clot activator in order to precipitate the fibrinogen, and the free fatty acid concentration was measured by the enzymatic method using a Hitachi 7150 (Japan). It was centrifuged for 10 min at 2,500–3,000 rpm, and then 0.5 mL of the serum was mixed with 50 µL each of distilled water. The sample was then mixed with the SICDIA NEFAZYME test reagent and kept in cold water for 5 min before analysis.

4) Glucose

The blood sample was centrifuged at 2,500–3,000 rpm for 10 min, and the glucose concentration of the supernatant was analyzed by the enzymatic method using an ADVIA 1650 (Bayer HealthCare, Ltd., Tarrytown, NY, USA).

5) Nitric Oxide

NO analysis was performed by the Griess Reagent [(1% sulfanilic acid + 5% H_3PO_4) + (0.1% naphthylethylene diamine dihydrochloride + distilled water)] method using a commercial kit (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemicals, USA). This method involves the conversion of nitrate to nitrite by a nitrate reductase, and the optical density at 540 nm is then measured. NOx = nitrite (NO_2) + nitrate (NO_3)

Statistical analysis

All results obtained from this study were represented as the mean ± standard deviation. Independent t-tests were conducted in order to determine the differences between inline skating and cycling at each exercise intensity. The level of statistical significance was set at $p < 0.05$, and all analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Results

The lactate, phosphorous, glucose, FFA, and total NO levels after inline skating and cycling are shown in Table 2. The lactate level was significantly higher after cycling at 55% HR_{max} than after inline skating at the same intensity (3.58 ± 0.45 mmol/L vs. 2.61 ± 0.73 mmol/L, respectively; difference, 0.97 mmol/L; $p < 0.05$). The lactate level was also higher after cycling at 85% HR_{max} than after inline skating at the same intensity (7.58 ± 1.17 mmol/L vs. 5.06 ± 1.70 mmol/L, respectively; difference, 2.52 mmol/L; $p < 0.05$).

The phosphorous level was significantly higher after cycling at 55% HR_{max} than after inline skating at the same intensity (4.33 ± 0.64 mg/dL vs. 4.17 ± 0.38 mg/dL, respectively; difference, 0.16 mg/dL; $p < 0.05$). The phosphorous level was also significantly higher after cycling at 85% HR_{max} than after inline skating at the same intensity (4.65 ± 0.53 mg/dL vs. 4.41 ± 0.33 mg/dL, respectively; difference, 0.24 mg/dL; $p > 0.05$).

The glucose level was significantly higher after cycling at 55% HR_{max} than after inline skating at the same intensity (89.80 ± 13.19 mg/dL vs. 86.00 ± 8.40 mg/dL, respectively; difference, 3.80 mg/dL; $p < 0.05$). The glucose level was also significantly higher after cycling at 85% HR_{max} than after

Table 2. The amounts of lactate, phosphorous, glucose, FFA, and total NO produced by inline skating and cycling (Mean ± SD)

Variable		Resting	End of exercise
Lactate (mmol/L)	Inline skating (55% HR_{max})	1.25 ± 0.04	2.61 ± 0.73*
	Cycling (55% HR_{max})	1.27 ± 0.05	3.58 ± 0.45*
	Inline skating (85% HR_{max})	1.28 ± 0.06	5.06 ± 1.70#
	Cycling (85% HR_{max})	1.29 ± 0.06	7.58 ± 1.17#
Phosphorus (mg/dL)	Inline skating (55% HR_{max})	3.81 ± 0.34	4.17 ± 0.38*
	Cycling (55% HR_{max})	3.87 ± 0.60	4.33 ± 0.64*
	Inline skating (85% HR_{max})	3.73 ± 0.61	4.41 ± 0.33
	Cycling (85% HR_{max})	3.77 ± 0.38	4.65 ± 0.53
Glucose (mg/dL)	Inline skating (55% HR_{max})	80.60 ± 7.20	86.00 ± 8.40*
	Cycling (55% HR_{max})	84.20 ± 11.74	89.80 ± 13.19*
	Inline skating (85% HR_{max})	84.20 ± 9.80	92.80 ± 10.90#
	Cycling (85% HR_{max})	83.20 ± 9.81	97.30 ± 18.68#
Free fatty acid (uEq/L)	Inline skating (55% HR_{max})	327.10 ± 162.04	628.70 ± 521.01*
	Cycling (55% HR_{max})	323.20 ± 178.39	575.30 ± 482.02*
	Inline skating (85% HR_{max})	299.90 ± 267.42	525.60 ± 367.95#
	Cycling (85% HR_{max})	306.10 ± 99.05	494.30 ± 223.01#
Total nitric oxide (**µmol**/L)	Inline skating (55% HR_{max})	37.01 ± 11.84	41.04 ± 38.61*
	Cycling (55% HR_{max})	35.99 ± 8.47	45.34 ± 26.85*
	Inline skating (85% HR_{max})	34.05 ± 19.01	47.98 ± 38.62
	Cycling (85% HR_{max})	37.69 ± 29.12	48.47 ± 33.32

HR_{max}, Heart rate maximum; the resting levels did not differ significantly between the inline skating and cycling experiments.
** $p < 0.05$; tested between inline skating (55% HR_{max}) and cycling (55% HR_{max}) by the independent t-test*
 # $p < 0.05$; tested between inline skating (85% HR_{max}) and cycling (85% HR_{max}) by the independent t-test

inline skating at the same intensity (97.30 ± 18.68 mg/dL vs. 92.80 ± 10.90 mg/dL, respectively; difference, 4.5 mg/dL; $p < 0.05$).

The FFA level after inline skating at 55% HR_{max} was significantly higher than after cycling at the same intensity (628.70 ± 521.01 µEq/L vs. 575.30 ± 482.02 µEq/L, respectively; difference, 53 µEq/L; $p < 0.05$). The FFA level was also higher after inline skating at 85% HR_{max} than after cycling at the same intensity (525.60 ± 367.95 µEq/L vs. 494.30 ± 223.01 µEq/L, respectively; difference, 31.3 µEq/L; $p < 0.05$).

The total NO level was significantly higher after cycling at 55% HR_{max} than after inline skating at the same intensity (45.34 ± 26.85 µmol/L vs. 41.04 ± 38.61 µmol/L, respectively; difference, 4.3 µmol/L; $p < 0.05$). However, the total NO level after exercise at 85% HR_{max} did not differ significantly between cycling and inline skating (48.47 ± 33.32 µmol/L vs. 47.98 ± 38.62 µmol/L, respectively; difference, 0.49 µmol/L; $p > 0.05$).

Discussion

This study aimed to investigate the differences between lactate, phosphorous, glucose, FFA, and total NO production between cycling and inline skating at intensities of 55% HR_{max} and 85% HR_{max}.

Fatigue is defined as reduced function of all or part of an organ due to prolonged exercise or repeated stimulation, resulting in a lack of energy or power for exertion or maintenance of muscular contraction activity (17). The causes of muscle fatigue have been asserted to be metabolite accumulation and depletion of energy substrates, which has resulted in much research on the relationships of metabolite accumulation to progressive exhaustion and energy depletion using lactate and phosphorous accumulation as an index for fatigue (17-18). Therefore, the levels of lactate and phosphorous were observed during this study to compare the levels of fatigue between different types of exercise at different physical intensities. Cycling produced higher levels of both lactate and

phosphorous than inline skating at both 55% and 85% intensity ($p < 0.05$). This shows that cycling produces more fatigue than inline skating at the same intensity.

During cardiovascular activity under conditions of adequate oxygenation, glycogen undergoes glycolysis to pyruvate, which enters the mitochondria and is oxidized to carbon dioxide and water to produce energy. However, when exercise exceeds one's fitness level, the contracting muscle cannot receive sufficient oxygen, which causes improper combustion and the conversion of pyruvate to lactate (9). In addition, phosphorous is usually formed by the hydrolysis of phosphate creatine (PCr) during high-intensity exercise, but the low level of phosphorous produced by inline skating in our study can be explained by low PCr hydrolysis, as a lower-body exercise like cycling would be expected to produce lower values than a full-body exercise such as inline skating.

Glucose levels were high after both inline skating and cycling at both 55% HR_{max} and 85% HR_{max}. Cycling uses fewer muscles than inline skating, which means that the muscles in use are acting at a higher intensity; we can therefore conclude that cycling requires higher glucose usage than inline skating. In addition, continuous low-intensity aerobic exercise is more effective than high-intensity exercise at accelerating lipid metabolism (9). Although the exercises were performed at the same HR_{max} levels, inline skating requires more muscle usage than cycling and therefore produced greater increases in FFA mobilization and blood flow, resulting in a higher amount of FFA being transferred into the mitochondria to producing ATP through β-oxidation.

The blood flow and shear stress produced by exercise improve the structure and function of the blood vessel. The release of NO and prostacyclin in response to shear stress reportedly suppresses the recurrence of stenosis and formation of atheroma and increases vasodilation (19). NO production was significantly greater during cycling than during inline skating at 55% HR_{max}. This may be because the peddling motion of cycling requires higher oxygen consumption than the gliding motion of inline skating, resulting in higher NO production.

Our study has some limitations. First, because we studied only college students recruited from 1 university at Yonsei, Seoul, Korea, the study sample did not represent the entire Korean college student population. Second, the sample size of this study was not large ($n = 10$). For these reasons, larger studies with a broader sampling base should be carried out in the future to determine the physiological differences among diverse types of exercise, such as inline skating and cycling, performed at equal intensities.

Conclusion

We conclude that cycling produced a more negative metabolic reaction than inline skating at the same intensity in terms of lactate, phosphorous, glucose, FFA, and total NO production. The differences in these metabolic parameters may be attributable to the fact that cycling involves more concentrated active muscle usage than inline skating.

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Pseudomonas aeruginosa osteomyelitis of the pelvic bones after retropubic surgery: A case report

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Abstract

Osteomyelitis of the pubic bone is a rare and very uncomfortable complication after pelvic surgery.

A 69-year-old diabetic man presented with acute incapacitating pelvic pain, gait disturbance, and low-grade fever three months after Millin's retropubic prostatectomy was performed for an adenoma. Pelvic radiography showed a change on the symphysis bone suggesting a periosteal inflammatory reaction. Cephalosporin antibiotic therapy led to improvement, and the patient was discharged after seven days for further home care. Ten days later, the patient was admitted with a worsening general condition, high fever, severe pelvic pain, and extremely reduced mobility. In the pubic area, tumefaction of lower surgical scars and an erythematous, immobile tumor were visible. After the patient was admitted, the tumefaction was incised using curettage, and the detrital content was sent for microbiological and histopathological analyses. Pelvic radiography and computed tomography scans showed osteomyelitic changes with complete destruction of the pubic symphysis. Cultures revealed Pseudomonas aeruginosa that was sensitive to ciprofloxacin. The patient was discharged from the hospital after one month in good general condition, walking with a cane.

With proper treatment according to microbiological culture combined with open surgical therapy, patients with pubic bone osteomyelitis can recover fully from this serious complication.

Key words: Osteitis pubis, pubis osteomyelitis, retropubic surgery, Millin's prostatectomy.

Introduction

The term "osteitis pubis" was first used by E. Beer in 1927 to describe idiopathic postoperative complications after suprapubic prostatectomy that are characterized by severe pain in the pelvis; a wide, plodding pace; and bone destruction at the edges of the frontal bones [1]. Osteitis pubis is a well-recognized painful inflammation that affects the anterior structures of the pelvic belt. Its causes remain controversial. In recent decades, despite various theories, the diagnostic criteria and optimal treatment of pubic osteitis remain contradictory. Some authors believe that many published cases of pubic osteitis are actually unidentified pubic osteomyelitis. It is generally accepted that osteitis pubis is a noninfectious, self-limited, relatively benign condition. Infectious osteomyelitis of the pubic symphysis is very uncommon and can mimic osteitis pubis. Pubic osteomyelitis caused by Pseudomonas aeruginosa has been described extremely rarely in the literature [2].

We reiterate the assumption that osteomyelitis of the pubic symphysis can be mistaken for pubic osteitis. Here, we report the case of a patient who was treated for osteitis pubis, but was correctly diagnosed with pubic osteomyelitics after microbiological and histopathological analyses supplemented by radiographs and CT scans.

Case Report

A 69-year-old diabetic patient on oral antidiabetics smoked more than 20 cigarettes a day for over 30 years. He was admitted with stomach pains; weakness in his hips, symphysis and entire pelvic belt; and movement difficulty.

Three months before admission, the patient had undergone Millin's retropubic prostatectomy for prostate adenoma. On the seventh day after the operation, drainage was discovered at the wound; a swab showed Pseudomonas aeruginosa susceptible to ciprofloxacin. Further conservative treatment with ciprofloxacin treated the infected wound, and the patient was discharged on the sixteenth day after the operation with normal urination and a healed surgical wound. For the next three months, the patient attended regular check-ups and reported a good general condition with no subjective complaints. After three months, he began complaining of dull pain in the wound and the hip and difficulty with movement. The patient also reported subjective problems with urination, as well as occasional burning and frequent urination. Because the condition was not ameliorated by nonsteroidal antirheumatics and occasional aspirin, the patient was admitted for hospital examination.

Upon the patient's admission, palpable pain sensitivity was found in the area of the rectus abdominus and in the frontal area with no change in the wound. Lab analyses showed the following: SE,80 mm / h; WBC, 9.1 g / L; RBC, 4.49 G / L; glycemia, 7.3 mmol / L; urea, 6.4 mmol / L; creatinine, 106 mmol / L; and acid phosphatase, 5.0 U / L. The urine sediment contained the following: a mass of bacteria, substantial amounts of salt urate, leukocytes, and 6 to 8 erythrocytes. E. coli was found in the urine culture. We performed radiography of the pelvis and hips. In addition to degenerative changes in the hips on the right side, radiography revealed a discreet bone defect on the upper edge of the symphysis (Figure 1). The orthopedic surgeon who was consulted considered the findings normal.

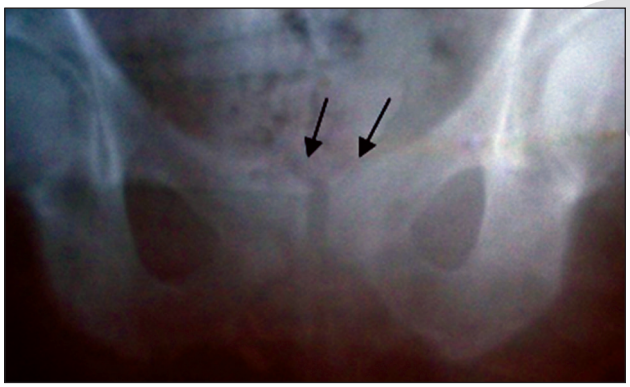

Figure 1. The arrows indicate the defect of the upper edge of the symphysis

The patient was treated for 7 days with ceftriaxone in the infusion solution, in accordance with the urine culture and antibiogram. On the third day of hospitalization, the patient's temperature was 37.10 C; for the rest of the period, the patient was afebrile. He was discharged in good general condition after 7 days, and oral ciprofloxacin was prescribed.

After two weeks, the patient returned with the following complaints: deterioration of general condition, a temperature of 38.40 C, uncertain and difficult gait with outstretched legs, and pain in the pelvic band along the hips and spine, in the abdominal wall and in the inner thigh. A flank mass 5 cm in diameter was discovered around the frontal abdominal region in the lower section of the wound. The mass had an irregular surface and was clearly circumscribed, mobile and very painful on palpation. The skin above the tumefaction showed signs of hyperemia, and the surgical scar tissue appeared stretched and thinned.

Upon admission, local anesthesia was administered and a wide incision was made along the scar, winding down over the symphysis to the Retzius space. Little drainage containing hemorrhagic detritus was obtained. We performed curettage widely to the lateral edges and the depth of the wound. The obtained material was sent for microbial and histopathological analyses with sequestration. Pseudomonas aeruginosa was found in the content of the wound.

The lab findings included the following: SE, 96; WBC, 7.6 g / l; RBC, 4.59 g / L; glycemia, 17.2 mmol / L; creatinine, 124 mmol / L; and urea, 7.2 mmol / L. In the urine sediment, a substantial amount of uric acid, leukocytes, 20 to 25 erythrocytes and some bacteria were found. Histopathologic analysis indicated chronic osteomyelitis. Upon repeat radiography of the pelvis and hips, destructive osteolytic changes in the pubic symphysis bone were seen (Figure 2).

A computerized axial tomography of the pelvis was performed. Soft tissue lesions with irregular contours 54 X 33 mm in diameter were identified subcutaneously and in front of the pubic symphysis, which is in contact with the pubic bone on the medial line. The structural changes in the pubic bone were more pronounced on the left. On the other pelvic bones, no pathological changes were found (Figures 3 and 4).

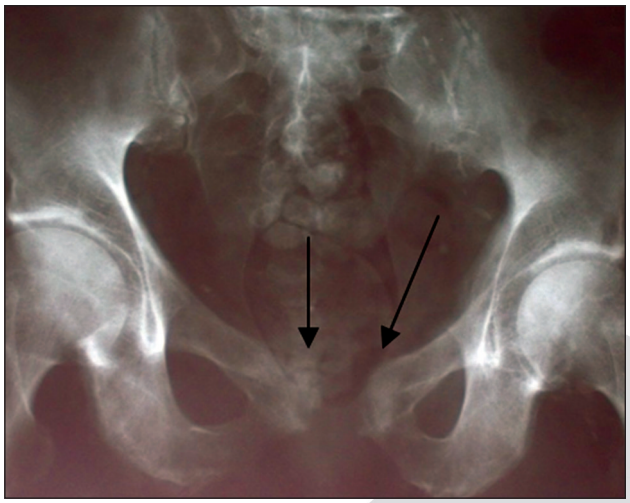

Figure 2. The arrows indicate osteolytic changes in the symphysis bone

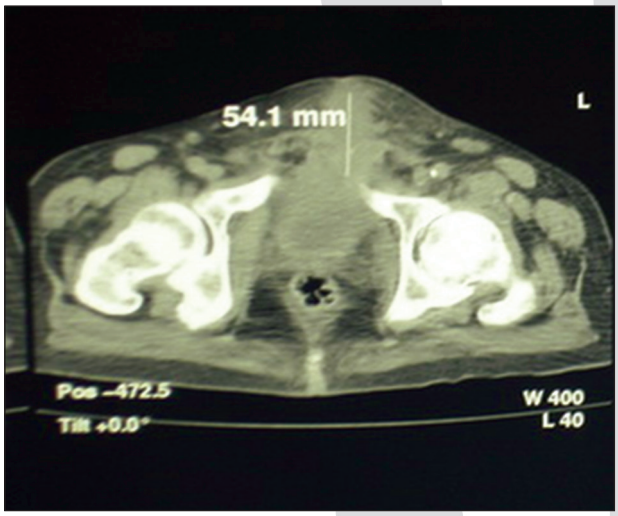

Figure 3. Soft tissue lesions

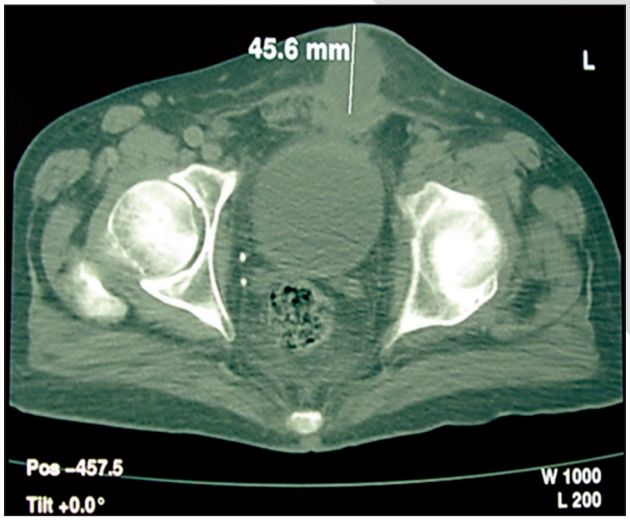

Figure 4. In contact with the pubic bone on the medial line

Lesions a 25 mm diameter corresponding to phlegmon infiltration can be seen in the left iliac fossa at the pelvic muscles (Figure 5).

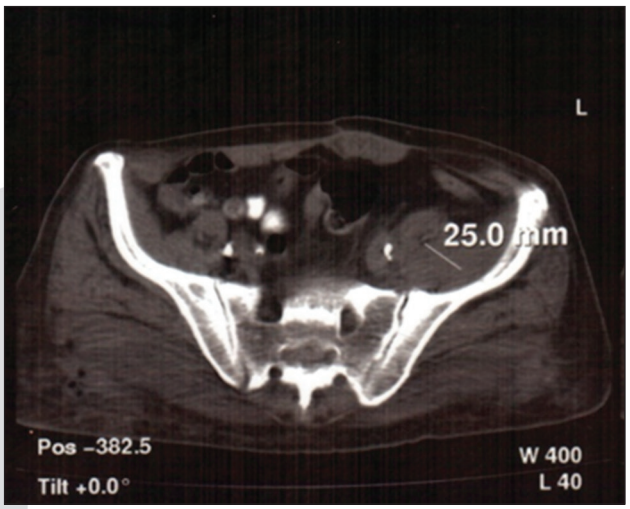

Figure 5. Lesions in the left iliac fossa at the pelvic muscle

After admission, treatment included infusion solutions and, based on the antibiogram results, ciprofloxacin 100 mg X 3 was started intravenously.

The new findings from the culture and antibiogram confirmed the infection's susceptibility to ciprofloxacin.

On the third day, the patient was afebrile, and he remained so until the end of hospitalization. The wound was cleaned regularly until fresh granulation appeared, after two weeks. The pain in the patient's stomach and hips ceased after seven days. The patient's difficult, waddling gait with a cane and difficulty rising persisted, but with much lower intensity. The patient was discharged after 27 days. He was treated with ciprofloxacin i.v. for three weeks; at discharge, oral ciprofloxacin was prescribed at a dose of 2 X 500 mg for the next 5 weeks, followed by a dose of 2 X 250 mg for the next three weeks. Control radiographs of the pelvic bones 2 months later showed that the destruction of the pubic symphysis bone had halted (Figure 6). After 12 weeks, the radiograph showed osteosclerotic edges at the ends of the pubic symphysis bones (Figure 7).

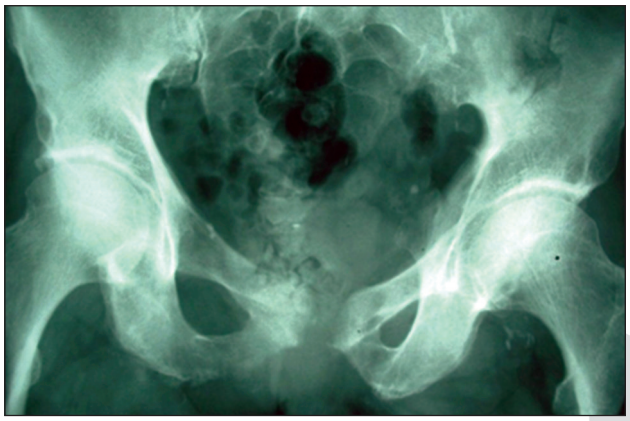

Figure 6. Control radiographs of pelvic bones, two months after the patient's release from the hospital

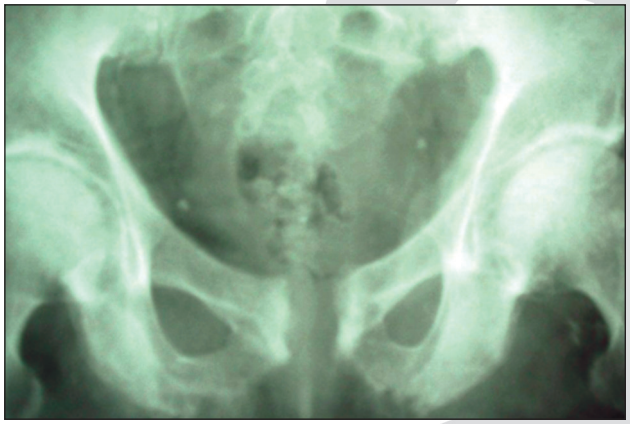

Figure 7. Osteosclerotic edges of the pubic symphysis bones

The control radiographs of the pelvic bones 11 months after the surgical incision and curettage clearly showed the sclerotic edges of the spaced frontal bones (Figure 8.).

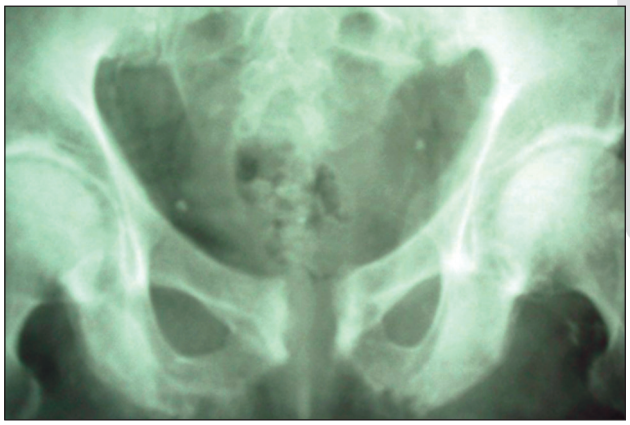

Figure 8. Sclerotic edges of the spaced frontal bones

For the following 11 months, the patient was in good condition and experienced no palpatory pain sensitivity or other changes in the symphysis area. He walked with a cane at a discrete, plodding pace. Laboratory assessments were performed after one month after the patient's hospitalization. Aside from variable erythrocyte sedimentation rate values and blood glucose levels, the results were normal. The patient's urine was cultured at intervals of 2 to 3 months, and the results were sterile.

Discussion

Osteomyelitis of the pubic symphysis bones is a rare condition that accounts for less than 1% of all cases of osteomyelitis.

Retropubic surgery on the prostate and bladder neck that opens the Retzius space widely creates a potential condition for the onset of this disease, which is caused by micro- or macrotrauma of the periosteal or bone structure of the pubic symphysis while infectious agents are present in the immediate vicinity.

Unlike osteitis pubis, osteomyelitis of the pubis is a frequently described condition that is distinguished by its noninfectious nature and unknown cause. Osteitis pubis has occurred after trauma in athletes [3]; after gynecological or urological surgery [4]; and in pregnant women or women after childbirth [5].

The characteristic radiographic findings of osteitis pubis include a widespread, uneven articular line and subchondral sclerosis. During pregnancy, ligamentary weakness caused by increased movement of the symphysis joint surfaces can lead to this condition. Osteitis pubis may be caused by microtrauma in athletes, most commonly in soccer (rugby) players and athletes, who strain stabilizing ligaments during recurring micromovements.

Because the thigh adductor muscles adhere to the upper branch of the pubic bone, the patient experiences pain in the inner thigh.

Some authors argue that weak circulation caused by damage to the frontal part of the venous plexus leads to thrombosis and circular obstruction, thus causing osteitis pubis.

Osteomyelitis of the pubic bone is caused by bacteria and most often occurs after gynecological, urological [6] or surgical intervention. It has

been described after the implantation of devices to treat incontinence and after suprapubic and inguinal hernia surgeries.

Osteomyelitis of the pubic bone has been described in hemodialysis patients with compromised immune systems when frequent intravenous catheter treatment leads a metastatic infection.

This phenomenon has also been described during urological surgery of the bladder neck and prostate [7, 8], after prolonged use of a catheter, after cardiac catheterization, during pregnancy and after birth [9].

Osteomyelitis pubis has been described after radiotherapy for carcinoma of the bladder and cervix, after kidney transplantation, and in association with excessive use of intravenous drugs, and a case of spontaneous osteomyelitis pubis caused by Pseudomonas aeruginosa has now been described.

The infectious causes of osteomyelitis pubis, as determined by a microbiological examination of the inflammation, include the following: Mycobacterium tuberculosis, Klebsiella pneumoniae, Salmonella indiana, Brucella, Psudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus similans, Proteus mirabilis, Pneumococcus, and Candida albicans [10].

Conclusion

Osteomyelitis of the pubic symphysis is a rare disease that is essentially a bacterial infection caused either by injury to the symphysis or by predisposing factors, such as impaired immunity or diabetes mellitus. This disease should be distinguished from osteitis pubis, but the possibility always exists that osteitis pubis is only a prelude to the much more difficult and complicated osteomyelitis pubis. Proper use of diagnostic radiography and computed tomography and histopathological and microbiological sample analysis is required for diagnosis. Treatment for this disease requires surgery and a compulsory long-term course of antibiotics based on an antibiogram.

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Left bundle branch block disappeared after coronary angiography: A case report

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Abstract

Cardiac resynchronization therapy is a main therapy for the left bundle branch block. We report on the clinical course of a patient and the disappearance of left bundle branch block after the coronary angiography despite withholding cardiac resynchronization therapy.

Key words: Left bundle branch block, coronary angiography.

Case report

A 79-year-old woman was referred to the cardiologist because of dyspnea for 5 months and was hospitalized in March 27th, 2012. She had a history of hypertension for 20 years. At her first visit to the outpatient department，the electrocardiogram (ECG) showed a complete left bundle branch block. When she was admitted to the inpatient department, the ECG showed a complete left bundle branch block (Figures 1-4), Chest radiography showed an enlarged heart shadow, mild multiple valve regurgitation and diminished cardiac function. Blood electrolytes showed: sodium 146mmol/l，potassium 3.2mmol/l, calcium 1.92mmol/l, N-terminal pro-brain natriuretic peptide 21328pg/ml. The patient's symptoms were improved after received standard heart failure therapy and potassium supplement. We planned to implant CRT device to correct the left bundle branch block. Before the implantation, we gave her coronary angiography to exclude coronary heart disease.

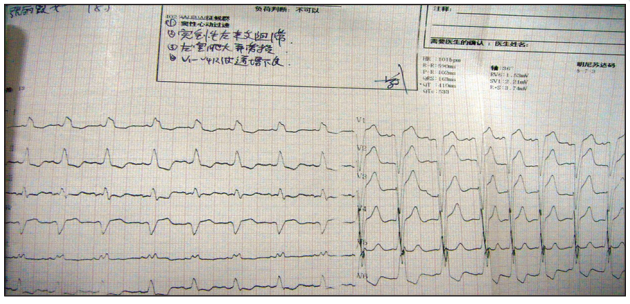

Figure 1. The ECG before the coronary angiography

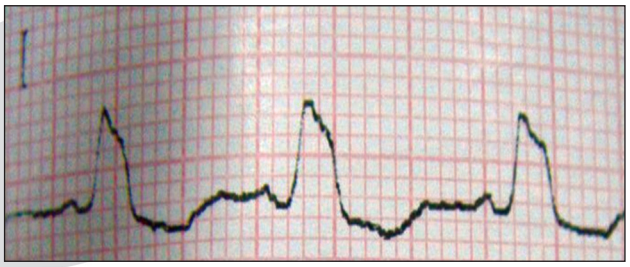

Figure 2. Lead I before the angiography

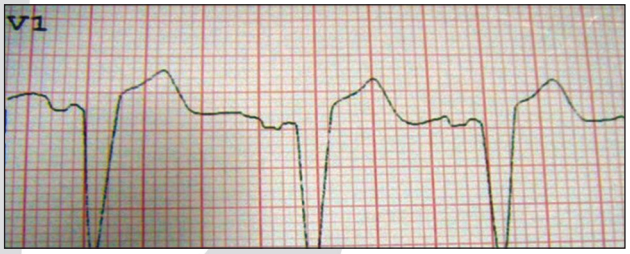

Figure 3. Lead v1 before the angiography

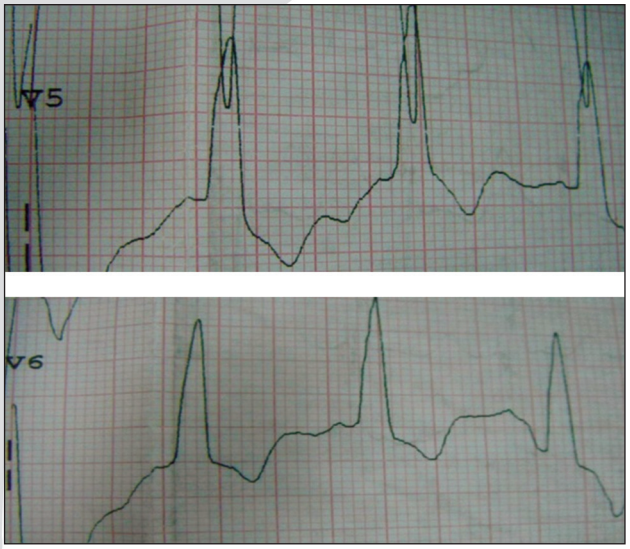

Figure 4. Lead v5,v6 before the angiography

The coronary angiography has been finished on April 1st, 2012 . The angiography showed that the patients' coronary distribution is right dominant, which satisfied coronary heart disease. Most lesions involved diagonal branch artery: plaques were seen; 90% stenosis was detected at the opening of D1 group and 80% stenosis was detected at the

proximal; 60% stenosis was detected at the opening of D2 group. Shortly after the angiography, the ECG (Figures 5-7) showed the left ventricular hypertrophy and the abnormality of ST-T segment. The left bundle branch block disappeared and the QRS duration was 115ms. The ECG in the next day also revealed no left bundle branch block. In the end, the patient's clinical symptoms improved remarkably with drug therapy. She left hospital without the CRT device implantation.

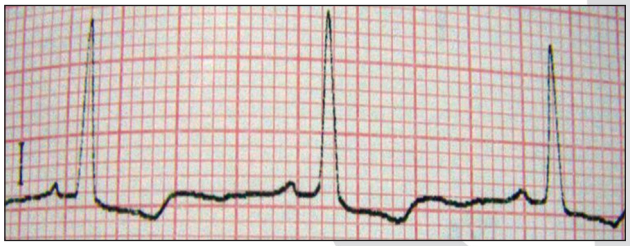

Figure 5. Lead I after the angiography

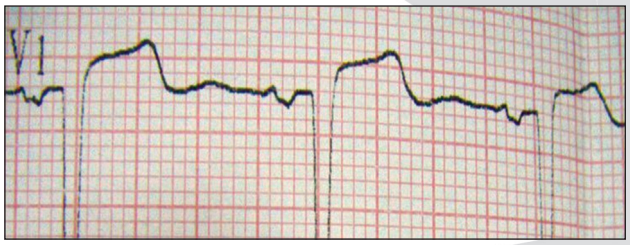

Figure 6. Lead v1 after the angiograp

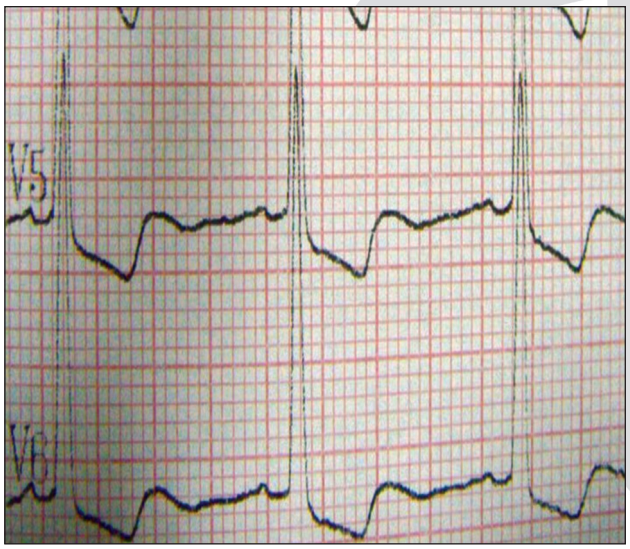

Figure 7. Lead v5,v6 after the angiography

Discussion

Left bundle branch is difficult to block. Most cases associate with organic abnormality. In this case, there are following possibilities that lead the disappearance of left bundle branch block after the angiography.

First, left bundle branch block can be the result of branch ischemia, therefore, the disappearance of bundle branch block could be contributed by the recovery of blood supply caused by coronary angiography. In general, left posterior branch blood is supplied by both left anterior descending artery and right posterior descending artery. Left anterior branch and septal branch are mainly supplied by left anterior descending artery and septal artery of left coronary artery. Sporadic plaques were seen during the whole way of the patient's anterior descending artery and its most lesions involved diagonal branch artery. Thus, probably, on the one hand, the patient's left bundle branch is mainly supplied by anterior descending artery or its main branch-diagonal branch artery; On the other hand, coronary angiography improves all the artery supply so that cure the left bundle branch block indirectly.

It has been described that the ECG of the patient with left bundle branch block complicated with coronary heart disease recovers after the Coronary Artery Bypass Grafting.[1] In this case, the patient's left bundle branch block is associated with the nutrient supply disruption caused by coronary lesions, so that the obstacle of the nerve electric stimulation conduction and metabolism abnormality occurs. There is another case: the left bundle branch injury was reversible in the patient with acute inferior myocardial infarction.[2] In that case, the complete left bundle branch block disappeared after the right percutaneous transluminal coronary angioplasty.

Second, it is possible that the left bundle branch block found before the angiography is paroxysmal. It stopped after the angiography immediately. Since we did not do the 24h dynamic electrocardiogram, it is very likely to record twice left bundle branch block. In addition, our ECG was not performed instantly before the angiography.

Finally, as the patient's symptoms of the heart failure is controlled after the therapy of ACE inhibitor, diuretic and digitalis. The ventricular pressure could somewhat be lower so that it weakens the strength to stretching the branch. The conductive function of the branch might be recovered before the angiography. Up to now, we have not found

corresponding articles that report heart failure could induce left bundle branch block. Contrarily, it is described that reversible left bundle branch block induced congestive heart failure.[3] According to this theory, it is very likely that in this case, as the left bundle branch block disappears, her heart function improves accordingly and her symptoms ameliorates.

The planned CRT implantation canceled terminally for this patient, which prevented her from operative pain and economic burden. This case illustrates that coronary angiography affects curing bundle branch block. Further, for the complete bundle branch block patients with the coronary heart disease, shall we recommend the etiological treatment like the stent implantation or the bypass grafting before the CRT?

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Late cardiac complications after combined modality therapy for Hodgkin's lymphoma

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Abstract

Combined modality therapy (chemo plus radiotherapy) was curative treatment for Hodgkin's lymphoma during the past 40 years. However, combined modality approach were associated with late sequelae among long-term survivors, including second cancers, heart, lung disease, and thyreoid dysfunction.

Case report: We present a case of a 54-year-old man who develop three serious complications, coronary heart disease, mitral valve regurgitation and cardiomyopaty, 11 year after combined modality therapy for Hodgkin's lymphoma. The role of mediastinal radiotherapy plus antracycline based chemotherapy in developing cardiovascular complications, is discussed.

Conclusion: Given the possibility of therapy-induced serious late cardiac sequelae recommended life-long cardiological follow-up of Hodgkin's lymphoma survivors.

Key words: Cardiac complications, combined modality therapy, coronary artery disease, valvular disease, cardiomyopathy, Hodgkin's lymphoma.

Introduction

Hodgkin lymphoma (HL) is one of the most curable malignancy in adult because of its sensitivity to modality treatment. The radiotherapy (RT) still has a role in the treatment early and advanced HL (1). More precisely, combined modality therapy is a standard treatment within limited and intermediate stage HL patients. In advanced disease RT is confined to patients having large residual posthemotherapy masses (2). The radiation field included normal organs, such as breast tissue, heart, lung and thyroid, and coronary arteries, which were at risk for long-term complications. Therefore, the long-term survivors of the lymphoma are at significantly increased risk for many late complications. The most serious late effects faced by these patients include the development of a second malignancy or cardiovascular disease (3) .

Late antracycline-induced carddiomyopathy usually appears within 1 years to decades after chemotherapy (4). The principal risk factor for anthracycline-induced cardiomyopathy is highly dependent on cumulative doses. Cardiomyopathy and congestive heart failure, which are late antracycline cardiac effects usually occurring after doses greater than 550 mg/m^2.

Radiation-induced cardiotoxicity is usually observed 10 - 15 years after RT, sometimes later. The risk is particularly high in patients treated before the age of 40 years. Also the risk of cardiotoxicity increases when greater than 65% of the heart is irradiated. Combined modality abroach, successive application antracycline and radiotherapy is associated with increased cardiac injury (4).

The most common heart pathology is pericarditis, ischaemic heart disease, arrhythmias, congestive heart failure due to cardiomyopathy, and valvular heart disease. Radiotherapy to the thorax induces damage to the endothelium of the microvasculature, causing hypoperfusion or ischaemia. The cause of valvular fibrosis is still unknown. As to the toxicity of chemotherapy, anthracycline causes damage to the myoepithelium, strongly dependent on the cumulative dose. The risk of myocardial infarction, angina pectoris, congestive heart failure and valvular disorders is increased up to sevenfold in survivors of Hodgkin's lymphoma (5).

The ischaemic heart disease increase rapidly 10-15 years after radiotherapy, and its incidence is increased by the usual risk factors, such as smoking, hypertension, obesity or diabetes (6) . Gaya et

al. believes that the cardiovascular complications are probably under-reported, as they occur long after cured patients have been discharged from follow-up (7). This report presented a 54-year-old man who over the years developed three serious pathology of the heart 11 year after combined modality treatment for HL.

Case report

We report the case of a 54-year-old man with a history of coronary artery disease, heart failure, was admitted to hospital in September 2011, for planned mitral valve replacement.

In 1998, Hodgkin's lymphoma of nodular sclerosis subtype in III B clinical stage in the patient were discovered. He was a non-smoker, had a normal lipid profile, no history of heart disease and presented no risk factors for coronary artery disease.

Clinical, laboratory and imaging examination excluded any comorbidity. Cardiac evaluation consisted of physical examination, ECG, chest x-ray, and echocardiography. The size od left ventricle was normal with EF 60%. Combined modality treatment consisting of 6 cycle ABVD (Adriablastin, Bleomycin, Vinblastin, Dacarbazine) followed by extended field radiotherapy, were aplied. The applied cumulative antracycline dose was 470 mg/m^2. With chemiotherapy patient achieved a partial remission with no evident cardiac injury. Radiotherapy was delivered in fractional doses of 1.8 Gy 5 days per week, with a total dose of 36.0 Gy to the mediastinum. A radiation field was covered about 65% of cardiac shadow. With combined modality treatment after one year, patient entered complete remission.

Before three years first symptoms began like chest pain described as unstable angina pectoris and soon after that the patient had myocardial infarction of inferoposterior location (wall), which was treated with conservative drugs-thrombolysis. After that, coronary angiography was performed which showed that existed residual stenosis of 90% in Cx artery after thrombolysis.

The implantation of one metallic stent in the Cx artery was performed and an excellent angiographic result was achieved. During the same hospitalization an echocardiographic exam was done and showed the scar of posterior left ventricular wall.

Four months later due to frequent occurrence of chest pain coronary angiography was redone. There was seen in stent restenosis at the site of previously implanted stent and the balloon dilatation (POBA) on Cx artery was done as well as implanting of another drug eluting stent in the distal segment of the LAD stenosis, which was described by 80%. Another described bifurcation stenosis of 60% in proximal LAD-D1 artery was left for later treatment.

Control echocardiogrphy exam done in our hospital pointed out to significant mitral regurgitation and akinesia of basal inferior wall and hypokinesia of basal and medial part of posterior wall. Left ventricle was of borderline size, reduced overall systolic function around 40%.

Due to maintenance of persisting symptoms and postinfarction unstable angina pectoris in september 2009. heart surgical revascularization was made with implantation of triple coronary artery venous bypass and mitral valve repair.

Postoperative echo exam revealed the existence of residual mitral regurgitation grade 2-3 +, and the left ventricle size was borderline with EF 40%. Few months later the patient was hospitalized again for congestive heart failure provoked by cardiac arrhythmia. Then echo exam showed dilated ischemic cardiomyopathy with significant mitral regurgitation, EF 25%.

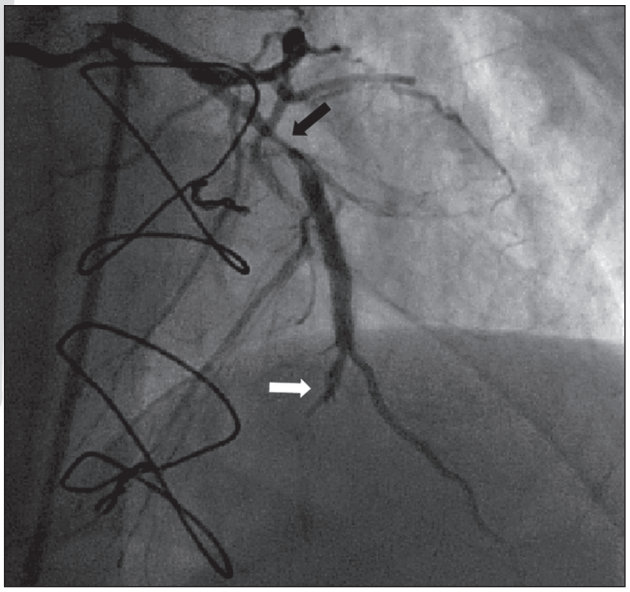

Figure 1. Coronary angiography showed stenosis LAD (50-70%) in the proximal segment (black arrow). Passable graft in the medial segment (white arrow)

Patient got a new drug therapy and next 15 months had no symptoms. In mid 2011 year permanent chest pain independent of effort appeared again. Repeated coronary angiography revealed stenosis LAD in the proximal segment (Figure 1) and occlusion of RCA in the proximal segment (Figure 2). Then echocardiography exam showed severe grade 4+ mitral regurgitation (Figure 3), and the surgical mitral valve replacement was indicated this would mean a second high-risk operation on a patient with massive mediastinal fibrosis. At operation patient died due to cardiac arrest. Histopathology of mitral valves showed fibroid degenerative changes with calcifications.

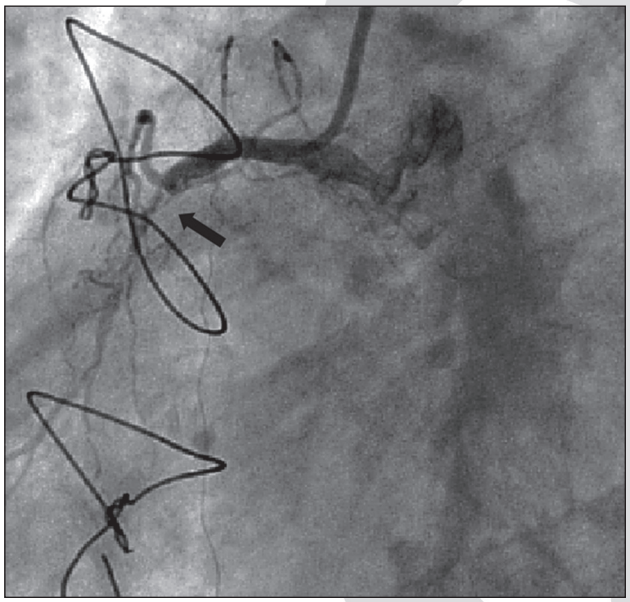

Figure 2. Coronary angiography showed occlusion of RCA in the proximal segment (black arrow)

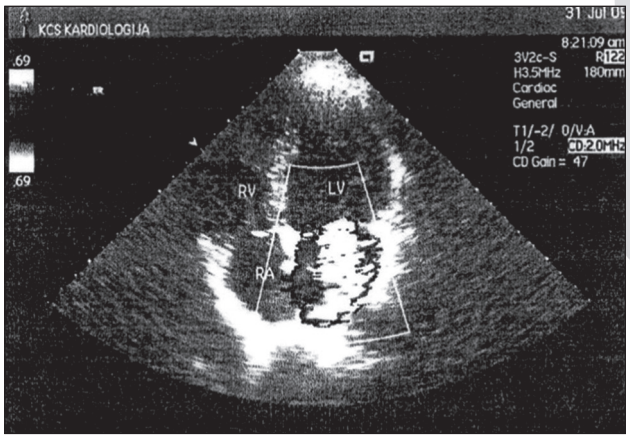

Figure 3. Echocardiography showed significant mitral regurgitation and akinesia of basal inferior wall and hypokinesia of basal and medial part of posterior wall

Discussion

HL is a malignancy that primarily affects adolescents and young adults. With combined modality treatment 80-85% of HL patients achieve long term remission and can be considered cured. Owing to the patients young age at diagnosis and the high curability of HL, survivors are at high risk for the development of chronic health conditions as a consequence of their treatment. In particular, increased risk of cardiac disease and cardiac death are important late sequelae of treatment caused by combined modality therapy (due to both radiation therapy and the use of anthracyclines) (5,8). Cardiotoxicity following chemotherapy is mainly associated with the use of anthracycline. This drug induced damage to the myoepithelium and cardiotoxicity is strongly associated with the cumulative dose, occurring after doses greater than 550 mg/m^2 (4).

Approximately 50% of HL patients have mediastinal disease and may require mediastinal RT, with exposure of cardiac structures to radiation. In fact, mediastinal radiotherapy for HL can cause damage to different anatomic structures of the thorax wall, lung, oesophagus and the heart.

Postiradiation heart diseases affecting the coronary arteries, pericardium, myocardium, conduction system, and myocardial valves (5,7,9,10,11). Pericarditis, angina, myocardial infarction and arrhythmias are the most frequent causes of morbidity, with myocardial infarction being the most common fatal complication (6) .

Most of the literature relates to the treatment of HL, as patients with this disease tend to be young and live long enough to manifest late cardiac complications, with ischaemic heart disease being the most common. According to the study Gaya et al. the incidence of ischaemic heart disease does not increase rapidly until 10 years from RT (7).

The incidence of valvular dysfunction has been reported to increase during the second decade after mediastinal radiotherapy for Hodgkin's lymphoma. After 20 years, 6–15% of HL patients have clinically important, moderate or severe valvular regurgitation in the aortic or mitral valve, and a few present with aortic stenosis (9) . Sclerotic lesions and calcification are typical changes of the cardiac valves in aproximately 37% of patients with previous mediastinal radiotherapy (3).

Progression of coronary arteries disease and valve fibrosis may contribute to progressive deterioration of cardiac valve function, with progression to heart failure and death.

Crestanello et al. reported 22 patients underwent mitral and tricuspid valve repar with previous mediastinal RT. During the time, severe dysfunction of the repaired valve developed in 32% of survivors, and 16% required further surgery. Therefore, they suggested valve replacement might be prefereble in the patients after mediastinal RT because of the limited durability of repairs (12). This approach is confirmed by the events shown with our patient who severe disfunction of the repaired valve developed one year leiter.

Herquet and coworkers observed that out of 141 lymphoma patients treated using anthracycline-based chemotherapy, only one after many years developed clinical cardiomyopathy (4).

Several studies has been reported increased mortality of cardiac disease after antracycline based chemiotherapy plus mediastinal RT for HL (4,8). Our patient had mediastinal disease and 10 years after combined therapy develop three cardiac complications whose result was a progressive heart failure. Complete remission HL has existed for 12 years, but coronary heart disease (unstable angina pectoris, myocardial infarction), congestive heart failure due to cardiomyopathy, clinically important valvular regurgitation, were life-threatening.

Aleman et covorcers in the study on 1474 survivors of Hodgkin lymphoma concluded that anthracyclines further increase the elevated risks of congestive heart failure and valvular disorders from mediastinal radiotherapy. Development of cardiomyopathy in our patient contributed to the Doxorubicin, which was applied to the cumulative doses of 470 mg/m^2 . This pathological conditions had in our patient a long latency and therefore are present many years after treatment of HL.

Improving disease control while reducing the toxicity of treatment has been a major objective of HL trials for more than two last decades (13).

Galper et al. have shown that the diagnosis of cardiac complications and the use of cardiac procedures occurs 10 years or greater after mediastinal irradiation. This suggests that these effects are slow to develop and may be partially preventable with the control of other cardiac risk factors in the time before the development of overt cardiac disease. The type of screening, the need to repeat it and at what interval, and whether all irradiated patients should be screened needs to be determined, they are considered (14). The Netherlands study group suggest the next interval in screening program for radiotherapy induced cardiovascular disease in HL survivors. Patients should be screened for coronary artery disease and cardiomyopathy starting 5 years, for valvular disorders 10 years, after combined modality therapy (15,16) .

Given the progressive nature of valvular dysfunction and left ventricular remodelling 20 years after treatment, Wethal et al. recommend life-long cardiological follow-up of HL patients treated with combined modality therapy (3).

Therapy guidelines with involved field RT was applied in EORTC – GELA (Group d'Etude de Lymphomes Adultes) randomised trial (13). New treatment strategies combining chemotherapy with involved field RT have only recently been proposed by the ESMO Guidelines Working Group for Hodgkin's lymphoma (2,16). Although the new radiation strategy with radiation involved field used in the past few years, HL patients treated with extensive radiation fields will still present for cardiac surgery in the coming years.

Conclusion

Cardiovascular complications induced with *combined* modality therapy progress slowly. That is very important to conduct long-term follow-up of patients with mediastinal irradiation and combination chemotherapy including antracycline. In fact, continued long-term follow-up of survivors, and preventative therapy for specific late cardiotoxicity are important steps in improving the survival and quality of life of patients who have been cured of HL.

Accurate prognostic factors are needed for identification of those patients that have a good prognosis and might be susceptible to overtreatment and therefore provide the strategies that will minimize the number of anthracycline based chemotherapy cycles, effective dose reduction to cardiac structures and decrease late toxicities without compromising the good results of overall survival.

Acknowledgments

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Management of end-stage liver disease with complicated large incisional hernia and refractory ascites: Transjugular intrahepatic portosystemic shunt

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Abstract

Incisional hernia is one of the common complications after surgery in patients with end-stage liver disease (ESLD). However, most ESLD patients with refractory ascites do not qualify for hernia repair. We report the first case of applying transjugular intrahepatic portosystemic shunt in an ESLD patient with a large complicated incisional hernia. This procedure may be a prudent treatment option for incisional hernias in ESLD patients.

Key words: Incisional hernia, ESLD, cirrhosis, portal hypertension-complication, TIPS.

Introduction

Incisional hernia is a common complication after laparotomy, with its incidence varying from 3% to 13% [1]. Open and laparoscopic surgical repairs are good methods for patients with clear symptoms [2][1]. However, patients with cirrhosis carry a significant risk of adverse outcomes after abdominal wall hernia repair compared with those without cirrhosis, particularly those who have undergone emergent surgery [3][2]. Transjugular intrahepatic portosystemic shunt (TIPS) is known to be an effective interventional procedure for the treatment of complications related to end-stage liver disease (ESLD), such as refractory ascites and variceal bleeding [4]. Recent research has shown that TIPS may become the procedure of choice for ESLD patients with complicated umbilical hernias and medically refractory ascites [5]. The aim of this study was to explore TIPS as a novel approach for ESLD patients with complicated incisional hernias.

Case report

In September 2011, a 45-year-old woman with a history of hepatitis B and liver cirrhosis was admitted to our unit because of a large abdominal mass and hematemesis. The patient experienced variceal bleeding and ascites 4 years prior to admission, for which she was treated with splenic artery ligation. Three years before being admitted to the hospital, the patient noticed a retractable mass on the mid-abdominal line that was gradually increasing until it became unable to retract. The patient had no signs of abdominal pain, distention or any other symptom of intestinal obstruction. However, the non-pulsatile and non-tender mass was prominent whenever she was in an upright position, thereby significantly affecting her daily life. Drug therapy resulted in little symptomatic improvement. At her physical examination, a 20-cm-long surgical scar in her mid-abdominal line and a large mass measuring 25 cm × 18 cm × 12 cm over the left abdominal wall were observed (Figure 1A). The complete blood count test revealed an RBC count of 1.73×10^{12}/L and a hemoglobin count of 42 g/L. Her liver functions were normal except for the albumin concentration, which was determined to be 29.6 g/L. The AFP concentration was 255.7 ng/mL. The ascites was transudate, and no tumor cell was found. Abdominal CT with contrast revealed hepatocellular carcinoma, liver cirrhosis, large-volume ascites and a massive incisional hernia (Figure 2).

The surgeons from our department concluded that the patient was not suitable for both laparoscopic and open repairs of the hernia because of her liver disease. In addition, the patient was unwilling to accept HCC treatment due to personal reasons.

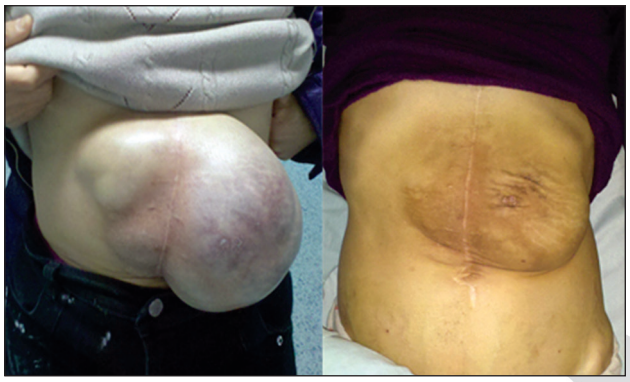

Figure 1. Fronterior view of incisional hernia before and after TIPS. 1A:incisional hernia with the patient in a upright position before TIPS. 1B:incisional hernia three days after TIPS.

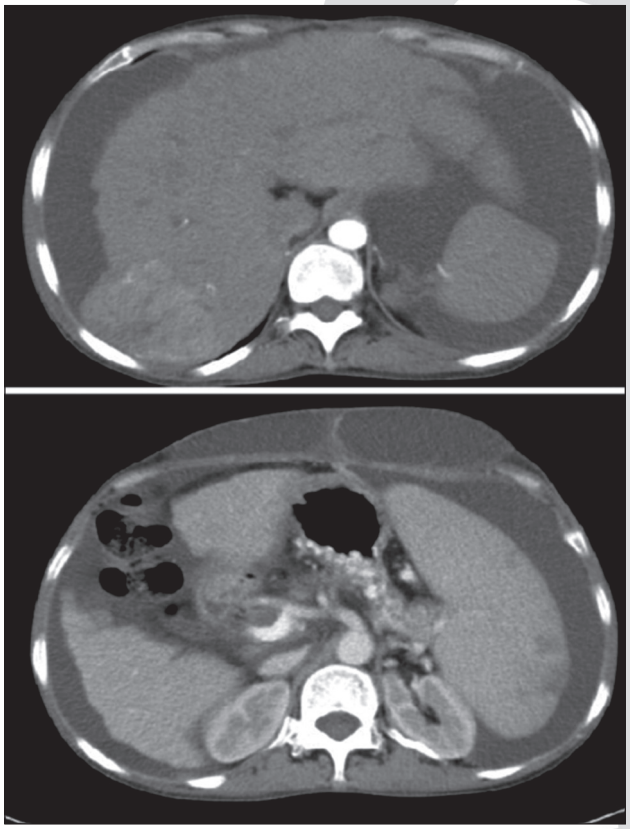

Figure 2. Abdominal contrast-enhanced CT images. 2A:CT in the arterial phase demonstrates a high attenuation region in the location of segment VII. 2B:CT in the portal phase showing incisional hernia and signs of portal hypertension.

Finally, TIPS was undertaken with the patient's consent to prevent variceal bleeding and to treat the hernia. During the procedure, hepatic artery angiography demonstrated a typical HCC manifestation in the VII segment of the liver (Figure 3A), thus confirming the CT findings. The shunt was created from the middle hepatic vein to the intrahepatic branch of the portal vein (Figure 3B). The intrahepatic channel was then dilated by an 8 mm × 60 mm balloon (Cordis Europa N.V., Roden, The Netherlands), and a 10 mm × 70 mm wall stent (Boston Scientific, Galway, Ireland) was deployed into the channel. Finally, the portal pressure gradient was reduced from 31 mmHg before TIPS to 14 mmHg after the procedure. The entire procedure lasted for approximately 1 h, with the patient being exposed to the radiation dose of 620 mGy.

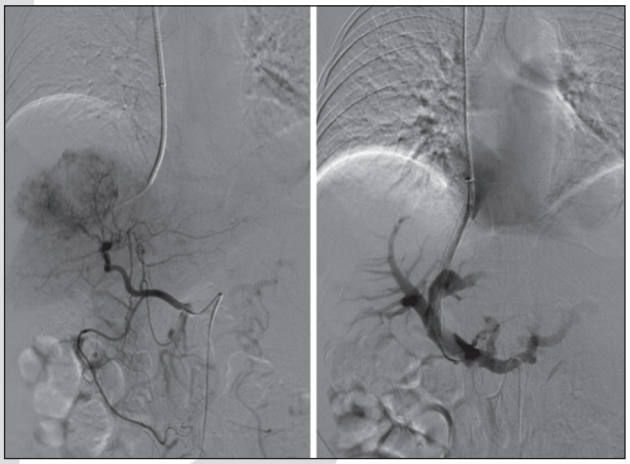

Figure 3. Hepatic artery angiography and portal venography. 3A:Selective hepatic artery angiography confirming hepatocelluar carcinoma. 3B:Decrease in the pressure within the portal vein immediately after TIPS

After TIPS implant, the patient's urine volume increased from 900 to 3000 mL/day; the hernia started to deflate 3 days later (Figure 1B). Through a telephone follow-up in March 2012, the patient declared that the hernia had significantly improved compared with its preoperative state but said she felt uncomfortable in the right upper abdominal area. The patient died of liver failure from HCC in May 2012.

Discussion

The literature provides scant data about patients with cirrhotic liver disease and incisional hernias. To our knowledge, this is the first report on the application of TIPS in an ESLD patient with an incisional hernia. Research has shown that increased intra-abdominal pressure associated with ascites and attenuation of abdominal wall fascia

and muscular structure lead to the formation of hernias [6][3]. Untreated abdominal wall defects in cirrhotic patients may grow into an immense size, often developing pressure that may cause necrosis of the overlying skin, skin breakdown, ascetic leak and, potentially, bacterial peritonitis. Optimal management of cirrhotic patients with incisional hernias and refractory ascites remains unclear. A small hernia without any symptoms may be treated with a conservative management plan.

A recent meta-analysis indicated that both open and laparoscopic approaches have a similar incisional hernia recurrence rate but that the laparoscopic approach is associated with fewer wound infections and complications, a shorter hospital stay and less frequent postoperative infections [7,8]. Moreover, the use of mesh has significantly reduced the recurrence rate of incisional hernias, which ranges from 2% to 36% after the application of mesh repair, compared with 12%–54% after suturing [7][4]. Incisional hernia repair cannot be completely considered as a low-risk procedure. Major complications may still occur after the repair of a large incisional hernia, such as mesh infection and enterocutaneous fistula [1][5]. Circumstances surrounding herniorrhaphy can certainly affect the outcome of repair. If ESLD patients have poor liver function and carry a high risk of adverse outcomes after an operative intervention, they are expected to be of high operative risk and to have a high incisional hernia recurrence rate after the repair. At the same time, refractory ascites poses a unique dilemma to the operative management of an abdominal wall hernia, because inadequate control of the ascites increases the rate of wound complications and recurrence of the hernia. Preoperative TIPS in conjunction with semi-elective repair appears feasible, particularly for patients with spontaneous umbilical rupture, and postoperative TIPS placement effectively controls the ascites without additional complications [9][6]. In this case, the hernia deflates and, more importantly, quality of life improves after the creation of the shunt. ESLD patients with contraindications for operation can also benefit from TIPS. Data regarding optimal management choices and long-term effects are limited. We have explored the feasibility of applying TIPS to treat an ESLD patient with a large incisional hernia and determined the procedure to be a prudent treatment option.

Acknowledgement

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Endovascular repair of subclavian artery pseudoaneurysm occurring after axillobifemoral bypass (case report)

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Abstract

Case presentation: In our article we present a relatively rare occuring complication of a vascular surgical method and an uncommonly used treatment for it. In the presented case we used an aorto-bifemoral bypass on a patient with Leriche syndrome. The implanted Y-graft had gotten infected and we were forced to remove it. After suturing the abdominal aorta, we implanted an axillobifemoral bypass to secure the circulation of the lower limbs. The graft occluded later, but 37 months after the surgery a rather large pseudoaneurysm appeared at the origin of the graft in the right subclavian artery. Another surgery was necessary to prevent embolisation, rupture and compression. Instead of the conventional surgical method (resection, interposition) we did an endovascular surgery. We excluded the false aneurysm by inserting a covered stent, using catheter technique, into the right brachial artery and therefore prevented the before mentioned complications.

Conclusions: This new minimaly invasive method is very useful for high risk patients to prevent the injury of the other anatomical structures in this region and also to minimize the blood loss and complications of prolonged anesthesia used in open surgeries.

Key words: Aortic graft infection, axillo-bifemoral bypass, false aneurysm, covered stent.

Introduction

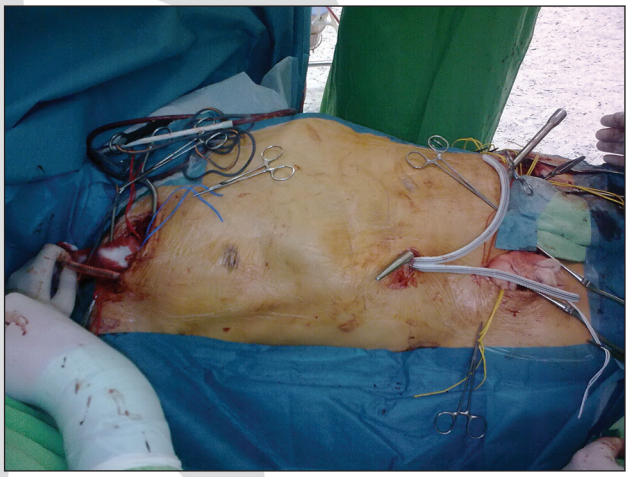
The search for minimal invasivity was not left out in the field of vascular surgery either. In 1974 Andreas Grüntzig performed the first percutaneous transluminal angioplasty on a superficial femoral artery (1). One year later Julio Palmaz reported in his article about the use of a stent to prevent restenosis (2). In 1991 Parodi was performing an exclusion of an infrarenal aortic aneurysm via stentgraft implantation using catheter technique, which has become a routine procedure in specific medical centers in our country as well (3,4,5). The endovascular methods present a lower stress for patients with comorbidities. The operation time and degree of exploration are lowered substantially, the blood loss is kept to a minimum, rehabilitation time shortened.

Case report

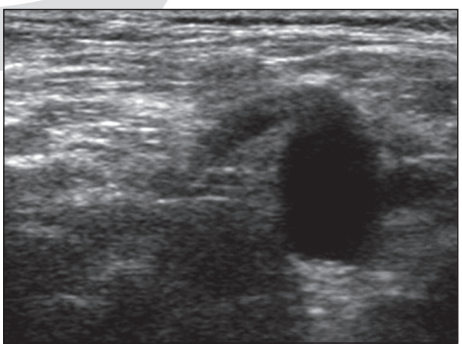
Diagnostics

We performed an aorto-bifemoral bypass on a 72-year-old man suffering from Leriche Syndrome in december 2007. The patient history contained smoking, hypertension and ischaemic heart disease. The superficial femoral arteries were occluded, therefore the distal anastomoses were made on the profound arteries. After 3 days of intensive care we discharged the patient on the 7th day of hospitalization. We removed the sutures on the 12th day. The patient had no fever. His wounds were healing per primam intentionem. His walking distance increased to 300 m. The patient came in for a control check up on the 12th week after the operation, when we immediately admited him with high fever and pus leaking in both inguinal regions. His lab results showed high leukocytosis, highly elevated CRP and septic anaemia. After admission we immediately opened up his wounds again. The inflammation proceeded in the deep regions including both grafts. We took microbiological samples, after which we started him on empiric clindamycin 3x600 mg iv. The bacteriological results showed a growth of staphylococcus aureus, which was susceptible to our treatment. During the operation we performed a debridement of the wounds and left a suck-flush drain behind. The CT scan made the next day showed a severe perigraft reac-

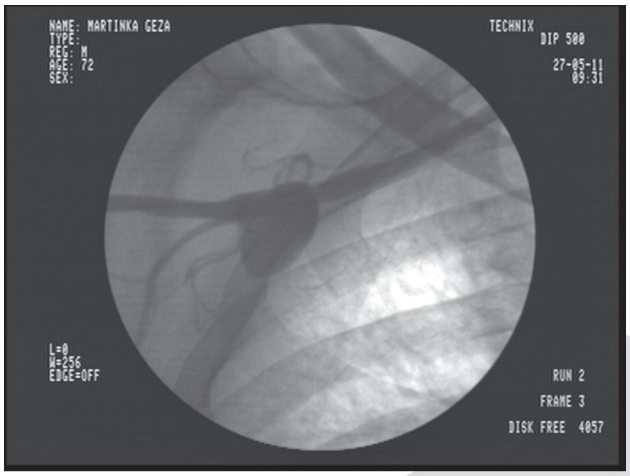
tion, which was obviously caused by the pus of the Y-graft. After a short preparation we performed a laparotomy. We searched for the functioning graft in the retroperitoneum and it was microscopically septic as well. After injecting one and a half ml Na-Heparin intravenously we managed and infrarenal exclusion and took the prosthetic graft out without causing a major bleeding. We made the suture on the terminal aorta with a 4/0 Prolen suture line. We flushed the pelvis a few times with a mixture of betadine and physiological saline solution. Around the aortic suture we placed an omentum lobe with a handle on it. After draining and closing up the abdominal cavity we opened up the distal anastomoses aswell and therefore extracted the entire infected graft. After that we looked for the subclavian-axillar border underneath the collar bone. Here we placed the proximal anastomosis of the axillo-bifemoral bypass. Finally the distal parts of the graft have been tunneled lateral to the anterior superior iliac spine, done by going lateral to the sartorius and anastomosed to the profunda well beyond the previous repair (picture 1). After a 2 week care the patient left the hospital cured. 3 months later he was readmitted because of an abscess above the left subumbilical branch of the graft, which included the left inguinal region as well. We extracted that graft part and secured circulation of the left limb with an axillo-femoral silver graft on the left side. This way the lower limb circulation was obtained on both sides with two axillo-profound bypasses. The patient had no symptoms for one and a half years. After that the distal part of the right axillo-femoral graft branch became septic and closed up. We extracted the lower 2/3 of the graft and performed an overbridge from left to right with a femoro-femoral silver cross-over. The patient had no symptoms for a long period of time. 37 months after the implantation of the right axillo-bifemoral bypass the patient complained of a pulsating growth under the right collar bone. The lab results showed no sign of infection. The ultrasound showed a 3 cm large aneurysm of the anastomosis (picture 2). The structure was anatomically in a difficult place and the risk of trauma was high (lesion of the vein or brachial plexus), so we decided to fix it by using the endovascular method. After a prophylaxis of 1g Cefazolin i.v., we prepared the brachial artery over the cubital groove. A puncture was made after the patient received 5000 NE Na-Heparin. After inserting a 9 Fr sheeth we introduced a guide wire in the subclavian artery until it passed the aneurysm. Then we made an angiogram over the sheeth using the road mapping technique. Finally we inserted a 7mmx50mm Viabahn expanded PTFE (e-PTFE) stent graft (WL Gore and Assosiates, Flagstaff, Arizona) (picture 3, 4). On the fluoroscopic image nativ X-Ray we saw the good position of the covered stent (picture 5). There was no endoleak and no aneurysm sack on the angiogram. (picture 6). We closed the puncture hole of the brachial artery with only one 6/0 Prolen nod suture. After two days the patient left the hospital cured. On the control physical exam the patient showed no symptoms. The upper limbs were pulsating well. The pulsating heamatoma was healed, the growth under the collar bone dissapeared. On the postoperative CTA there was no leaking or graft migration (picture 7).



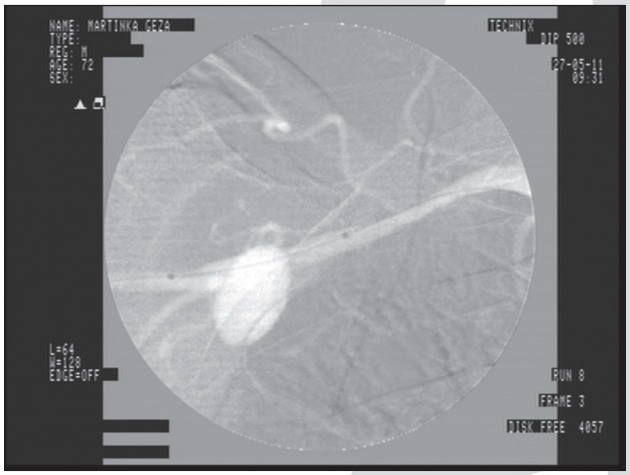
Picture 1. Axillobifemoral bypass operation



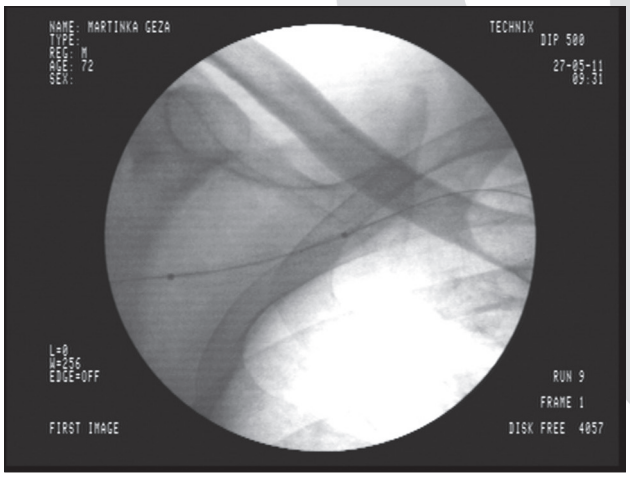
Picture 2. Ultrasound picture of the right subclavian pseudoaneurysm



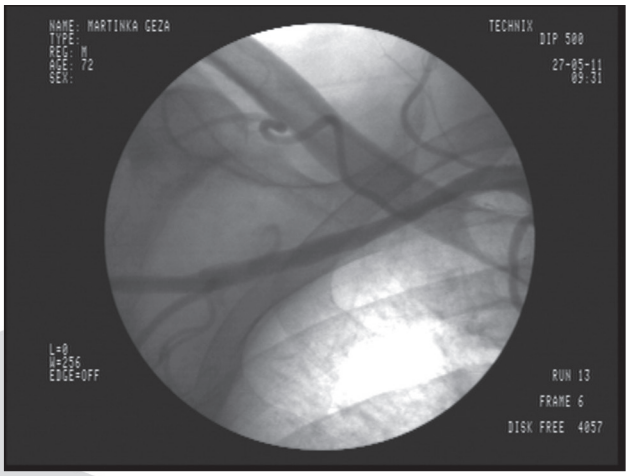
Picture 3. Intraoperative angiogram showing the pseudoaneurysm



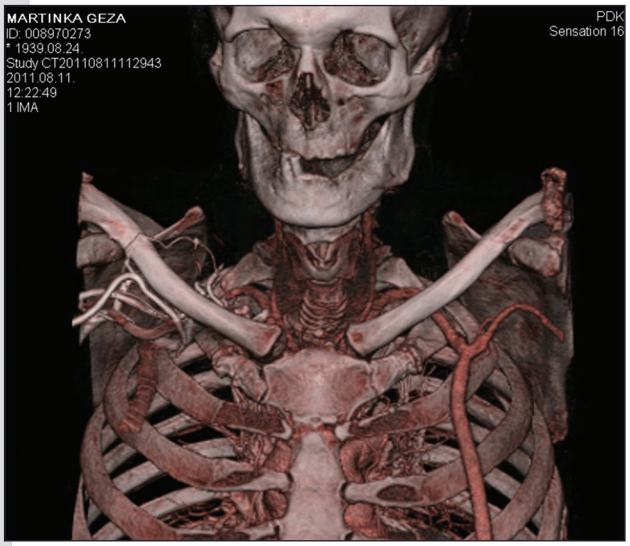
Picture 4. Position of the guide wire



Picture 5. Fluoroscopic image of the expanded covered graft



Picture 6. Intraoperative completion angiogram with the graft in good position



Picture 7. Postoperative CTA with the stentgraft in the right subclavian artery

Discussion

From literature data is known that after performing aortobifemoral bypass an infection of the graft occurs in 1-2 %. Even today the mortality rate of that complication is very high 10 to 25%. (6). There are many ways of treatment of graft infections, from conservative treatments to radical surgical interventions (7,8). For the solution of septic grafts there are local and extraanatomical treatments. For the elimination of the septic condition a radical extraction of the infected graft is necessary. The most trustworthy but also most straining local method is the reconstruction using an autologous graft using the superficial femoral veins. (9). Also an accepted method is the

in situ solution with a silver graft , but also the well known cryopreserved homograft (10). From the extraanatomical solutions the axillobifemoral bypass is the most known (11, 12). The expanded operation is not recommended for older patients, who already had multiple operations, have comorbidities, are in a septic condition because of a long lasting graft infection, are in a weak condition and/or suffer from an acute bleeding. In our case we also looked to find the optimal, minimally invasive method for the treatment of the infection of the aorto-bifemoral graft and the aneurysm of the right subclavian anastomosis that occured after the axillo-bifemoral bypass at an elderly, comorbid patient. True aneurysms of the subclavian artery are extremely rare in comparison to aneurysms of other vessels. In the literature we can usually read about iatrogenic aneurysms and their treatment on patients with degenerative connective tissue diseases like Marfan syndrome or Ehlers Danlos syndrome. Many publications talk about traumatic lesions of this region. (13). In our case we chose a Viabahn covered stent for the treatment of the pseudoaneurysm during the endovascular treatment because of the location under the collar bone. This endoprosthesis is a durable, biocompatible PTFE graft and a combination of a nitinol stent, which follows the tortuosity of the vascular system perfectly (14). During the endovascular treatment no blood transfusion was needed. In comparison to the open surgery the exclusion had no complications, no vein or nerve damage had occured. The duration and the stress ammount of the operation were lowered substentially. This method could therefore be an alternative to the classical open vascular oparation.

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The effect of the gamma knife on intracranial Rosai-Dorfman disease: A case report

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Abstract

Intracranial Rosai-Dorfman disease (RDD) is an extremely rare disease and is usually treated by surgical resection. The effectiveness of Gamma Knife for intracranial RDD has never been reported before. In this article, we present a case of intracranial RDD which received Gamma Knife treatment. The unfavourable effect revealed intracranial RDD may be insensitive to Gamma Knife.

Key words: Rosai-Dorfman disease, gamma knife.

Introduction

Rosai-Dorfman disease (RDD) is a rare non-neoplastic benign lymphoproliferative disease, and characterized by prominent painless cervical lymphadenopathy accompanied by fever and leukocytosis[1]. Intracranial RDD is extremely rare and usually treated by surgical resection [2]. The effectiveness of Gamma Knife for intracranial RDD has never reported before. Here we present a case of intracranial RDD which received Gamma Knife treatment. The imaging founding, pathology, effectiveness of Gamma Knife and current treatment are overall discussed.

Case History

A 40-year-old man presented with headache over 2 months. He had no history of focal neurological deficits. Neurological examination did not found any abnormality. Extensive laboratory workup including blood test, biochemical examination and immunological studies showed no abnormality. Magnetic resonance imaging (MRI) revealed an enhanced mass in pre-central gyri area [Figure 1A] with bone destruction in computerized tomography (CT) [Figure 2]. We recommended to total resect the mass, but the patient refused surgery and chose Gamma Knife treatment (therapeutic doses of the lesion edge isodose curve was 50%, the edge dose 800cGy split treatment interval of 12 days). 24 months after primary treatment, there was no significant change in MRI [Figure 1B]. However, the patients underwent craniotomy and total mass resection due to epileptic seizures [Figure 1C]. Postoperative pathological examination confirmed the mass as intracranial RDD. Postoperative course was uneventful and there were no recurring seizures and any other complications at 1 year follow-up after surgery.

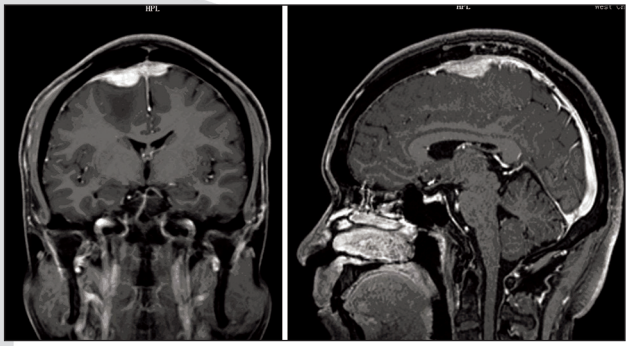

Figure 1. A MRI revealed an enhanced mass in pre-central gyri area with obvious edema and "dural tail" sign.

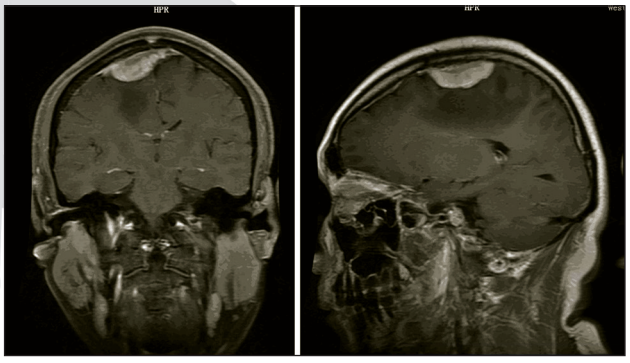

Figure 1. B MRI revealed there was no significant change of the mass at 24 months follow-up after Gamma Knife treatment.

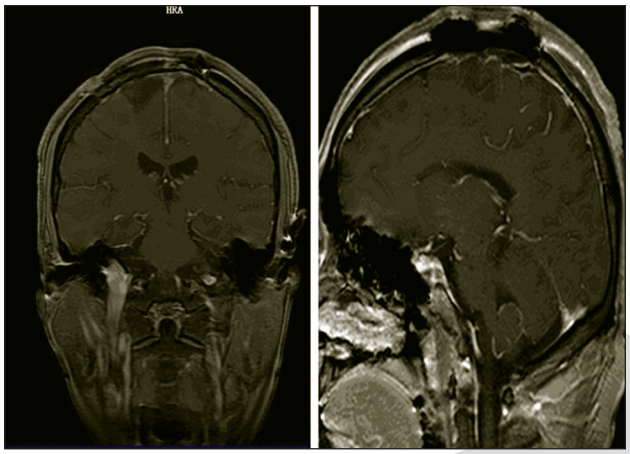

Figure 1. C The mass was totally resected.

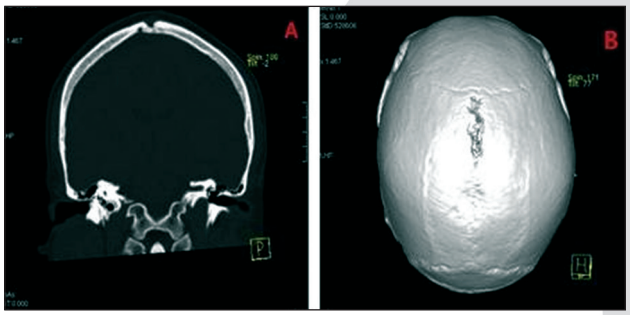

Figure 2. There was an obvious bone destruction in CT and 3D-CT reconstruction

Discussion

RDD is a rare non-neoplastic benign lymphoproliferative disease, characterized by painless bilateral cervical lymphadenopathy [3]. Primary intracranial RDD without lymphadenopathy is extremely rarely, occurs in less than 5% of RDD patients[4, 5]. As almost all of the reported intracranial RDD appear to be dural based, intracranial RDD is commonly thought to represent meningioma on neuroimaging studies[6].

The diagnosis of intracranial RDD is mainly based on pathological examination [7]. Histopathologically, RDD is characterized by a variety of chronic inflammatory cells dominated by lymphocytes and plasma cells. There are also scattered giant foamy macrophages and histiocytes engulfing lymphocytes, plasma cells and polymorphonuclear leucocytes, fibrosis and emperipolesis may occur, scattered in fibrotic background [Figure 3]. In immunohistochemical examination, RDD have positivity for S100 protein and CD68, but negativity for CD1a and EMA[Figure 4].

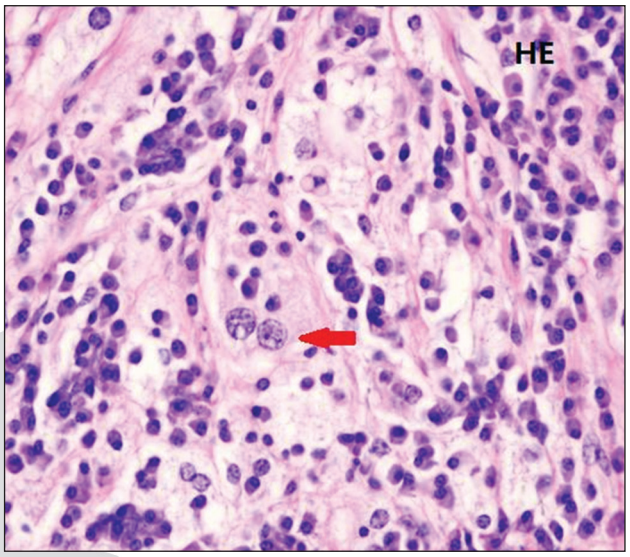

Figure 3. There were scattered giant foamy macrophages and histiocytes engulfing lymphocytes (arrow), plasma cells and polymorphonuclear leucocytes in histopathological examination.

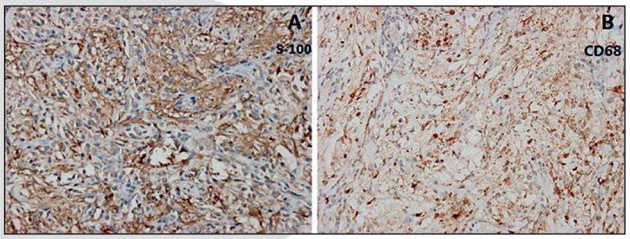

Figure 4. In immunohistochemical examination, RDD had positivity for S100 protein and CD68.

In our case, the dural based enhanced mass is also difficult to differentiate from meningioma. However, some specific features can help to identify RDD, including: 1) obvious edema [Figure 1A]; 2) hypointensity on T2 MRI compared with isointensity or hyperintensity of meningioma [Figure 5].

Surgical resection is the mainstay therapy for intracranial RDD. The hormone and radiation therapy have been reported, but the effect were uncertain[8]. The effect of Gamma Knife for this disease has never been reported before. As our patient did not have any neurological deficits and signs at first, we performed Gamma Knife treatment when he refused surgery. At 24 months after Gamma Knife treatment, epileptic seizures happened. There was no significant change in imaging. There was also no change of KI67 on immunohistochemistry in intracranial RDD after Gamma Knife [Figure 6]. We hypothesize intracranial RDD may be insensitive to Gamma Knife at current dose. But whether

Gamma Knife at other dose will take effect on intracranial RDD is indefinite. In addition, it is strange that this mass in pre-central gyri area did not cause any neurological abnormality, and there is obvious edema around intracranial RDD. As the result, the pathophysiology of intracranial RDD should also be further investigated.

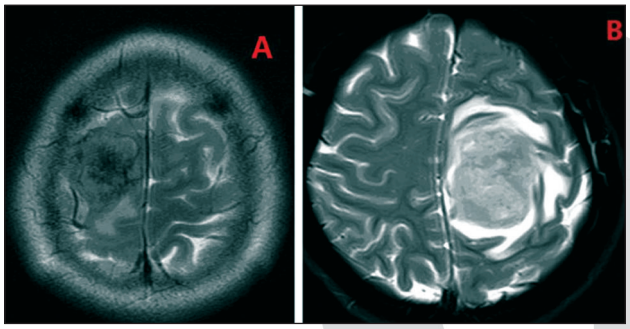

Figure 5. A RDD was hypointensity on T2 MRI; B: meningioma was hyperintensity on T2 MRI.

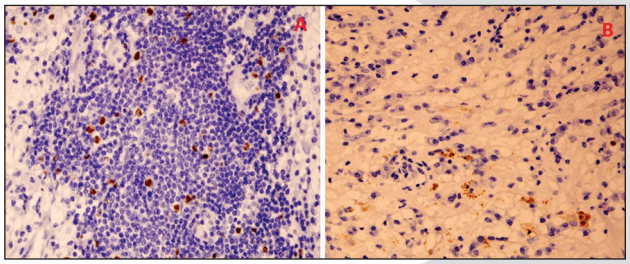

Figure 6. No change of KI67 (A: cell proliferation) and Caspase-3 (B: apoptosis) were found on immunohistochemistry.

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Laparoscopic splenectomy in patients suffering from thrombosis artery lienalis

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Abstract

Authors has decide for this case study because of specific indications for operative procedures – laparoscopic splenectomy. Laparoscopic splenoctomy is very rare made at occlusion of celiac trunk and spleen infarction, because in largest number of cases classic spleenoctomy was made, and the nature of this ailment like in this particular patient is very rare. Laparoscopic spleenectomy is specific surgical procedure and it demands good well defined indication. Developments of laparoscopy surgery and technics as well as advantages of this procedure lead surgeons to try possibilities of using this surgical technics within different spleen ailment. This case study presents patients ailment and the authors decided to perform laparoscopic spleen surgeon.

Key words: splenectomy, laparoscopic, splenic infraction.

Introduction

In last few years aproach to surgical threatment of splenic ailment is experiencing change and it's cause are well-known advantages of laparoscopic procedures compared to conventional. Laparoscopic procedures are implementing in usual surgical program, and one of them is the laparoscopic splenectomy. Due to the extreme vascular structures and characteristic anatomic relations spleen in performing this surgery the surgeon is faced with very specific problems. Advantages of laparoscopic surgery in general like minor operative trauma, shorter hospitalization and quicker return to normal activities initiated surgeons to apply this procedure on spleen ailments. 4.

Given anatomical and physiological specificity of spleen indicate that the laparoscopic technique has narrow indication area than classic surgery. The best patients for laparoscopic splenectomy are patients with idiopathic thrombocytopenic purpura (ITP), where the spleen is usually normal weight or slightly enlarged and without major anatomical anomalies (4,7). Sometimes laparoscopic splenectomy is performed within malignant spleen ailment. (5).

Case report

J.N. 26-year old patient admitted to hospital because of suspicion of splenic infarction. Amnestic dates: patient stated that she had a spontaneous abortion month ago. Lab tests results: Rbc 2,62 WBC Hgb 83,3 Hct 0,24 Plt 428 Na 134 K 3,6 Ca 2,15 Gluc 6,0 Urea 1,4 Kreat 47 Bili T 24,2 D Bili 10,2 I Bili 14,0 AST 53 ALT 39 CK 63 LDH 305 CRP 123. Koagulog. verified elevated levels of fibrinogen, and trombelastogram verified diathesis.

CT angiogram: shows occlusion on celiac axis, as well as first part of art. Lienalis. Test shows and hepatosplenomegaly and major splenic infraction.

Due to impending rupture of the spleen indication for surgery is set. Laparoscopic surgery is done under general anesthesia by the principle of laparoscopic surgery, and the spleen is removed in total by minimum median laparotomy. The postoperative course was normal.

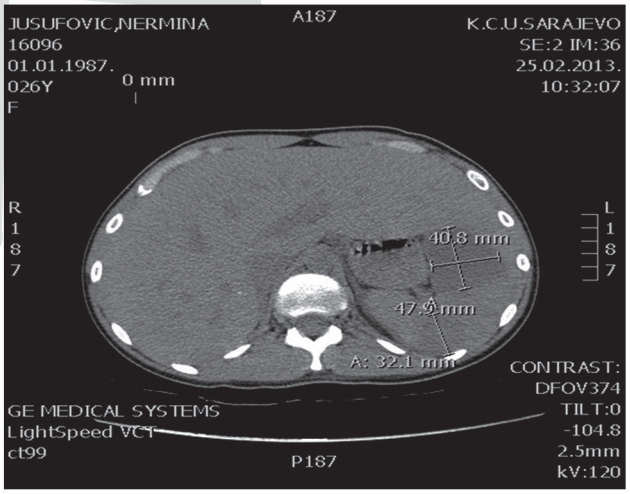

Figure 1. CT image zone splenic infarction

Discussion

The CT abdomen in our patients indicating extensive ischemia in the stage of progression and revascularization of art. hepatica set the indication for surgery because of the possibility of spontaneous rupture of the spleen. In order to prevent possible complications indicates a splenectomy. Given the size of the spleen, CT findings and lab there, we decided to conduct a laparoscopic procedure, which will speed up the rehabilitation and patient quickly restore life activities**.**

Conclusion

Laparoscopic splenectomy is a newer procedure that one group of authors called the "gold standard" in the treatment of hematologic diseases. The surgery takes longer and requires a set of instruments that is in contradiction to the fact that in favor of a shorter postoperative period and faster recovery activities. Everything is likely to laparoscopic splenectomy its further development, operator experience and technology to find its proper place in the indications for surgery.

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Outcomes of rescue therapy in patients with autoimmune hepatitis and primary biliary cirrhosis overlap syndrome after failing response to ursodeoxycholic acid monotherapy: A case series study

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Abstract

Background: Controversy exits in the treatment of autoimmune hepatitis (AIH) and primary liver cirrhosis (PBC) overlap syndrome. The study aims to report the rescue treatment outcome of patients with AIH/PBC overlap syndrome who failed response to ursodeoxycholic acid (UDCA) monotherapy.

Methods: Patients who were diagnosed as AIH/PBC overlap syndrome and failed response to UDCA monotherapy in Beijing 302 Hospital from January 2004 to December 2011 were retrospectively studied.

Results: In a total of 10 patients, 7 responded well to persistent combination therapy of UDCA and prednisolone, 3 of whom relapsed due to the discontinuation of prednisolone, and in the other 3 patients who failed response to combination therapy of UDCA and prednisolone or azathioprine, 2 showed decreasing biochemical indicators under the addition of azathioprine and 1 was treated successfully with Chinese herbals.

Conclusions: Rescue therapy of combination of UDCA and corticosteroid is efficacious in most Chinese patients with AIH/PBC syndrome, and discontinuation of corticosteroid needs to be weighed cautiously. For resistance to this regimen, adding-on azathioprine can control the condition in some cases, and traditional Chinese medicine is a choice when and where appropriate.

Key words: Autoimmune hepatitis, primary biliary cirrhosis, overlap syndrome, rescue therapy.

Introduction

Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis are typical autoimmune liver diseases. The term "overlap syndrome" refers to the coexistence of two autoimmune liver diseases in the same patient, and AIH/PBC overlap syndrome is the most common form [1]. Regarding the treatment, immunosuppression is a standard and highly effective therapy in AIH, and ursodeoxycholic acid (UDCA) is recommended to reduce disease progression in PBC, however, there are no evidence-based recommendations for treatment of AIH/PBC overlap syndrome [2].

In several studies, UDCA has been proved to be efficacious in improving the clinical manifestations and biochemical profiles for some patients with this syndrome, and immunosuppressive therapy should be used if UDCA is ineffective [3-8]. Corticosteroids and azathioprine, are primarily used in the immunosuppressive therapy. In this study, we report the rescue treatment outcomes of patients diagnosed as AIH/PBC overlap syndrome after failing response to UDCA monotherapy.

Patients and Methods

Patients

Patients who were diagnosed as AIH/PBC overlap syndrome and failed response to UDCA monotherapy in Beijing 302 Hospital from January 2004 to December 2011 were retrospectively studied. The diagnosis was made when patients fulfilled two out of three specific criteria as follows [9]. The

AIH criteria included (i) ALT levels > 5×the upper limit of normal, (ii) IgG levels > 2×the upper limit of normal or positive SMA, and (iii) a liver biopsy with moderate or severe periportal or periseptal lymphocytic piecemeal necrosis, and the PBC criteria comprise (i) ALP levels > 2× or GGT levels > 5×the upper limit of normal, (ii) positive AMA, and (iii) a liver biopsy showing florid bile duct lesion. Exclusion criteria included coinfection with hepatitis A, C, D, E, Epstein-Barr virus, cytomegalovirus or human immunodeficiency virus; the presence of other forms of liver diseases such as alcoholic liver disease, drug hepatitis or Wilson's disease. The study was approved by the ethics committee of Beijing 302 Hospital.

Serological markers

Serum autoantibodies, including antinuclear antibody (ANA), smooth muscle antibody (SMA) and antimitochondrial antibody (AMA) were tested using indirect immunofluorescence with the standard methods (Euroimmun Medizinnische Labordiagnostika AG, Germany), and sera were considered to be positive when they produced a reaction at a dilution of ≥1: 80. The M2 fraction of AMA was detected using an enzyme linked immunosorbent assay (ELISA) kit (Euroimmun Medizinnische Labordiagnostika AG, Germany). Immunoglobulin (Ig) assay were taken with the mothod of immunological turbidimetry (Diasys Diagnostic Systems, China). The normalized levels of IgG and IgM were respectively 7.23-16.6 g/L and 0.63-2.77 g/L.

Biochemical profiles, including alanine transarninase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) were measured using standard laboratory procedure. The normalized levels of ALT, AST, TBil, GGT and ALP were respectively <40U/L, <40U/L, <17.1umol/L, 7-32U/L, and 40-150 U/L.

Liver histology

Liver biopsy was performed for definite diagnosis, and fibrosis stage was evaluated by an experienced pathologist. Fibrosis stage was defined as S0 (no fibrosis), S1 (portal fibrosis to be enlarged, localized perisinusoidal and intralobular fibrosis), S2 (periportal fibrosis, several fibrous septa with lobule structure remained), S3 (numerously fibrous septa companied lobule structure distortion, without cirrhosis), and S4 (cirrhosis).

All the 10 patients with AIH/PBC overlap syndrome had concomitant histological features of both PBC and AIH; in particular bile duct lesion, interface hepatitis and plasma cell infiltration were detectable in each liver specimen, as required by the above inclusion criteria.

Results

Baseline characteristics

10 patients with detailed clinical, biochemical and histological data were eventually included in our study. Table 1 listed out the relevant clinical, biochemical and histological information for these patients at diagnosis. All patients had elevated levels of ALT and ALP or GGT and were positive for ANA and AMA or AMA-M2. Though the ALT level of Patient 3 (P3) did not exceed the diagnostic borderline of AIH, evident overlap features of AIH and PBC existed in the liver biopsy specimen, and moreover, UDCA monotherapy was not effective in improving the condition. Based on the evidence, the diagnosis of AIH/PBC overlap syndrome was made.

All of these patients in our study received UDCA monotherapy after diagnosis, but they failed to respond to this treatment regimen after a period of time. The rescue therapy regimens and biochemical profiles before initiation of rescue therapies were listed in Table 2. All of these regimens were based on attending physicians' own discretion.

Treatment outcomes

Figure 1 described the change of biochemical profiles in P1 to P4 after initiation of combination therapy with UDCA and prednisolone. They achieved satisfactory biochemical response after this regimen was adopted. Figure 2 described the biochemical change with the diversity of regimens in P5 to P7 after initiation of rescue therapy with UDCA and prednisolone. During the persistence of treatment with UDCA and prednisolone, the condition of P5, P6 and P7 gradually became stabilized, however, after prednisolone was stopped owing to decisions by individual clinicians, the liver enzymes failed to show further decreases and deteriorated in all of the three patients, and ascites developed in P5 in March., 2008. Eventually,

Table 1. Baseline characteristics of patients at diagnosis

Number	Sex	Age (year)	ALT (U/L)	TBil (umol/L)	ALP (U/L)	GGT (U/L)	IgG (g/L)	IgM (g/L)	ANA (titer)	SMA (titer)	AMA (titer)	AMA-M2	Time of diagnosis	Fibrosis stage in histological assessment
1	male	68	76	43.9	535	699	52.0	9.8	1: 1280	(-)	1: 640	(+)	Apr., 2009	S4
2	male	57	300	20.1	482	461	20.5	5.1	1: 320	(-)	1: 320	(+)	May., 2004	S2
3	female	29	284	71.0	562	417	28.8	16.0	1: 1280	(-)	1: 1280	(+)	Jul., 2005	S1
4	female	51	215	21.5	775	367	16.8	4.7	1: 160	(-)	1: 160	(+)	Aug., 2004	S2
5	male	49	207	37.3	885	1085	21.8	8.9	1: 320	(-)	1: 640	(+)	Jan., 2004	S4
6	female	43	311	34.7	599	442	33.4	12.3	1: 640	(-)	1: 1280	(+)	Oct., 2006	S2
7	female	31	52	32.7	347	286	34.5	7.9	1: 1280	(-)	1: 640	(+)	Oct., 2007	S3
8	female	53	174	28.0	381	413	29.0	5.7	1: 1280	(-)	1: 640	(+)	Mar., 2007	S2
9	female	50	232	49.9	195	1998	25.6	4.9	1: 320	(-)	1: 160	(+)	Apr., 2007	S2
10	male	49	198	24.3	798	1203	27.5	8.8	1: 640	(-)	1: 640	(+)	Oct., 2008	S2

ALT, alanine transarninase; TBil, total bilirubin; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; Ig, immunoglobulin; ANA, antinuclear antibody; SMA: antismooth muscle antibody; AMA, antimitochondrial antibody.
(+), positive; (-) negative.

Table 2. Biochemical profiles before initiation of rescue therapies

Number	ALT (U/L)	TBil (umol/L)	ALP (U/L)	GGT (U/L)	Rescue therapy regimens	Starting time of rescue therapy
1	70	56.7	484	741	UDCA 10mg/kg/day+prednisolone 25mg/day	Jul., 2009
2	445	23.4	527	578	UDCA 10mg/kg/day+prednisolone 25mg/day	Jan., 2005
3	391	45.4	509	442	UDCA 10mg/kg/day+prednisolone 20mg/day	Jan., 2006
4	167	22.3	675	681	UDCA 10mg/kg/day+prednisolone 20mg/day	Nov., 2004
5	310	40.5	796	969	UDCA 15mg/kg/day+prednisolone 30mg/day	Jun., 2004
6	362	30.1	667	514	UDCA 10mg/kg/day+prednisolone 20mg/day	Dec., 2006
7	76	27.5	343	292	UDCA 10mg/kg/day+prednisolone 20mg/day	Dec., 2007
8	326	31.1	280	531	UDCA 15mg/kg/day+azathioprine 50mg/day	May., 2007
9	307	38.8	1449	2239	UDCA 15mg/kg/day+prednisolone 30mg/day	Aug., 2007
10	202	25.0	838	1132	UDCA 10mg/kg/day+prednisolone 25mg/day	Jun., 2009

ALT, alanine transarninase; TBil, total bilirubin; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; UDCA, ursodeoxycholic acid.

P5 and P6 transferred to another hospital and thus dropped out, and P7 died of upper gastrointestinal bleeding in Jul., 2010.

Figure 1. Change of biochemical profiles in Patient 1 to Patient 4 after initiation of combination therapy with UDCA and prednisolone

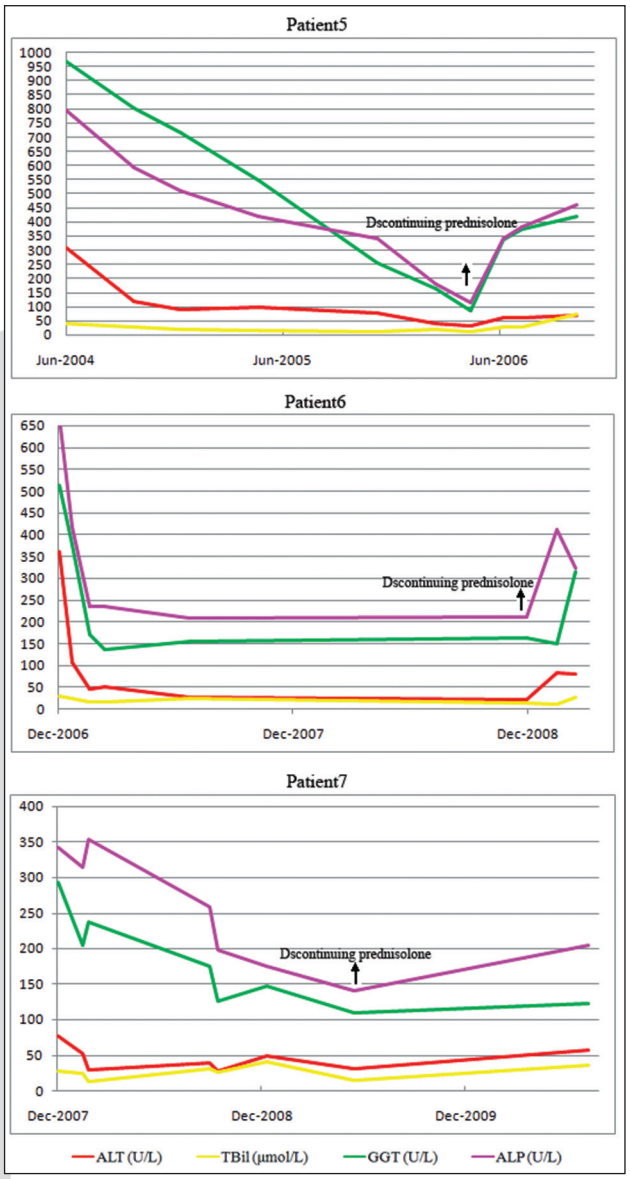

Figure 2. Biochemical change with the diversity of regimens in Patient 5 to Patient 7 after initiation of rescue therapy with UDCA and prednisolone

The levels of ALT, TBil, ALP and GGT in P9 and P10 were rising within 3 months from start of the combination therapy and as this result, azathioprine was added on. After that, biochemical indicators in both of the two patients began to descend and the detailed changing process was described in Figure 3. P8 was treated with combination of UDCA and azathioprine as salvage, but still responded poorly, and the levels of ALT, TBil, ALP and GGT were respectively persistently rising to 326 U/L, 31.1 umol/L, 280 U/L, 531 U/L within one month. Based on claim of this patient and evaluation for the condition, azathioprine was stopped and Chinese herbals were prescribed by a physi-

cian of traditional Chinese medicine in our hospital as subsequent therapy, and after a period of treatment, the condition of this patient was under control with satisfactory clinical and biochemical response. In Jun., 2011, relative biochemical indicators of this patient were as follows: ALT 14 U/L, ALP 85 U/L, GGT 94 U/L, TBil 18 umol/L.

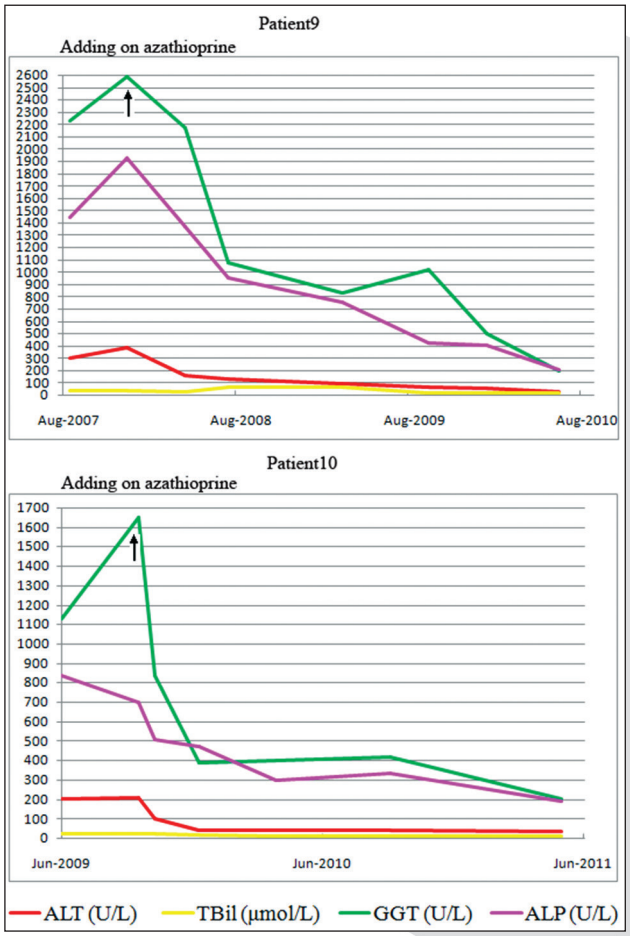

Figure 3. Biochemical change with the diversity of regimens in Patient 9 and Patient 10 after initiation of rescue therapy with UDCA and prednisolone

Discussion

Treatment of AIH/PBC overlap syndrome has been reported by several studies. However, due to the lack of standardization and variations in the populations under study, the characteristics of AIH/PBC overlap syndrome vary between studies [3,6,7,8,10], and recommendations remain controversial though UDCA and/or immunosuppressive medications have been evaluated [6]. Some studies disclose that UDCA monotherapy can make patients with overlap syndrome achieve satisfactory clinical and biochemical response [3]. Other researchers suggest that UDCA and immunosuppressive combination therapy would be more effective in improving biological indicators for AIH/PBC overlap patients than either UDCA or immunosuppressive therapy administered separately [11]. For these patients who have poor response to UDCA monotherapy, it has been suggested that combination therapy with UDCA and corticosteroids may be the treatment of choice [12].

In this present study, excluding P8, P9 and P10, persistent combination therapy of UDCA and prednisolone as salvage was effective in most of patients who had failed response to UDCA monotherapy. However, to the best of our knowledge, when to stop corticosteroid has not been widely demonstrated up to now. 3 included patients who had benefited after prednisolone was added on relapsed when it was stopped, which indicated that discontinuation of prednisolone could aggravate the disease even if the condition seemed to have been under control.

Previous studies showed that more patients diagnosed as AIH/PBC overlap syndrome with liver fibrosis failed to respond to corticosteroid therapy compared to patients without fibrosis, and concluded that the presence of liver fibrosis might increase the likelihood of treatment failure [13]. However, in our study, 3 patients who had the upper stage of liver fibrosis (S3-4) responded well after prednisolone was added on, and this outcome agreed with the observational result from another study on AIH treated by budesonide [14]. Thus, we inferred that the extent of liver fibrosis might have few impacts on the efficacy of corticosteroid in AIH/PBC overlap syndrome.

For patients resistant to combination therapy of UDCA and corticosteroids or azathioprine, other agents such as cyclosporine-A and mycophenolate mofetil as substitution were used but their efficacy in these patients was not often satisfactory [15]. In this study, resistance to combination therapy of UDCA and azathioprine happened in P8, and Chinese herbals were used and played an important role in improving the condition of this patient. Regarding treatment for autoimmune liver diseases with Chinese herbals, some studies reported their validity [16]. So, Chinese herbals could be adopted when corticosteroids and azathioprine were both in vain, but they must be prescribed by a specialist.

In conclusion, rescue therapy of combination of UDCA and corticosteroid is efficacious in most Chinese patients with AIH/PBC syndrome, and discontinuation of corticosteroid needs to be weighed cautiously. For resistance to this regimen, adding-on azathioprine can control the condition in some cases, and traditional Chinese medicine is a choice when and where appropriate.

List of abbreviations

AIH: autoimmune hepatitis; ALP: alkaline phosphatase; ALT: alanine transarninase; AMA: antimitochondrial antibody; ANA: antinuclear antibody; GGT: gamma glutamyl transferase; PBC: primary biliary cirrhosis; SMA: antismooth muscle antibody; TBil: total bilirubin; UDCA, ursodeoxycholic acid.

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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
Left margin	20 mm
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)

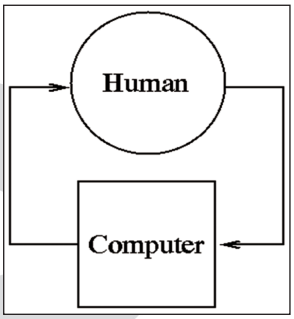

Figure 1. Text here

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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