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Bladder paraganglioma during pregnancy characterized by bladder bloody tamponade with normotension: A case report and review of the literatures

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Abstract

Paragangliomas of urinary bladder are rare neoplasms coming from the chromaffin tissue. Patients with bladder paragangliomas may have similar clinical symptoms as pheochromocytomas and/or some special syndromes such as microscopic or gross hematuria, paroxysmal hypertension caused by catecholamine releasing during micturition. Moreover, bladder paraganglioma during pregnancy is intriguing but extremely rare. Here, we report a 28-year-old woman with a bladder paraganglioma at 24 weeks gestation during her second pregnancy. Her symptoms were characterized by repeated painless gross hematuria and acute urine retention with normal blood pressure and unelevated plasma catecholamine level. Ultrasound and CT (computer tomography) scan both detected the tumor, cystoscopy and pathologic results of biopsy finally confirmed the diagnosis of bladder paraganglioma. The tumor was successfully removed by partial cystectomy after pregnant termination. Additionally, a brief review of eight other cases was studied.

Key words: Bladder tumor, Bloody tamponade, Normotension, Paraganglioma, Pregnancy

Introduction

Paragangliomas (extra-adrenal pheochromocytomas) can develop from tissues wherever there are chromaffin cells, probably from the skull base to the bladder. Paragangliomas locating in urinary bladder are rather rare, comprising only 1% of all pheochromocytoma cases and 0.06% of all tumors in the urinary bladder[1]. Furthermore, bladder paraganglioma combined with pregnancy is extremely rare. The authors searched the litera-

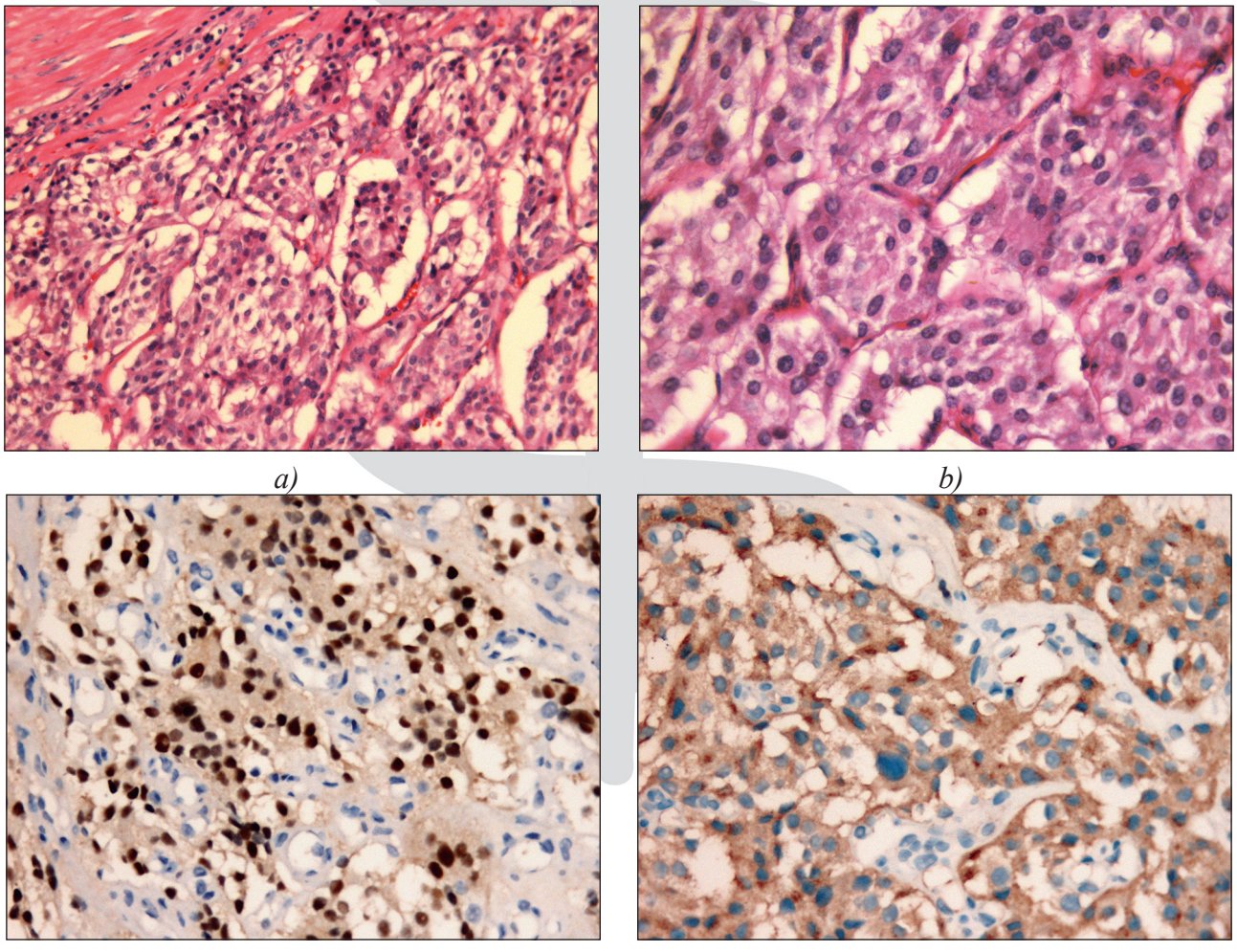
Table 1. Systematic review of the bladder paraganglimoas during pregnancy in reported literature

Time	Age	Diagnosis time	Main symptom	CA Level	Location in bladder	Pregnancy outcome	Treatment
2002[2]	29	before pregnancy	hypertension	elevatory	unknow	terminate	Partial cystectomy
2000[3]	17	After terminate	Macroscopic hematuria; preeclampsia	normal	Left posteroinferior	terminate	Partial cystectomy
1997[4]	17	unknow	Dysuria, microscopic hematuria	unknow	unknow	unknow	Surgery
1995[5]	33	20w pregnancy	ventricular tachycardia	elevatory	unknow	delivery	surgery
1992[6]	40	8w pregnancy	Molar pregnancy Vaginal bleeding	unknow	unknow	Total hysterectomy	Partial cystectomy
1991[7]	19	30w pregnancy	hypertension	elevatory	Left lateral	delivery	surgery
1977[8]	20	after delivery	hypertension	elevatory	dome	delivery	Partial cystectomy
1976[9]	18	after delivery	headache, flushing, palpitations, choking	elevatory	anterior	delivery	Partial cystectomy

tures on bladder paragangliomas during pregnancy published in English and gave a brief review (table 1).Giving the searching results from PubMed, Embase and the Cochrane library, only 8 cases reported in English are available and no new report has been added to the database for this recent decade[2-9]. To our knowledge, the present case is the first bladder paraganglioma during pregnancy with total silence catecholamine-related symptom except for bladder hemorrhage reported in the literature.

1. Case report

A 28-year-old woman, G2P1, at 24 weeks gestation, came to our emergency room after being ruled out the possibility of placenta increta. Repeated painless gross hematuria and dysuria were her main complaints. Abdominal ultrasound and CT scan (after her deciding to terminate pregnancy) both detected a tumor with marked vascularity in urinary bladder. After admission, the bleeding was stopped by some medical therapies, cystoscopy was performed and an irregular solitary mass, protruding to the lumen with congestive mucosa nearby, was found in the right lateral of the bladder. Pathogical and immunohistochemical study established the diagnosis of paraganglioma (Figure 1). The plasma levels of norepinephrine and epinephrine before operation were within the normal range (norepinephrine 221 ng/L and epinephrine<25 ng/L). One week after odinopoeia the patient underwent a partial cystectomy under general anesthesia, and the tumor, a 2.5*2.0*2.0cm material mass, was resected successfully. Blood pressure during operation was almost normal and stable. The period of convalescent was uneventful.



a) *b)* *c)* *d)*

Figure 1. Pathology of the bladder neoplasm. Histological examination revealed a typical "zellballen pattern" Organoid nests around with delicate fibrovascular stroma.(a: HE x 200 and b: HE x400); Strong positive S-100 protein(c) and CgA(d) immunoreactivity of the tumor cell cytoplasm.

Follow-up is persisted every 3 months, the patient now have stable blood pressure with normal catecholamine secretion and no complaint of hematuria or anyother lower urinary tract symptom.

2. Disscusion

Although pheochromocytoma during pregrancy is rare, establishment of the accurate and quick diagnosis in these special characters is critically important since the maternal and foetal outcomes can be improved significantly by early diagnosis: maternal and foetal mortality drop to 2% and 10% from 50% respectively, owing to the right antenatal diagnosis rate from 25% before 1970 to current 83% [10].

The typical symptoms of bladder paragangliomas, which arise from excessive catecholamine secretion during micturition or bladder distension (urinate attack), are distinguished by paroxysmal hypertension with/or without gross or microscopic hematuria. It is the most important clue to suspect bladder paraganglioma. The disease would be highly suspected by detection of the elevated plasma or urine catecholamine levels during hypertension attacks or after micturtion [11]. Because of possibly serious side effects, many pharmacodynamic tests, such as glucagon stimulation test and clonidine suppression test, should be avoided in pregnant women [12].

Several techniques could be able to evaluate the location of the tumor. Abdominal ultrasound is convenient and noninvasive, but the diagnostic sensitivity for small tumors is limited. CT scan and MRI have similar sensitivities (90–100%) and specificities (70–80%)[12]. However, MRI is recognized better than CT scan during pregnancy because it is free from radiation. Cystoscopy is one most important approach in differentiating the hematuria source and indicating tumor location. Transurethral biopsy during cystoscopy is not recommended owning to the possibility of increasing catecholamines release and causing hypertensive crisis when paraganglioma is suspected [13]. While our patient had no symptom related with urination and hypertension before, therefore paraganglioma was not suspected before the biopsy procedure.

With respect to treatment, surgical resection of the total neoplasm is the definitive treatment. Partial or radical cystectomy with or without pelvic lymph node dissection is the standard operation procedure [11], both open surgery and laparoscopic procedure can been chosen. TUR (transurethral resection) is not recommended because of the difficulty to remove the tumor completely and potential danger inducing hypertension crisis [13]. General anesthesia and invasive hemodynamic monitoring is necessary during surgery.

Similar to non-pregnant patient, ten to fourteen days preparation including adequate alpha- and selective beta-adrenergic antagonists before surgery are definitely important to prevent hypertensive crisis and consequent risks to the mother and foetus [12]. Moreover, the patient, before 24 weeks of gestation, is recommended to remove tumor as early as the adequate medical treatment is taken; while after 24 weeks of gestation, the patient should be treated with preoperative preparation drugs for a prolonged period until the fetus is mature. Tumor excision may be performed at the time of cesarean delivery or separately after delivery [10, 11]. Vaginal delivery is not recommended since it may stimulate the massive release of catecholamines from the tumor and bring a higher mortality rate of 31% compared with cesarean section (19%) [14].

The present case presented as the typical pathologic characteristics of bladder paraganglioma. Generally speaking, these submucosal tumors usually locate at the dome or trigone of the bladder. They may invade the muscle bundles of the bladder wall with poorly circumscribed boundary. The “zellballen” characteristic pattern in microscopic examination is the typical histologic feature. Immunohistochemical study confirms the diagnosis by neuroendrocrine markers, like neuron-specific enolase, chromogranin, synaptophysinand and S-100 protein which are similar to adrenal pheochromocytoma. [1,4]

About 15% of bladder paragangliomas were reported to be malignant, higher than adrenal peochromocytomas. Consensus has been reached that malignancy of paraganglioma is determined by the biological behavior rather than the histological features. Clearly, the presence of tumor in sites where chromaffin tissue is not normally found and/or the infiltration of neighbour organs can make the definite diagnosis of malignancy [1, 13]. Follow-up for a lifetime is mandatory for these

patients, including monitoring the blood pressure and the catecholamine concentrations and, if possible, taking I^{131}-MIBG scanning. Finally, genetic screening for hereditary pheochromocytoma, such as VHL syndrome, multiple endocrine neoplasia types 2A and 2B, type 1 neurofibromatosis, succinate dehydrogenase B or D genes mutations, should not be forgotten since these conditions are commonly associated with young patient [10, 11].

Because of the limited cases reported, the pathogenesis and biological behavior of these tumors are not clear, more reports and researches are still needed.

Acknowledgement

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Efficacy of hepatitis B vaccine in adult HIV/AIDS patients

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Abstract

Introduction: The aim of this study was to evaluate the efficacy of Hepatitis B vaccination and the influence of immunologic stage in HIV/AIDS patients.

Methods: Ninety-four HIV-infected individuals without immunity to HBV (81% men) and 12 isolated anti-HBc positive HIV/AIDS patients (83% men) were analyzed. Age, gender, CD4 cell count/HIVRNA level at the moment of first introduction and before HBV vaccination, serological parameters of hepatitis B infection, response to hepatitis B vaccination of patients were collected retrospectively from medical records. Efficacy of vaccination was compared with control group consisting of 89 healthy volunteers without immunity to HBV. Hepatitis B vaccine as registered for adults was injected to three groups at month 0-1-6. Anti-HBs titers were checked one month after completion of vaccine series. All analyses were performed using SPSS version 15.0.

Results: Protective level of anti-HBs was found in 42.5% patients without immunity to HBV, in 58.3% patients from isolated anti-HBc positive group and in 85.3% subjects from control group (respectively, $p<0.001$,$p=0.03$). In seronegative patients, no difference was detected between mean CD4 cell counts conducted at the time of diagnosis ($p=0.06$) and before vaccination in responders and non-responders to hepatitis B vaccine ($p=0.25$). In the same subjects, a review of the effect of CD4 levels determined as of the time of first diagnosis on the efficacy of vaccination, made by plotting a ROC curve, revealed a CD4 cut-off value of 376/mm^3 for the vaccine response.

Discussion: In our country with intermediate prevalence for HBV infection, in the light of the impact of HIV parameters on vaccination, HBV vaccination should be performed before CD4 cell count starts to decline or after HIV RNA suppression and immune recovery are confirmed in HIV/AIDS patients.

Key words: Efficacy, Hepatitis B vaccine, HIV/AIDS.

Introduction

The hepatitis B virus (HBV) is one of the most common infectious diseases worldwide. Among HIV infected patients, HBV infection prevalence is approximately ten times higher than in general population, due to shared routes of transmission.,

The course of hepatitis B is negatively influenced by HIV infection. Liver-associated mortality is about 15 times higher than in HIV-negative patients [1, 2]. Therefore, all HIV-infected patients with negative hepatitis B serology should be vaccinated. However, the immune response to hepatitis B vaccine is frequently suboptimal in this population. The aim of this study was to evaluate the efficacy and the effective factors of hepatitis B vaccination in HIV/AIDS patients.

Materials and methods

262 HIV/AIDS patients admitted between January 2006 and December 2011 were included in the study. Age, gender, immunological/virological status (CD4 cell count/HIV RNA level), serological parameters of hepatitis B infection, response to hepatitis B vaccination of patients were collected retrospectively from medical records.

165 HIV/AIDS patients with HBsAg, anti-HBc IgG and anti-HBs negative are named and termed as “serologic negative cases”, while 41 HIV/AIDS patients with HBsAg and anti-HBs negative, anti HBc-IgG positive and HBV-DNA negative are named and termed as “isolated anti-HBc positive cases”, and 56 patients were HBsAg negative and Anti-HBcIgG and Anti-HBs positive. 94 of serologic negative cases and 12 of isolated anti-

HBc positive cases are included in this study after completion of Hepatitis B vaccination scheme. Efficacy of vaccination was compared with "control group" consisting of 89 healthy volunteers with HBsAg, anti-HBc IgG and anti-HBs negative. All HIV-infected cases and control group subjects included in the study are considered and treated as anti-HCV negative.

For all cases included in this study, HBsAg, anti-HBc IgG, anti-HBs, and anti-HCV measurements were effected by micro-ELISA (General Biologicals Corp, Taiwan), while HBV-DNA was measured by quantitative real time PCR (Rotorgene 6000), and CD4 cell count was determined by standard flow cytometry (*FACScalibur, Becton Dickinson*). Viral load was measured with Cobas Amplicor HIV-1 (*Roche*).

Euvax B vaccine produced by Sanofi-Pasteur, 20 μg, as registered for adults, was injected to three groups at month 0-1-6. Anti-HBs titers were checked one month after completion of vaccine series. The patients with anti-HBs <10 IU/l after completion of the vaccination scheme were accepted to be non-responders to vaccine, and those with anti-HBs ≥10 IU/l were accepted to be responders thereto. One year after the date of vaccination, anti-HBs levels were assessed again.

All analyses were performed by using graphpad prism 5.0 and SPSS version 15.0 software. Data were described using mean ± standard deviation (SD) (or median and range). In statistical analysis of the study results, the groups were compared by using unpaired student t test and One-way ANOVA post hoc Tukey test results. The methodology applied in data analysis containing the frequency values was Chi-square statistical analysis test, while logistic regression was applied in analysis of anti-HBs of multi-variables. The effect of CD4 level determined at the time of first application of patients on anti-HBs positivity was studied, and a ROC curve was plotted. A p value less than 0.05 was considered significant.

Results

Age-related characteristics of all three groups are presented in Table 1 hereinbelow. According to the Chi-square test, the groups have similar characteristics in terms of age and sex (p=0.42).

An anti-HBs of protective level has been detected in 40 out of 94 (42.5%) serogenative HIV/AIDS patients after Hepatitis B vaccination, and in 7 of 12 (58.3%) isolated anti-HBc positive cases, and in 76 subjects (85.3%) from control group (Figure 1).

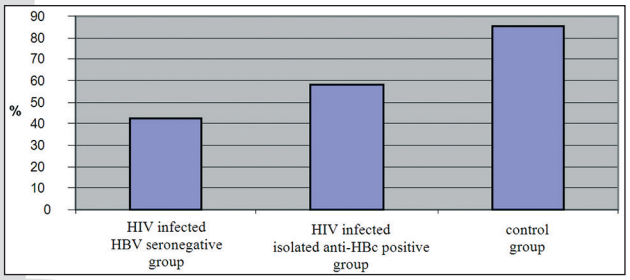

Figure 1. Responses of HIV/AIDS cases and control group subjects to Hepatitis B vaccination

A comparison of seronegative and isolated anti-HBc positive cases in terms of response to vaccination revealed statistically significant difference (respectively, p<0.001; Odds ratio 4.8 and p=0.03; Odds ratio 4.12). A significant difference was not detected between responses to vaccination of seronegative and isolated anti-HBc total positive cases (p=0.36; Odds ratio 1.89).

In 26 of seronegative cases (27.6%), and 5 of isolated anti-HBc cases (41.6%), and 49 of control group subjects (55%), following Hepatitis B vaccination, anti-HBs is found to be ≥100 IU/l. In statistical assessment of these percentages, a significant difference was detected by Chi-square test between serogenative cases and control group subjects (p<0.001; Odds ratio 3.2), while a significant difference could not be found between isolated anti-HBc cases and control group subjects (p=0.53).

Average CD4 counts, determined as of the time of first diagnosis, of responders and non-responders to Hepatitis B vaccination were respectively 327.3 ± 31.15/mm^3 and 316.4 ± 35.59/mm^3, with no significant difference between them

Table 1. Average age of patient and control groups

	Control group	Isolated anti-HBc positive	Serological negative
Mean ± SD	35.07±8,91	40,00±13,64	37,23±10,30

(p=0.06). Average CD4 counts of the same cases, determined before Hepatitis B vaccination, were respectively 422.8±31.68/mm^3 and 418±32.52/mm^3, again with no significant difference between them (p=0.25). Nor has a difference been found between responders to vaccination (mean ± SD, 37.23±1.629) and non-responders to vaccination (mean ± SD, 38.76±1.538), when the effect of age of the patients in this group on the response to vaccination was studied (p=0.50; F=1.2).

A ROC curve is plotted in the course of studying the effect of CD4 levels of HIV-infected serologic negative patients determined as of the time of first diagnosis on post-vaccination anti-HBs response. Accordingly, as the cut-off value for an adequate anti-HBs response, CD4 level is determined as 376 per mm^3 with 52% sensitivity and 72% specificity. By Spearmen correlation test performed according to this cut-off value, a positive correlation is detected between CD4 level as of the time of first diagnosis on one side and response to vaccination in the other side.

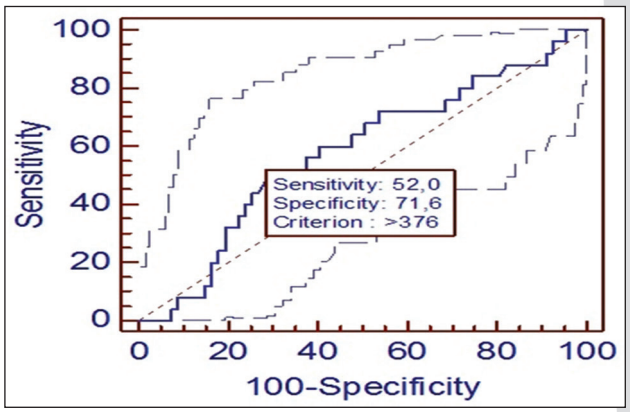

Figure 2. Effect of CD4 levels of serologic negative patients determined as of the time of first diagnosis on the post-vaccination anti-HBs response

While average HIV RNA level of responders to Hepatitis B vaccine in serologic negative group was 221600±192800 copies/ml, average HIV RNA level of non-responders was 122500±70930 copies/ml, with no statistically significant difference between them (p=0.60, F=5.9).

In 21 out of 40 cases of serologic negative group responding to Hepatitis B vaccination, anti-HBs measurements are repeated one year later, and the response to vaccination is determined to continue in 12 of them (57%).

Discussion

High HBV infection rates have been described among HIV/AIDS patients worldwide. Despite recent increases in incidence, Turkey is still among low prevalence countries in Europe for HIV/AIDS. According to the statistical data provided by the Ministry of Health, as of December 2011, there are 5224 HIV infection cases in our country with a population of 75 millions. On the other hand, prevalence of chronic HBV infection varies geographically, and Turkey is a country with intermediate prevalence (4%), having high and low prevalence (0% – 9.9%) in distinct geographical areas [3]. The single study conducted in our country with respect to prevalence of chronic HBV infection among HIV/AIDS patients has revealed a co-infection rate of 4% [4].

Due to the negative influence of HBV co-infection, all HIV-infected patients with negative hepatitis B serology should be vaccinated. Vaccination is performed at 20 µg at months 0, 1 and 6 as recommended by manufacturers. However, due to immunosuppression, the vaccine may be less effective. Approximately 34-63% of HIV infected patients have a primary response to vaccine [5, 6, 7, 8]. The response to the vaccine detected in 42.5% of seronegative HIV/AIDS cases in our study is also similar with the results of the previous studies.

Isolated anti-HBc serologic profile may signify either a false positive result or a prior HBV infection in the distant past with loss of anti-HBs. The response to vaccine in HIV-infected isolated anti-HBc cases was 40.7 - 62% in the previous studies and was also similar (58.3%) in our patients [9, 10].

Factors associated with impaired HBV vaccine response in HIV infected patients may include high level of HIV RNA, low CD4 cell count and HCV co-infection. We concluded that efficacy of the routine vaccination scheme was lower among HIV/AIDS patients in comparison to healthy volunteers. Although there was no statistically significant difference with stage of HIV infection and response to hepatitis B vaccine, the response to vaccine is influenced by the CD4 cell count and level of HIV-RNA. In the course of review of the effect of CD4 cell count in HIV-infected serologic negative patients on post-vaccination anti-HBs response, a ROC curve was plotted, and as cut-off value, CD4 level was

detected as 376 per mm^3. According to this cut-off value, a positive correlation was detected between low nadir CD4 cell count and response to vaccine. Therefore, patients with CD4 cell counts of less than 376/mm^3 who are not on HAART may receive HAART first and hepatitis B vaccination thereafter. However, neither low CD4 count nor high HIV viral load should be used as a justification to delay vaccination of high-risk persons [11, 12]. Fonseca et al. have also concluded that the best strategy for hepatitis B vaccination in HIV/AIDS patients would be to use a double dose as a primary series when CD4 level is ≥ 350 [6]. Because of low response to HBV vaccine in HIV infected patients, there are numerous reports describing a variety of dose schedules. In these reports, either additional doses of vaccines or double-dose vaccination (40 μg) at 3 vaccination time points (months 0, 1, 6) may help to improve response rates to HBV vaccination [5, 6, 7].

Post-vaccination testing for anti-HBs is recommended and vaccine non-responders should undergo repeat immunization with a full series. Double-dose re-vaccination (40 μg) at 3 vaccination time points (months 0, 1, 6) may help to improve vaccination response rates [13, 14].

Anti-HBs ≥10 IU/L is generally accepted as a marker of protection against HBV infection in general population. However, higher anti-HBs titers are generally desired since antibody levels decline over time [15]. Loss of protective immunity is seen in up to 30% of immunized HIV infected patients each year following anti-HBs response [14]. In this study, loss of protective immunity was found 43% one year later. Hepatitis B infection can be performed in HIV/AIDS patients when anti-HBs is <10 IU/l. Therefore, in HIV/AIDS patients who show an immune response to hepatitis B vaccine, level of anti-HBs should be measured periodically.

In conclusion, we came to the conclusion that efficacy of the routine vaccination scheme was lower among HIV-infected patients in comparison with healthy volunteers. In our country with intermediate prevalence for HBV infection, in the light of impact of HIV parameters on vaccination, HBV vaccination should be performed before CD4 cell count starts to decline (if the patient is not taking HAART) or after HIV RNA suppression and immune recovery are confirmed (if the patient is starting HAART).

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Children with Cerebral palsy have greater demodex density than age-matched control group

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Abstract

Demodex are tiny, wormlike mites that live in the hair follicles and sebaceous glands of various mammals. Under normal conditions they are classified as commensals and not harmful. However, in certain situations such as impaired immune system, intense stress, or malnutrition, the mites can reproduce rapidly, causing symptoms in sensitive hosts that range from mild irritation to severe rosacea, and in rare cases a life-threatening condition.

Nutrition is an indispensable component of vigorous immune response. Malnutrition is the most common cause of immunodeficiency worldwide and develops in case a body is not sufficiently satisfied with its nutritional needs. The severity of malnutrition can be quantified by comparing standardized norms to body's height and weight.

Many studies have been reported that the nutritional deficiencies due to the difficulties in chewing and swallowing, and spasticity is a main cause of growth retardation in patients with cerebral palsy. So they tend to lose the strength of the immune system as a result of the malnutrition. In the literatüre, although there are recent reports indicating the increase in the density of demodex, especially in immune compromised patients, but no studies showing the role of malnutrition on demodex density. Therefore, we aimed to investigate the density of *demodex* in children with cerebral palsy which is essentially very predisposed to malnutrition. To the best of our knowledge this is first clinical study investigating the demodex density in the children with cerebral palsy and the effect of malnutrition on the prevalence of these mites.

Key words: Children, cerebral palsy,demodex mites, malnutrition.

Introduction

Demodex (Dd) are tiny, wormlike mites that live in hair follicles and sebaceous glands of various mammals. Two species living on humans have been identified as *Demodex folliculorum* (DF) and *Demodex brevis* (DB), which are both frequently referred to as the face mites[1,2]. Because they are primarily found in the face around nose, eyelids, cheeks, forehead, nasolabial fold and eyebrows, but also occur elsewhere on the body. As the name says, *DF* is found in hair follicles, while *DB* lives in sebaceous glands connected to hair follicles. So, it is believed that *Dd* mites are a common residents of hair follicles and sebaceous glands in humans after birth[3,4].

As a word *Demodex* comes from the Greek, demos- fat, and dex- worm, which defines general characteristics. The mites vary in size from 0.1mm to 0.4mm long. Mites do not invade internal organs. Under normal conditions they are classified as commensal rather than parasite and not harmful. When large numbers of these mites are found on humans, the infestation is named as demodicosis[3,4]. However, in certain situations, such as an impaired immune system, intense stress, or malnutrition, the mites can reproduce rapidly, causing symptoms in sensitive hosts that ranges from mild irritation to

rosacea-like severe and widespread inflammation, and-in rare cases-a life-threatening condition. As can be seen in almost all ages, the incidense increases as people age, and is very rare in children[5].

Nutrition is an indispensable determinant of vigorous immune responses. So malnutrition is the most common cause of immunodeficiency worldwide and develops in case a body is not sufficiently satisfied with its nutritional needs. It can turn a normally simple and mild illness to turn persistent, recurrent, severe and sometimes fatal. Even the deficiency of single nutrients can result in an altered immune response to foreign substances. Micronutrients like iron, copper, zinc, folic acid, and vitamins A, C, E, all have important influences on immune response[6]. Although *Dd* mites are normal inhabitant of the skin of most adults, the authors have led to the hypothesis that their density can rise as a result of the undernourishments that allow mites to proliferate.

Cerebral Palsy (CP) is a nonprogresive clinic syndrome characterized by abnormal muscle tone, reflexes, or motor development and coordination resulting in the disturbance of motion and posture. It is also known as the most common chronic motor failure of childhood[7,8]. The classical symptoms are spasticity, involuntary movements, unsteady gait, problems with balance, and soft tissue findings consisting largely of decreased muscle mass. The disorder is commonly accompanied with eating problems, seizures, dysarthria or other communication disorders, mental retardation, learning disabilities, urinary-fecal incontinence, and behavioral disorders[7,8]. Growth and developmental deficiencies are seen at high rate[9,10]. Many studies have been reported that the nutritional deficiencies due to the difficulties in chewing and swallowing, and spasticity is a main cause of growth retardation in patients with CP[11,12]. In addition, cellular and humoral immunity can easily be weakened by the malnutrition, leading to the overgrowth of opportunistic organisms[13,16].

The severity of malnutrition can be quantified by comparing standardized norms to children's height and weight, calculating your body mass index (BMI) or measuring skin-fold thickness of the upper arm. The measurement of feeding efficiency also provides the basis for early identification of children who cannot be adequately nourished without ancillary feeding[17].

The children with CP tend to the weakness of the immune system due to the chronic malnutrition. In the literature, there are some recent reports indicating the increase in the density of demodex, especially in immunocompromised patients[18-22]. Therefore, we aimed to investigate the density of demodex in patients with CP that is essentially very predisposed to malnutrition. To the best of our knowledge this is first clinical study researching the effect of malnutrition on the demodex density in the patients with CP.

Patients and Methods

The ethics committee of Mersin University, Faculty of Medicine, approved the study and written informed consent was obtained from all patients and controls. Sixty children with CP who applied to outpatients clinic at the Mustafa Kemal University, Pediatrics Neurology Department, and 50 healthy subjects for comparing in similar sex and age between on August 2011 and January 2012, were included in the study. The children who were diagnosed as CP before and firstly also enrolled the study.

Anthropometric measurement is a noninvasive, quantitative technique for determining an children's feeding status by measuring and analyzing specific dimensions of the body, such as height and weight. In the study, the malnutrition rate based on both the weight for height (WFH) and the weight for age (WFA) which are used widely to identify underdeveloped children[2]. Anthropometric measurements were both taken from the patients and controls, and then categorized. The children whom WFH and WFA values was 80-89% of the standard figure, was accepted as mild degree malnutrition, 70-79% was moderate malnutrition, <70% was severe malnutrition, and 90-100% was normal-weight children respectively[21].

A variety of methods such as skin scraping, cellophane tape, punch biopsy, and standardized skin surface biopsyare are used for measuring of the *Dd* density. Standardized skin surface biopsy (SSSB) is an appropriate, noninvasive sampling method by which it is possible to collect the superficial part of skin with the contents of the pilosebaceous follicle. To sample, cyanoacrylate adhesive is little dropped on a clean slide and will be kept up to 45-60 seconds on the relevent region of skin. It is then

gently removed and a lamel is closed after leaving a drop of glycerin on the sample to obtain a better image amongs superficial dead cells, remnants of the hair and the fat. The examination was performed by a microscope at the magnification of ×10 and ×40 within 1 h of sampling. The number of mites was counted as a living mite per centimeter square. Five and above numbers/cm^2 was accepted as the positive *Dd*[23,24]. The children with CP and controls were examined for dermatological lesions and symptoms such as pruritus, redness, scaling, telangiectasia, pustules and papules on the face. Five samples of SSSB were gained from the forehead, both cheeks, jaw and nose. Diagnosis of *Dd* mites is accomplished by viewing the samples under a microscope. Statistically analyze was performed with SPSS for Windows versa.

Results

A total of 60 children with CP and 50 age and sex matched healthy controls were included in the study. Of the patients, 35 (58.3%) were male and 25 (41.7%) were female. Gender was aquel in the control subjects (25 male (50%) and 25 (50%) female). The mean age for the CP group was 6.50±4.38 (range from 1-15 years) and 6.21±3.76 (range from 1–16 years) for the controls. All participants was examined before taking samples and no dermatological signs or no symptoms were seen in each.

According to the anthropometrical mesuarement, the mean value of WFH was 89.7±18.1and WFA was 70.0±20.4 in CP group. Mean value of WFH was 103.8±11.3 and WFA was 97.9±9.7 in control group.There is statistically a significant difference between two groups in terms of both WFH (p= 0.001) and WFA (p=0.001) (Table 1).

While in CP group, 20 (33.3%), 13 (21.7%), 15(25%) and 20 (33.3%) of the patients was determined as severe malnutrition, moderate malnutrition, mild malnutrition, and normal, respectively. In control group, only 5(10%) of the children was determined mild malnutrition, others was normal (Table 2). There was statistically significant between *Dd* positive (84.44±13.04) and nonpositive patients (97.20±16.84) in regarding to WFH (p=0.029) and WFA. The positive Dd (≥ 5 mites/cm^2) was found in 9 of the patients with CP (Figure 1). Of 9 patients with positive *Dd*, 7 (77.8%) was females and 2 (22.2%) was males. But none of the controls had positive samples. In aspect of positiveness, the difference between patients and controls was statistically significant (*P*=0.001) (Table 2). All positive samples were detected from the cheeks in 9 of 60 (15%) children with CP, and SSSB samples taken from the forehead, nose, jaw were found the negative of whom had less than five *Dd*/cm^2 (< 5 mites/cm^2). *Dd* density was statistically significantly higher in CP groups than in controls.

Table 1. Evaluation of the groups in terms of age, WFH, and WFA

	CP groups	Control groups	P
WFH(%)	89.7±18.1	103.8±11.3	0.001
WFA(%)	70.0±20.4	97.9±9.7	0.001
Age(year)	6.5±4.3	6.2±3.7	0.714

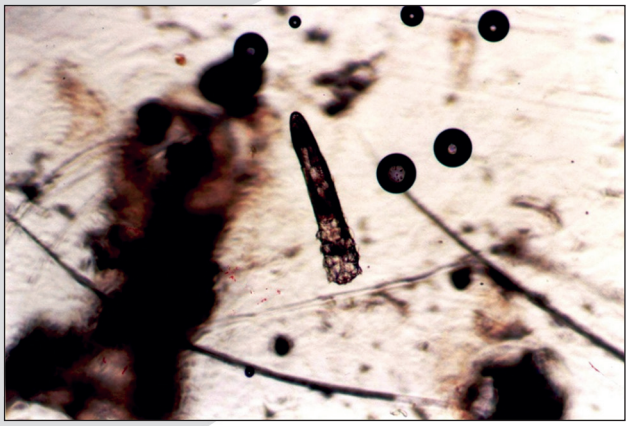

Figure 1. The appearance of Dd in a skin surface biopsy: Adult of Dd (Original magnification 100X)

Table 2. Evaluation of the groups for presence of demodex and malnutrition

n		CP groups		Control groups		Total		P
		%	n	%	n	%		
Demodex	Positive	9	15	-	-	9	8.2	0.001
Malnutrition	Severe	20	33.3	-	-	20	18.2	0.001
	Moderate	13	21.7	-	-	13	11.8	
	Mild	15	25	5	10	20	18.2	
	Normal	12	20	45	90	57	52.8	

Discussion

The *Dd* mites have been implicated as a cause of many human skin disorders such as rosacea, pustular folliculitis, acne vulgaris[25,26]. Their infestation may also be associated with immuno supression, such as in children with leukemia and HIV infection[18,19], or more severe in its clinical presentation[22]. Nutting et al. was reported that the appereance of *Dd* at healthy skin changed by age and reached 100% in elderly persons[3]. Although frequent in adults, *Dd* is rarely found on the skin of children[27]. *Dd* infestation has also been determined in immunocompetent eight children presenting with facial erythema and papulopustules aged ten months to five years[18]. Recent reports of *Dd* in association with congenital or acquired immunodeficiency suggest that proliferation of *Dd* is allowed by host immune dysfunction[22,28-30].

All types of CP are characterized by abnormal muscle tone, reflexes, motor development and coordination. The classical symptoms are spasticities, spasms, other involuntary movements, unsteady gait, problems with balance, consisting largely of decreased muscle mass. Secondary conditions can include eating problems, seizures, dysarthria, sensory impairments, mental retardation, learning, disabilities, urinary and fecal incontinence. Children with CP are obviously at high risk of undernutrition due to various reasons[11,31,32]. Approximately a one-third of children with CP are undernourished. Hung et al. found that undernutrition was a very common problem for these children[33]. In a study, seven children with severe CP and growth failure were compared with children of the same weight in respect of their eating efficiency. As a conclusion, it is reported that the children with cerebral palsy took 2-12 times longer to chew and swallow a standard amount of food and 1-15 times longer for solid food than did their weight-controls. Even long meal times do not compensate for the severity of these children's feeding impairment. In our study varying degrees of malnutrition also detected in 80% of CP patients (Table 2).

The poor nutrition of children with CP was probably due to inadequate food intake because of severe feeding problems such as oromotor dysfunction and spasticity[11,31,32]. If severe nutritional problems left untreated they may even cause the impairment of the immune system, cognitive problems, and neuromuscular disability[34-38]. With insufficient nutrition, the next major problem is severe and chronic infections, particularly diarrhea, parasitic infections that may also exacerbate the malnutritional status. Cellular response is impaired by the atrophy of the tymus, lymph nodes and tonsils, decreased CD 4 response, phagosytosis and release of secretory IgA in malnutrition[39-40].

Dd, which is a saphophytic mite of human pilosebaceous units, can be found anywhere on the skin. The cause of the clinical features in *Dd* infestation is still not known. The hypotheses include immunological deficiency or abnormal immunological reaction of the skin to the parasite[30]. Incidence of *Dd* were reported as 13.8% of mild malnutrition group, 25.8% of moderate malnutrition group and %36.3 of severe malnutrition group in a study carried out to determine the incidence of *Dd* in malnourished children with immune system dysfunction[41]. In our study, all patients with positive *Dd* density was malnourished and, 3 (33.3%) mild malnutrition, 5 (55.6%) moderate malnutrition and 1 (11.1%) had severe malnutrition. Parallel to the degree of malnutiriton, not increasing positivity for *Dd*, connected to shortage in number of patients with malnutrition.

Dd infestation is not finalized that what is the role in the pathogenesis of skin diseases in humans. That current hypothesis that there may be an increase in the number of *Dd* as a result of immun deficiency or *Dd* may provoke the formation of cutaneous lesions as a result of abnormal imunologic reaction of the skin. Demodex infestation is most probably caused by external and internal factors such as sebum gland dysfunction and T-cell suppression[42]. Generally the *Dd* do not penetrate the basal membrane and therefore are not fully exposed to the skin's immune system. So *Dd* is only responsible for the erosion of the epithelium[43]. With eating the human cells, it can penetrate into the dermis[44]. *Dd* produce a humoral factor, which causes selective suppression of T lymphocytes, and this factor blocks the local immune response. In our study determination of the positive *Dd* in 15% of patient with CP may be explained by an immune comprimised effect of malnutrition.

The studies in adults, it is reported that *Dd* located all over the world without distinction of race and increase the frequency with increasing age in healthy

subjects[45,46]. There are presence of *Dd* at rosasea like lesions of immunsupresif children who's age between 15 mounth and 10 year[20,29]. In our study, there was no statistically significant difference between demodex positive and negative group regarding the age of the cases that was between 1-16 (p=0.756).

According to gender in pediatric patient group, there are certain reports indicating *Dd* was more frequent in girls[20,28]. In our study, incidence of positive *Dd* was detected in 77.8% females and 22.2% males. This is consistent with the literature.

In adult studies demodex detected from different area of the body, for example face, nasolabial sulcus, chine, forehead, scalp, back, trunk. The most frequently reported in nasolabiyal sulci and in the forehead[3,47,48]. In our study, *Dd* was detected all part of face gained samples, but only positive results were from the cheek, not other area. This result may be due to the study carried out on children in which it is seen rarely. In conclusion, Having high *Dd* density in children with CP may be related malnutrition via immunsupresion. In the study it was proposed that the increase in *Dd* apperence migth be grounded in wakening of immun system as a result of malnutrition in children with CP. There is a need for further studies with larger groups to investigate this relationship more thoroughly.

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Geometry function evaluation of high dose rate Ir-192 flexi source based on Monte Carlo (MCNPX) simulation

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Abstract

Two approximations of point source and line source are considered in TG-43U1 protocol to calculate the geometry function. The accuracy in geometry function calculation provides more accuracy in the other TG-43U1 dosimetry parameter determination.

In this study, the accuracy of geometry function of these two approximation sources on the Ir-192 high dose rate Flexi source were evaluated by using nuclear calculating Monte Carlo MCNPX code.

The Monte Carlo N-particle MCNP version x code was used to calculate the three dimensional dose distribution of a brachytherapy seed. The geometry function has been determined using tally F4 at different distances and several angles with respect to the source longitudinal axis.

The comparison of results shows that at angles close to the source longitudal axis, from θ=0 to 15° and θ=165 to 180°, the values evaluated from different methods are not in agreement with each other. The findings also show that in this angle span, the point source approximation is better than line source approximation. The result for angle span θ=15 to 165° shows that the difference between the line and point approximations and MCNPX is acceptable and line approximation is much better than point approximation.

Due to the importance of the distance less than 1.5 cm in brachytherapy and the effect of geometry function on the other brachytherapy dosimetry parameters especially interpolation and extrapolation of radial dose and anisotropic function, so the accuracy of geometry function calculation improve the accuracy of the other brachytherapy dosimetry parameter. Therefore it is proposed that for small distances and near angles, the geometry function be evaluated with calculating nuclear code such as MCNPX to improve the brachytherapy dosimetry accuracy and radiation therapy.

Key words: Geometry Function, High Dose Rate, Ir-192, MCNPX, Brachytherapy.

Introduction

Brachytherapy with low-energy photon-emitting sealed sources is one of the important options for the treatment of cancer. High dose rate (HDR) brachytherapy is a highly extended practice in clinical brachytherapy. Accurate radiation dosimetry is essential to achieve local control of the tumor while avoiding an unacceptable risk of normal tissue Complications. For this reason, the American Association of Physicists in Medicine (AAPM) formed Radiation Therapy Task Group 43 to standardize both the dose calculation formalism and the required data for each seed that was available for clinical use at the time. [1, 2, 3]

Two methods of obtaining the required data are widely accepted by the Med. Phys. community: direct measurements of dose rates in water equivalent material surrounding the seed, usually with LiF thermo luminescent dosimeters, and Monte-Carlo simulation of the transport of the photons emitted by the seed. [1]

For the purpose of treatment planning, dose distributions around cylindrical seeds are consider asymmetric. Within the context of clinical brachytherapy dose calculations, the purpose of the geometry function is to improve the accuracy with which dose rates can be estimated by interpolation from data tabulated at discrete points. Physically, the geometry function neglects scattering and attenuation, and provides an effective inverse square-law correction based upon an approximate model of the spatial distribution of radioactivity within the source. Because the geometry function

is used only to interpolate between tabulated dose-rate values at defined points, highly simplistic approximations yield sufficient accuracy for treatment planning. [4]

Accuracy in the calculation of the geometry function yields accuracy in the interpolation and extrapolation of the radial dose and anisotropy functions. However, this function is the only dosimetry parameter in TG-43U1 protocol which is determined through calculations rather than physical measurements.

Materials and Methods

Brachytherapy dosimetry parameters

According to the AAPM TG-43U1 protocol, the necessary dosimetry parameters for brachytherapy sources include: air-kerma strength (S_k), dose-rate constant (Λ), geometry function ($G(r,\theta)$), radial dose function ($g(r)$) and anisotropy function ($F(r,\theta)$). These parameters are integrated in Eq (1) to calculate spatial dose distribution, $\dot{D}(r,\theta)$, according to the coordinate system shown in Fig. 1. [4]

$$\dot{D}(r,\theta) = S_K \Lambda \frac{G_X(r,\theta)}{G_X(r_0,\theta_0)} g_X(r) F(r,\theta) \quad \text{..... (1)}$$

Where X=P for point source approximation and X=L for line source approximation.

$$S_K = \dot{K}(d).d^2 \quad \text{..... (2)}$$

$$\Lambda = \frac{\dot{D}(r_0,\theta_0)}{S_K} \quad \text{..... (3)}$$

$$G_P(r,\theta) = r^{-2} \quad \text{.....(4a)}$$

$$G_L(r,\theta) = \begin{cases} \dfrac{\beta}{L \sin\theta} \\ \left(r^2 - L^2/4\right)^{-1} \end{cases}$$

$$f\,\theta \neq 0^\circ \; f\,\theta = 0^\circ \quad \text{..... (4b)}$$

$$g_X(r) = \frac{\dot{D}(r,\theta_0)}{\dot{D}(r_0,\theta_0)} \cdot \frac{G_X(r_0,\theta_0)}{G_X(r,\theta_0)} \quad \text{..... (5)}$$

$$F(r,\theta) = \frac{\dot{D}(r,\theta)}{\dot{D}(r,\theta_0)} \cdot \frac{G_X(r,\theta_0)}{G_X(r,\theta)} \quad \text{..... (6)}$$

where L, r, β and θ are the parameters shown in the coordinate system used for brachytherapy dosimetry calculations (Figure 1). In Eq (2), d is the calibration distance; $\dot{K}(d)$ is the air kerma rate at distance d from the source center on the transverse plane. In dose calculations, the reference point is selected at r_0=1 cm, on the transverse axis bisecting the source (θ = 90). [4,5]

For point source approximation, the activity distribution is considered as a dimensionless point with an isotropic dose distribution around the source, therefore the geometry function $G_p(r,\theta)$ is calculated from Eq (4a).

However in line source approximation, radioactivity is assumed to be uniformly distributed along a one dimensional line-segment with active length L. The geometry function is calculated using Eq (4b) where β is the angle subtended by the tips of the hypothetical line source with respect to the calculation point, P(r,θ), and is calculated using Eq (7).

$$\beta = \theta_2 - \theta_1 = \tan^{-1}\left(\frac{x + L/2}{y}\right) - \tan^{-1}\left(\frac{x - L/2}{y}\right) \quad \text{..... (7)}$$

Where x= rcosθ, and y= rsinθ. [4]

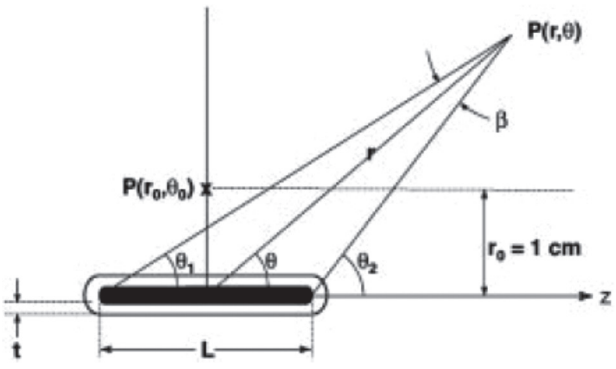

Figure 1. Coordinate system used for brachytherapy dosimetry calculation. [4]

The Monte Carlo MCNPX code

The Monte Carlo N-particle MCNP version x code was used to calculate the three dimensional

dose distribution of a brachytherapy seed. The MCNP code used in this study is a general purpose Monte-Carlo radiation transport code which can simulate coupled neutron–photon–electron transport in three dimensions through complex geometries constructed as Boolean combinations of planes, spheres, cones, and cylinders. The detailed photon physics treatment includes photoelectric absorption; K- and L shell fluorescence, Auger emission, coherent and incoherent scattering. [1, 6]

Monte Carlo method has been used to define the anisotropy dose function, the radial dose function, and the dose calculation close to the source in brachytherapy and in this study was used to evaluate geometry function. [7, 8]

The seed geometry was simulated as shown in Figure 2. The geometry function has been determined using tally F4 at different distances and several angles with respect to the source longitudinal axis. The medium inside and around the seed has been considered as vacuum in order to disregard the absorption and scattering in the seed and the surrounding media, therefore purely representing the effect of the activity distribution and inverse square law. [9]

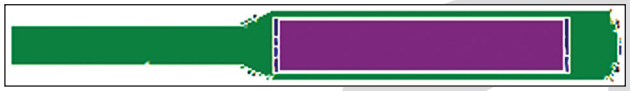

Figure 2. Flexisource Ir-192 simulated by MCNP code

Ir-192 Source

The source used in this study is the Flexisource Ir-192. The Flexisource consists of a 3.50 mm long Ir-192 core with a diameter of 0.60 mm enclosed in a 0.85 mm diameter AISI 304 stainless steel capsule (density of 8.02 g/cm3). The tip of the encapsulation is assumed to be a 0.108 mm thick conical section with a half angle of 23.6° and the radius of the face being 0.17 mm. The conical section is attached to a 0.49 mm long solid cylindrical section followed by a 3.6 mm long hollow section with an inner diameter of 0.67 mm. Following the hollow section is a 0.40 mm long solid conical section with a half-angle assumed to be 24°. Attached to the conical section is a 5 mm long section of AISI 304 stainless steel cable. The active length of this source is 3.50 mm. [10]

Radial Averaged Difference (RAD)

The radial averaged difference (RAD) is one of the best criterions to compare the MCNPX evaluation with point and line approximation source calculation. The radial averaged difference (RAD) is the squared difference of point/line approximation calculation and MCNPX evaluation that is averaged for different distances from source center at the specific angle. The closeness of RAD to zero shows the lesser difference between the point/line approximation source calculation and MCNPX evaluation. With regard to above explanation,

$$RAD = \frac{\sum_{i=1}^{n} (O_i - T_i)^2}{n} \quad \text{..... (8)}$$

Which O_i is MCNPX evaluation and T_i is the point/line approximation source and n is the number of data on a specific angle.

Results and Discussion

Physically, the geometry function neglects scattering and attenuation, and provides an effective inverse square-law correction based upon an approximate model of the spatial distribution of radioactivity within the source. In dose calculations, the reference point is selected at r_0=1 cm, on the transverse axis bisecting the source (θ=90), so the approximation source calculations and MCNPX results normalized to reference point value and to neutralize the inverse square law effect, the normalized value multiply to r^2. Then, the comparison values should be near to 1.

The normalized and neutralized geometry function values have been compared at various distances from the source center (radius) and for angles θ=0 to 180°. To show the results of different method, the behaviors of normalized and neutralized geometry function for different angles versus distances from source center are demonstrated in Figures 3-10.

Due to symmetrization of the source activity, the results of different method are also symmetry about the transverse axis bisecting the source (θ=90°). So to prevent the repetition, the curves of θ=105 to 180° is omitted. The following curves show that at near distances of the capsule wall, the line approximation calculation and MCNPX eval-

uation for angle span of θ=0 to 45° are higher than 1 and for θ=60 to 90° are lesser than 1. The curves also show that for θ<30°, there are the unacceptable difference between approximation methods and MCNPX but for 30°<θ<90° there are the good overlapping between the approximation calculation and MCNPX evaluation. All the result of θ=0 to 90° is repeated for θ=90 to 180°.

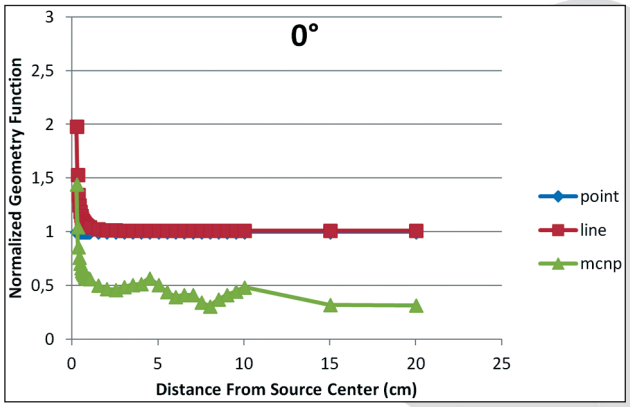

Figure 3. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 0°

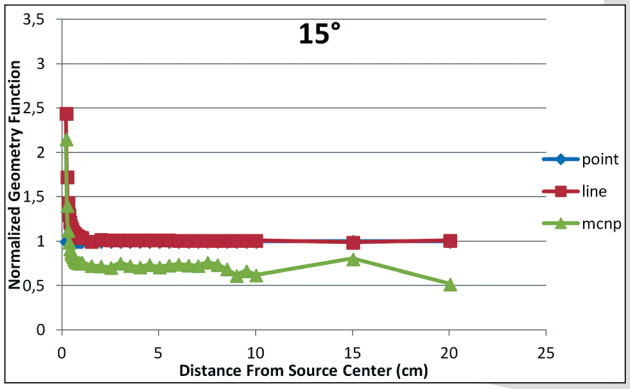

Figure 4. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 15°

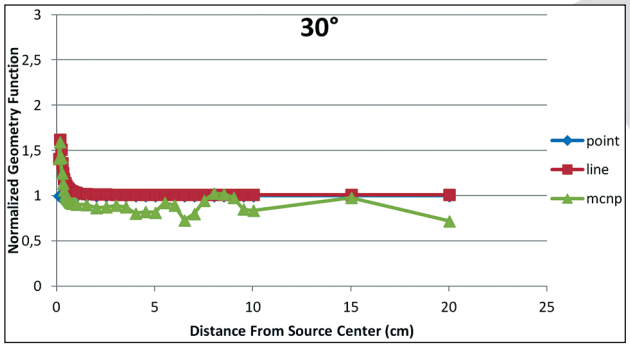

Figure 5. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 30°

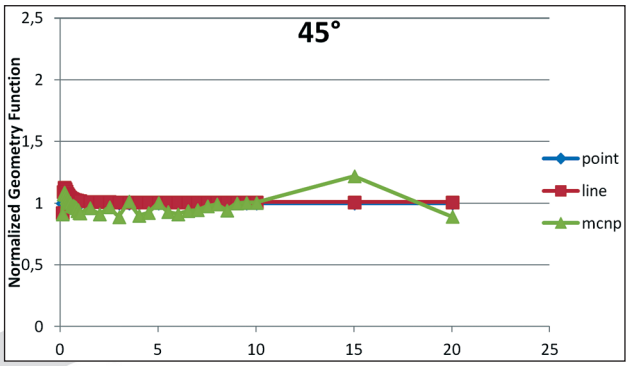

Figure 6. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 45°

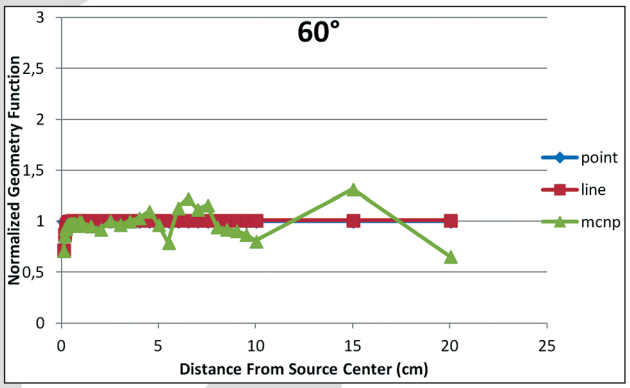

Figure 7. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 60°

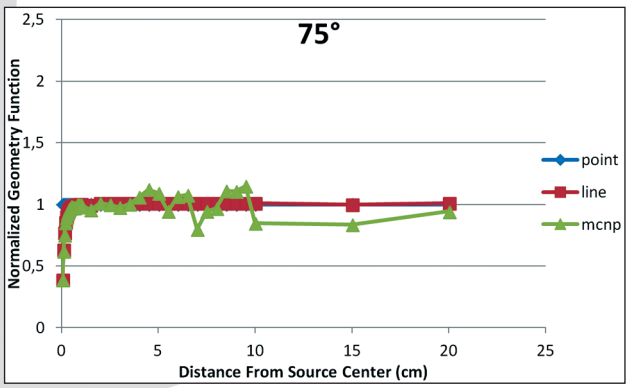

Figure 8. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 75°

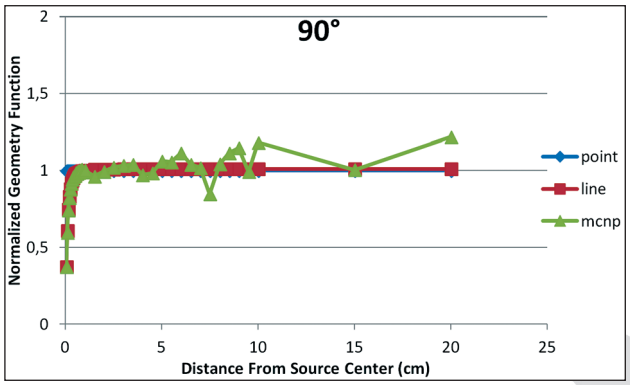

Figure 9. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 90°

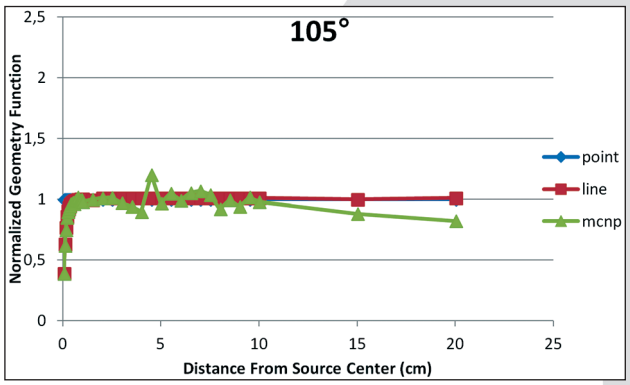

Figure 10. r^2 neutralized and $G(r_0, \theta_0)$ normalized geometry function calculation of point and line approximation and MCNPX evaluation in 105°

The comparison of results shows that at angles close to the source longitudal axis, from θ=0 to 15° and θ=165 to 180°, the values evaluated from different methods are not in agreement with each other. The radial averaged difference (RAD) between the MCNPX evaluation and point source approximation calculation for θ=0, 5, 10 and 15° are 0.24, 0.17, 0.09, and 0.08 respectively. The radial averaged difference (RAD) between the MCNPX evaluation and line source approximation calculation for θ=0, 5, 10 and 15° are 0.29, 0.22, 0.13, and 0.09 respectively. The radial averaged difference (RAD) of the source approximations and MCNPX in θ=165 to 180° is like the θ=0 to 15°. These results show that for the angle span up to the 15° from the source longitudal axis, the difference between the source approximations and MCNPX is high and the approximations are not valid enough. The findings also show that in this angle span, the point source approximation is better than line source approximation.

The comparison of the result for the other angle spans shows that for θ=15 to 60° and θ=120 to 165°, the radial averaged difference (RAD) between the line approximation and MCNPX is from 0.04 to 0.012 and for point approximation is from 0.07 to 0.014. The comparison of the results for θ=60 to 120° which is near transverse axis, show the difference between 0.007 to 0.003 and 0.02 to 0.009 for line and point source approximation, respectively. The result for angle span θ=15 to 165° shows that the difference between the line and point approximations and MCNPX is acceptable and line approximation is much better than point approximation.

The behavior of RAD versus different angles is shown in the Fig. 11. The comparison between the line and point approximations curve reveals that the line approximation is better than the point model. The minimum point of the curve is near the transverse axis bisecting the source which demonstrated the best agreement among the different methods and the maximum is near the longitudal axis of the source which demonstrated the worst agreement.

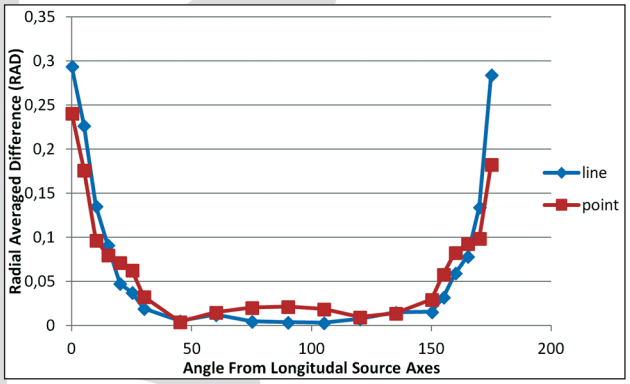

Figure 11. Radial Averaged Difference (RAD) of point approximation with MCNPX and line approximation with MCNPX

Conclusion

The comparison between the point and line approximation calculation and MCNPX evaluation show disagreement in the angles near to the source longitudal axis. The results also show that for the distances near to capsule wall, the difference between the point approximation and MCNPX is higher than line approximation model, then; the line model is better approximated than point. For the angles near to the source longitudal axis and

small distances from the capsule wall, there are difference between the line approximation and MCNPX. Due to the importance of the distance less than 1.5 cm in brachytherapy and the effect of geometry function on the other brachytherapy dosimetry parameters especially interpolation and extrapolation of radial dose and anisotropic function, so the accuracy of geometry function calculation improve the accuracy of the other brachytherapy dosimetry parameter. Therefore it is proposed that for small distances and near angles, the geometry function be evaluated with calculating nuclear code such as MCNPX to improve the brachytherapy dosimetry accuracy and radiation therapy.

Acknowledgment

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Prosthetic rehabilitation of a patient with iliac graft after mandibulectomy and partial glossectomy

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Abstract

Mandibular reconstructions improve esthetic and function of patients with mandibular oncologic defects. After reconstruction patients need to have prosthetic rehabilitation for mastication, swallowing, speech, psychological and esthetic. This clinical report describes the prosthodontic rehabilitation of a woman patient with iliac graft after mandibulectomy and partial glossectomy. The mandibular residual alveolar ridge presented a typical anatomy with a healed, discontinuous defect on the left side, extending to the midline, which displayed leftward displacement. During reconstruction surgery, the partially resected tongue had been attached to the crest of the iliac graft. A surgical plate composed by prosthodontists was inserted during the operation in order to reshape sulcular region. Prosthetic rehabilitation was completed with maxillary and mandibular telescopic over dentures. The patient presented significant improvements in oral function and psychosocial activities and no prosthetic complications.

Introduction

Oral cavity tumors are a common indication for mandibular resection; other indications include congenital abnormalities, osteomyelitis, and osteoradionecrosis (1). Injuries such as motor vehicle accidents and firearm and other traumas also damage the soft and hard tissues of the mandible (2).

As a result of a segmental mandibular defect, deviation toward the affected side causes serious deficiencies in chewing, drinking, swallowing, and speech functions; compromises aesthetics; and has negative psychosocial effects on the patient. Numerous studies have shown that oral rehabilitation of patients with large segmental defects remains challenging. The method of bone reconstruction has important effects on functional and aesthetic results. The iliac bone, fibula, and scapula are preferred donor sites and result in good survival rates (3).

After soft- and hard-tissue reconstruction, patients require prosthetic rehabilitation to achieve optimal oral functioning, especially mastication, swallowing, and speech. One challenge in defect prosthetics is the rehabilitation of a patient who has lost part of the tongue. Such patients have difficulties with deglutition, speech, and saliva control. Furthermore, swallowing dysfunction contributes to nutritional deficiency (4). Anatomical, functional, and aesthetic aspects must be taken into account when performing reconstruction and prosthesis placement. Normal speech, deglutition, mandibular movement, and facial contours must be achieved.

This report presents the case of a patient who underwent iliac graft reconstruction after unilateral mandibulectomy and partial glossectomy, and received a removable partial denture retained by telescopic crowns.

Patient history and clinical evaluation

A 49-year-old woman presented to the Department of Prosthodontics of Karadeniz Technical University (KTU) for mandibular reconstruction and prosthetic rehabilitation. At presentation, the patient was dissatisfied with her maxillary partial denture and complained of the poor appearance and adaptability of her mandibular removable denture. She had undergone two radical surgeries at KTU and the Istanbul University Faculty of Medicine 18 years previously to resect a small part of the tongue and left mandible due to a tumor. After partial glossectomy and mandibulectomy at the Istanbul Faculty of Medicine, reconstruction of the bone defect was attempted with an iliac crest graft (Figure 1).

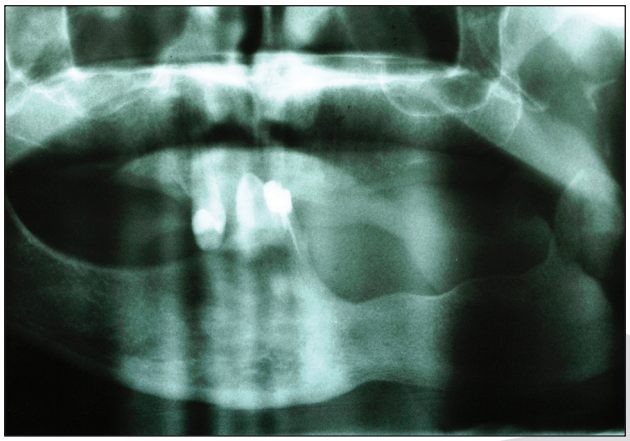

Figure 1. Panoramic radiograph after partial glossectomy and mandibulectomy

Extraoral clinical examination revealed leftward chin curvature and sinking of the left mandibular region due to soft-tissue shrinkage after iliac graft reconstruction surgery. The oral aperture was limited, and intraoral examination revealed the presence of only the mandibular right canine and lateral incisors and maxillary right canine, which lacked mobility and tenderness after periodontal therapy. The mandibular residual alveolar ridge presented an atypical anatomy with a healed, discontinuous defect on the left side, extending to the midline, which displayed leftward displacement. During reconstruction surgery, the partially resected tongue had been attached to the crest of the iliac graft, resulting in complete obliteration of the lingual sulcus on the affected side and severely compromising tongue function. Thus, the patient had difficulties with swallowing, speech, and saliva control (Figures 2a-2b-2c).

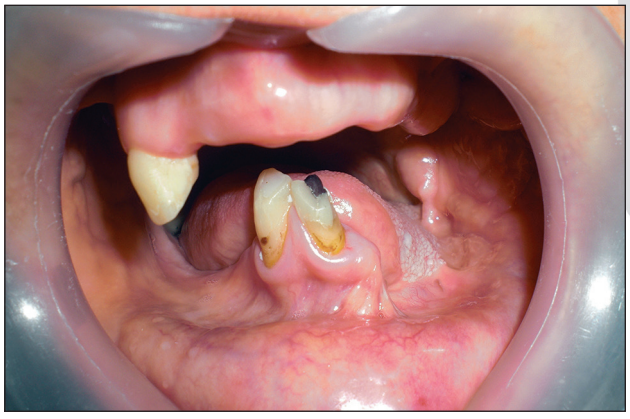

Figure 2a. Intraoral view, the partially resected tongue had been attached to the crest of the iliac graft

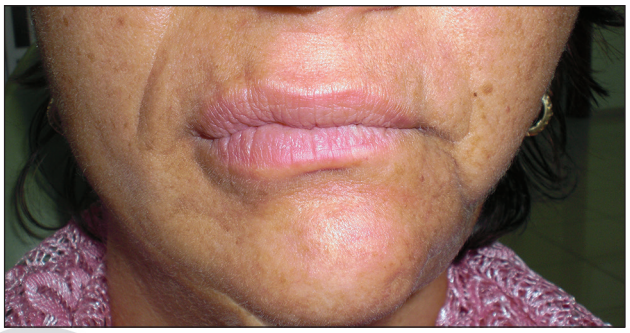

Figure 2b. Extraoral frontal view, the left mandibular region due to soft-tissue shrinkage after iliac graft reconstruction surgery

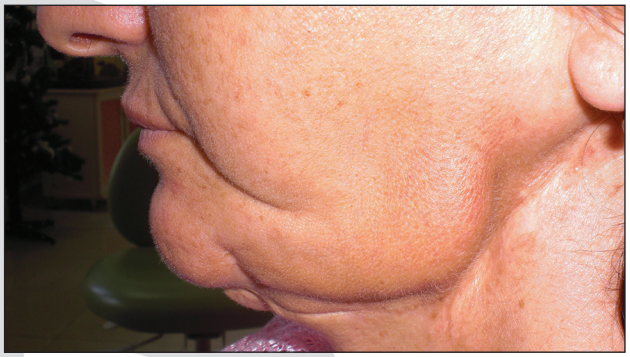

Figure 2c. Extraoral lateral view

Surgical reconstruction

Resection surgery for a tumor that included the mandible and tongue had altered the muscles of the tongue and the mouth floor. To eliminate these dysfunctions and facilitate prosthetic treatment, soft-tissue reconstruction surgery was performed on the tongue and left mandible attachment in the Department of Oral and Maxillofacial Surgery of KTU. A surgical plate fabricated by prosthodontists was inserted to reshape the sulcular region. The surgical procedures proceeded without complication and healing occurred properly (Figures 3a-3b).

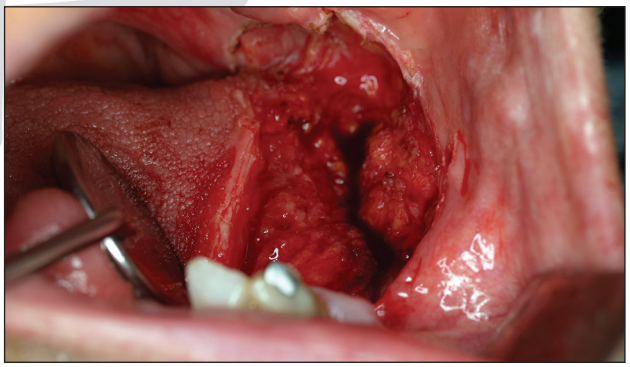

Figure 3a. Soft-tissue reconstruction surgery was performed on the tongue and left mandible attachment

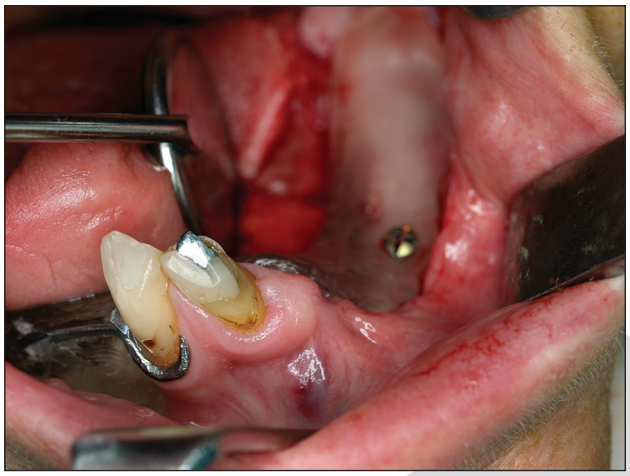

Figure 3b. A surgical plate fabricated by prosthodontists was inserted to reshape the sulcular region

Prosthesis fabrication

During the following visits, a treatment plan was discussed with the patient; the preferred approach called for the use of a telescopic crown–retained removable denture, which can be a valid treatment option for patients with limited finances and a large defect.

After periodontal and endodontic evaluations, the maxillary and mandibular canines and the mandibular lateral incisor, which had been selected to serve as abutments for the telescopic crown, were prepared with diamond burs to form a chamfer margin and to create sufficient space for the metal cap of the telescopic crown–retained overdenture. Coping impressions were made using metal stock trays and condensation silicone impression material. Molding, casting, adjustment, and polishing of the metal copings were performed according to conventional techniques (Figure 4a). Irreversible hydrocolloid material was used to obtain an impression of the removable denture with an individual tray, which was fabricated from self-curing acrylic resin. The metal framework of the mandibular telescopic crown–retained removable denture was adjusted to the mandibular defect. Bite registration was then conducted and transferred to the articulator. After arrangement of the artificial teeth, a try-in was conducted to check retention, stability, and occlusion. Finally, the restoration was finished using conventional procedures (Figure 4b).

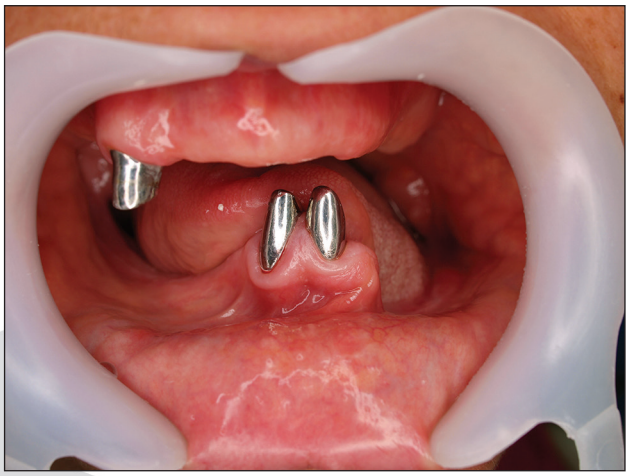

Figure 4a. Metal copings

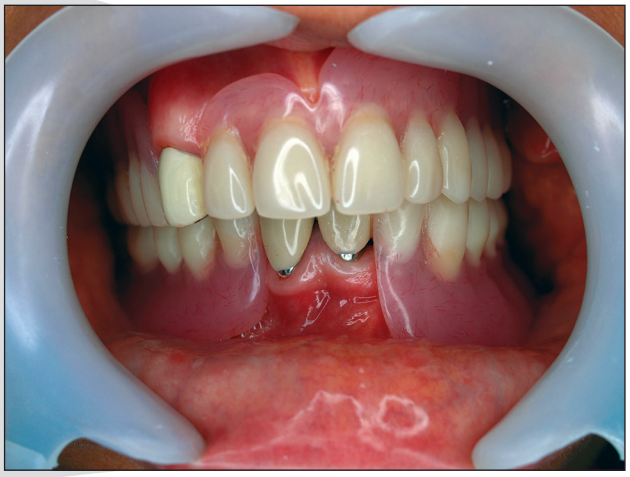

Figure 4b. Final restoration

Discussion

The resection of oral pathologies can lead to significant facial deformity; impaired oral functions such as speech, swallowing, and saliva retention; and concomitant psychological problems. Moreover, the loss of teeth and alveolar and basal bone can significantly impair mastication. The rehabilitation of a patient who has lost all or part of the tongue presents a challenge in defect prosthetic applications. Such patients may experience difficulties with deglutition, speech, and saliva control, as well as swallowing dysfunction, which contributes to nutritional deficiency (4). Because our patient's tongue had been attached to the left mandible due to a partial glossectomy, surgical soft-tissue reconstruction with a plate was performed before prosthetic procedures.

Selection of the best treatment plan depends on several diagnostic factors and the patient's prefe-

rence. Several treatment plans to restore function and aesthetics, ranging from the placement of a conventional removable partial denture to the use of implants, were generated and discussed with our patient. The patient feared surgery because of her experience with previous operations, financial limitations, and dissatisfaction with the existing conventional clasp-retained removable partial denture. Thus, instead of placing an implant-retained prosthesis and a conventional clasp-retained removable denture, we fitted a telescopic crown–retained removable partial denture after surgical reconstruction of the tongue.

Overdentures can be anchored using slips on the bar connecting to the implant, ball attachments, or magnets. Precision attachments have also been used for years in removable and fixed prosthetics, and have contributed to the success of removable partial dentures and overdentures. However, a previous study found that precision attachment–retained dentures tended to concentrate more stress at the terminal abutment tooth than did telescope-retained dentures (5).

As an alternative to precision attachment– and implant-retained restorations, a removable partial prosthesis may be a treatment option owing to its satisfactory functional stability, good retention, and oral comfort. Considering these requirements, a telescopic crown–retained removable partial denture is an alternative to a conventional clasp-retained removable partial denture, which results in inefficient mastication, generates periodontal inflammation, and increases abutment mobility due to the presence of clasps. Proper oral hygiene and ideal occlusal loading between the abutment teeth and alveolar bone should be ensured (6). In addition to clinical experience, previous long-term follow-up studies (7,8) have indicated a good prognosis and rigidity of telescopic crown systems. Although some researchers (9) have pointed out the aesthetic problems of telescopic crown–retained dentures due to the exposed cervical metal collar, especially when anterior teeth serve as abutments, most clinicians prefer this approach because root-supported overdentures, which are an alternative to extraction and the use of complete dentures, preserve the alveolar ridge and prevent bone loss. In the case presented here, the patient's teeth were preserved through the placement of a telescopic crown–retained removable partial prosthesis.

Conclusion

The primary objectives of prosthodontics must be the preservation of oral health in existing tissue, and the achievement of optimal oral function and patient satisfaction with the replacement of lost structures. In the present case, the patient had previously undergone a poorly planned restoration after reconstruction surgery due to a tumor. Our restoration with a telescopic crown–retained removable partial denture satisfied the realistic expectations of a patient who exhibited fatigue, feared surgery, had financial limitations, and was dissatisfied with the existing conventional clasp-retained removable partial denture.

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Electro-neurological patterns in preeclampsia: Clinical EEG evaluation and power spectral analysis in the third trimester of pregnancy

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Abstract

Background: Preeclampsia is a serious pregnancy-specific disease, a multi-system disorder in which the cerebrovascular changes and neurological complications remain an important cause of maternal mortality.

Objective: This study was undertaken to quantificate the patterns of brain electrical activity in preeclampsia using both classical and spectral electroencephalographic (EEG) analysis.

Methods: We obtained EEG data for forty women with clinical and paraclinical features of preeclampsia, in the third trimester of pregnancy. Visual analysis of EEG traces was followed by the absolute power spectrum evaluations of four different EEG frequency bands (alpha, beta, theta, delta). The results were compared with those of forty healthy (normotensive) pregnant women.

Results: After preliminary visual analysis, our study revealed EEG abnormalities in 31 cases in the preeclamptic group compared to healthy pregnant women, consisting in generalized and focal slowing of the background activity, intermittent slowing/ polymorphic delta activity, spikes and slow-wave complexes. Power spectrum analysis in the preeclamptic group showed a significant decrease of absolute power in the high frequency EEG bands (alpha and beta) and a significant increase of absolute delta power ($p< 0,05$) especially over the posterior cerebral regions.

Conclusion: Our findings indicate that multichannel EEG analysis can be used as a flexible tool to detect brain dysfunction in preeclampsia before cerebrovascular changes result in irreversible brain dysfunction.

Key words: EEG, hypertensive disorders, preeclampsia, pregnancy, power spectra.

Introduction

Hypertension is the most common medical problem in pregnancy, complicating up to 15% of pregnancies and accounting for about a quarter of all antenatal admissions which makes it an important threat to public health in both developed and developing countries (1). Hypertensive disorders in pregnancy remain a leading cause of maternal, fetal, and neonatal morbidity and mortality. Pre-eclampsia is an idiopathic multisystem disorder of pregnancy, characterized by gestational hypertension and new-onset proteinuria occurring in the second half of pregnancy (2, 3) and can present as late as 4-6 weeks postpartum. This complex condition is characterised by suboptimal uteroplacental perfusion associated with a maternal inflammatory response and maternal vascular endothelial dysfunction with associated vasospasm (4). Preeclampsia produces multiple systemic derangements that can involve a diversity of organ systems including hematologic, hepatic, renal, and cardiovascular systems as well as the central nervous system (5, 6). Perhaps the most feared complication of preeclampsia is eclampsia itself, defined by the occurrence of one or more generalized convulsions and/or coma in the setting of preeclampsia and in the absence of other neurologic conditions (7). Although numerous organs are affected by hypertension in pregnancy, cerebrovascular involvement is the direct mechanism of death in ≈40% of patients (8).

To better understand the cerebrovascular mechanism(s) involved, a number of neuroimaging tech-

niques have been used that include angiography, computed-tomographic (CT) scanning, magnetic resonance imaging (MRI), positron emission tomography (PET/sPET) and Doppler velocimetry (9). These tests are used to measure brain anatomy or structure, but many of them are costly and, in some cases, there are risk factors associated with the procedures.

By contrast, the EEG (Electroencephalogram) does not assess the structure of the brain, but evaluates the manner in which a particular person's brain functions. The pattern of electrical activity produced on an EEG can be used to help diagnose a number of conditions that affect the brain. In addition to this, Quantitative Electroencephalogram (QEEG), also known by the acronym BEAM (Brain Electrical Activity Mapping), is a non-invasive technique for topographic display and analysis of brain electrophysiological data. Once a sample of the electrical activity data of the patient's brain has been collected, the proprietary software of the acquisition device performs a computerised transformation of the raw, analog brain waves into digital form, which can be analysed by the computer. Further, the report includes full colour topographic maps and tables of data that can be used for more detailed analysis, providing a sensitive and specific method to detect subtle variations in the activity of the brain (10).

The aims of this study was to acquire a better understanding of brain injury in preeclampsia. In this perspective, for the first time, we used both classical visual analysis of the EEG and BEAM to investigate the spatiotemporal aspects and electrical patterns of brain activity in pre-eclamptic patients and healthy pregnant control women during their third trimester of pregnancy.

Materials and methods

Participants

This study was carried out at the Department of Obstetrics, Second Clinic of "Cuza Voda" University Hospital in Iasi, Romania, a state facility specialized in high-risk pregnancies serving as a tertiary referral center. Eligible women were identified by the research coordinator and/or the medical staff involved in project, inclusion criteria being definition of a preeclampsia. In our study, we recruited 40 pre-eclamptic women, in the third trimester of pregnancy, age between 18 and 40 years, from the obstetric unit. The diagnosis of preeclampsia was based on the presence of elevated systolic blood pressure of at least 140 mmHg and diastolic blood pressure of at least 90 mmHg at two occasions at least six hours apart after the 20th week of gestation, in association with proteinuria of at least 300 mg/24 h in absence of urinary tract infection. Severe preeclampsia was diagnosed based on the presence of preeclampsia together with at least one of the following: systolic blood pressure >160 mmHg and/or diastolic blood pressure >110 mmHg, renal impairment (oliguria or elevated blood creatinine levels >135 $\mu mol.L^{-1}$ or proteinuria > 5g/day), hematologic abnormalities (thrombocytopenia-platelet count < $100.109.L^{-1}$ and microangiopathic hemolysis), epigastic pain, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count, and right-upper quadrant pain), eclampsia or other neurological impairment (headache, visual disturbances, hyper-reflexia, nausea and vomiting). Patients were managed according to basic protocol for preeclampsia. Healthy pregnant (control subjects, n=40) whose pregnancies had been uncomplicated and normotensive with normal laboratory tests were also recruited. All the women were nonsmokers and had no history of chronic hypertension, diabetes or renal, cardiac, and vascular disease. In both groups exclusion criteria were: prepregnancy convulsive disease (epilepsy), a history of coma, alcohol or substance abuse, toxic exposures, a known cerebrovascular accident, demyelinating diseases or other neurological or psychiatric disorders known to influence brain functions. Patients were also asked about traumatic brain injury and brain surgery in the past, and current use of medication. The study was approved by the local ethical committee and conducted in accordance with the Helsinki Declaration. All participants of the study were informed about the non-invasive nature and purpose of the investigations, and written consent from each subject was obtained prior to the study.

EEG data acquisition

EEG/BEAM recordings were performed in Ambulatory Care Unit (Functional Exploration Laboratory) of Sf.Spiridon Hospital, Iasi, Romania. Only one measurement was performed for

each subject in both groups. EEG recordings were always assessed following overnight or morning abstinence from caffeine (such as coffee, tea, cola, and chocolate) and non-prescripton medication that could affect brain's usual electrical activity and cause abnormal results. Subjects were seated in a comfortable reclining chair in a dimly-lit, sound-attenuated room, and they were instructed to close their eyes, relax, but stay awake and minimize eye and body movement. The standard ''only eyes closed condition'' was being used. Vigilance controlled recording sessions lasted 15–20 min, and as soon as drowsiness patterns appeared in the record, the subjects were aroused by auditory stimuli (tapping). All EEGs were recorded using the Nihon Kohden digital EEG 2100 (Nihon Koden, Tokyo, Japan) and signals were continuously represented online on a computer screen. Data were recorded from 16 scalp sites using Ag/AgCl coated plastic cup electrodes (surface electrodes), attached with paste at the following positions of the International 10-20 System in a hard-wired bipolar montage: Fp2-F8, F8-T4, T4-T6, T6-O2, O2-P4, P4-C4, C4-F4, F4-Fp2; Fp1-F7, F7-T3, T3-T5, T5-O1, O1-P3, P3-C3, C3-F3, F3-Fp1, using linked earlobe electrodes as the reference. Electrode-impedances were kept below 5 kV. The EEG was amplified (0.3 to 60 Hz bandpass filter), digitized (200 Hz), and stored on hard disk for offline processing. After each EEG recording, subjects were asked for the presence of side effects, such as sleepiness, fatigue, lassitude, and nausea.

EEG data analysis

Analysis of the EEG consisted of four stages: (1) removal of artifacts (2) preliminary visual analysis (3) calculation of power spectra, and (4) determination of the statistical significance of differences between conditions/groups. Elimination of artifacts and identification of all others was accomplished by a qualified technologist. Most typical artifacts were caused by eye movements, drowsiness, actual sleep, muscle contractions, bad channels, and clipping. A preliminary visual EEG analysis was assessed according to clinical criteria, and each EEG was reevaluated by a certified and experienced staff of neurophysiologists without knowledge of clinical informationand blinded to the patients' diagnoses. From the recorded data, 20 artifact-free epochs of 2.56 sec (512 data points) duration recorded in the eyes-closed but awake state were selected based on visual inspection of EEG. All epochs were recomputed against common average reference. The signal in each epoch was baseline corrected and filtered using theta (4.0–8.0 Hz), alpha (8.0–12.0 Hz), beta (12.0–25.0 Hz), and delta (1,0–4.0 Hz) bandpass filters. For each epoch and every scalp location the power spectrum was calculated by converting the raw EEG signal from the time domain into the frequency domain using Fast Fourier Transformation (FFT). Therefore, spectral analysis of EEG included a FFT transformation in which the absolute power (the total amount of brain activity at each electrode; μv^2) of four different EEG frequency bands (alpha, beta, theta, delta) was estimated.

Statistical Analyses

Differences between groups were investigated by using t-tests, including a comparison of absolute power in the delta, theta, alpha and beta bands of the QEEG. Data were expressed as mean ± SD. Mean, standard error of the mean, and standard deviation were determined for quantitative variables. Relative risks with the 95% CIs were also determined. A P value of < 0.05 was considered statistically significant. All analyses were performed using MS EXCEL 2010 (Data Analysis) software.

Results

All participants completed the experiment. Relevant characteristics of the two groups of women included in the study are shown in Table 1. The groups did not differ by mean years of age (preeclampsia 26,96; control 27,04) or parity (percent multiparous: preeclampsia 24%; control 25%) at index pregnancy. There were no statistically significant differences in weight and body mass index (BMI) in patient profiles between groups. By definition, systolic and diastolic blood pressures were elevated in the preeclamptic group compared with the healthy pregnant control groups.

EEG visual analysis

A preliminary visual inspection and analysis was carried out in order to establish the objectives. The whole group of preeclamptic patients shows EEG abnormalities in 31 cases, without any

Table 1. Clinical characteristics of participants

Data	Controls (n=40)	Standard eror	Standard deviation	Confidence level (95%)	Statistical analysis-P	PE (n=40)	Standard eror	Standard deviation	Confidence level (95%)
Current age* (years)	27,04	1,44508	7,22541	2,982505	0,49236	26,96	1,47815	7,39076	3,050755
Weight* (kg)	83,88	3,43429	17,1714	7,088041	0,31855	83,96	3,40121	17,006077	7,019763
Height* (cm)	168,04	1,66681	8,33406	3,44013	0,79914	168,4	1,61554	8,0777472	3,334330
BMI* (kg/m2)	29,576	1,01495	5,07479	2,094773	0,3783	29,5008	1,05284	5,2642362	2,172969
Current SBP* (mmHg)	118,32	2,34307	11,7153	4,835868	0,75324	158,2	4,45784	22,289235	9,200543
Current DBP* (mmHg)	71,64	1,30639	6,53197	2,696265	0,876	105,2	3,01544	15,077246	6,223581
Age of gestation* (weeks)	35,48	0,44015	2,20075	0,908428	0,938	34,96	0,44899	2,2449944	0,926688

**Results are given in means; Abbreviations: SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure; PE-Preeclampsia.*

abnormal EEG in control group. The main EEG abnormalities found in the preeclamptic group are shown in Table 2.

Generalized slowing with dominant frequency of rhythmic background activity below 8 Hz or anteriorly prominent delta waves was highlighted (7 cases). We found focal slowing of the dominant posterior background activity-namely, parieto-occipital lobes-with right hemisphere location in 12 cases. Intermittent slowing was also present (6 cases), consisted in bursts of generalized slowing (2-4 sec), especially polymorphic delta. Another finding was the continuous slowing with generalized polymorphic delta activity (greater than 80% of the recording). In 4 cases EEG shows sudden bursts of electrical activity (spikes) and slow-wave complexes in left temporo-occipital region associated with background attenuation.

EEG power analysis

The brain topography of power spectra along the antero-posterior (A-P) axis was studied in the group of preeclamptic woman with EEG abnormalities (in the preliminary analysis) compared with control group. Table 3 shows the mean values of absolute power (μV^2) for alpha and beta EEG bands for the various positions of the electrodes in the preeclamptic and control groups.

Absolute alpha power was reduced for the majority of the electrodes for the preeclamptic group, and that the differences were statistically significant over parietal (P3,P4) and occipito-temporal regions (O1,O2,T5,T6). Absolute beta power, was reduced over parietal (P3,P4) and occipital (O2) regions in the preeclamptic group. Table 4 shows the mean values of absolute power (μV^2) for delta and theta EEG bands for the various positions of the electrodes in the preeclamptic and control groups.

Patients with preeclampsia showed, as compared with controls, a significant increase in absolute delta power over parietal (P3,P4) and occipital (O2) regions. Absolute delta power was also increased for the majority of the electrodes over frontal (FP2,F3,F4,F7) regions. No statistically significant differences were found between the preeclamptic and control groups for the absolute theta power.

EEG clinical analysis

EEG analysis was corroborated with the clinical findings. Clinical examination of preeclamptic women have revealed various neurologic symptoms such as headache, visual disturbances,

Table 2. Clinical characteristics and EEG findings for preeclamptic women's with abnormal recordings

PE patients	Current age (years)	Age of Gestation (weeks)	EEG findings	SBP (mmHg)	DBP (mmHg)	Neurological symptoms*
1	33	37	Generalized slowing	168	94	+
2	24	33	Focal slowing	147	98	-
3	21	35	Intermittent slowing	146	110	+
4	34	37	Spikes/slow-wave complexes	161	108	+
5	33	36	Generalized slowing	148	104	+
6	20	34	Focal slowing	151	96	-
7	29	35	Generalized slowing	154	112	+
8	28	35	Intermittent slowing	147	93	+
9	36	36	Generalized slowing	162	99	+
10	22	33	Focal slowing	150	100	+
11	23	32	Focal slowing	151	102	+
12	21	34	Focal slowing	154	107	+
13	27	34	Focal slowing	156	95	-
14	29	35	Spikes/slow-wave complexes	148	100	+
15	35	36	Continuous slowing	160	104	+
16	32	33	Intermittent slowing	148	94	-
17	25	32	Generalized slowing	155	95	+
18	25	37	Focal slowing	152	92	-
19	28	37	Focal slowing	146	98	+
20	31	39	Spikes/slow-wave complexes	159	110	+
21	20	36	Intermittent slowing	155	105	+
22	26	35	Focal slowing	142	93	+
23	34	39	Generalized slowing	149	95	-
24	22	33	Focal slowing	146	94	+
25	21	36	Intermittent slowing	145	95	+
26	29	37	Generalized slowing	155	110	+
27	30	39	Spikes/slow-wave complexes	165	100	+
28	19	33	Focal slowing	147	95	-
29	26	32	Intermittent slowing	145	95	+
30	22	36	Focal slowing	150	100	+
31	36	38	Continuous slowing	160	110	-

*Abbreviations: EEG- Electroencephalography; PE-Preeclampsia; SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure; *Blood pressure and neurological symptoms at the time of EEG recordings: (+) neurological symptoms were present/(-) neurological symptoms were absent.*

confusion, hyper-reflexia, myoclonia, nausea and vomiting. The presence of one or more of these symptoms were associated with abnormalities detected by analysis of EEG patterns. Neurologic symptoms were present in 23 patients with EEG abnormalities. The mean systolic blood pressure/mean diastolic blood pressure values were 152,32±6,52 mmHg/ 100,09 ± 6,22 mmHg in the group of preeclamptic woman with EEG changes. Furthermore, the observed EEG abnormalities were not closely associated with severity of hypertension. Table 2 shows the association between EEG abnormalities, blood pressure values, and neurologic symptoms in the preeclamptic group.

Discussion

In the first part of this study, visual inspection of electroencephalographic tracings in the preeclamptic group, showed a variety of changes in majority of cases, compared to healthy pregnant women. The most commonly reported EEG abnormality was slowing of background activity. Generalized slowing is generally nonspecific, an abnormal

Table 3. The mean values of absolute power (μV^2) for alpha and beta EEG bands for the various positions of the electrodes in the preeclamptic and control groups

	Alpha band			Beta band		
Electrode	**CG**	**P**	**PE**	**CG**	**P**	**PE**
FP1	36,17	0,402	37,22	7,86	0,054	7,92
FP2	35,84	0,953	36,21	8,93	0,098	7,89
F3	40,14	0,324	37,06	8,94	0,056	8,99
F4	40,65	0,178	46,77	9,19	0,321	9,16
F7	41,58	0,298	37,57	8,32	0,293	8,35
F8	42,13	0,283	38,12	8,16	0,123	8,08
C3	43,65	0,817	29,77	9,19	0,747	10,10
C4	43,16	0,906	29,27	9,66	0,122	9,64
T3	23,08	0,521	28,53	15,34	0,058	17,22
T4	22,75	0,377	22,28	8,67	0,058	8,65
T5	55,22	0,045*	42,39	11,29	0,660	10,41
T6	56,16	0,039*	47,64	10,32	0,914	10,21
P3	83,92	0,041*	45,35	8,11	0,046*	8,06
P4	78,15	0,026*	44,04	8,25	0,047*	6,21
O1	122,35	0,041*	71,32	9,17	0,338	4,22
O2	117,66	0,011*	70,26	9,68	0,032*	4,34

Abbreviations: PE-Preeclampsia; CG-control group. Asterisks (*) indicate statistically significant differences (t test, $p<0.05$) in the mean values between the two groups.

Table 4. The mean values of absolute power (μV^2) for delta and theta EEG bands for the various positions of the electrodes in the preeclamptic and control groups

	Delta power			Theta power		
Electrode	**CG**	**P**	**PE**	**CG**	**p**	**PE**
FP1	30,64	0,116	31,26	10,69	0,128	9,06
FP2	48,57	0,086	48,67	13,14	0,142	11,16
F3	31,12	0,054	32,94	22,17	0,065	20,93
F4	31,55	0,063	32,06	21,22	0,067	21,13
F7	46,93	0,065	47,16	17,18	0,071	18,66
F8	49,93	0,149	47,06	16,47	0,052	16,12
C3	33,85	0,069	33,86	15,83	0,110	17,56
C4	34,78	0,269	33,54	16,26	0,077	17,28
T3	33,15	0,229	36,37	10,65	0,079	10,41
T4	32,78	0,132	32,88	11,22	0,060	11,45
T5	33,76	0,250	38,57	11,45	0,091	11,87
T6	33,86	0,054	35,62	13,26	0,067	13,46
P3	31,87	0,038*	39,32	15,68	0,376	15,72
P4	33,56	0.045*	46,13	15,35	0,125	17,32
O1	31,32	0,160	49,22	14,65	0,120	16,22
O2	32,74	0.033*	49,15	14,61	0,053	15,32

Abbreviations: PE-Preeclampsia; CG-control group. Asterisks (*) indicate statistically significant differences (t test, $p < 0.05$) in the mean values between the two groups.

pattern appearing as an indicative of diffuse brain dysfunction, representing the most common finding in encephalopathies of various etiologies, including metabolic, hypoxic, endocrinologic, degenerative, and inflammatory encephalopathies (11). Focal slowing activity seen in EEG recordings in preeclamptic patients, is also nonspecific as to etiology, and is the most common abnormality associated with focal lesions of any type, including vascular (hypertension), neoplastic subdural collections, traumatic, and infectious (11). In this context , the EEG slowing of background activity in the preeclamptic group may be related to metabolic and hypertensive encephalopathy. It has been generally assumed that neurologic complications of pre-eclampsia are thought to be similar to posterior reversible encephalopathy syndrome (PRES), a variant of hypertensive encephalopathy in which an acute and excessive elevation in mean arterial pressure causes forced dilatation of the cerebral arteries and arterioles, loss of cerebral blood flow (CBF) autoregulation, BBB (blood-brain barrier) disruption, and vasogenic edema formation (12).

Another finding in the present study was intermittent rhythmic delta activity, usually occurs at frequencies of 2-2.5 Hz with relatively sinusoidal, stereotypic, bilaterally synchronous waveforms appearing in short bursts (13, 14). Although the mechanisms for production are understood incompletely, intermittent rhythmic delta activity has many etiologies, including vascular, metabolic, toxic, hypoxic, or various diffuse or focal intracranial diseases (13, 14). Polymorphic (arrhythmic) slowing that we found, are typically associated with focal attenuation of normal rhythms. Marshall et al. (15) noted that these lesions typically requires involvement of white matter and were correlated with structural and hypoxic lesions. The present findings may be another argument that some of the etiologic mechanisms involving in the pathogenesis of neurological complications in preeclampsia can be represented by cerebral vasoconstriction or vasospasm hypertensive encephalopathy, cerebral edema or infarction, and metabolic encephalopathy. The EEG recording in 4 preeclamptic women shows spikes and slow-wave complexes, that are considereted as epileptiform patterns, associated with epilepsy or other neurologic conditions. However, the term epileptiform is only descriptive and does not necessarily imply that the pattern is epileptogenic (16). Supporting our observations, Brussé et al. (17) previously showed that EEG may detect epileptiform abnormalities (spikes, sharp waves, spike and slow-wave complexes, polyspike and slow-wave complexes) in patients with severe preeclampsia and eclampsia.

We found no EEG abnormalities after visual inspection in healthy controls. The findings of the current study are consistent with those of Brussé et al. (17) and Keunen et al. (18) who found no significant differences during third trimester pregnancy and six month postpartum in healthy women, an argument that EEG is normal in the third trimester pregnancy. According to these studies, it appears that EEG changes seen during normal pregnancy indicate a pre-existing or more recent brain's unknown injury.

EEG analysis based on spectral absolute power of the separate frequencies showed interesting changes in preeclamptic patients with primary visual EEG abnormalities compared to controls. The current results showed a decrease of power in the high frequency EEG bands. Topographic brain maps revealed a temporal, parietal and occipital reductions for absolute alpha power. Multiple relevant studies have shown the thalamus and regions of occipital and parieto-occipital cortex to be involved in alpha rhythm generation. Also, it had been established that alpha band activity can be used as an index of cortical activity, implying a negative correlation with cerebral blood flow (19). This findings of our study are consistent with the concept that pre-eclampsia is a state of over-perfusion, associated with the syndrome of reversible posterior leukoencephalopathy , in which changes in blood pressure can result in an imbalance of the capillary and cellular perfusion pressures, leading to vasogenic edema (18, 20). Therefore, reduction of alpha power may be an indicator of increased cerebral blood flow in the bilateral occipital cortex, including primary and association visual areas. This may suggest that more common visual symptoms, such as blurred vision, double vision, photophobia, reported by women with pre-eclampsia, can be associated with an abnormality in cerebral perfusion pressure. Beta power spectrum reduction found in our study can be an indicator of cortical integrity in preeclampsia, because loss of beta activity, whether diffuse or focal, indicates

compromised cortical function (21). Furthermore, Jordan et al. (22) found that decreases in beta activity was associated with reductions in cerebral blood flow. For delta frequency, increases in activity occurred in absolute power in preeclamptic patients. Delta activity in the electroencephalogram is thought to reflect the depth or intensity of sleep. In addition, in brain imaging studies,delta activity recorded at the scalp was shown to correlate negatively with cerebral blood flow in the thalamus, cerebellum or orbito-frontal cortex (23). Put together, reduction of beta power and delta's increase in preeclampsia can be attributed to cerebral blood flow reductions. Therefore, these observations represent an possible argument in support another alternate theory proposed to explain the pathogenesis of hypertensive encephalopathy : vasospasm, which causes local ischemia, arteriolar necrosis, and disruption of the blood-brain barrier.

As far as we know, no relevant study has been conducted to evaluate EEG power spectra changes in preeclampsia. Therefore we cannot compare the results of our analyses regarding the absolute power of different EEG frequency bands.

EEG abnormalities found in the preeclamptic group, regarding topographic distribution, were more frequent in the posterior cerebral region. According to some opinions on pathophysiology of PRES, it seems that one mechanism by which the posterior brain region is more susceptible to edema in preeclampsia is because of enhanced BBB permeability in that region compared with the anterior cerebrum in response to acute hypertension (24). Also, the posterior circulation is more susceptible to this type of damage, because there is less sympathetic innervation of the ventrobasilar vasculature to protect the parenchyma from rapid increases in arterial blood pressure (25, 26, 27). The regional differences of EEGs indicate that neurologic involvement in preeclampsia is not only a global phenomenon but also a local brain process with a different regional involvement of neuronal populations.

Neurological symptoms or signs in the group study were related to EEG abnormalities. This findings is consistent with the concept that neurological complaints in a woman with pre-eclampsia are associated with an abnormality in cerebral perfusion pressure. Headache, confusion, and some common visual disturbances may be warning signs of hypertensive encephalopathy, which can accompany severe preeclampsia. These symptoms are associated with over-perfusion (28), which can provide the pathological base of focal or generalized EEG abnormalities.

There was no significant correlation between presence of EEG changes and severity of hypertension. One explanation of this evidence is that posterior reversible encephalopathy syndrome is a variant of hypertensive encephalopathy with diverse causes including pregnancy. The difference between hypertensive encephalopathy and PRES is that PRES can develop without a significant elevation in blood pressure (29).

The occurrence of cerebral edema and EEG abnormalities without severe high blood pressure during pre-eclampsia suggests that autoregulatory breakthrough is not necessary but may be more related to diminished autoregulatory capacity or enhanced BBB permeability, or a combination of both (24).

Conclusions

There remain many unanswered questions regarding the pathogenesis of the cerebral manifestations of pre-eclampsia. However, further work needs to be done in this field, in order to study the effects of pre-eclampsia on background cerebral electrical activity and to identify an accurate method for predicting which women will progress to the more severe forms of neurologic complications or eclampsia. The interpretation of the results of our study regarding type and localization of EEG anomalies, in conjunction with the previous studies, would suggest that EEG (electroencephalography) can be a sensitive method to detect brain dysfunction in preeclampsia before ischemic or cerebral hyperperfusion conditions result in irreversible brain dysfunction. Also, the present findings may be an argument to perform more detailed QEEG analysis during the third trimester in preeclampsia, in order to understand the early EEG phenomena which may precede the neurological complications of eclampsia. Moreover, further investigation on large groups of patients is required to determine whether this EEG anomalies reflects the presence of subclinical vascular changes as coexisting aetiologic factors in the development of neurologic complications in preeclampsia.

Authors' contributions

All authors contributed to the collection and assessment of the cases. All authors contributed to the analysis and interpretation of the data. All authors contributed to the revising of the article. Also, all authors read the final draft and give their approval of the final version.

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Protective effects of ebselen against iron-induced cardiotoxicity in rats

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Abstract

Objective: Ebselen, a substance with glutathione peroxidase-like activity, shows antioxidant, anti-inflammatory, neuroprotective and immunomodulatory effects. The purpose of this study was to find out the effects of ebselen against iron-induced cardiotoxicity in rats.

Material and Method: After obtaining ethical committee approval, fifty-six male Wistar Albino rats were divided into seven groups. Serum iron, ferritin, lactate dehydrogenase, creatine kinase and creatine kinase-MB (CK-MB) levels were measured biochemically. Iron accumulation in cardiac tissue samples was measured using inductively coupled plasma atomic emission spectrometry (ICP-AES). Comparison of iron accumulation histopathologically performed using Prussian blue stained sections by semi-quantitative method.

Results: Serum LDH levels in ebselen+iron, iron+deferoxamine and iron+deferroksamine+ebselen groups were significantly hihger compared to control group, while significant difference was observed between serum CK-MB level of iron+deferroksamine+ebselen group and control samples ($p<0.001$). Serum iron levels in iron+deferoxamine and iron+deferroksamine+ebselen groups were highly significanly hihger than controls, and serum ferritine levels in iron, ebselen+iron, iron+deferroksamine and iron+deferroksamine+ebselen groups were significantly higher compared to control group ($p<0.001$). Iron levels of cardiac tissue measured by ICP-AES in iron, iron+deferroksamine and iron+deferroksamine+ebselen applied groups were highly significantly different compared to those in controls ($p<0.001$), while a relatively less significant difference observed between ebselen+iron group and control ($p < 0.05$). Iron accumulation was found to be decreased in ebselen and deferroksamine given groups when histopathologically compared to iron applied group.

Conclusion: In conclusion, obtained findings indicated the efficacy and utility of ebselen treatment on iron induced cardiotoxicity. In this regard, ebselen might contribute to treatment in β-thalassemia and sickle cell patients, and those in need of frequent blood transfusion.

Key words: Ebselen, heart influences, iron induced toxicity.

Introduction

Serum iron level is increased in thalassemia, hemochromatosis, treatment of certain types of anemia, frequent blood transfusion, and African-type diet (1). In iron overload cases iron tends to accumulate in tissues such as liver, heart, gonads, pituitary, thyroid, skin and pancreas, which might result in clinical conditions including hepatocellular cancer, diabetes mellitus, cirrhosis and hypertension (2). Increased iron concentrion has been associated with lipid peroxidation, which was reported in patients with thalassemia and in iron treated animals (3,4). Myosit membranes and sarcoplasmic membranes are in direct contact with extracellular fluid; thus, these membranes might be extremely affected by iron toxicity. Previous studies showed that sarcolemma membrane damage in rat myocard is associated with levels of iron and concentrations environmental oxygen (5,6). Cytoplasmic membrane and mitochondria are susceptible and directly affected by acute iron poisoning that might cause many clinical manifestations such as metabolic aci-

dosis and hypotension. In this study, ebselen, with its antioxidant and anti-inflammatory effects, will be studied. Obtained findings are intended to contribute treatment of diseases characterized with iron overload as in β-thalassemia, and will contribute novel treatment approaches.

Material and methods

Animals

Fifty six male Wistar Albino rats weighing 280–300 g were used in this study. Rats were housed in a continuously ventilated room at a mean temperature of 22±2 °C with a lighting period of 12 h dark and 12 light. Throughout the study, the animals had free access to standard pellet rat chow and drinking water. The experiments were performed in accordance with Guide for the care and use of Laboratory Animals (National Research Council, 1996). The protocol of this study was approved by Ethics Committee of Mustafa Kemal University.

Experimental design

Animals were divided into 7 groups. Since ebselen is dissolved in 3% dimethyl-sulfoxide (DMSO) a DMSO group was created.

1. Control Group (C) (n:8): as control each rat was given intraperitoneally (i.p.) 0.5 ml saline per day for seven days.
2. Iron Group (F) (n:8): Iron was administrated at a dose of 100 mg/kg/dat i.p. for seven days
3. Ebselen Group (E) (n:8): 5 mg/kg/day Ebselen added to 0.3 ml of saline was given i.p. for seven days
4. DMSO group () (n:8): 0.25 cc/day DMSO was given i.p. for seven days
5. Iron+Ebselen Group (FE) (n:8): 100 mg/kg/day of iron, 5 mg/kg ebselen dissolved in 0.3 cc saline was given i.p. as 100 mg/kg/day for seven days
6. Iron+Deferroksamine Group (FD) (n:8): 100 mg/kg/day of iron and 100 mg/kg/day of deferroksamine added to 0.3 cc of saline were given i.p. for seven days
7. Iron+Deferroksamine+Ebselen Group (FDE) (n:8): 5 mg/kg/day ebselen, 100 mg/kg/day iron and 100 mg/kg/day of deferroksamine added to 0.3 cc of saline were given i.p. for seven days.

Collection and processing of samples

The rats were anesthetized with ketamine/xylazin (90/10 mg/kg, i.m.) and a midline incision was performed. Blood samples were collected from portal vein and centrifuged and stored at -70ºC untill analysis. Rats were sacrifised by blood draining. After a midline dissection heart was taken out and weighed. Cardiac tissue sample was obtained from apical site and tissue samples sent to the Experimental Research Laboratory of Mustafa Kemal University for iron levels' measurement by ICP-AES. Remaining cardiac tissue and blood samples stored at -85º C untill analysis.

Biochemical Analyses

Serum iron, ferritin, lactate dehydrogenase, creatine kinase and creatine kinase-MB (CK-MB) levels were measured biochemically. Serum CK, LDH activities, and iron levels were determined by spectrophotometric methods with using an autoanalyzer, Abbott Architect c8000 chemistry analyzer (Abbott Diagnostics Division, Abbott Laboratories, USA). Serum CK-MB activity was determined by immunoturbidimetric methods with using an autoanalyzer, Abbott Architect c8000 chemistry analyzer (Abbott Diagnostics Division, Abbott Laboratories, USA). Serum Ferritin level was determined by Chemiluminescent Microparticle Immunoassay (CMIA) methods using an immunoanalyzer, Abbott Architect i2000 immunoassay analyzer (Abbott Diagnostics Division, Abbott Laboratories, USA) .

Histopathological and Histochemical Analyses

Rats were divided into seven groups (C, E, F, FE, FDE, DMSO, FD). After rats were sacrified, organs harvested. The middle third of the hearts was exampled for histopathological processes. The cardiac tissue samples were fixed in 10% formalin overnight and underwent routine tissue processing procedure. The tissues were embedded in paraffin, sectioned with microtome (5 micrometer per section), and stained hematoxylin-eosin and Prussian blue stain. Histopathological sections examined with the light microscope in terms of cellular disorganization inflamation and necrosis. Iron accumulation was evaluated histopathologically by comparison of Prussian blue stained sec-

tions by semi-quantitative method. The iron deposition in myocites was graded as negative (0), mild (+), moderate (++) and severe (+++) as described in the literature (Table 1) (7,8).

Table 1. Grading of iron accumulation in myocites

Grade	Iron accumulation
0	None
+	Mild
++	Moderate
+++	Severe

Measurement of iron accumulation in cardiac tissue by ICP-AES

Iron accumulation levels in tissues were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) instrument.

Heart tissues samples weighed into Teflon vessels were treated with nitric acid (8 mL, Suprapur, E. Merck) and hydrojen peroksit (2 mL, Suprapur, E. Merck), transferred into a microwave oven (CEM, Mars-Express) and heat-treated at 170° C for 1 h. After cooling the sample solutions were diluted and the iron content was determined by ICP-AES (Varian Liberty-ll), using wavelengths of 259.940 nm. ICP-multielement standart solution-IV (1000mg/L Certipur, Merck) was used as iron standart solution. The iron concentrations in different groups are summarised in Table 1.

Stock solutions of the analyte 1 mg/ml were prepared from Merck Titrisols. The stock solutions of the matrix components 10 mg/ml were prepared by dissolving the corresponding chlorides in hydrochloric acid.

Statistical analysis

Statistical analyses were carried out using SPSS 16.0 for Windows statistical package (SPSS Inc., Chicago, IL). Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $P<0.05$ was considered statistically significant. All values were expressed as mean values±standard deviation (SD).

Results

Biochemical Findings

Comparison of seven groups in terms of evaluation of five studied parameters (CK, LDH, CK-MB, Serum iron, Serum ferritine) was as follows;

CK (U/L) levels: No significant difference was observed between groups.

LDH (IU/L) levels: Serum LDH levels in ebselen+iron (842.62±77.30), iron+deferoxamine (1142.62±159.45)andiron+deferroksamine+ebselen (661.50±68.23) groups were significantly hihger compared to control group ($p < 0.001$).

CK-MB (U/L) levels: serum CK-MB levels in iron+deferoxamine group (677.12±68.70) was significantly different ($p<0.05$), while iron+deferroksamine+ebselen group (486.62±49.85) showed highly significantly higher levels than control samples ($p<0.001$).

Serum Iron (mikrogram/dL) levels: Serum iron levels in iron (1088.25±110.83), iron+deferoxamine (767.00±35.91) and iron+deferroksamine+ebselen (574.12±33.10) groups were highly significanly hihger than controls ($p < 0.001$); while, a significant difference observed between ebselen+iron group (415.62±55.30) and controls ($p < 0.05$).

Serum Ferritine (nanogram/mL) levels: serum ferritine levels in iron (44.42±8.04), ebselen+iron (40.50±6.91), iron+deferroksamine (30.37±3.53) and iron+deferroksamine+ebselen (30.12±2.86) groups were highly significantly higher compared to controls ($p < 0.001$). Biochemical findings are shown in Table 2.

Histopathological Findings

The iron deposition in heart tissues wasn't observed in Control (C) and Ebselen (E) groups. There was mild iron deposition in myocytes in DMSO and iron+deferroksamine+ebselen (FDE) groups. Moderate iron deposition was detected in myocardial cells of iron (F) and iron+deferroksamine (FD) groups. Severe iron deposition was observed in myocytes of F groups rats (Figure 1). Except for samples obtained from F groups' rats, in which yellow-brown pigmented material (iron deposition) within isolated myocardial cells was observed, there were no obvious histopathologic findings in hematoxylin-eosin stained heart tissue sections.

ICP-AES Analyses' Findings

Measurements of iron levels in cardiac tissue by ICP-AES revealed that; iron (3.52±0.32), iron+deferroksamine (2.45±0.26) and iron+deferroksamine+ebselen (2.12±0.27) applied groups were highly significantly higher than compared

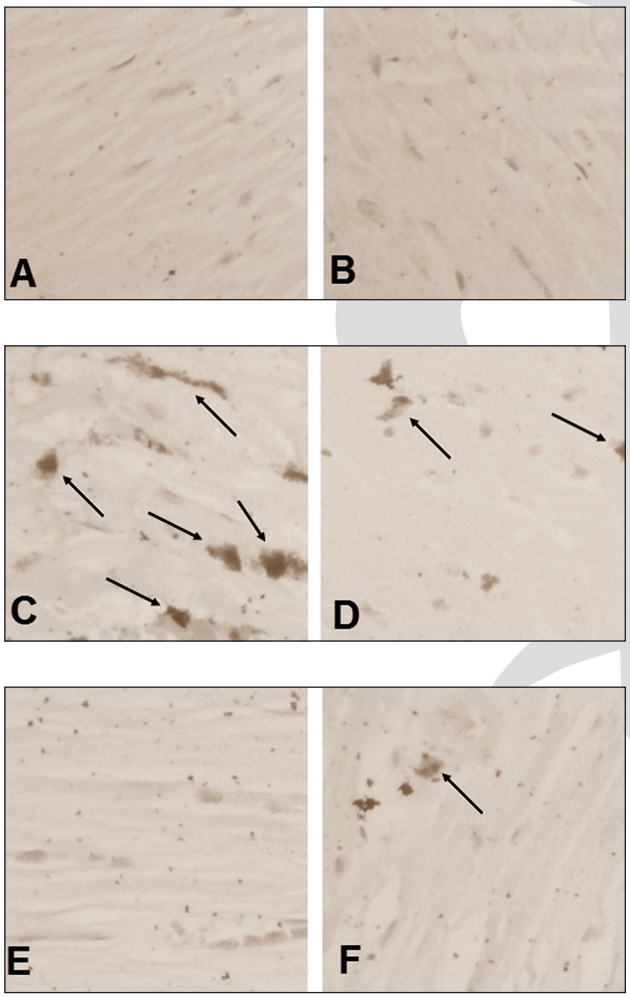
Table 2. The levels of CK, LDH, CK-MB, Serum Iron, Ferritin enzyme activities (mean ± SEM)

Group	CK (U/L)	LDH (IU/L)	CK-MB (U/L)	Serum Iron (mg/dL)	Serum Ferritin (ng/mL)
Control (n=8)	2454.42±263.92	1766.57±84.46	1208.85±60.27	157.14±17.79	2.85±0.34
DMSO (n=8)	2276.00±341.27	1834.12±70.72	1171.62±66.77	149.75±4.47	3.37±0.26
Iron (n=8)	2972.25±343.77	1879,62±80,98	1038.20±145.87	1088.25±110.83[a]	44.42±8.04[a]
Ebselen (n=8)	2142.87±213.39	1683.62±108.29	1261.25±131.22	162.00±17.70	3.37±0.26
Ebselen+ Iron (n=8)	1728.87±268.63	842.62±77.30[a]	781.25±141.96	415.62±55.30[c]	40.50±6.91[a]
Iron +Deferroxamine (n=8)	1719.50±254.84	1142.62±159.45[a]	677.12±68.70[c]	767.00±35.91[a]	30.37±3.53[a]
Iron+ Deferroxamine +Ebselen(n=8)	1436.25±246.59	661.50±68.23[a]	486.62±49.85[a]	574.12±33.10[a]	30.12±2.86[a]

ppm*

[a]: p < 0.001 compared with control group.
[b]: p < 0.01 compared with control group.
[c]: p < 0.05 compared with control group.

to those in controls ($p < 0.001$), while a relatively less significant difference observed between ebselen+iron (1.76±0.21) group and control ($p < 0.05$) (Table 3).



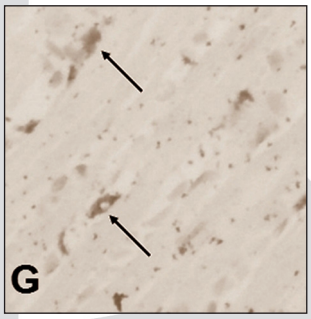


Figure 1. Iron accumulation is shown with black arrows as black deposits in histopathological sections (Prussian blue, 200X).
A. Control group (C); Negative (0)
B. Ebselen group (E), Negative (0)
C. Iron group (F); Severe (+++) iron accumulation
D. Iron+Ebselen group; Moderate (++) iron accumulation
E. DMSO group; Mild (+) iron accumulation
F. Iron+deferroxamine+ebselen group; Mild (+) iron accumulation
G. Iron+deferroxamine group; Moderate (++) iron accumulation

Table 3. Heart tissue FE levels in rats (mean± SEM)

Group	Tissue Fe*(g)
Control (n=8)	0.78±014
DMSO (n=8)	0.90±0.04
Iron (n=8)	3.52±0.32[a]
Ebselen (n=8)	0.90±0.58
Ebselen+Iron (n=8)	1.76±0.21[c]
Iron +Deferroxamine (n=8)	2.45±0.26[a]
Iron+ Deferroxamine +Ebselen(n=8)	2.12±0.27[a]

ppm*

[a]: p < 0.001 compared with control group.
[b]: p < 0.01 compared with control group.
[c]: p < 0.05 compared with control group.

Discussion

Free radicals are atoms or groups of atoms formed during certain metabolic pathways (9). Iron accumulation in parenchymal organs such as liver, heart, tyroid, hypofisis and pancreas is involved in formation and catalysation of free oxygen radicals (6). Oxidizing feature of iron was firstly introduced in 19th century; since then, several studies dealing with oxydant-antioxydant balance have been performed (10,11.) Cardiotoxic effects of iron are attributed to LDL oxidation and endotelial dysfunction, and these have been reported to be associated with serum ferritine and iron levels (12). Iron accumulation in cardiac tissue occurred in familial hemochromatosis, thalassemia and transfusion patients (13,14). There are studies examining cardiac and liver involvement following 100-200-400 mg of i.p. administration (15,16).

In the present study, cardiotoxic effects of iron evaluated by biochemical, histopathological and ICP-AES Histopathologically significant iron accumulation was observed in 100 mg/kg of iron applied groups for 7 days, compared to controls. Serum CK-MB levels of iron+deferroksamine and iron+deferroksamine+ebselen groups were significantly lower compared to control cases and control samples. Tissue iron levels' measurements revealed that iron levels in iron+deferroksamine and iron+deferroksamine+ebselen groups were singinificanly lower compared to iron administered group, which might be attributable to effects of chelation and antioxidant treatments.

Besides phlebotomy, chelation, angiotensin converting inhibitors, beta blockers and heart transplantation, novel treatments such as calcium channel blockers and antioxidant treatments have been recommended, in iron overload related cardiomyopathy (17). Phlebotomy is a treatment method requiring long-term follow-up. Deferoxamine, a chelator of free iron, is an antioxydant-like acting cardioprotective agent and it increases survival in patients with *thalassemia* (18,19). In cases suffered from iron overload, iron, which is normally absent in cardiac tissue, accumulates in myocites, macrophages and his bundle. Therefore, iron accumulation might result in cardiac arythmias and even death (20). A previously conducted study showed iron accumulation in myocites using hematoxylin-eosin and Prussian blue stain (21). Hemochromatosis, a condition leading iron accumulation in myocardium and papillary muscles of hearts, causes cardiomyopathy. Iron overload-related cardiotoxicity is reported to be reduced by use of antioxidant-like effects of acetaminophen in addition to use of chelators (22). Histopathological comparison of iron applied group and ebselen or ebselen+deferroxamine administered groups in terms of iron accumulation, in present study, revealed significantly decreased iron levels in ebselen and ebselen+deferroxamine given groups.

Ebselen, with its peroxidase-like activity, reduces endothelial cell damage caused by hydrogen peroxide. Therefore, ebselen, thanks to its antioxidant effects, might be protective for clinical conditions caused by oxidative stress (23). Iron has effect of catalyzing free oxygen radicals. A study by Davis and Bartfay examining effects of ebselen on free oxygen radicals in iron given rats revealed that ebselen decreased iron levels in cardiac tissue; thus, it is able to prevent cardiotoxicity (24). Essential role of ebselen for preventing mitocondrial damage, myocardial damage secondary to ischemia and reperfusion was reported in other studies dealing with effects of ebselen on cardiomyopathy (25,26). CKMB and LDH levels were increased in cardiomyopathy generated with Daunorubisine in rats and activities of these enzymes were found to be decreased in the group treated with ebselen (23). In the present study, decreased iron accumulation and decreased levels of CKMB and LDH were observed in cardiac tissue in ebselen administered groups. These findings were considered to be significant in terms of protective effects of ebselen on iron overload related cardiomyopathy.

Conclusion

Iron accumulation in cardiac tissue, which was detected to be decreased with ebselen treatment, was showed using biochemical, histopathological and ICP-AES analyses. In respect of findings obtained in this study, besides chelation treatment, use of ebselen as antioxydant agent is thought to *increase survival* in iron overload cases such as thalassemia and sickle cell patients involving tissue damage due to oxidative stress.

Note

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Assessment of neonatal intensive care unit and operating room staff's skill about ventilation and chest compression

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Abstract

Background: Mortality and morbidity rates in neonate are very important. Asphyxia is one of the most important problems during the neonatal period. The optimal ventilation and chest compression can prevent neonatal mortality. This study examined practical skills of NICU and operating room personnel in ventilation and chest compression on neonates and also the available instruments were evaluated.

Methods: Fifty six nursing and midwifes and OP Personnel staff's were selected. We evaluated each candidate during performance of two rescuers CPR on a neonate manikin, using a checklist.

Results: Availability of the main equipment related to ventilation and cardiac massage was moderate but accessible. Also, 44.6% of them had good capacity of performing ventilation in neonate with Ambo bag, and 50% of them had good capacity of cardiac massage. The evaluation was conducted using check list by two examiners.

Conclusion: In spite of good skill, 50% of the staffs were weak or moderate in giving cardiac massage and ventilation. For many of the staff conducting classes on neonate's rehabilitation and process of ventilation and cardiac massage is necessary.

Key words: Neonate ventilation, chest compression, cardiac massage

Introduction

More than 10 million children die every year in the world [1]. Rate of neonates' mortality is considered as a main health index which is 4-5 neonates in a thousand in the developed countries and 12-25 in a thousand in the developing countries. Over the last two decades in Iran there has been a major waning trend in infant mortality rates with 63.5, 43.5, and 26.7 per 1000 live births in 1988, 1994 and 2000, respectively[1-5]. At the present time, the need for resuscitation is greater in the neonate than in any other age group. The Neonatal Resuscitation Program (NRP) was developed by the American Academy of Pediatrics and the American Heart Association and endorsed in 1987 and revised [6-8].

Current attention to the differences in health status has led to more research on the health of different groups in developing countries [2, 9]. Proper function of the resuscitation equipment before the delivery in the neonates ward is necessity. Also neonates ward staff's skill in performing the basic cardio pulmonary resuscitation is very important. After the initial neonate care, monitoring of neonate respiration, heart beat and color is recommended. One of the main executions in the neonates with respiratory and cardiac problems, and color is the proper ventilation [10-15] the improper respiration, heart rate below 100 beat/ minute, and the central cyanosis which leads to Asphyxia in neonate, could be recovered by proper ventilation, and/ or treatment. Therefore proper ventilation with the help of ambo bag, for initial establishment of self respiration, maintaining of cardiac output and prevention of neonates mortality is very important [13, 16-17].

Success in recovery of the patients from the critical condition and of asphyxia is related to the availability of resuscitation equipment, the air way control instruments and even to the staff's skill [18-23]. The other studies showed that despite of

workers intension in learning the basic neonates' resuscitation and having good knowledge, there is inadequate skill in proper performance of different stages of resuscitation [23]. The studies show that despite of staff intension in learning the basic neonates' resuscitation and having good knowledge, there is inadequate skill in proper performance of different stages of resuscitation [23-26].

The aim of this study is to monitor the two main features of resuscitation: the availability of the equipment and the staff's skill in performing ventilation and cardiac massage in the neonates in 2007-2008.

Materials and methods

The subjects of this descriptive study were midwifes and nurses at NICU and operating room. The Boali, Fridonkenar and Ayatollah Taleghani hospitals at Mazandaran provinces were selected randomly. All of the nurses', midwife and operating room technicians who were involved in resuscitation entered the study after obtaining consent letter from them. Fifty six subjects were selected. The data were collected in questionnaire comprising three sections. One section on the demographic information with 4 questions about the attitude towards the equipments used for neonate resuscitation, the second section consisted a check list (selected from a book on neonate resuscitation) about the condition of equipments at the time of delivery [27-28] and the third section, was a standard check list of American Heart Association and the book of neonate resuscitation [9, 27]. Its reliability was confirmed by the faculty members of the Cardiac Department. The questionnaire was completed by two researchers. The staff's skill level in performing ventilation and cardiac massage was determined on the infant Manikin (Laerdel) and designated as good, moderate and weak. The check list consisted three sections: first on the ventilation with Ambo bag; second, 5-applied step in improving the neonate ventilation; and third on performance of cardiac massage. Each section had 5 division and questions. Resuscitation was performed on manikin and evaluated by giving score designated as 0 to 5. Classification of the skill levels for each step of the cardiac massage or ventilation was done by the three-point scales, where 4-5=good, 2-3=moderate, and 0-1=weak. Over all the skill level was designated as follow: 60-70=excellent, 45-60=good, 30-45=moderate and less than 30=weak. For the completeness and readiness of the resuscitation equipment the scores were designated as follow: 4= being completed and prepared; 3= being completed and available; 2= being defected; and 1= un-available.

The minimal and maximal scores of ventilation equipment and cardiac resuscitation drugs were considered 12 and 36 respectively. The equipments were categorized as good (more than 30), moderate (20-30) and weak (less than 20). The attitude of the staff towards readiness of the equipments, drugs and air way devices was designated as completely agreed to completely disagree and each answer was evaluated by 1 to 5. The minimal and maximal scores were considered 5 and 25 respectively, therefore more than 20 good, 10-15 for moderate, and less than 10 for weak were designated.

The durability of the research tools was evaluated using repeated test, in a preliminary study, and the correlation index of above 75% was accepted.

In this study the descriptive statistical indices, such as mean and standard deviation for the qualitative variables and X^2 test and Fisher exact tests were used for statistical analysis. Level of $P<0.05$ was evaluated significant.

Results

In this study 49 (87.5%) were female and 7 (12.5%) were male, mean age of 27.3±8 yr (20-46 years), mean experience 6±2.5 years. Of them 15 (27%) were midwife, 17 (30%) nurse, and 24 (43%) operating room staff. Also 40 (71%) of them attended neonate resuscitation work shop unofficially approximately 2 sessions each 90 for min, 9 (16%) did not attend any relevant workshop, and only 7 (13%) spent the official workshop of 2-3 days period. At least 5 months to 3 years had been passed since the date of attending the official or unofficial workshops. The subjects under study, 18 (32%), 14 (25%) and 24 (43%) were working in the neonates' ward, NICU and operating room, respectively.

The findings revealed that the mean of staff confronting with the need of neonate resuscitation was as follow: for 7 staff, 11 cases; generally for 25 (45%), less than 5 cases; for 6 (10%) between 5-10 cases

and for 25 (45%), more than 10 cases. The success of resuscitation was reported 20% to 70%. Attitude of the subjects towards needs of prepared equipments and the required drugs for air way management and neonate resuscitation is given in table 1.

The readiness and availability of the equipment needed for neonate ventilation, air way management and the drugs at NICU are given in the table 2.

The score designated for the main equipments of ventilation at the neonate wards and NICU units was medium and available. The anesthetic bag and the guide wire (Stylet) were not available but the self extending bag was ready and available. The condition was the same at all hospitals under study. Based on the check list on the ventilation, its condition is given in the table 3.

Table1. Participant's attitude about neonate resuscitation equipment at the NICU and operating room

Frequency	Completely agreed	Agreed	Somewhat agreed
	Number (%)	Number (%)	Number (%)
Readiness of radiant warmer and heat wasting preventing machine	55 (98.2)	1 (1.8)	-
Readiness of ventilation system with positive pressure for neonate	53 (94.6)	3 (5.4)	-
Readiness of tube, laryngoscope and suction	42 (75)	11 (19.6)	3 (5.4)
Availability of adrenaline drugs, the increasing volume bicarbonate and Naloxan	39 (69.6)	15 (26.8)	2 (3.6)

Table 2. Readiness neonate resuscitation drugs and ventilation equipments

Readiness Equipment	Good, Complete and ready	No complete but available
-Suction	+3	
-Suitable source of oxygen	+3	
-Suitable size airway		+2
-A suitable anesthesia bag	+3	
-Suitable round and anatomical mask		+2
-Laryngoscope with curve blade and property bended		+2
-Proper size tracheal tube		+2
-Guide of TT (stylet)		+0
-Epinephrine 1/10000		+2
-Volume expander		+2
-4.2% sodium bicarbonate		+2
-Naloxan		+2

Table 3. Participant, s skill's in performing ventilation in neonates at the neonate wards of the hospitals under study

Skill Ventilation variables	Good (4-5)	Moderate(2-3)	Weak (0-1)	SD mean
	Number (%)	Number (%)	Number (%)	
1) Checked the proper function of Ambo bag	19 (32.9)	27 (48.2)	10 (17.9)	3±1.1
2) The position of rescuer and mask placement on face	35 (62.5)	14 (25)	7 (12.5)	3.6±1.4
3) Rhythm and number of ventilation alone	23 (41)	25 (44.7)	8 (14.3)	2.9±0.9
4) Proper pressing of bag and proper volume of ventilation	25 (44.7)	21 (37.5)	10 (17.8)	2.93±0.085
5) Chest lifting, and looking at during inhalation	24 (42.9)	19 (33.9)	13 (23.2)	2.9±0.7

Results indicated that 44.6% of the staff has good skill in performing ventilation and 55.4% of them possess medium or weak.

This study shows the neonates wards, NICU and operating room staff's skill based on the 5-applied step performance in correction and improvement of ventilation when ventilation with mask did not lead to chest rising, which is given in the table-4.

Results revealed that 42.9% of the staff had good skill, 26.7% with medium skill in performing the 5-applied-step performance and 30.4% were weak.

Skill of the staff in performing external cardiac pressure by two routine methods in the neonates is given in the table-5.

Mean of skill score obtained by all of the subjects was 47.2±8. The mean score obtained by the NICU, operating room and by the neonates ward staff were 42.7±7, 59.7±14 and 27.8±7.2 respectively. In fact the skill of the staff at NICU, and operating room was medium and of the neonates ward was weak.

Discussion

Findings indicated that 42% to 50% of the staff at neonate wards had good skill in performing ventilation and cardiac massage and the rest with medium or weak skill. All of the study subjects agreed on the readiness of all ventilation equipments presented at the neonates' wards and NICU, but the score of the readiness for main ventilation equipments was medium and available.

Though the self extending bag was completely ready but the air way equipments had defect and not completely ready. Amrollahi et al. in a study found that 68.9% of the staff in performing pulmonary ventilation with Ambo bag and mask possessed weak skill and only 1.9% of them possessed good skill [22, 29-30]. It was in contrast with our findings. The reason of difference is because in that study the trainees had no training experience of resuscitation and ventilation in their educational programs. While our study subjects had gone under official and non official training for at least 2 hours during their education or demonstrated the neonates being resuscitated. The other study found that even despite of proper training, the efficiency declines with time, which is probably

Table 4. Distribution of the study subjects based on the staff's skill in correction and improvement of ventilation based on the 5-applied step performance

Ventilation correction Variables of ventilation correction	Good (4-5) Number (%)	Medium (2-3) Number (%)	Weak (0-1) Number (%)
1) Replace the mask properly	27 (48.2)	16 (28.6)	13 (23.2)
2) Position improves head tilt and chin lift	28 (50)	18 (32.1)	10 (17.9)
3) Mouth and then nose suctioned	32 (57.1)	15 (26.8)	9 (16.1)
4) Remove the obstruction caused by tongue airway	27 (48.2)	17 (30.4)	16 (28.6)
5) Increases bag pressure	20 (35.7)	14 (25)	22 (39.3)
Perform the above mentioned items in order	10 (17.9)	11 (19.6)	35 (62.5)
Total	6: 144=24 (42.9)	6: 91-15 (26.7)	6: 105= 17.5 (30.4)

Table 5. Distribution of the staff under study based on their skill in performing external cardiac massage

Skill The variables related to cardiac massage	Good Number (%)	Medium Number (%)	Weak Number (%)
1) Selects the place of massage properly	22 (57.1)	17 (30.4)	7 (12.5)
2) Assure of two fingers positions during massage	28 (50)	25 (44.6)	3 (5.4)
3) Assure of two thumbs position during massage	22 (39.3)	27 (48.2)	7 (12.5)
4) The number and depth of massage	28 (50)	20 (35.7)	8 (14.3)
5) Ratio of the number of massage to respiration	30 (53.6)	12 (21.4)	14 (25)
Total	28 (50)	20 (35.7)	8 (14.3)

due to forgetting the techniques and lack of practicing. The Other studies indicate that the skills of ventilation and resuscitation decline by time [23, 30-21]. Bagheri et al. (2003) reported that 71% of the subjects who were operating room and anesthesiology technicians and medical student trainees had weak skill and 7.5% of them with good skill [18-32-35]. In our study, 50% of the subjects had medium to weak skill in doing ventilation and cardiac massage. Results of the two above mentioned studies indicate that majority of the study subjects have problem in performing resuscitation. But the difference is noticed in those who are weak in performing; their number is less in our study compared with the other studies. It is related to the pass of time and the priority given in the recent years to the neonates' resuscitation and/or more controlling of the staff helping the neonates with ventilation problems or need ventilation and cardiac massage.

Because attitude of all study subjects towards the readiness of the equipments used in ventilation and cardiac massage was absolutely positive. The score of the readiness of the main equipments was medium and available. It seems that, due to weak resuscitation skills of the subjects, and emergency need for ventilation as a vital factor in neonate's life support, repeating of the resuscitation educational program and especially focusing on the manner of giving ventilation and cardiac massage is necessary.

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Influence of Nifedipine on gingiva

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Abstract

Background\Aim: Noninflammatory hyperplastic growth of gingiva induced by calcium channel blockers, mostly nifedipine, is often seen in everyday practice. Nifedipine is a drug which induces gingival fibroblasts to produce higher quantity of collagen that causes gingival overgrowth.

Methods: In order to establish an association of nifedipine and gingival hyperplasia, experimental model was used. Wistar rats were given water solution of nifedipine in different daily doses, using specially designed cannula. At the begining of the experiment, before the application of nifedipine and in the determined time period, gingival volume was measured. The volume of lower incisors interdental central papillas, represented multiplied values of vertical hight, mesio-distal width and bucco-lingual depth, expressed in millimeters.

Results: The results indicated that gingival hyperplasia was more excessive in the experimental animals, which were given higher doses of the drug for longer time period. The analysis of nifedipine influence to patients gingiva demonstrated occurrence with hyperplasia and inflammation of gingiva.

Conclusion: Nifedipine induces gingival fibroblasts to produce collagen that causes hingival hyperplasia.

Key words: Nifedipine, gingival hyperplasia.

Introduction

Calcium antagonists, pharmacologically defined as calcium channel blockers, have been in use for many years in the treatment of cardiovascular diseases (1).

According to their therapeutic effects, these drugs can be divided into the ones for the treatment of angina pectoris, systemic hypertension and the ones for the treatment and prophylaxis of supraventricular arrhythmia.

According to their chemical structure, the drugs of this group can be divided into the derivatives of phenylalkylamine, benzodiazepine and dihydropiridine. The moste used dihydropiridines are amlodipine, nicardipine and nifedipine.

Besides the well-known positive effects of nifedipine in the treatment of cardiovascular diseases, there still exists the lack of knowledge about the adverse effects, which may occur in periodontium, genitals and skin (2).

Pharmacokinetically, calcium channel blockers are orally active agens. They are characterized by high first pass effect, high plasma-protein binding and extensive metabolism (3). Their administration during longer period of time might have negative effects on periodontal tissues and they might generate gingival hyperplasia, which in the later phase causes the destruction og deeper periodontal tissues. As a consequence of periodontal destruction, there occurs tooth migration and tooth loss.(4) Calcium cannel blockers can also change immune response of periodontal tissues to dental plaque bacteria and in that way initiate and stimulate the onset and progression of inflammatory process.(5)

In 1939, Kimball published data that indicated close relation between gingival overgrowth and the use of some drugs (6). The first case of gingival overgrowth cused by nifedipine was reported in 1987. by Lederman.

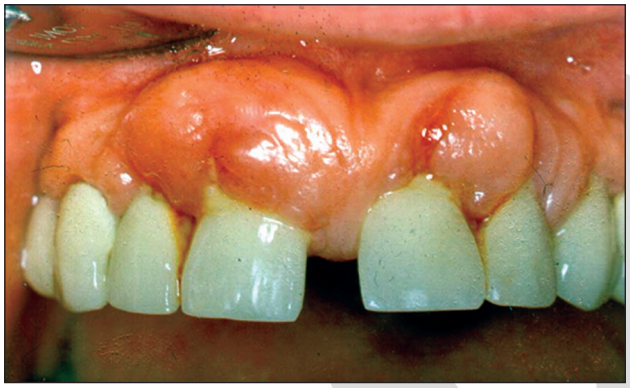
Gingival hyperplasia as a result of nifedipine use is usually generalized and front teeth interdental papillas are most affected. In the regions without teeth, hyperplasia was not registreted. Sometaimes gingival overgrowth might be so massive that the whole tooth is covered with the tissue which may cause masticatory problems.(7, 8)

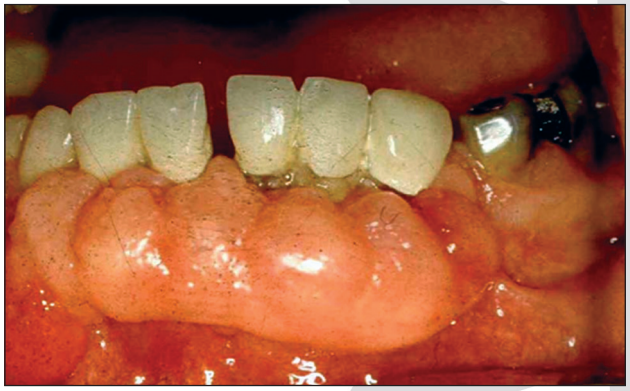
Hyperplastic gingiva is usually of pale pink color, with compact consistency and tiny granular surface.(9, 10, 11, 12)

There are literature data (13) that nifedipine stimulates gingival fibroblasts to produce collagen, thus causing gingival hyperplasia.

Gingival enlargement increases gingival sulcus depth, and as a result gingival pocket is formed.

(Pictures 1 and 2). It makes the adequate maintenance of oral hygiene almost impossible and gingival inflammation occurs. The reason for gingival enlargement is hyperplastic process by drug use, and inflammation is a secondary complicating factor. (14, 15)





Picture 1. and 2. Nifedipine induced hyperplasia

The aim of this study was to explore the influence of nifedipine on experimental animals gingiva, depending on dosage and time period.

Methods

The research was performed on 50 male Wistar rats, aged 6 weeks, weight between 150 and 250g, divided into 3 group of 15 animals. Other 5 animqals were sacrificed at the beginning of the experiment in order to record the basic state of gingiva, i.e. the state of gingiva before the administration of nifedipin.

Experimental animals received water solution of nifedipine by special cannula in daily doses of 10 and 15 mg (nifedipine dissolved in 0, 5ml of saline) and the control group received only saline solution without nifedipine. During the experiment, animals were fed regularly.

Gingiva was measured at the beginning of the experiment, before nifedipine administration, and in time intervals of 3, 6 and 9 weeks. The volume of central papilla was obtained as the result of multiplication of vertical height, mesio-distal width, and bucco-lingual depth in millimeters.

Measurment was performed by special millimetric graduated probe at the beginning of the experiment and after 3, 6 and 9 weeks, which was the overall time of nifedipine administration to experimental animals. After 3 weeks, 5 animals from each group were sacrificed , after 6 weeks another 5 from each group and after 9 weeks the remaining 15.

The obtained values were expressed as mean values, and were processed by adequated statistical methods with standard deviation and standard error.

Results

The size of central interdental papilla was monitored at beginning of the experiment and in the determined time intervals during the experiment. At the beginning of the experiment, the volume of lower incisor central papilla was 12 mm3.

The dose of nifedipine of 10mg/d after 3, 6 and 9 weeks, did not cause statistically significant change in the volume of lower incisor central papilla of the experimental animals (Table 1).

Table 1. Influence of the lower drug dose (10mg) on the size of lower incisor central papilla in the experimental animals during time period of 3, 6 and 9 weeks

Time period	n	x-+-SD
3 weeks	5	22.40+-1, 96
6 weeks	5	28.60+-7.58
9weeks	5	29.60+-8.16

The dose of nifedipine of 15mg/d, after 3, 6 and 9 weeks, caused statistically significant change in the volume of lower incisor central papilla of the experimental animals.

Larger dose of the drug caused change in the volume of lower incisor papilla of the experimental animals during the time interval of 6 and 9 weeks, compared to the state after 3 weeks of drug administration. This confirmed that lower incisor central papilla was enlarged after 6 weeks of drug administration and then stagnated during another 3 weeks period (Table 2).

Table 2. Influence of the higher drug dose (15mg) on the size of lower incisor central papilla in the experimental animals during time period of 3, 6 and 9 weeks

Time period	n	x-+-SD
3 weeks	5	26.00+-4, 47
6 weeks	5	43.20+-3.92*
9weeks	5	43.20+-3.92*

**p<0, 001*

There was no change in the volume of lower incisor central papilla, comparing 6 and 9 weeks of higher dose administration, so the value of t-test was zero.

The second aim of this study was to compare the volume of lower incisor central papilla in the experimental animals at the beginning of the experiment, when Wistar rats received 0, 5 ml og saline solution (control group), lower dose (10mg/d), and higher dose (15mg/d) of the drug in the defined time intervals.

During the first 3 weeka, comparing the lower and the higher dose of the administered drug, there was no statistically significant change in the volume of lower incisor central papilla, in contrast to the control group, where statistically significant change occurred, comparing the values measured by standard deviation and by t-test (Table 3).

Table 3. Comparison of the size of lower incisor central papilla of the experimental animals after the administration of the control, lower and higher drug dose during time period of 3 weeks

Drug dose	n	x+-SD
Control dose	5	11.40+-2.24
Lower dose	5	22.40+-1.96*
Higher dose	4	26.00+-4.47*

**p<0, 01*

During 6 weeks time period, statistically significant change in the volume of the lower incisor central papilla was noticed, particularly comparing to the higher dose grup and the control group (Table 4).

After 9 weeks, statistically significant change was obvious between the lower and the higher dose of the drug (p<0, 05), the lower dose and the control, with somewhat higher standard deviation (p<0, 01) and the most significant change occurred between the higher dose and the control (p<0, 001) for 9 weeks time period (Table 5).

Table 4. Comparison of the size of lower incisor central papilla of the experimental animals after the administration of the control, lower and higher drug dose during time period of 6 weeks

Drug dose	n	x+-SD
Control dose	5	11.40+-2.24
Lower dose	5	28.40+-7.58*
Higher dose	5	43.20+-3.92*

**p<0, 01*

Table 5. Comparison of the size of lower incisor central papilla of the experimental animals after the administration of the control, lower and higher drug dose during time period of 9 weeks

Drug dose	n	x+-SD
Control dose	5	11.40+-2.24
Lower dose	5	29.60+-8.16*
Higher dose	4	43.20+-3.92*

**p<0, 001*

Discussion

The results of this study demonstrated that there was more significant gingival hyperplasia at the experimental animals, whish were given the higher dose of the drug during the longer time period. During the 6 weeks time period, lower and higher doses of the drug casued changes in the volume of lower incisor central papilla, compared to the control group. Similar results were obtained during the 9 weeks time period, when more significant changes occurred in the volume of lower incisor central papilla for the lower and higher doses of drug, compared to the control(16).

Gingival overgrowth casued in the experimental animal model indicated that nifedipine had a significant role in interdental papilla enlargement of Wistar rats. These findings were in accordance with the results derived from the experiments on Sprague-Dawley animals, in which the dependence between gingival growth and the dose of the drug was also demonstrated after nifedipine administration.(17) Gingival growth may also be a consequence of the increased number of fibroblasts in the tissue, induced by nifedipine (18).

Collagen decomposition is important for physiological remodeling of connective tissue. Nifedipine participation in collagen production may increase the imbalance of collagen degradation.

Investigations performed by Myrales GJ, in 1999. (19, 20) regarding fibroblasts of healthy and hyperplastic gingiva approved the exstence of different fibroblast subtypes, which variously reacted on stimulus, including drugs. These investigations confirmed the findings of this study.

Latest investigations of Morton RS et al. In 1999. showed that nifedipine in conbination with dental plaque bacteria reduced interleukin 6 secretion and increased it in the presence of interleukin 1 beta.

Interleukin 6 is a cytokine with more than one effect. It has positive effect on the activation and differentiation of B cells into plasma cells, which secrete immunoglobulins. Interleukin 6 is a differentianting factor for cytotoxic T cells, and a growth factor for B cells, T cells, and for mesenchymal cells. (21, 22) It has also been proved that interleukin 6 has stimulation effect on growth and metabolism of connective tissue cells like fibroblasts, thus it is assumed that it has pathogenic role in the diseases where transformation occur on fibroblasts(23).

Gingival fibroblasts secret considerable amount of interleukin 6 with or without stimulation, so they might be one of primary interleukin 6 sources in animals that received nifedipine. (24) It is also possible that nifedipine stimulatory effect is not directly manifested only on the fibroblasts of gingiva, but that it also causes a complex interaction with dental plaque and inflammatory cytokines (25, 26).

It is approved that there are quntitative and qualitative differences in the reaction of gingival fibroblasts, concerning the increase or the decrease of interleukin 6 secretion.

Inside fibroblast population there are phenotypic variations, thus it is assumed that nifedipine effect on a population of fibroblasts depends on relative presence of those subtypes that react to nifedipine(27, 28). If such fibroblasts exist, the presence of nifedipine will cause the increased secretion of interleukin 6, resulting in the proliferation of fibroblasts and the increase of their activity, as well as the increase of extracellular matrix.

Although according to the obtained data, bacteria do not directly increase interleukin 6 secretion, they act synergistic with nifedipine in secretion increase.

Conclusion

Based on the data from this study, it is possible to conclude:

- Higher dose of nifedipine induces overgrowth of lower incisor central papilla in experimental animals;
- Length of administration also has the influence on lower incisor central papilla overgrowth;
- Lower incisor central papilla overgrowth was registered during the first 6 week of drug administration and then gingival growth stagnated.

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Is mean platelet volume an activity marker in patients with familial mediterranean fever?

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Abstract

Objective: The aim of this study is to investigate the correlation between mean platelet volume (MPV) and the clinical disease activity indices of Familial Mediterranean Fever (FMF).

Material and Method: Files of the 90 cases diagnosed with FMF were screened considering Tel-Hashomer Clinical Criteria. Forty-eight patients referred with attack (group 1), 42 patients presented in the remission (group 2) and 66 healthy persons as the controls (group 3) were included to the study. Demographic characteristics, white blood cell count (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), platelet count, and MPV levels of the patients evaluated were recorded using the computerized patient database. **Results**: In this study, average of the CRP, MPV and PLT values in group 1 and group 2 were found significantly higher than in group 3 ($p<0.001$). A positive correlation was defined between MPV and PLT values, CRP values and disease severity scores, and between CRP and MPV values in the group 1 and group 2. MPV and PLT values were positively correlated with the disease severity score in the patients in group 1.

Conclusion: We concluded that MPV is may be useful for assessment of the disease activity and as a corroborative test for CRP, ESR, and WBC in FMF. The current status of clinical and sub-clinical inflammation with CRP and MPV values which we think to concordantly increase are important pre-atherosclerotic markers providing a prediction of atherosclerosis.

Key words: Familial Mediterranean Fever, CRP, MPV, Aterosclerosis.

Introduction

FMF is an autosomal recessive disease, and an autoinflammatory hereditary disease characterized by a fever emerging as periodical attacks and involvement of serous membranes such as peritonitis, pleuritis, and arthritis (1,2). The disease is most common seen in Turk, Jew, Arab and Armenian ethnic groups, the populations of Mediterranean origin (3,4). The diagnosis of FMF is primarily based on clinical symptoms and manifestations. There is not any specific laboratory test used in diagnosis of FMF disease. However, acute phase reactants such as CRP, ESR and WBC are known to increase with the attack in the inflammatory diseases and return to normal after the attack (2,5,6).

Long-term effects of subclinical inflammation on the patients with FMF are not completely defined. However, in a few study increasing of atherosclerosis is reported as the long-term effects of this auto-inflammation (7,8).

MPV is a marker of platelet size and it can reflects changes of the platelet stimulation and production. MPV is associated with platelet activity. It is a biologically important variable since the larger platelets are potentially more likely to be thrombotic events (9,10). Because of MPV is a marker of the

platelet activation, it plays an important role in pathophysiology of atherosclerotic diseases, and it can be used as an indicator of atherosclerosis (10,11). In this study, we aimed to compare the MPV values of the FMF patients in attack and remission period with the MPV values of healthy individuals and therefore to investigate the relationship between inflammation and MPV. In addition, the current study evaluate MPV is whether or not an activity marker in patients with FMF.

Material and methods

Records of the patients diagnosed with FMF and followed up in the Mustafa Kemal University Faculty of Medicine (Hatay, Turkey), Department of Internal Medicine between October 2009 and March 2011 were retrospectively studied. In addition, the persons referred our clinic and defined healthy were accepted as the control group and data belonging to them was obtained from the recordings. Files of 90 patients diagnosed with FMF between 18 and 58 years old were screened. Ninety patients (49 males and 41 females) with FMF and 66 healthy controls (34 males and 32 females) were enrolled into the study. The diagnosis was established in all the patients considering Tel-Hashomer Clinic Criteria (12). All the cases diagnosed with FMF were receiving colchicine therapy. The cases examined in the study were designed as three groups. The first and second groups were taken as the patients groups, while the third one was assigned as the control group. The FMF cases presented with acute attack (n=48) were evaluated in group 1, with remission phase (n=42) the second group and healthy persons (n=66) constituted the controls in the group 3. Acute attack and remission phase were determined based on clinical (fever, abdominal pain, arthritis) and laboratory (CRP, ESR, WBC) findings. Demographic data was examined. Information about the onset age of the disease, age of diagnosis, duration between the disease onset and diagnosis (diagnosis delay time), Tel-Hashomer severity score (13), dosage of the colchicine therapy and duration of the treatment were recorded. Data of the patients were collected using the computerized patient database. Complete blood count (CBC) samples from the patients and controls were obtained. CBC analysis is performed in the central laboratory of our hospital with a Coulter analyzer device. The standard tubes used in CBC included certain amount of ethylenediaminetetraacetic acid (EDTA). Automatically measured MPV, PLT, CRP, ESR and WBC values of the patients were recorded. CBC parameters, CRP and ESR values of the healthy persons were obtained from the database of the same computer. Normal platelet count is range from 150000 to 400000 microliter. Platelet count above 400.000/μL was considered as thrombocytosis and that of under 150.000/μL as thrombocytopenia. Normal MPV is in the 6.5 to 11.6 femtoliter range. The study was approved by the Ethics Committee of Mustafa Kemal University.

Statistical analysis

Mean and standard deviation (SD) were calculated for continuous variables. The normality of the variables was analyzed by Kolmogorov-Smirnov test. Chi-square (2 c) test with Yates Correction evaluated associations between the categorical variables. The mean values of three groups were compared by using ANOVA followed Post Hoc test Bonferroni. Intercorrelations between variables were computed through the Pearsons Correlation Analysis. ROC (Receiver operating characteristic) curve and AUCs values of CRP, ESR and WBC was determined for patients with attack and without. Two-sided p values were considered statistically significant at Pd0.05. Statistical analyses were carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Ninety patients (54.4% male, 45.6% female), 66 healthy controls (51.5% male, 48.5% female) were enrolled into the study. Forty eight patients (54.1% male, 45.9% female) in group 1 and 42 patients (54.8% male, 45.2% female) were in group 2. No significant difference considering age and gender was found between the patients and the controls. Median age of the group1 was 28.8 (range 18-58), group2 was 30.4 (range 18-52) and the controls were 29.6 (range 20–55).

In Group 1, fever was found in 92.5%, abdominal pain in 86.4%, joint pain in 86.4%, chest pain in 62.5% and erysipelas like rash in 31.2% of the

patients; respectively. In Group 2, fever was found in 70% abdominal pain in 84.2%, joint pain in 75%, chest pain in 40% and erysipelas like rash in 15% of the patients; respectively. There was not a significant difference between the two groups in terms of mean age of the disease onset, mean age of the diagnosis, diagnosis delay time, positive familial history, and mean colchicine dose used by the patients.

Demographic characteristics of the patients are shown in Table 1. No person had a PLT value under 150.000/μL in group1, group 2 and group 3. Of 48 patients from Group 1, thrombocytosis was found in 6 FMF patients having inflammation, while platelet values were in the normal range in Group 2 and Group 3. MPV values were under the <6.5 fL in 3 of 48 cases in group 1 and 5 of 42 cases in group 2, while the persons in group 3 had MPV values at normal levels. Mean MPV and PLT values were found significantly higher in group 1 and group 2 than in group 3 (MPV values were 8.8±0.5 vs 6.9±0.3 (p<0.001) and 8.3±0.4 vs 6.9±0.3 (p<0.001); respectively. PLT values were 361±45 vs 277±81 (p<0.001) and 355±52 versus 277±81 (p<0.001); respectively). MPV and PLT values were quantitatively higher in group 1 compared in group 2, although there was not a statistically significant difference between the two groups (p=0.56, p=0.90; respectively). Comparison of the blood parameters between the groups is seen in Table 2. A positive correlation was defined between MPV, PLT, CRP and ESR values of the patients in group 1 and the disease severity score (r=0.55, r=0.49, r=0.41, r=0.38; respectively). A positive correlation was found between CRP and MPV values of group 1 and group 2 (r=0.44, r=0.25; respectively). No correlation was defined between colchicine usage duration and MPV values in group 1 and group 2 (r=0.05, r=0.04; respectively). The correlations between the patient groups are shown in Table 3.

Table 1. Demographic characteristics of FMF patients

Parameter	Group1(n=48)	Group 2(n=42)	p value
Gender (M/F)	26/22	23/19	0.935
Age at onset (years)*	15.87±3.75	16.78± 3.90	0.465
Age at diagnosis (years)*	24.65±5.98	25.42±6.89	0.771
Delay in diagnosis (years)*	9.37±2.23	10.66±2.61	0.984
Colchicine dose (mg/day)*	1.12±0.34	1.06±0.30	0.992
Positive family history	27 (%56.25)	22 (%52.38)	0.675
Disease severity score	7.8±3.12	6.0±2.42	0.032

**mean ± SD*
χ2: Chi-Sqare test
t: Student's t test for independent samples

Table 2. Comparison of blood count parameters among the groups

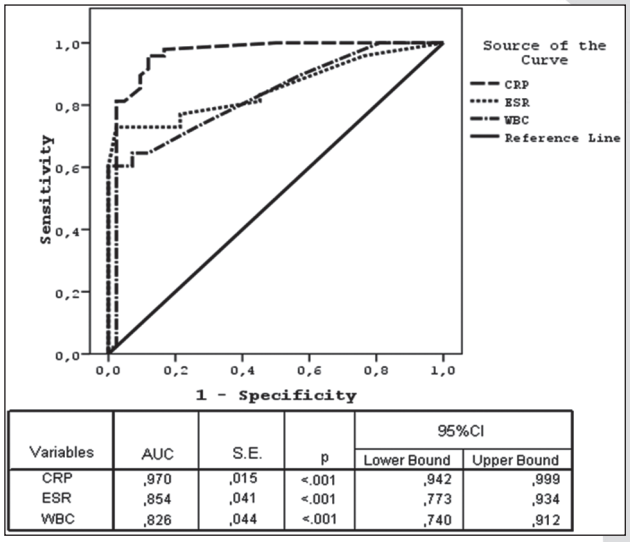
Parameter	Group 1 (n=48)	Group 2 (n=42)	Group 3 (n=66)	ANOVA (F; p)	Group comparisons by Post Hoc Bonferroni Test (p value)*
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
MPV (fL)	8.8±0.55	8.3±0.41	6.9±0.30	7.54; p<0.001	1–2 (0.56), 1-3 (<0.001), 2–3 (<0.001)
PLT (×10³/μL)	361±45	355±52	277±81	18.25; p<0.001	1–2 (0.90), 1-3 (<0.001), 2–3 (<0.001)
WBC (×10³/μL)	11.8±6.1	7.8±4.2	7.5±3.6	6.43; p<0.001	1–2 (<0.001), 1-3 (<0.001), 2–3 (0.84)
CRP (mg/L)	48.5±9.6	6.1±1.8	1.2±0.24	21.32; p<0.001	1-2 (<0.001), 1-3 (<0.001) 2-3 (<0.001)
ESR (mm/h)	32.3±8.1	12.5±4.3	11.8±3.9	10.61; p<0.001	1-2 (<0.001), 1-3 (<0.001) 2-3 (0.782)

$\bar{x} \pm SD$*: mean ± SD*
**p<0.05 is significant*
F: One way ANOVA test

Table 3. Correlations between variables of patients in the group1 and group 2

Variables	Group 1 (n=48) r; p	Group 2 (n=42) r; p
MPV-PLT	0.50; =0.001	0.41; <0.001
MPV-Disease Severity Score	0.55; <0.001	0.11; 0.412
PLT-Disease Severity Score	0.49; =0.01	0.08; 0.641
MPV-CRP	0.44; <0.001	0.25; 0.03

r: *Pearson correlation coefficien*
**p<0.05 is significant*



Variables	AUC	S.E.	p	95%CI	
				Lower Bound	Upper Bound
CRP	,970	,015	<.001	,942	,999
ESR	,854	,041	<.001	,773	,934
WBC	,826	,044	<.001	,740	,912

Figure 1. ROC (Receiver operating characteristic) curve and AUCs values of CRP, ESR and WBC For patients with attack and without.
AUC: Area Under Curve
SE: Standard Error of AUC
p: Significant value

Discussion

In the present study, FMF disease in which systemic inflation is known to play an important role in the pathogenesis was demonstrated to be correlated with MPV. A correlation was found between MPV elevation and severity of the disease in the patient groups with and without inflammation. In addition, MPV was demonstrated to be correlated also with acute phase proteins. Means of MPV and PLT values were found significantly higher in FMF patients in the attack and remission periods than in the healthy controls group. Thrombocytosis was found in 12.5% (6/48) of the patients with inflammation, while no thrombocytosis was defined in the controls and in the group of patients without inflammation. So far, three different studies investigating the association between the disease and MPV in FMF patients were published. Of these, two were conducted with pediatrics (14,15) and one with adults (16). In the study with adults, patients in the attack phase were not evaluated, whereas in our study, association of patients both in the attack and remission phase with MPV was studied. In a study made in children by Makay et al., FMF patients were studied in two groups as the patients presented with acute and remission phase similar to our study. MPV values of the FMF patients presented with acute phase were found significantly lower than the patients with remission phase and higher than the healthy controls. No significant difference was defined between the MPV values of the patients with remission phase and the control group (14). In another study by Arıca et al. with pediatrics (15), FMF patients were studied in two groups as the patients presented with and without acute phase. In that study, MPV values were found significantly higher in FMF patients from both groups compared to the healthy controls. That study includes a result parallel to our study. In another study by Çoban and Adanır (16), again the relationship between FMF and MPV were examined. MPV values in the FMF patients with remission period were higher than in the control group of healthy volunteers. It is seen that similar results with our study were obtained in that study by this aspect. However, our study is more detailed, because it included the FMF patients with acute period also with the patients with the remission period and demonstrated MPV values of both patient groups were higher than the healthy persons. Increased patelet activation and aggregation are known to be closely associated with atherosclerotic events (10,17). During atherogenesis, platelet aggregation and migration of smooth muscle cells from the media to endothelium and consequent proliferation are the early events. Atherosclerosis develops as a consequence of lipid accumulation in the

vessel wall, a co-existent inflammatory response and proliferation of smooth muscle cells; endothelial dysfunction can be added to this pathogenic triad (18,19). Platelet size is associated with the function and activation of the platelet. Large volume platelets contain more granules and aggregated more quickly with collagen than the smallvolume platelets. Since these large platelets have thromboxane A2 in higher levels, they express glycoprotein 1b (adhesion molecule) and glycoprotein 2b/3a (aggregation molecule) receptors in greater amounts (20-22). Elevated MPV values showing a larger platelet volume are considered as an indicator of the activation and recognized as the predictor of an increased risk for atherosclerotic disease (10,11,23,24). FMF is accepted as a self-limiting, hereditary, inflammatory disease that progresses with repeating febrile attacks. Acute phase reactants such as CRP, ESR and WBC are known to increase in patients presented with acute period due to iflammation and return to normal after the attack (25,26). However, there are several studies indicated that inflammatory activity continues between the attacks and CRP values of the FMF patients in the remission period, and asymptomatic FMF carriers are higher than the healthy controls. In their study, Lachmann et al. concluded that the chronic sub-clinical inflammation state continues also in the remission period of the FMF patients (27). Korkmaz et al. also came to a parallel conclusion and reported the sub-clinical inflammation state continues also in the remission period (28). Endothelial disfunction which is known to be associated with sub-clinical inflammation is thought may trigger the cytokine production (29,30). There are studies reporting cytokine level increases during the acute attack in the FMF patients (31,32).There are studies indicating platelet volume may be affected by the cytokine increasing during the attacks. Increased cytokine production and endothelial disfunction stimulate production of the large platelets in volume. Cytokines such as interleukin-3 and interleukin-6 affects megakaryocytes, leading production of the larger and more reactive platelets (33-35). Systemic inflammation is an important factor in onset and development of atherosclerosis. In several studies, a significant correlation has been found between the increase of CRP, which is a marker of systemic inflammation and atherosclerotic events (36-38). In this study also CRP values of the FMF patients with acute and remission period were found significantly higher compared to the healthy individuals. This suggests a sub-clinical inflammation state continues also in the remission period. Besides CRP levels, MPV values were also defined as significantly higher compared to the healthy controls and this is important in respect to demonstrate an association between the clinical and sub-clinical inflammation and MPV values. Specially, an inflammatory response that has a peak in attack period with continuing chronic sub-clinical inflammation suggests there can be a close relationship between FMF disease and atherosclerosis. Therefore, our study argues that MPV value can be an important marker in respect to provide us to predict the risk for atherosclerosis and take the necessary measures in FMF patients in the early period. MPV which is known as a component of the CBC test, does not require an additional cost. Main limitation of this study is retrospective design. Second, all the patients in the study were on colchicine treatment. We know that colchicine decreases cytokine levels in FMF (39). In conclusion, this study demonstrates CRP, MPV and PLT values of the FMF patients are significantly higher than the values of the control group of healthy persons. Besides the patients with acute period, finding of higher CRP, MPV and PLT values also in the patients with remission period compared to the healthy persons is important in suggesting the existence of a chronical sub-clinical inflammation. In this study, we suggest that MPV is an cost-effective test which may be useful for assessment of the disease activity and as a corroborative test for CRP, ESR, and WBC in FMF. In the light of all this information, we believe systemic clinical and sub-clinical inflammation state and CRP and MPV values which we think to increase in parallel are important preatherosclerotic markers providing a prediction for atherosclerosis. We think it would be useful to examine CRP and MPV values especially in the FMF patients with frequent attacks, to study risk factors of atherosclerosis and to take the necessary steps.

Conclusion

We concluded that MPV is may be useful for assessment of the disease activity in FMF. The current status of clinical and sub-clinical inflammation with CRP and MPV values which we think to concordantly increase are important pre-atherosclerotic markers providing a prediction of atherosclerosis.

Note

This study was presented in "European Society for Emergency Medicine (EuSEM), EuSEM 2012 7. European Congress on Emergency Medicine, 8. Emergency Physicians Association of Turkey (EPAT), National Emergency Medicine Congress. 3-6 October 2012. at Antalya, Turkey.

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Depression and acute coronary syndrome

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Abstract

Objective: Epidemiological studies suggest that beside classic factors for development and prognosis of acute coronary syndromes, depression and anxiety are important as well. Considering the potential link between depression and coronary heart disease, depression is not always either cause or effect of coronary artery disease (CAD). They both occur simultaneously in the same person due to a common pathophysiological mechanism, possible genetic dysfunction of serotonin receptors. Epidemiological data suggest that in the ward with 25 beds, a cardiologist will probably see four patients with major depression and five with minor forms of depression. The numbers are similar for hospitalized patients who have experienced myocardial infarction (MI), unstable angina, coronary bypass, angioplasty or heart failure.

Methods and Results: We enrolled 30 patients of both sexes (19 males or 63.3% and 11 women or 36.7%), mean age 61.75 ± 84 years. After the division of the entire group of coronary patients by level of depression we got four groups - 15 patients without depression (50%), mild depression: 8 patients (26.67%), with moderate depression: 5 patients or 16.67%, and with severe depression: 1 patient or 3.33%.

Conclusions: In our study, a correlation was found between the duration of hospitalization and degree of hyperglycemia and depression and anxiety that exacerbate progression of CAD. Increased depression leads to increased anxiety and higher blood glucose levels - both additional risk factors for the progression of CAD. Increase in anxiety in patients diagnosed with the CAD increases the risk of MI, lethal outcome of coronary disease and sudden coronary death.

Key words: Acute coronary syndrome, depression.

Introduction

During the last decades there is increasing evidence that psychological factors, including depression, lack of social support, anger, job related stresses could have effect on prognosis in cardiovascular patients. Recently number of studies pointed out that depression is a big psychosocial problem.[1,2] It is important to note that certain amount of negative emotional reaction should be expected in acute coronary syndrome. Physician should expect everyday anxiety from the patient, irritability and sadness. Physician should respond on those reactions with support, warmth and to give hope for recovery.

Usually those basic emotions ameliorate adaptation and progress. For example, the fear from the heart attack in the future could stimulate acceptation of the proposed therapy. However, for some patients the fear is disproportional in comparison to the treatment and it is not adaptive such as the case with the intensive fear which leads to insomnia, atypical chest pain and avoiding of low-risk activities.[3] Unlike the sudden fear which is a short-term reaction, the behaviour caused by serious illness needs time for development. Some patients affected by sadness or by stopping the usual activities react with the acceptation as a normal adjusting process while the others are not adaptable with the loss; the emotional healing process is slow like a curing of the infected wound. This unresolved grief could be the first sign of a depression. Long stay in intensive care unit or numerous re-hospitalizations could also trigger the depression. Epidemiological data suggest that on the medical ward with 25 hospital beds, cardiologist would probably see four patients with major depression and five with minor depression form. Numbers are similar for hospitalized patients with myocardial infarction, unstable angina pectoris, and coronary by pass, angioplasty or heart failure.[4,5]

During the last decade of rapid growth recorded evidence to suggest that various psychological factors, including depression, lack of social sup-

port, anger, stress at work, can affect the prognosis of cardiac patients. More recently, the increasing number of well-designed study has been published in medical journals and cardiology, drew attention to the great depression as a psychosocial risk.

Thrombus formation is a key factor in the rapid progression of CAD and in the occurrence of acute MI and unstable angina. The hypothesis of an association between depression and increased platelet activation in particular appears to explain the link between depression and mortality from heart diseases. Although serotonin itself is a weak agonist for platelet aggregation, serotonin can potentiate the effect of other agonists in the induction of platelet aggregation. Serotonin may further encourage the formation of thrombus by induction of coronary vasoconstriction of damaged vessels. During many years, platelets were used in psychiatric researches as a model of serotonin pre-and post-synaptic function in the brain.[1,2]

Aims

The first aim of the study was determination of the incidence and severity of the depression in patients with acute coronary syndrome and the second was to examine their's correlation with the risk factors for coronary artery disease (hypertension, hyperlipidemia, inflammation).

Methods

We included in the study patients who were hospitalized in the Clinic for the cardiology Clinical Centre Nis with acute coronary syndrome (unstable angina pectoris – UAP, myocardial infarction without ST segment elevation –NSTEMI and myocardial infarction with ST segment elevation – STEMI).

In all patients routine laboratory examinations were done in Central laboratory Clinical Centre Nis. From markers of necrosis we followed troponin I, CK-MB, from inflammation markers CRP and white blood cells count were followed and lipid profile – triglycerides, cholesterol, LDL and HDL fractions. Standard 3 channel ECG, echocardiography and invasive examinations were done.

In the examined group we followed 30 patients age 61.75±84, 19 men (63.3%) and 11 women (36.7%). The average age of male subjects was 64.19 ± 11.02 years and 61.19 ±10.14 years for female, the difference was not statistically significant (t = 1.870, p> 0.05) (Table 1).

Table 1. Characteristics of the study population by age and gender

Gender	n	%	Age		
			Min	Max	X ± SD
Male	19	63,3	40	79	64,19 ± 11,02
Female	11	36.7	42	76	61,19 ± 10,14
Total	30	100			61,75 ± 12,84

Evidently, the most common risk factors were: hypertension with 66.7%, 46.8% with heritage, smoking with 50.1%, diabetes mellitus with 30.0%, and lipid disorders with 32.8%. Obesity was present with 23.3% (Table 2).

Table 2. Risk factors in the study population

Risk factor	n	%
Hypertension	20	66,7
Diabetes melitus	9	30,0
Smoking	15	50,0
Lipid disorders	17	32,8
Obesity	7	23,3
Heritage	19	63,3

In Table 3 heart rate and blood pressure are presented.

To obtain additional data we used questionnaires:

1. The general questionnaire to obtain sociodemographic data and data about the disease progression (length of illness, number of hospitalizations), hereditary diseases, association with psychological or stress disorders.
2. Beck Depression Inventory – questionnaire to measure the intensity of the depressive symptoms. It has 21 question and offered answers are 4-scaled (0-3). The final score is the sum of all answers. According to the score we could divide depression on 4 groups – without, mild, moderate and severe depression.
3. State and Trait Anxiety Inventory (STAI) – the questionnaire has 40 questions which measure the intensity of two anxiety forms – actual anxiety (state anxiety) and anxiety as a personal characteristics (trait anxiety). According to the score both types of anxiety could be divided into mild, moderate and intensive. With special tables we could

transform primary scores into Z- scores (0-100) which allow comparing those two forms of anxiety.

4. Health Locus of Control – questionnaire which measure where patient puts centre of the control of the disease – internal (he thinks that he is dominantly responsible for his health and disease progression) and external (he thinks that the external factors determine if he will be seek or he will be healed as faith, physician and other people). With processing the results we get the intensity scores for (a) internal control centre, (b) control centre associated with the fait/coincidence, (c) doctors, (d) other persons.

The data were processed in SPSS 12 program, including T test, Chi-square test, ANOVA and correlation analysis.

Table 3. Values of heart rate, systolic and diastolic blood pressure

	n	Values		
		Min	Max	X ± SD
Heart rate	27	35	110	79,22 ± 18,34
Systolic pressure	26	80	190	143,27 ± 30,06
Diastolic pressure	26	20	140	85,35 ± 21,64

Results

A group of subjects consisted of 30 patients of both sexes (19 males or 63.3% 11 women or 36.7%), mean age 61.75 ± 84. After the division of the entire group of coronary patients by level of depression we got 4 groups - 15 patients without depression (50%), mild depression: 8 patients (26.67%) with moderate depression: 5 patients or 16.67% with severe depression: 1 patient or 3.33%. Statistical analysis showed a significant correlation among the variables presented in the Figure 1. In the study population, with the number of hospitalizations the severity of depression increased. This is presented in the Figure 2. Patients who had severe anxiety traits had the lowest values of internal locus of control, and differed from the others in a statistically significant level (F=5379, P=0.011) (Figure 3). Correlation analysis revealed a statistically significant positive correlation of severity of depression and current anxiety. This was shown in Figure 4. Correlation analysis revealed a significant correlation between the intensity of the current depression and anxiety, as well as blood glucose levels, and a negative correlation with the level of cholesterol. (Table 4).

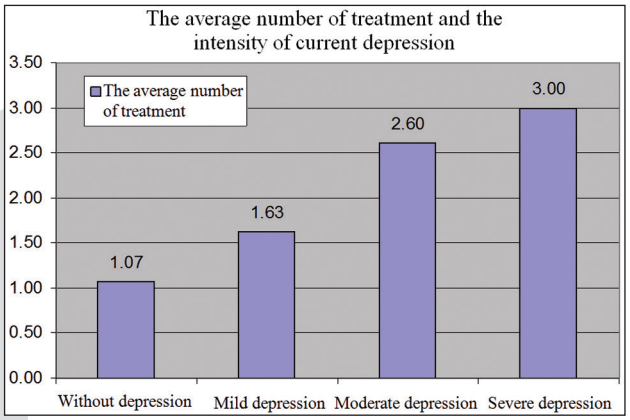

Figure 1. The average number of treatment and the intensity of the current depression

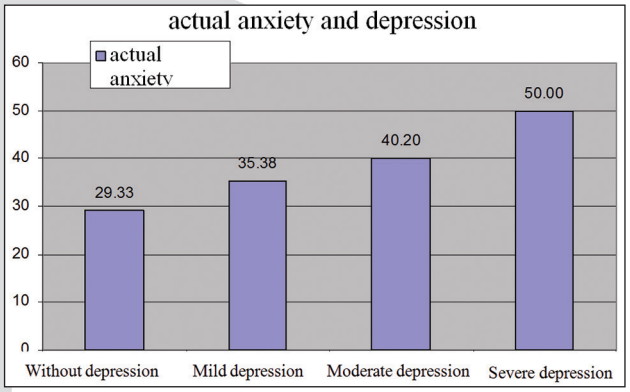

Figure 2. Incidence of actual anxiety and depression

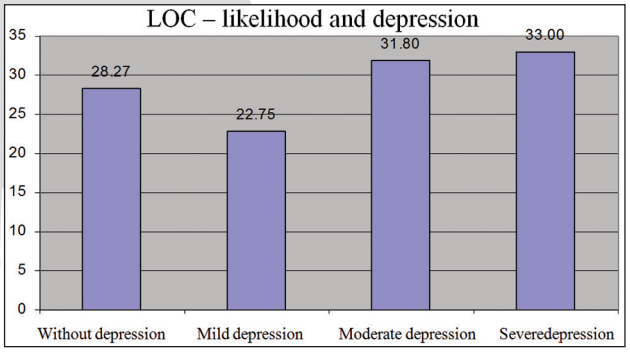

Figure 3. Average score of internal health locus of control and anxious personality traits

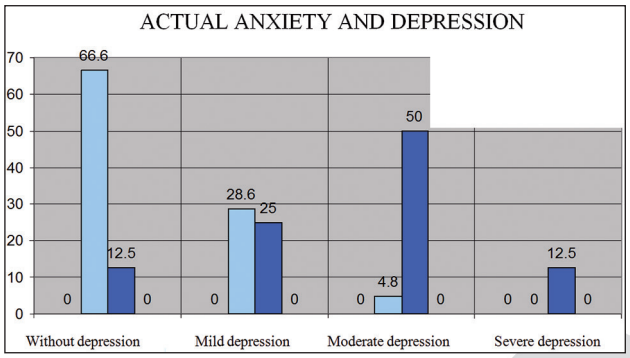

Figure 4. The current anxiety and depression

Table 4. Correlations of depression

	Pearson correlation coefficient	P value
Actual anxiety	0.592	0.001
Cholesterol	-0.382	0.045
Glycemia	0.452	0.014

Discussion

In order to understand possible relationship between depression and coronary artery disease we should consider possibility that the depression is nor cause nor the result of the coronary artery disease (CAD) but they coincide in the same person because they have the same pathophysiological mechanism. It is possible that genetic dysfunction of serotonin receptors induces depression development and in platelets it could induce the risk of thrombotic events.[1, 4, 5]

Two others common pathophysiological mechanisms are low intensity chronic inflammation and low intake of omega-3 fatty acids. Low intensity chronic inflammation is integral part of coronary artery disease. In patients with coronary artery disease markers of inflammation as CRP, IL6 or soluble intracellular adhesion molecules are high and are associated with worse prognosis. They predict development of CAD in healthy population.

There is an interesting relation between depression and regulation of the immune function and inflammation. Meta-analyses of studies on patients without CAD concluded that major depression is associated with high leucocytes count, high CD4/CD8 ratio, and increase of haptoglobine, prostaglandin E2, IL 6, lower natural killer cell cytotoxicity and low response on mitogenes. Authors concluded that there are evidence that major depression is associated with one immune activation of reminiscent acute phase in inflammation response.[5, 6]

Clinical data on inflammation have parallel in experimental studies. Animal experimental studies suggest that immune activation can induce depression similar behaviour and, conversely, that chronic stress induces release of pro-inflammatory cytokines in the brain with consequent systemic responses. How is it done? Pro-inflammatory cytokine-1 and tumor necrosis factor alpha produced by activated immune cells, promote the production of interleukin-6 from leukocytes, adipocytes or endothelial cells. Interleukin-6 induces a broad systemic effects, including liver production of acute phase proteins (such as C-reactive protein) inhibition lipoprotein lipase activity, and increased platelet aggregation in response to ADP and epinephrine. Interestingly, systemic pro-inflammatory cytokines circulating in the blood also induce interleukin-1 activity in the hippocampus and hypothalamus, which acts as a messenger and SRES stimulate serotonin and norepinephrine neurotransmission and the release of corticotropin-releasing factor.

One possible explanation for bigger prevalence and prognostic impact of depression in patients with coronary heart disease is that the immune activation associated with progression of coronary artery disease score induces depressive episodes in susceptible patients. The psychological concept of "vital exhaustion," defined as a combination of excess fatigue, irritability and low morale may be particularly relevant for understanding the relationship between immune activation and symptoms of depression. Big epidemiological study of the elderly without coronary disease was found, although the C-reactive protein was associated with symptoms of depression and fatigue symptoms, the relationship remains independent of cardiovascular covariates and measures of physical weakness for symptoms of fatigue. It happens that the patient communicates vital exhaustion symptoms may be more likely to have concomitant immune activation than with the more common symptoms of depression. Finally, there is a statement about the normalization of inflammatory markers after antidepressant treatment.[6]

In the discussion, we expect that higher level of depression and anxiety lead to higher blood glucose levels - both additional risk factors for the progres-

sion of coronary artery disease with an increase in the anxiety in already diagnosed patients which increases the risk for myocardial infarction, lethal outcome and sudden coronary death. In addition to the direct effects on myocardial vulnerability, anxiety can lead to diabetes, hypertension and hyperlipidemia. Chronically increased catecholamine levels were shown to increase the level of lipoprotein lipase induced hyperglycemia and increase blood pressure.

There is a hypothesis that the relative deficit of omega-3 fatty acids may explain the link between depression and heart disease. Population-based studies show that reduction in dietary omega-3 relative to omega-6 is associated with increased incidence of cardiovascular disease and depression. Although it was documented that red blood cells have reduced levels of omega-3 in depressed patients in comparison with a normal control, it remains to be proven in depressed cardiovascular patients. Furthermore, in addition to its antiplatelet, anti-arrhythmic, anti-inflammatory, and anti-triglyceride action, it appears that omega-3 may have antidepressant effects, especially in depressed bipolar patients.

In terms of potential behavioural explanations of the link between depression and prognosis, it can be assumed that the reduction of interest, self-esteem, sense of control and the hope of found in depression is transformed into poorer adherence to a modification of risk factors and treatment. Recent evidence suggests that either because of their motivation or their physicians' reaction, depressed patients may have a reduced benefit of revascularization procedures. Role of quitting smoking in depressed patients is particularly complex. In a way, the nicotine is a self-administrating antidepressant for many patients. Patients with previous depression are less likely to succeed in stopping smoking, and may experience relapse or recurrent episodes of depression in an attempt to quit smoking. Only three antidepressants: buproton, nortiptiline and moclobemide have been successful in smoking cessation. Having this in mind, non-depressed patients with previous episodes of depression who are trying to quit smoking must be strictly monitored in the evaluation of symptoms suggestive of recurrent or relapsing of depression.

Considerable epidemiological evidences support a link between chronic emotional stress and coronary heart disease / CHD /. Emotional factors related to atherosclerosis and adverse cardiac events include primarily disorders such as depression, anxiety, anger, and hostility. It is now well established that depression is associated not only with the incidence of CHD but also with the prognosis of patients with the disease. In meta-analyzes relating to the role of depression in the development of coronary artery disease, Regulies found that individuals with clinical depression have > 2.5 fold increased the risk of myocardial infarction or coronary death than the general population. In the patients with established coronary heart disease, major depression is not only a significant predictor of mortality after acute myocardial infarction, but also the level of depressive symptoms has a dose-dependent relationship with cardiac mortality over several years of monitoring. There is no evidence that could recommend systematic screening for depression in CAD patients. Self-reported questionnaire similar to BDI is quick and easy but it is too sensitive, and it gives many false positive diagnoses. An interesting perspective can be developed soon and it is called - Patient Health Questionnaire. However, systematic screening can overcome already scarce mental health resources available for patient search psychiatric help. Instead, we need cardiologists to ask a few questions about mental health problems during regular visits. It can also encourage the patient to accept the referral to psychiatrist when necessary. Some simple questions can be used to open a chapter of emotional distress without using the word depression, which often leads to rejection.[7]

Conclusions

The connection between depression and coronary artery disease may be reflected in three different types of relationships. First, depression may directly cause cardiac mortality through biological or behavioural mechanisms. Second, depression may be a consequence of the systemic complications of cardiovascular disease or its treatment. Finally, both depression and heart disease may share common genetic and pathophysiological cause and have no causal correlation between each other.[6]

In our study, a correlation was found between the duration of hospitalization and degree of hy-

perglycemia and depression and anxiety that exacerbate progression of CAD. Increased depression leads to increased anxiety and higher blood glucose levels - both additional risk factors for the progression of coronary artery disease. Increase in anxiety in patients diagnosed with the CAD increases the risk of MI, lethal outcome of coronary disease and sudden coronary death.

In conclusion, depression is common in patients with diagnosed cardiac disease. It is associated with increased incidence of cardiac death and re-hospitalization, as well as continuing with chronic depression. But what kind of depression increases the risk of cardiac events? Research suggests that the diagnostic distinction between major and many minor forms of depression may not be relevant in the assessment of cardiac risk. The question may not be which the normal level of depressive symptoms is but where is the border where medical attention is needed. To prevent the adverse impact of depression on the prognosis of acute coronary syndrome should be promptly implemented psychiatric therapeutic support.

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Comparison of 2-octyl-cyanoacrylate with suture and the classic technique for colon anastomosis in rats

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Abstract

Aim: The aim of the present experimental study was to compare the effects of 2-octyl cyanoacrylate (2-OCA) with suture and classic technique in colon anastomosis in rats.

Materials and Methods: Three groups, each including 20 rats were formed. The anastomoses in Group 1 were treated with the simple closure technique. Group 2 anastomoses were glued using 2-OCA with suture. Only laparatomy was performed in Group 3. The anastomosis sites including 2 cm distal and proximal colorectal segments were taken from the sacrificed rats to test explosion pressure and measure the hydroxyproline levels in the tissue.

Results: The explosion pressures analysis on the 3rd postoperative day showed that Groups 2 and 3 showed significantly higher than Group 1. Hydroxyproline levels on the 3rd postoperative day were significantly higher in Groups 2 and 3 than Group 1. Likewise, hydroxyproline levels on the 8th postoperative day were also significantly higher than Group 1.

Conclusions: We conclude that using 2-OCA with suture in rat colonic anastomosis improves, and in fact has a positive influence on, the healing process. As a consequence, the use of 2-OCA with suture in colonic anastomosis in the clinical situation appears to be justifiable.

Key words: Colonic anastomosis, 2-octyl cyanoacrylate, anastomosis leakage.

Introduction

Colonic anastomosis is one of the most frequent operations in surgical practice. The success rates of anastomoses are highly dependent on the surgical technique.[1].

The tissue adhesives may prove advantageous that they do not result in potentially dangerous pressure during tissue approximation. Furthermore, unlike the sutures they do not cause a potential tract between the lumen and serosa of the bowel.[2]

The aim of this experimental study was to compare the effects of 2-OCA with suture and classic technique in colon anastomosis in rats.

Materials and methods

This study was completed at the Biomedical Research Laboratories of the Medical School of Duzce University with the approval of the Institutional Animal Care and Use Committee.

Experimental animals

60 adult male albino rats with the weight range of 250-280 g were used. All of the animals were housed in metal cages under controlled environmental conditions with ambient temperatures of 21±2°C and 12-hour light/dark cycle. Standard procedures were followed for feeding the animals, water and food was not limited. None of the albino rats died during the study. Three groups, each including 20 rats were formed. The anastomoses in Group 1 were treated with the simple closure(end to end anastomoses) technique. Group 2 end to end anastomoses were glued using 2-OCA (Dermabond®, Ethicon Inc. Summerville, NJ) with suture. Group 3 were performed only laparatomy. In each group, 10 rats were sacrificed on the 3rd day and the remaining 10 on the 8th day.

Surgical Procedures

After shaving the abdominal area of all the rats, the skins were sanitized using povidone-iodine and covered with a sterile cloth except for the incision site. All the rats were anesthetized with ether

and underwent laparatomy incisions of 2.5 cm on the abdomen under sterile conditions. Except for the control group, the right colon segment two cm in length and five cm distal to the ileocecal valve was resected with preservation of perfusion to the remaining colon in 40 rats.

The 20 rats in Group 1 were treated with 4 primary end to end anastomotic sutures using 5/0 Polyglactin (Vicryl®Ethicon Inc. Summerville, NJ). End to end anastomoses in Group 2 rats were done using 2-OCA with suture**.**

The surgery was completed by closing the abdomen. Rats were kept in isolated cages following the operation and were started oral feeding 6 hours after the operation. All the rats received standard feeding and watering until the next operation. From each group, half of the rats were anesthetized at 3rd day and the remaining at the 8th day of operation using high doses of diethyl ether (rats were placed in a covered container lined with paper towels soaked in diethyl ether. They remained there until rendered unconscious) Next, they were sacrificed using 50 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, and Istanbul). The anastomosis sites including 2 cm distal and proximal colorectal segments were taken from the sacrificed rats to test explosion pressure and measure the hydroxyproline levels in the tissue

Measuring the explosion pressure

Inside of the anastomosis colons were carefully cleaned and the distal ends were knotted using 3/0 thread. The proximal ends were attached to 3 mm polyethylene catheter using 3/0 thread to infuse air at constant speed. Anastomoses were observed for air leakage and the air pressure where air bubbles were recorded as the explosion pressure. Subsequently, 1 cm of tissue encompassing the 0.5 distal and 0.5 cm proximal ends of the anastomoses were resected. Explosion pressure measurements were done using patient monitors (Mindray PM–9000/Express Patient). [3].

Hydroxyproline Measurements

Hydroxyproline measurements were done using tissue chromatography technique. Half of the resected tissue was kept in 1 mL of isotonic sodium chloride solution at -22 °C to be later used for analysis of hydroxyproline levels. Tissue samples that were previously frozen at -22°C were thawed, dried, and weighed. 2 mL of 6N Hydrochloric acid was added to each tissue and incubated at 110 °C for 24 hours. 0.2 mL of the hydrolyzed tissue was mixed with 1.6 mL of deionized water. To this, 1 mL of 5-fold diluted borate and 0.3 mL Chloramine T was added. After 20 minute incubation, 1 mL Sodium Thiosulfate was added and mixed. Next, 1.5 g potassium chloride was added to the mixture and the tubes were kept in boiling water for 20 minutes. After the tubes were cooled down, 2.5g toluene was added to each and vortexes for 5 minutes and then centrifuged at low speed to eliminate undisolved material. 1 mL of the supernatant was mixed with 0.4 mL of Ehrlich reagent and incubated for 30 minutes. Spectrophotometric readings for hydroxyproline levels were done using Architect C8000 auto analyzer.

Statistical analyses

Data analyses were done using Statistical Package for Social Sciences (SPSS) software for Windows 11.5. Statistical results were presented as mean±standard deviation. The significance of the differences among group means was analyzed using One-Way ANOVA test. Whenever the one-way variance analysis showed a significant difference between means, we used Tukey post-hoc test to determine the group that was different than the others. Data were analyzed with 95% confidence interval and $p<0.05$.

Results

60 adult Wistar albino rats were used for this study. They were divided into 3 groups and no demographic differences were noticed observed among the groups. Macroscopic leakage was not observed in any group.

The explosion pressure analysis on the 3rd postoperative day showed that Groups 2 and 3 showed significantly higher explosion pressures than Group 1 (208.20±14.05, 224.20±12.20, 141.40±26.16 mmHg, respectively) ($p<0.001$) (Table 1).

Hydroxyproline levels on the 3rd postoperative day were significantly higher in groups 2 and 3 than in group 1 (2.53±0.07, 2.59±0.07, 1.62±0.06 mcg/100g tissue, respectively) ($p<0.001$) (Table 3).

Table 1. Comparison of explosion pressures (Mean± Standart Deviation)

mmHg	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	P value
3. POD*	141.40±26.16	208.20±14.05	224.20±12.20	P<0.001
8. POD*	152.20±22.7	211.00±13.6	222.20±13.5	P<0.001

** POD: Postoperative day*

Table 2. Comparison of tissue hydroxyproline levels (Mean± Standart Deviation)

mcg/100g tissue	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	P value
3. POD*	1.62±0.06	2.53±0.07	2.59±0.07	P<0.001
8. POD*	2.86±0.08	3.42±0.10	2.63±0.11	P<0.001

** POD: Postoperative day*

Likewise, hydroxyproline levels on the 8th postoperative day were also significantly higher than group 1 (3.42±0.10, 2.63±0.11, 2.86±0.08 mcg/100g tissue, respectively) (p<0.001) (Table 2).

Discussion

The explosion pressure was statistically lower in group 1 compared to group 2 and group 3, while difference between group 2 and group 3 was not significant. Despite that the levels of hydroxyproline which has an important role in wound healing were statistically similar in group 2 and group 3, it was significantly lower in group 1 compared to group 2 and group 3. Compared to conventional tissue glues, use of cyanoacrylate provides the advantage of being ready for use, obviation of the need for additional procedures, prevention of operative contamination and short anesthesia time.

Although the rate of occurrence has significantly reduced, in line with technical improvement, anastomotic leakage is still a major complication after colonic surgery, having a reported incidence of up to 5% in elective and 10–15% in emergency surgery[4-7]. In order to decrease the rate of complications, alternative techniques for anastomosis are continually being sought. However, experimental studies are conflicting and randomized clinical trials are lacking[8-9]. Seidenberg et al.[10] and Uroskie et al.[11], studying cyanoacrylate in gastrointestinal anastomosis, showed a high rate of leakage, exceeding 30%–60%. However, in our study incidence of anastomotic leakage was not observed (0%), there was no difference in the incidence of anastomotic leakage all groups.

Kanellos *et al*.[12] previously used fibrin glue in order to increase the strength of sutured anastomosis. They applied fibrin glue around the sutured anastomosis and found that the mean the explosion pressure of the anastomosis increased significantly despite the unchanged leakage rate.

Our study was carried out in order to investigate the influence of 2-OCA with suture on the healing of colonic anastomosis in the rat. Mechanical strength, hydroxyproline levels and histologic appearance of the anastomosis were evaluated for this purpose and results were compared with conventional sutured anastomosis. Although the application of 2-OCA with suture resulted in increased adhesion formation around the anastomosis on day 3, the difference was not prominent on day 8. Similarly, in 2001 Weiss and Haj used *n*-butyl-2-cyanoacrylate for gastrojejunal anastomosis in rats and also observed no difference between *n*-butyl-2-cyanoacrylate and control groups[13].

In this study, the strength of the anastomosis was expressed as the explosion pressure, and *in vivo* measurement was used. Application of *n*-butyl-2-cyanoacrylate did not result in an elevation of the explosion pressure during the first 7 days of healing, contrary to expectations [13]. Statistically significant lower the explosion pressure was observed on day 3 and day 7. As a consequence, the sealed anastomosis can be considered as being weak during the critical period of healing, which is a concern because this is the time when leakage threatens. In contrast, one should also consider the previous comments of Byrne *et al*.[2] that the explosion pressure might not be correlated with the integrity of the anastomoses and the clinical outcome.

Although numerous suturing techniques have been devised, the main function of the sutures/staples is to hold the ends of the intestines together until the tissue healing occurs. In this regard the denominator of the many suturing techniques is to place the sutures optimally equidistant from each other and not to tie the knot too tight which may compromise tissue perfusion[15].

One of the theoretical alternatives to conventional suturing is to glue the ends together which may overcome the disadvantages related to the sutures. We found decreased anastomosis leakage, hence better wound healing with combined use of 2-OCA and suture compared to other methods in our study. In accordance with our hypothesis, anastomosis leakage was not observed and wound healing was not problematic at the end of the study.

Nursal et al.[1] reported that at the postoperative seventh day, which represents the late phase of the healing in the rat, there was no difference between the groups regarding gross perianastomotic changes, and hydroxyproline concentration. But, in our study, hydroxyproline levels were found lower in group 1 compared to group 2 and 3 on 3rd and 8th days.

Conclusions

In conclusion, the healing is better with the composite use of 2-OCA and suturing for colon anastomosis than the use of 2-OCA or sutures *per se*. The outcome may differ depending on the clinical situations. Therefore, we conclude that using 2-OCA with suture in rat colonic anastomosis improves, and in fact has a positive influence on the healing process. As a consequence, the use of 2-OCA and suture in colonic anastomosis in the clinical studies appear to be justifiable.

Conflicts of interest

We would like to submit our manuscript titled "Comparison of 2-Octyl-Cyanoacrylate with Suture and the Classic Technique for Colon Anastomosis in Rats" for your consideration as a publication in The Journal of Surgical Technique and Case Report**.** We accept all authors do not have any financial relationship with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript.

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The importance of graded chronic pain scale and algometry in myofascial pain and fibromyalgia diagnostics

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Abstract

The aim of this research was to assess the clinical applicability, interconnection, the possibilities and significance of algometry and graded chronic pain scale in myofascial pain and fibromyalgia diagnostics.

Methods: The study was carried out on 60 subjects, divided into one experimental and one control group. The algometrical measurements were conducted in order to determine the threshold of pain in masticatory muscles bilaterally. The locations of measurements in both groups for musculus temporalis were the anterior, mid and posterior fascicles. For muscles masseter both the insertions and its body, the mid portion of the muscle. In the experimental group for the psychosocial assessment of pain related disturbances and the degree of chronic pain was carried out using the graded chronic pain scale (GCSP).

Results: There are statistically significant differences between the subjects from the control and experimental groups in the threshold of pain of m. masseter (t=14.29) and m. temporalis (t=11.97). 87.50% of patients with fibromyalgia had a disability of the IV degree, and 46.67% of the patients with myofascial pain had degrees I and II. There are statistically significant differences in the GCPS results between the subjects with different diagnoses (χ^2=30.82, p=0.00003). Spearman rank correlation coefficient confirmed that the graded chronic pain scale values are in a statistically significant correlation with the values of algometrical measurements of the threshold of both muscles (-0.55 i -0.58).

Conclusion: Carefully interpreted results of the GCPS combined with properly calibrated digital algometer are clinically relevant and reliable instruments for the identification of patients with a high level of disturbances, pain and dysfunctionality.

Key words: GCPS, algometry, myofascial pain, fibromyalgia.

Introduction

Complex anatomy, physiology and neurobiology of the head and neck region demand a specific approach and a combination of different complex procedures in diagnostics of painful chronic myofascial syndromes (myofascial pain and fibromyalgia). Yet, a wholly acceptable, unique diagnostic model and pain pathogenesis in muscle dysfunction are still subject to controversy and remain an aim for future research.

Pain is a complex, highly personal, and multidimensional experience influenced by a variety of biological and psychosocial factors (1-4). Consistent with the biopsychosocial model, research findings indicate that clinical diagnosis alone is often insufficient to explain observed levels of pain and disability. Therefore, the pain assessment in patients with painful chronic myofascial syndromes is remarkably complex. A number of researchers claim that the patients with fibromyalgia and various subtypes of the TMD have different characteristics of chronic pain compared to healthy subjects, a higher degree of dysfunctionality and psychosocial disturbances related to pain (5-9). A precise diagnostic assessment of chronic myofascial pain and fibromyalgia, as well as of the dysfunctionality and disability degree, affects the proper therapy choice and can be highly dependent on the presentation of chronic pain (10-13).

The graded chronic pain scale (GCPS) is one of the instruments of psychosocial assessment (Axis II) within the RDC/TMD protocol (14,15). Its applicability and validity have been proven (16) in a number of studies; yet, few studies have dealt with the interconnection of the scores of chronic pain severity and the algometrical measurements results.

Most researchers agree that algometry is a good method to monitor and document the pressure pain

threshold or level of pain in patients with muscle dysfunction (myofascial pain and fibromyalgia) and tension-type headache (17-20). It is non-invasive, repeatable, precise, economical and highly applicable. The repeatibility and validity of algometrical measurements are significant in patients with TMD and in asymptomatic subjects (17,21,22). A large number of studies confirm the positive correlation and interdependency between algometry and other pain assesment instruments (9,23). However, due to numerous inconsistencies regarding the measurement metodology and pain presentation in contemporary scientific literature in the field, there has appeared an initiative for establishing a generally accepted, normed protocol on the usage of instruments for pain assesment in different myofascial pain syndromes.

The aim of this researcher was to assess the clinical applicability, the potentials and significance of graded chronic pain scale as an instrument in diagnostics of varios chronic myofascial pain syndromes, and to compare it with algometrical values of the pain sensitivity level.

Materials and Methods

The study included 60 subjects, divided into two groups. There were 30 subjects in the control group (15 women and 15 men) of the mean age of 39.53±6.53. The experimental group had 30 subjects (16 women and 14 men) of the mean age of 42.77±11.57. All the subjects were between 18 and 60 years of age. The research was carried out at the Dental Clinic of the Medical Faculty in Novi Sad from 2009 to 2011.

At present the diagnosis is based on the history and clinical examination of the patient clinical criteria for diagnosis: The criteria for inclusion of myofascial pain of the masticatory muscles were those of Okeson (1996) (24). Therefore, the two subgroups of patients with myofascial pain as described by Dworkin and LeResche (1992) were assembled (14). Diagnosis of fibromyalgia had been set up at Clinic of Medical Rehabilitation of Novi Sad. Inclusion criteria were the classification criteria for fibromyalgia developed by the American College of Rheumatology (ACR, 1990) (25).

In addition, in order to be included, the subjects had to feel pain symptoms (a duration of more than 3 months) in at least one masseter or one temporal muscle, not pregnant, no acute pain caused by dental disease (e.g. pulpitis, severe periodontal disease), ear infection, neurological disorders, atypical pain, acquired immune deficiency syndrome (AIDS), neoplasms and chronic systemic diseases affecting the joints. Patients with spontaneous or triggered TMJ pain and neuropathic pain were excluded. The specific criteria used to constitute the three patient groups were the followings:

The myofascial pain group consisted of 15 subjects (age 38.53±10.01; ratio 46.67% female). The group with diagnosis myofascial pain with limited mouth-opening consisted of 7 subjects (age 40.71±12.63; ratio 57.14% female). Only in the group of 8 subjects diagnosed with fibromyalgia, average age 52.5±7.05, a significantly higher percent of women (62.5%) is noticed with regards to the one of men (37.5%).

Before starting, the patients were fully informed about the experimental procedure and gave their verbal consent prior to participation in the study.

Methods

The GCPS assesses pain intensity and interference with daily activities (15). On the GCPS, study participants rated on scales from 0 = "no pain" to 10 = "pain as bad as could be" their current pain and average and worst facial pain in the past six months. The mean of these three ratings, multiplied by 10, is the characteristic pain intensity (CPI) score. Participants also rated on scales from 0 = "no interference" to 10 = "unable to carry on any activities" the degree of facial pain interference with daily activities, recreational/social/family activities, and work/housework activities in the past six months. The mean of these three ratings, multiplied by 10, is the pain-related activity interference score. The GCPS also assesses the number of days of significant activity limitation due to pain in the past six months. Based on all three variables, the GCPS can be used to classify individuals into chronic pain grades: 0 = no pain, I = low pain intensity and low pain-related disability, II = high pain intensity and low pain-related disability, III = moderate pain-related disability, and IV = severe pain-related disability.

Algometer Wagner Instrumments (CT, FPIX 10, SAD, 2007), dimensions: 2 3 / 4 "WX 4" HX

1 1 / 4 “d. were used, consecutively, to determine the pain threshold and pain tolerance pressure by the masticatory muscles.

The testing on patients was conducted under the exact same time and space conditions. The measurement places for m. temporalis were anterior, middle and posterior bundle, and for m. masseter those were both grips and the body, that is the surface bundle. A probe with a surface area of 1cm² was applied to the this point with certain intensity which is expressed in kgf/cm². Patients were asked to notify the investigator when they started to feel pain (pain threshold) and when they could no longer bear the pain (pain tolerance threshold). The pressure at each point was recorded on the algometer display, wich enabled enhanced precision in measuring. The measurements were done three times and there was a time lag of 5 min between the measurements. Since this instrument has its software support, statistically significant values were calculated directly on the computer.

Statistics

Trace measurements and data analyses were performed in blind conditions. After testing the normality of the distributions, we compared and analysed the quantitative mean values the standard error of the mean in kgf/cm2 for the algometry. Groups were compared by Student’s *t* test and Pearson’s χ^2 test. When Analysis of variances (ANOVA) indicated a significant difference, was carried out as post-hoc test (risk at 5%). Tukey’s test war used for multiple comparisons. Spearman’s test was performed to assess the level of correlation between algometry and Graded Chronic Pain Scale pain-related disability. Statistical analysis was carried out using SPSS software 8.0. The significant level was set at p of 0.05.

Results

Table 1 shows the differences in the algometrically measured pain threshold values of m. masseter and m. temporalis between the two groups. Student’s *t* test confirmed that there are statistically significant differences in the pain threshold values for m. masseter and m. temporalis between the control and experimental groups.

Testing the differences with the use of variance analysis, it has been confirmed that there are statistically significant differences in the values of pain threshold in m. masseter in subjects with different diagnoses (F=39.452, p=0.00000). Tukey’s test confirmed that there are statistically significant differences (p=0.000144) in the pain threshold values for m. masseter between subjects with fibromyalgia and those with diagnosed myiofascial pain, as well as between the subjects with fibromyalgia and myiofascial pain with limited mouth opening (p=0.000063). There are no statistically

Table 1. Differences in the pain threshold levels of m. masseter and m. temporalis between the two groups of subjects

	Control	Experimental	t-value	p-value
	$\bar{X} \pm SD$	$\bar{X} \pm SD$		
PPT m. masseter	4.91 ± 0.91	2.05 ±0.60	14.29*	0.000000*
PPT m. temporalis	5.21 ± 1.15	2.46 ±0.51	11.97*	0.000000*

**- statistical significance; t-values – results of the Student’s t-test; p-values – degree of statistical significance; $\bar{X} \pm SD$ - mean value and standard deviation; PPT – pressure pain thresholds*

Table 2. Differences in the values of the pain threshold of the M. Masseter in the subjects with different diagnoses

	Pain threshold of m. masseter				
Diagnosis	n	$\bar{X} \pm SD$	p-value		
MFP	15	2.44 ± 0.35	0.068465		0.000144*
MFP with lim.	7	2.16 ± 0.37		0.000063*	
Fibromyalgia	8	1.22 ± 0.12			

**- statistical significance; p-values of the Tucky’s test; $\bar{X} \pm SD$ - mean value and standard deviation; MFP - myofascial pain; MFP with lim. - myofascial pain with limited mouth opening*

significant differences in the values of pain threshold for this muscle between the subjects diagnosed with myiofascial pain and myiofascial pain with limited mouth opening (Table 2).

The variance analysis confirmed that there are statistically significant differences between the mean values of the pain threshold in subjects with different diagnoses (F=7.1991, p=0.00312). Table 3. shows the differences in the mean values of the pain threshold for m. temporalis in subjects with different diagnoses. Pain threshold values in subjects diagnosed with fibromyalgia and with myiofascial pain (p=0.002220) are statistically significant. This also applies for the subjects with fibromyalgia and with myiofascial pain with limited mouth opening (p=0.021948). The results were acquired using the Tukey's test.

The Table 4 demonstrates that 87.50% of patients with fibromyalgia had a fourth level of disability, whereas not one patient with myiofascial pain and myiofascial pain with limited mouth opening belonged to this group. 53.33% of patients with myiofascial pain had a third degree of disability, and 46.67% had a first and second degree of GCPS. Depending on the different diagnoses, there are statistically significant differences in the GCPS results (χ^2=30.82, p=0.00003).

Spearman rank correlation coefficient confirms that the values of the graded chronic pain scale are in negative statistically significant correlation with the values of algometrically measured values of the pain threshold for both muscles (Table 5).

Discussion

Imprecise diagnostics, the lack of a universally accepted classification system and the use of different inclusive and exclusive criteria for defining

Table 3. Differences in the pain threshold levels of m. temporalis in subjects with different diagnoses

	PPT m. temporalis				
Diagnosis	**n**	$\bar{X} \pm SD$	**p-value**		
MFP	15	2.70 ± 0.39	0.289595		
MFP with lim.	7	2.48 ± 0.40		0.021948*	
Fibromyalgia	8	1.99 ± 0.52			0.002220*

**- statistical significance; p-values of the Tucky's test; $\bar{X} \pm SD$ - mean value and standard deviation; MFP - myofascial pain; MFP with lim. - myofascial pain with limited mouth opening;*

Table 4. Differences in the degree of disability in subjects with different diagnoses

Experimental	**Diagnosis**			
Disability degree	**MFP**	**MFP with lim.**	**Fibromyalgia**	**Total**
	n (%)	n (%)	n (%)	
I	3 (20.00%)	4 (57.14%)	0 (0.00%)	7 (23.33%)
II	4 (26.67%)	0 (0.00%)	0 (0.00%)	4 (13.33%)
III	8 (53.33%)	3 (42.86%)	1 (12.50%)	12 (40.00%)
IV	0 (0.00%)	0 (0.00%)	7 (87.50%)	7 (23.33%)
χ^2 test	χ^2=8.58*, p=0.03577*			
		χ^2=0.11*, p=0.011727*		
	χ^2=56.00*, p=0.0000*			

**- statistical significance; n - number of subjects; p-values - results of χ^2 test; MFP - myofascial pain; MFP with lim. - myofascial pain with limited mouth opening*

Table 5. Spearman rank coefficient correlation for different variables

	PPT m. masseter	**PPT m. temporalis**	**Disability degree**
PPT m. masseter	1.00	0.53*	-0.55*
PPT m. temporalis	0.53*	1.00	-0.58*
Disability degree	-0.55*	-0.58*	1.00

**- statistical significance; PPT - pressure pain tresholds*

similar subsets in different taxonomical systems all result in confusion and inability to compare previous observations and research results. The are some of the obstacles in expanding the knowledge and information on muscle dysfunctions. Yet, over the past few decades there has been a continuous scientific development in the field which can bring about progress and improve the clinical research protocols and instruments for chronic myofascial pain assessment.

Benoliel (2008) claims that pain in masticatory muscles represent a common feature of orofascial muscle dysfunctions and fibromyalgia, but that it differs in character. It is important determinant of diagnostic assessment and can be expressed through the pain threshold, which is defined as the lowest applied pressure to cause the sensation of pain (9,26).

The values of pain threshold in literature vary significantly in healthy subjects (27,28). Estimating the sensitivity and specific values of pain threshold for m. temporalis and m. masseter, a number of researchers concluded that they are remarkable in comparison to subjects with no pain, and that they can be measured in subjects of both sexes and all age groups (29,30). A number of studies demonstrate that the pain threshold is essentially lower in patients with fibromyalgia and orofascial muscle dysfunction than in healthy subjects (9,17-19,31). The results of our research prove this hypothesis. The subjects from the experimental group had statistically significant lower values of the pain threshold for both muscles than the healthy subjects from the control group (with the degree of certainty of 95%).

A smaller number of studies deal with scoring the pain threshold in the orofacial muscle dysfunctions and fibromyalgia. In our research, the obtained pain threshold values measured in m. temporalis and m. masseter were significantly different diagnostis ($p<0,05$). Such results are in accord with the research found in literature, where patients with fibromyalgia, apart from the lower pain threshold, also have a more number of manifestations of different disturbances (pain, sleep, fatique, etc) in comparison with diagnosed myofascial pain (9,26).

Another fundamental diagnostic characteristic for differentiating between myofascial pain and fibromyalgia is the heterotopic pain. In his research, Wright (2000) confirmed the view that the source, place and zone of transferred pain are consistent ad predictable. It is beneficial for the clinical practitioner during examination to use specific figures, showing pain sources and regions where pain can be most easily projected (projection areas). He claims that the heterotopic pain mechanisms are still unknown, and undoubtedly, they are not at all simple. He believes that this type of pain result from multiple mechanisms in CNS, two of them being central: convergence and central sensitization (32).

A great number of researchers confirmed in their studies that reproducibility and validity of algometry results are remarkable. Yet, they claim that it is crucial to have the equipment and standardized methodology (calibration and type of instruments, examiners and locations of measurement) (9,17,33). There are different commercially used hand algometers, simple and digital (electronic) algometers. Hand algometers have many drawbacks. It is hard to keep consistent paces on several testing periods, which results in frequent variability in results. Also, test results connecting the pain with the biological aspects in real time are limited. The introduction of digital algometers with the new generation software, suitable for measurement in orofascial region, created new, more precise possibilities for pain quantification.

The possibilities of chronic pain assesment using algometers are involuable. A frequent methodological approach in research within the chronic myofascial pain diagnostics was based on the comparison of algometrical measurements with numerical pain scales or the VAS (34). Few authors compared algometrical values with the GSCP and other parameters for psychosocial pain assessment. Our research compared chronic pain assessment through algometry and GSCP, and we obtained results which prove statistically significant correlation and the existence of negative correlational rank (-0.55 and -0.58).

The creators of this scale attempted to quantify the degree of psychosocial functions related to chronic pain as precisely as possible. In their three year research, von Korff (1992) described numerous painful states and their prognostic measurements. They created a scale to estimate pain using a combination of several scales for self assessment and continuous measurement of dysfunctions (15).

The ease of use was one of the most important criteria for including this instrument in RDC/TMD Axis II (14). It has been found to be a valid and reliable instrument for use as a self-completion questionnaire (35) and used in earlier pain studies (14,36).

Manfredini (2010) this may well be due to the relatively low psychosocial dysfunction found in the present non-patient population as there were no GCPS grades III and IV, which are groups of high disability caused by the facial pain condition. However, over 4% of the subjects had high intensity pain but low disability (10). This is a lower figure than in a study by Von Korff et al. (1990) who reported that in a community sample severe and persistent pain was found in 8% of the subjects and that high disability due to facial pain condition was found in 3% (37). In addition, it seems that multiple pain conditions predict more severe depression than do pain severity or persistence. Overall, the relationship between TMD pain and psychological variables appears to be very complex.

The advantages of GSCP are that it has clearly defined criteria, it is simple and quick, easy to calculate and distinguish between specific orofacial pain conditions. Within our research, 87.50% of the subjects from the group with diagnosed fibromyalgia had the IV degree disability and a high level of dysfunctionality, whereas 53.33% of the subjects with myofascial pain had the third degree, and the remaining subjects had the first and second degree. The subjects with different diagnoses demonstrated statistically significant differences in relation to the GSCP resulta (χ^2=30.82, p=0.00003). The subjects diagnosed with fibromyalgia all had a high level of sisability, while those with various orofascial muscle dysfunctions had a lower degree of disability, Thus, our results confirm the claim that the GSCP values can be a parameter for distinguishing between different chronic orofascial pain conditions. It should also be stressed that the values of this scale need to be carefully interpreted in evryday practical work.

Conclusion

We conclude that the usage of graded chronic pain scale in combination with properly calibrated algometer is undoubtedly a proper and necessary method for the complete chronic pain assessment, and, possibly, even the leading parameter in the diagnostics of muscular dysfunction. They are easy, quick and simple to use and the results obtained in this way are reliable and lasting. Therefore, it can be stressed that the use of these two instruments in everyday clinical practice would be of invaluable aid in resolving diagnostic uncertainties. In order to arrive at ever more relevant results, it is necessary to educate evaluators beforehand. Our suggestion would be to carry out a complete diagnostic assessment of patients with painful chronic myofascial syndromes using several pain assessment instruments, due to the complex nature of these disturbances. In this respect, future research should be aimed at completing and developing uniform, generally accepted diagnostic protocols for these orofascial region disturbances.

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Is there any relationship between serum neopterin level and adolescent and post-adolescent acne vulgaris?

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Abstract

Background: The pathogenesis of acne vulgaris includes sebaceous hyperplasia, follicular hyperkeratinization, bacterial hypercolonization, immune reactions and inflammation. However, the development of acne lesions is characterized by comedones, papules and pustules while nodules are occurred by a sequence of still unclear pathophysiological events. The aim of this study was to evaluate serum levels of neopterin and FSH, LH, prolactin, estradiol, progesterone, DHEA-S and testosterone in patients with adolescent and post-adolescent acne and control subjects.

Methods: The study comprised 30 patients with acne vulgaris lesions and 30 healthy individuals. The serum levels of FSH, LH, prolactin, estradiol, progesterone, DHEA-S, testosterone and neopterin in patients with acne were measured and they were compared with the control subjects.

Results: As the serum levels of FSH, LH, estradiol, progesterone, prolactin, testosterone and DHEA-S in patients with acne were comparable to those of control subjects; the neopterin levels in patients were significantly higher than those in control subjects (p=0.001). There were also no significant correlations between the number of comedones, papules, pustules, nodules, cysts and serum hormone levels in patients with acne. On the other hand, the serum level of neopterin significantly correlated with the number of pustules. Besides, the neopterin level was notably higher in adolescent patient group than in the post-adolescent and control subjects (p=0.001).

Conclusions: We have come to the conclusion that, neopterin can play a role in the pathogenesis of acne and especially in the adolescent patients it is also associated with pustules.

Key words: Acne vulgaris, Neopterin, Adolescent, Post-adolescent.

Introduction

Acne vulgaris is defined as a chronic inflammatory disorder of the pilosebaceous follicles. It occurs most commonly during adolescence as a sign of puberty. Although this disorder generally breaks up spontaneously before the age of 25, one forth of cases and especially in female patients it may last until 3. and 4.decades (1). Additionally, post-adolescent acne is discribed as the form of acne which is seen after the age of 25. The dominant skin lesions include comedones, papules and nodules which usually lead to scar formation (2).

Etiology of acne vulgaris is multifactorial (3-5). Hormonal changes, alterations in lipid composition, and abnormal response to local cytokines are all hypothesized that they play an important role in the pathogenesis (3,4). In addition, androgen stimulation of sebaceous glands may play a critical role as well. Acne vulgaris begins after the increase in androgen secretion and acne lesions associated with hirsutizm and menstrual abnormalities frequently appears on women with hyperandrogenic condition (2,6-8).

Neopterin is a non-specific marker of cellular immunity and associated with various clinical states such as allograft rejection, infections, autoimmune diseases, malignancies, heart and kidney failure, coronary artery disease and myocardial infarction (9). Monocytes and macrophages are considered to be the main source of neopterin in humans (10).

Neopterin can also play a role in the pathogenesis of acne vulgaris.

In this study, we sought to evaluate/investigate the serum levels of neopterin and follicular stimu-

lating hormone (FSH), leutinising hormone (LH), prolactin, estradiol, progesterone, dehydroepiandrosterone sulfate (DHEA-S) and testosterone that may play role in acne etiopathogenesis in patients with adolescent and post-adolescent acne vulgaris and compare the results with healthy control subjects.

Material and method

The study was conducted in Abant Izzet Baysal University Faculty of Medicine between October 2007 and June 2008. Before the study, a written informed consent was received from each patient and all the rules of good clinical practice were respected. Besides, the approval of the study was obtained by Local ethics committee.

Thirty (30) female patients were diagnosed with acne vulgaris in dermatology outpatient clinic and another 30 healthy female subjects whose age and gender were equal to the patients, were consented to participate in the study. Patients using topical and hormonal drugs for the treatment of acne vulgaris were not included in the study. Patient evaluation, dermatologic examination, and blood sampling were carried out on the 3rd and the 5th day of menstrual phase. Serum levels of FSH, LH, prolactin, progesterone, estradiol, DHEA-S, testesteron and neopterin were measured after sampling all patients and control subjects by solid-phase competitive method using chemiluminescence assays on an Immulite 2000 Siemens analyzer. Serum samples were stored at -70°C until the day of neopterin measurement. Comparison of age and the levels of FSH, LH, prolactin, estradiol, progesterone, DHEA-S, testosterone and neopterin in serum between the patients and the control groups were performed.

Neopterin levels were measured by DRG enzyme-immunoassay kit plates (DRG Instruments GmbH, Marburg, Germany). The plates were washed by Thermo Electron Corporation Well Wash AC (Thermo Fisher Scientific Inc., MA, USA), the absorbances were read by the Biorad Benchmark Plus Microplate Spectrophotometer (Biorad Laboratories Inc., CA, USA) and the results were calculated using the Biorad Microplate Analysis Software Version 5.2.1 for Windows. The reference range for neopterin was <10 nmol/L. The presence and number of comedones, papules, pustules, nodules and cysts of all patients were recorded.

Statistical Evaluation

For statistical analysis of the data, software package ″SPSS (Statistical Package for Social Sciences) for Windows 16.0″ was used. While continuous variables were expressed with mean ±, standard deviation and categorical variables were given with % (percentage). Continuous variables were compared with t-test, Mann Whitney U test; and categorical variables were compared with chi-square test. The relationship among neopterin, FSH, LH, prolactin, estradiole, progesterone, DHT, DHEA-S, testosterone levels and clinical findings (comedones, papules, pustules, nodules and cysts) was evaluated by means of Pearson correlation test. P value less than 0.05 was accepted as statistically significant. A statistical analysis was also carried out on a population subgroup as divided into adolescent and post-adolescent.

Results

The age and neopterin, FSH, LH, estradiol, progesterone, prolactin, testosterone, and DHEA-S serum levels of patients and control subjects are shown in Table 1. There was no statistically significant difference by age between patients with acne vulgaris and control subjects ($p>0.05$)(Table 1).

The serum levels of FSH, LH, estradiol, progesterone, prolactin, testosterone, and DHEA-S in patients with acne vulgaris were comparable to those of the control subjects ($p>0.05$ for all) (Table 1). However, neopterin levels of patients with acne vulgaris were significantly higher than those of the control subjects (1.63±0.70 nmol/L, 1.04±0.18 nmol/L, respectively) ($p= 0.001$) (Table 1).

There were no significant correlations between the number of comedones, papules, pustules, nodules, cysts and serum hormone levels in patients with acne vulgaris. On the other hand, the serum level of neopterin significantly correlated with the number of pustules ($r=0.46$, $p=0.01$) (Table 2). The clinical findings were correlated with neopterin, FSH, LH, prolactin, estradiol, progesterone, testosterone, and DHEA-S serum levels of patients with acne vulgaris. The number of pustules significantly correlated with neopterin level ($r=0.46$, $p=0.01$) (Table 2). There was no correlation between FSH, LH, prolactin, estradiol, progesterone, testosterone, and DHEA-S levels and

Table 1. Comparison of age and laboratory parameters in patients with acne and healthy subjects

	Patient (N= 30)	Control (N= 30)	P value
Age (years)	25,27 ±7,66	26,10±4,83	0,21
Neopterin (nmol/L)	1,63±0,70	1,04±0,18	**0,001**
FSH (mlU/mL)	6,79±1,79	6,73±1,60	0,58
LH (mlU/mL)	4,77±2,11	5,00±2,45	0,21
Estradiol (pg/mL)	50,30±27,22	50,31±31,82	0,46
Progesterone (ng/mL)	0,41±0,23	0,43±0,22	0,91
Prolactin (ng/mL)	16,43±6,34	14,85 ±6,50	0,67
Testosterone (ng/dL)	35,73±15,76	41,35±17,45	0,45
DHEA-S (μg/dL)	230,66±93,13	268,85±106,12	0,10

Table 2. Correlation of laboratory parameters with clinical parameters in patients with acne

		Comedones	Papules	Pustules	Nodules	Cysts
Neopterin (nmol/L)	Pearson Correlation	0,35	0,01	0,46	-0,10	-0,27
	Sig.(2-tailed)	0,06	0,98	**0,01**	0,59	0,15
FSH (mlU/mL)	Pearson Correlation	-0,09	0,06	0,27	0,10	0,18
	Sig.(2-tailed)	0,64	0,76	0,16	0,59	0,35
LH (mlU/mL)	Pearson Correlation	-0,01	-0,02	-0,15	0,23	0,21
	Sig.(2-tailed)	0,97	0,94	0,94	0,22	0,26
Estradiol (pg/mL)	Pearson Correlation	-0,12	0,14	0,04	0,00	0,14
	Sig.(2-tailed)	0,52	0,46	0,84	0,99	0,45
Progesterone (ng/mL)	Pearson Correlation	0,06	-0,02	-0,18	0,09	0,01
	Sig.(2-tailed)	0,77	0,92	0,36	0,63	0,98
Prolactin (ng/mL)	Pearson Correlation	0,11	0,06	0,07	0,03	-0,03
	Sig.(2-tailed)	0,56	0,77	0,70	0,88	0,86
Testosterone (ng/dL)	Pearson Correlation	0,14	0,29	0,02	0,35	0,37
	Sig.(2-tailed)	0,48	0,12	0,91	0,06	0,05
DHEA-S (μg/dL)	Pearson Correlation	0,10	0,26	0,04	0,33	0,33
	Sig.(2-tailed)	0,60	0,17	0,85	0,08	0,08

clinical findings (comedones, papules, pustules, nodules, and cysts) (Table 2).

Patients were also divided into two subgroups as adolescent group younger than/under the age of 25 (years old) and post-adolescent group older than/over the age of 25. Serum hormonal levels and neopterin level of adolescent group, post-adolescent group and control subjects were compared by Kruskall Wallis test. There were no statistically significant differences between adolescent, post-adolescent and control subjects with respect to the serum levels of FSH, LH, prolactin, estradiol, progesterone, testosterone, and DHEA-S. The neopterin level was significantly higher in the adolescent patient group than in the post-adolescent group and control subjects (p=0.001) (Table 3). However, neopterin levels in post-adolescent group were comparable to those of control subjects (p=0.38) (Table 3).

Discussion

Acne vulgaris is an exclusively follicular disease, with the principle abnormality of comedone formation. It is produced by the impaction and distention of the follicles with a keratinous plug in the lower infundibulum. The keratinous plug is caused by hyperproliferation and abnormal differentiation of keratinocytes is caused by unknown reasons (1,3). Complex mechanisms such as sebaceous hyperplasia, follicular hyperkeratinization, bacterial colonization, immune reactions and inflammation are involved in the pathogenesis of acne vulgaris (11,12). The combination of keratin, sebum, and

Table 3. Comparison of subgroup population as divided into adolescent and post-adolescent with acne and healthy subjects

	Groups	Mean Difference	Std.Error	P value
Neopterin (nmol/L)	Group 1- Control	1,00	0,13	**0,001**
	Group 1- Group 2	0,82	0,15	**0,001**
	Group 2- Control	0,18	0,13	0,38
FSH (mlU/mL)	Group 1- Control	0,61	0,53	0,48
	Group 1- Group 2	-1,08	0,61	0,18
	Group 2- Control	-0,47	0,53	0,64
LH (mlU/mL)	Group 1- Control	-0,25	0,73	0,93
	Group 1- Group 2	-0,03	0,84	0,99
	Group 2- Control	-0,22	0,73	0,95
Estradiol (pg/mL)	Group 1- Control	-7,52	9,29	0,69
	Group 1- Group 2	-15,01	10,72	0,35
	Group 2- Control	7,49	9,29	0,70
Progesterone (ng/mL)	Group 1- Control	-0,03	0,07	0,93
	Group 1- Group 2	-0,01	0,08	0,99
	Group 2- Control	-0,02	0,07	0,96
Prolactin (ng/mL)	Group 1- Control	1,59	2,05	0,72
	Group 1- Group 2	0,02	2,36	1,00
	Group 2- Control	1,57	2,05	0,72
Testosterone (ng/dL)	Group 1- Control	-4,97	5,30	0,62
	Group 1- Group 2	1,29	6,12	0,97
	Group 2- Control	-6,27	5,30	0,47
DHEA-S (μg/dL)	Group 1- Control	-28,64	31,77	0,64
	Group 1- Group 2	19,10	36,69	0,86
	Group 2- Control	-47,74	31,77	0,29

Group 1= adolescent acne patients Group 2= post-adolescent acne patients

microorganisms, particularly *Propionibacterium acnes*, leads to the release of proinflammatory mediators and accumulation of T-helper lymphocytes, neutrophils and foreign body giant cells. This, in-turn, causes the formation of inflammatory papules, pustules and nodulocystic lesions (1). Although commensally microorganisms such as *P. acnes and S. epidermidis* have been implicated in the pathogenesis of acne vulgaris, it is not an infectious disease. However, it is an important triggering factor for the inflammatory process. *P.acnes* plays a crucial role in the pathogenesis of acne and initiates the inflammatory process by producing chemotactic enzymatic factors (13,14).

Androgens, alterations in lipid composition and an abnormal response to local cytokines are all hypothesized that they play an important role. Androgen stimulation of the sebaceous glands is also critical. Acne begins after an increase in sebum secretion and frequently appears on women with hyperandrogenic condition, along with hirsutizm and menstrual abnormalities (1,2,8). However, we have observed no significant difference between patients and control subjects regarding hormonal status, namely FSH, LH, prolactin, estradiol, progesterone, testosterone, and DHEA-S. Since the patients and control subjects are in the same age range, we may assume that the sebaceous glands of the patient group are not exposed to higher levels of hormonal stimulation compared to those of the control subjects. Therefore, this leads to the idea that there should be other mediators or pathways playing a role in the pathogenesis of acne vulgaris, in addition to hormonal changes in adolescent and post-adolescent patients.

Neopterin is a non-specific marker of the activation of cellular immunity system and is associated with the activation of T cells and the activity of monocyte/macrophages. Monocytes/macrophages seem to be the main source of neopterin in

humans (9). Recent studies have revealed abnormal neopterin concentrations in the body fluids in various clinical states, such as allograft rejection, infections, autoimmune diseases, malignancies, heart and kidney failure, coronary artery disease, and myocardial infarction (9,15-17).

Higher levels of neopterin in patients with acne vulgaris and its significant correlation with the number of pustules emphasize the role of inflammation in acne vulgaris and the possible role of cellular immunity. Nevertheless, it is still not clear the reason of documented activation of cellular immune system by neopterin levels by a bacterial infection such as *P.acnes* or other triggering factors. Subgroup analysis of patients with acne vulgaris yielded that the serum level of neopterin in adolescent patients was higher than that in post-adolescent patients and control subjects, whereas serum level of neopterin in post-adolescent patients was comparable to that in control subjects. This finding also suggests that the mechanism of acne vulgaris in adolescent patients might differ from post-adolescent patients.

In conclusion, we have showed for the first time that the patients with acne vulgaris have higher levels of neopterin compared to the control subjects, whereas the highest level is in the adolescent patient group. Additionally, it has been observed that the levels of serum neopterin significantly correlate with the number of pustules. The exact pathogenesis of acne vulgaris and its association with neopterin remains to be elucidated in further clinical studies.

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Effect of L-glutamine on mercury chloride-induced neurotoxicity

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Abstract

Background: Citrulline is by-product in nitric oxide production from arginine. Nitric oxide has different functions in the body, including neuromodulary. L-Glutamine serves as a precursor of, excitatory amino acid, glutamate, and inhibitory neurotransmitter, γ-amino butyric acid. Glutamine supplementation improves survival rates in multiple system organ failure caused by sepsis or other extreme conditions.

Objectives: We have hypothezed that glutamine may be beneficial in protection of brain from injury caused by mercury chloride.

Methods: It was studied the possible beneficial role of glutamine in neurotoxicity induced by mercury chloride by measuring of lipid peroxidation and citrulline levels in the brain 1 hour after administration of mercury chloride to Sprague Dawley rats.

Results: Citrulline and lipid peroxidation levels were increased in the brain of intoxicated rats. Pretreatment of animals with L-Glutamine decreases significantly tissue levels of lipid peroxidation and citrulline compared to group treated with mercury chloride.

Conclusions: Our results suggest that nitric oxide over-production is involved in mechanisms of mercury chloride toxicity. L- Glutamine pretreatment decreases nitric oxide and free radical production in the brain and may be beneficial in neurotoxiciy induced by mercury chloride.

Key words: L-Glutamine, mercury chloride, brain.

Introduction and goal

Brain function is dependant on the aviability of many substrates including amino acids. Besides to structural role some of them are neuromodulators or precursors for synthesis of neurotransmitters. Glutamine is precursor for both: excitatory amino acid, glutamate, and inhibitory neurotransmitter, γ-aminobutiric acid (GABA). L-Glutamine functions as a precursor of purines and pyirimidines, protein synthesis, respiratory fuel and in ammonia detoxication (1). L-Glutamine supplementation improves survival rates in multiple system organ failure caused by sepsis and protects tissues from oxidative injury through glutathione (GSH) synthesis. (2).

Heavy metal toxicity is very frequent in the world. Mercury chloride ($HgCl_2$) is heavy metal with high distribution in many sources. Mercury exists in different molecular forms, as elemental mercury found in thermometers, thermostats, and dental amalgams. Mercury vapor is carried to all parts of the central nervous system as a lipid soluble gas. Inorganic mercury (mercury salts) is found in cosmetic products, laxatives, teething powders, diuretics, and antiseptics. Organic mercury is considered the most toxic and most frequent form of mercury exposure. Organic mercury is found in fish, pesticides, fungicides, insecticides, and thimerosal- containing vaccines and gamma globulin (3). Ethyl mercury also causes renal and central nervous system toxicity and is deposited in the liver, kidneys, skin, brain, spleen, and plasma. Ethyl mercury may actually be converted to inorganic mercury in the tissues in greater amounts and more rapidly than methyl mercury (4).

Many research studies shown that dietary factors, including protein, amino acids, glucose, fructose, fatty acids, vitamins, minerals, phytoestrogens, polyphenols, are either beneficial to health or contribute to the pathogenesis of many diseases partially through modulation of nitric oxide (NO) production. As known, glutamine supplementation improves survival rates in multiple system organ failure caused by sepsis or other extreme conditions. We have hypothezed that glutamine may be beneficial in protection of brain from injury caused by mercury chloride.

Methods

The research was conducted at the Department of Biochemistry, Faculty of Medicine, University of Nis. In Laboratory for Biomedical Research of the Faculty of Medicine in Nis during the three week experimental animals - male Sprague Daley rats weighing approximately 200 g each, were divided into 4 groups. In each group were 8 rats. First group was control group of rats and those consumed drinking water, second group of rats was treated with mercury chloride in a dose of 3mg/kg intraperitoneally (i.p.), third group of rats was treated with L-glutamine in a dose of 200 mg/kg per os, fourth group of rats was treated with $HgCl_2$ and L-glutamine in same doses such in previous groups. Acute toxicity was induced by administration of $HgCl_2$ in a dose of 3mg/kg (i.p.) and L-glutamine, 200mg/kg was administrated 1 hour before mercury chloride. One hour after administration of $HgCl_2$ animals were sacrificed and their brains were removed and dissected and frozen prior to measuring the lipid peroxidation and citrulline levels.

Malondialdehyde (MDA) is the most abundant aldehyde resulting from lipid peroxidation (LP) breakdown in biological systems and used as an indirect index of LP. The determination of MDA in brain homogenate is based on its reaction with thiobarbituric acid (TBA) which forms a pink complex with absorption maximum at 535 nm. Tissue lipid peroxidation level, as MDA, was measured by thiobarbituric acid reaction and expressed in nmol/mg (5).

Citrulline, as a measure for nitric oxide synthase activity, was measured on the basis of diacethylmonoxime reaction and expressed in μmol/mg (6). Protein concentration was determined by the method of Lowry (7).

The entire study was done with respecting the principles of International guidelines for biomedical research on animals.

Statistical significance of the results was determined by Students t-test.

Results

Measured values of MDA and citrulline were compared between different groups of animals, and the results were presented graphically.

On Figure 1 were shown the results which demonstrated that mercury chloride significantly increases MDA level in the rat brain ($p< 0.01$). In group of animals treated with L-glutamine brain tissue level of lipid peroxidation was significantly decreased ($p<0.05$). Pretreatment with glutamine decreases lipid peroxidation level compared to $HgCl_2$ group ($p<0.02$).

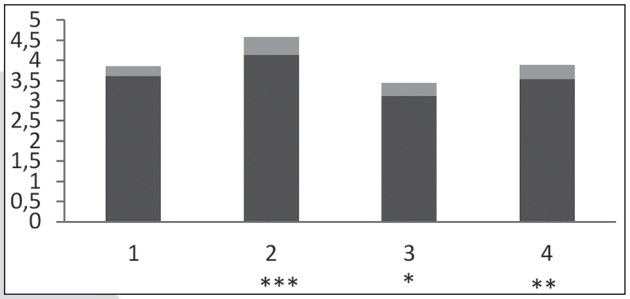

Figure 1. L-glutamine effect on brain MDA level
1- control group
2- group treated with mercury chloride
**** p<0.01 vs. control*
3- group treated with L-Glutamine
** p<0.05 vs control*
4- group treated with mercury chloride and L-Glutamine
*** p<0.02 vs mercury chloride gruop*

On Figure 2 was shown the level of citrulline in mercury chloride treated rats and it was significantly increased ($p<0.05$). L-Glutamine decreases citrulline level ($p<0.01$). In group of animals pretreated with L-glutamine citrulline level was decreased compared to $HgCl_2$ treated group ($p<0.02$).

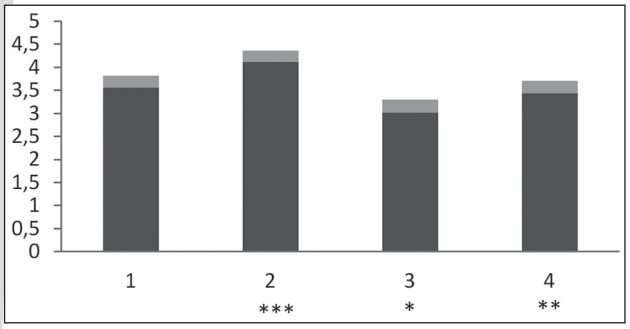

Figure 2. Glutamine effect on brain citrulline level
1- control group
2- group treated with mercury chloride
**** p<0.01 vs. control*
3- group treated with L-Glutamine
** p<0.05 vs control*
4- group treated with mercury chloride and L-Glutamine
*** p<0.02 vs mercury chloride gruop*

Discussion

It was suggested that metal-induced oxidative stress in cells could be partially responsible for the toxic effects of heavy metals (8). Free radicals. are molecules such as superoxide anion (O_2-), hydroxyl radical (HO·), nitric oxide (NO) and lipid radicals. In reactions between radicals and polyunsaturated fatty acids within cell membrane may result in a fatty acid peroxyl radical (R-COO·) that can attack fatty acid side chains and initiate production of other lipid radicals. End products of lipid peroxidation, including unsaturated aldehydes and other metabolites, have cytotoxic and mutagenic properties. Cells under oxidative stress display various dysfunctions due to lesions caused by reactive oxygen species (ROS) to lipids, proteins and deoxyribonucleic acid (DNA).

NO in brain is derived from neuronal niric oxide synthase (NOS) as well as from endothelial NOS (eNOS). NO is a potent mediator of various biological responses. Besides serving as a mediator of immune responses, a neurotransmitter and a signaling molecule. NO is an endothelium-derived relaxing factor and plays an important role in regulating vascular tone and permeability (9, 10, 11, 12, 13, 14, 15, 16).

There are three forms of nitric oxide synthase - a neuronal type called nNOS, an epithelial type called eNOS, and an inducible form called iNOS. NO production is a stress response and can lead to either tissue injury because of its radical chemistry, or be cytoprotective, protecting cells from damage. The first NO is a short-living chemical transmitter, which is freely diffusible across membranes. NO causes vascular dilatation in epithelial cells by controlling smooth muscle contractility. Glutamate is produced from glutamine and released by a synapse and activates the N-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors. This leads to an influx of calcium ions which in turn bind to calmodulin, activating the neuronal NOS. Activation of NMDA receptor inhibits glutamine synthase activity (17).

The activity of eNOS and nNOS is controlled by tetrahydrobiopterin and calcium (Ca) availability because these two cofactors are needed for the proper dimer formation of an active synthetase. The dependence on calmodulin has been used as a model to explain the role of glutamate in neurotoxicity in the central nervous system. The immediate effect of glutamate on neurons is its role in activating glutamate receptor, namely to pharmacological subtypes known as NMDA receptors. Glutamate receptors are selective for calcium ions. Neurons are particularly sensitive to impaired mitochondrial adenosine triphosphate (ATP) synthesis capacity, because neurons depend almost exclusively on the oxidative degradation of glucose and ketone bodies.

Glutamine is metabolic substrate and precursor for nucleotides, purine, pyrimidine and glutathione. Glutamine is one of the most important substrates for ammonia genesis and regulation of acid-base homeostasis. During the sepsis and catabolic states, such as trauma, hypoxia, major surgery and bone marrow transplantation as well as intense chemotherapy and radiotherapy, blood glutamine level is reduced (18, 2, 19, 20, **21**). Glutamine synthesis depending of the catalytic activity of glutamine synthetase. Nitric oxide inhibits glutamine synthetase by covalent modification by nitrozylation (17). According to Allen et al., mercury chloride, but not methyl mercury, inhibits glutamine synthetase activity in primary cultures of cortical astrocytes and decreases glutamine level (22).

Oxidative stress may contribute to the development of neurodegenerative disorders caused by mercury intoxication (23, 24). Exposure to mercury in vitro produced a concentration-dependent increase of ROS in different regions of the rat brain. Metallothionein and glutathione are carriers for heavy metals. Glutathione (GSH), an antioxidant, has specific roles in protecting the body from mercury toxicity. Glutathione, binding with mercury, forms a complex that prevents mercury binding to cellular proteins and damage to enzymes and tissue. Glutathione-mercury complexes have been found in the liver, kidney, and brain and appear to be the primary form in which mercury is transported and eliminated from the body. Mercury accumulates in the central nervous system primarily in astrocytes, the cells that provide the first line of defense for the central nervous system against toxic compounds (3).

L-Glutamine, the most abundant free amino acid in plasma, is a physiological inhibitor of NO synthesis in endothelial cell, intact blood vessels

and cerebral tissues, and therefore has been suggested to have a role in regulating cardiovascular function (25, 26, 27, 28, 29). According to Houdijk et al., dietary glutamine supplementation reduces plasma nitrate levels in rats (30).

Glutamine inhibits endothelial arginine synthesis via competitive inhibition of citrulline uptake and a decrease in argininosuccinate synthetase (ASS) activity or by inhibiting recycling of citrulline to arginine limiting arginine availability for NO production (31). Argininosuccinate synthetase catalyses the condensation of citrulline and aspartate to argininosuccinate, the immediate precursor of arginine. Glutamine metabolism to glucosamine inhibits endothelial nitric oxide synthesis by inhibiting pentose cycle and decreasing of cellular nicotinamide adenine dinucleotide phosphate (NADPH) concentration (**32**).

In the liver, citrulline is locally synthesized by OCT and metabolized by ASS for urea production. In most of the tissues producing NO, citrulline is recycled into arginine via ASS to increase arginine availability for NO production (33, 34). Macrophages can convert citrulline into arginine (32, 26, 21).

Plasma citrulline level became marker for intestinal diseases (35, 36, 37), but it may be a marker of diseases related to other citrulline producing cells, also.

Conclusion

Results of our study show that acute mercury chloride toxicity increases lipid proxidation level and nitric oxide production in brain tissue. L-Glutamine pretreatment of intoxicated rats decreases lipid peroxidation and nitric oxide production. These results indicate nitric oxide mediated brain injury. A beneficial effect of glutamine on toxicity is a result of interrelation between glutamine and NO production.

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Serum angiopoietin-1, angiopoietin-2, their receptor levels in patients with various gastrointestinal types of carcinomas

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Abstract

Objectives: The aim of this study was to investigate the importance of the measurements angiopoietins and their receptor in patients with various gastrointestinal system cancers in relation to angiogenesis.

Patients and Methods: We enrolled serum specimens of the 100 individuals with esophageal, gastric, pancreatic, colon and rectum cancer. Serum concentrations of Ang-1, -2, and Tie-2 were measured by enzyme-linked immunosorbent assay.

Results: Serum Ang-1 levels were found higher in patients with esophageal, gastric, colon, and rectal cancer according to control group and in patients with pancreas cancer ($p<0.001$, $p=0.03$, $p<0.001$, $p<0.001$, $p=0.03$; respectively). Serum Tie-2 levels were found higher in all patients than control group ($p<0.01$). We did not find differences between patient groups in terms of serum Ang-2 and Tie-2 levels ($p>0.05$).

Conclusions: These data suggest that expression of Ang-1 is implicated in tumor development in human gastric, colon and rectum carcinomas. Its production may assist in tumor angiogenesis.

Key words: Angiopoietins, cancers, angiogenesis.

Introduction

Cancer is one of the leading causes of cancer death worldwide (1). The high mortality of this disease is largely due to the lack of a screening strategy to detect early stage disease. Angiogenesis, the generation of new blood vessels, is a progressive physiological process and an important mechanism in the pathology of tumor progression (2, 3). The mechanism of angiogenesis includes direct production of angiogenic cytokines by plasma cells. Cytokines such as basic fibroblast growth factor and vascular endothelial growth factor were played a leading role in angiogenesis. Another important cytokines are the angiopoiteins (Ang) -1 and -2, and the tyrosine kinase receptor Tie2 expressed in endothelial cells. However, other known and unknown factors are also involved and are under investigation.

Angiopoietins (Angs) and their receptor are pro-angiogenic mediators in tumor angiogenesis (4). Thus, the effects of Angs on angiogenesis and tumor growth remain controversial. They don't seem to participate in the initial phase of vascular development, but rather play a critical role in angiogenic outgrowth, vessel remodeling. Growth of the vascular wall is regulated by Ang-1 binding to Tie2 receptor. By contrast, Ang-2 antagonizes Tie2 binding and induces vessel destabilization, which leads to the angiogenic sprouting (5, 6).

Ang-1 binds specifically to Tie2 causing activation by phosphorylation. Ang-1 is produced by endothelial cells and pericytes and is widely expressed in adult tissue, where it appears to have a stabilizing effect on blood vessels (5). The role of Ang-1 in tumor development is complex and studies have shown both pro- and anti-angiogenic effects with this growth factor. Ang-2 is expressed at sites of vascular remodeling (7) and promotes vessel destabilization (8). This appears to be accomplished by Ang-2 binding to Tie2 and therefore blocking Ang-1 binding. Ang-2 appears to be a non-signal transducing ligand and therefore disrupts normal Tie2 activation (9).

In tumors, a shift in the balance pro-ant anti-angiogenic factors is thought to occur; termed the 'angiogenic switch' resulting in an angigenic phenotype (10, 11). It has been proposed that a change in the ratio of Ang-1:Ang-2 in favour of Ang-2 might play a role in the switch (12). In some studies, Ang-2 was expressed ubiquitously in tumor epithelium of cancer specimens, whereas expression of Ang-1 in tumor epithelium was rarely detected. This observation suggests that a net gain in Ang-2 activity over Ang-1 activity might be an initiating factor for tumor angiogenesis (13-16). In various type of gastrointestinal tumors, there have not been any studies that establish a causal role for Ang-1, -2 and their receptor, especially in eusophageal, pancreatic, hepatic and rectal cancer types.

In this study, to define a putative role for the Ang-1, -2 and Tie2 system in various gastrointestinal cancer types, we investigated the expression of Angs and their clinical significance. We focused especially on the role possible mechanism resulting in tumor growth induced by Ang-1, -2 and Tie2 in tumor angiogenesis.

Materials and Methods

Patients

We recruited 100 patients with esophageal, gastric, pancreas, colon, rectum and hepatic cancer who had undergone gastroscopy, colonoscopy and rectoscopy by gastroenterologist. The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethics Committee. Descriptive data consisting of demographics, diagnosis, and clinical data were obtained from the medical records.

One hundred cancer patients, 27 of the patients with esophageal cancer (16 males and 11 females; median age 52.1 years, range 39 to 60 years), 30 of the patients with gastric cancer (15 males and 15 females; median age 48.4 years, range 40 to 58 years), 22 of the patients with pancreas cancer (10 males and 12 females; median age 47.6 years, range 40 to 60 years), 28 of the patients with colon cancer (15 males and 23 females; median age 55.1 years, range 50 to 68 years), and 27 of the patients with rectal cancer (14 males and 13 females; median age 55.1 years, range 46 to 69 years).

Twenty healthy individuals volunteered to determine a normal reference range for Ang-1, Ang-2, and Tie levels (10 males and 10 females; median age 47.2 years, range 39 to 70 years).

Laboratory measurements

Serum samples were obtained before surgical or other treatment. Serum samples were immediately centrifuged at 3000 rpm (4°C, 20 min), frozen and kept at -80°C until assay. Samples were processed by same biochemist in the laboratory.

Ang-1, -2 and Tie-2 levels were measured by enzyme-linked immunosorbent assay kits according to manufacturer's instruction (R&D System, Minneapolis, MN, USA). The absorbance was measured by optical densitometry with a 450 nm filter.

Statistical Analyses

Shapiro Wilk test of normality of variables control charts and histograms were drawn. The average of the data, standard deviation, median values were given. Comparisons between groups were made with Kruskal-Wallis one-way analysis of variance. Pairwise comparisons (post hoc) were performed with Bonferroni-corrected Mann-Whitney U test ($p<0.0034$ was taken as the limit of significance here. Spearman's correlation test was used for correlations within the groups. Tests bi-directional, and the significance limit was set at $p<0.05$. Analyses using SPSS 17.0 statistical package program assessed.

Results

We did not find differences between groups in terms of age ($p>0.05$).

Serum Ang-1 levels were found higher in patients with esophageal, gastric, colon, and rectal cancer according to control group and in patients with pancreas cancer ($p<0.001$, $p=0.03$, $p<0.001$, $p<0.001$, $p=0.03$; respectively) (Table 1).

Serum Tie-2 levels were found higher in all patients than control group ($p<0.01$). We did not find differences between patient groups in terms of serum Ang-2 and Tie-2 levels ($p>0.05$) (Table).

Ang-1 levels in the serum of only the gastric, colon and rectal cancer have found positive correlation between the groups ($p<0.001$, $r=0.73$, $r=0.49$, $r=0.61$).

Table 1. Serum levels of the control group and patients

Variables	Ang-1	Ang-2	Tie-2
Control Group	330.4 ± 163.9 (105-905)	38.1 ± 8.7 (22.7-56.4)	12.5 ±2.8 (9.2-19.0)
Patients			
Esophgaeal Cancer	659.2 ± 201.7 (285-936)	39.1 ± 9.7 (20.7-58.1)	36.2 ± 15.6 (10.6-78.4)
Gastric Cancer	1309.8 ± 237.9 (1003-1839)	33.5 ± 8.7 (18.5-504)	41.1 ± 5.8 (40-58)
Pancreatic Cancer	221.3 ± 41.6 (150-303)	33.5 ± 8.7 (19.4-49.3)	41.1 ± 5.8 (28.6-49.0)
Colon Cancer	1969 ± 651.0 (1211-3620)	48.6 ± 5.5 (38.9-58.6)	75.8 ± 12.2 (48.6-97.3)
Rectum Cancer	1124.8 ± 172.2 (905-1744)	33.1 ± 9.1 (19.6-54.2)	32.1 ± 7.0 (20.6-45.9)

Discussion

In the present study, we have demonstrated that significantly higher Angs and Tie2 are detected in various gastrointestinal system cancers patients. These findings over-expression of Angs and their receptor leads to increased tumor growth and angiogenesis.

Similar findings have been reported serum Angs levels in colonic, lung and ovarian cancers (17-19).

The role of in tumor angiogenesis remains to be elucidated. Moreover, it could not indicate whether production or consumption of these factors occurred across the tumor development, because blood samples were assayed. We have important changes in the levels of these markers within cells of tumor in relation to in patients with gastrointestinal cancer. To our knowledge, this is the first investigation the dynamics of the Angs and Tie2 in the tumor. Angs are a family of vascular growth factors with critical roles in postnatal angiogenesis. Ang-1 and Ang-2 both act on the Tie-2 tyrosine kinase receptor found on endothelial cells, but appear to play antagonist roles (20, 21).

We report that the effects of serum Ang-1 in angiogenesis and regulation of vascular permeability; over-expression of serum Ang-1 by gastric colon and rectum cancer cells stimulated tumor angiogenesis and serum Ang-1 achieved the permeability effects of tumor cells derived growth factors. This effect of Ang-1 may have been mediated by the endothelial cells, leading to overall vessel stabilization.

Angs in the regulation of angiogenesis and in their effects on tumor growth is reflected (22-27). Several studies have suggested that Ang-1 may be proangiogenic (28,29). In a study reported that on the effects of imbalances in Ang-1 and -2 expression in colon cancer cells (29).

Similar results were found proangiogenic effects of Ang-1 too us. Carlson and collegeaus (30) demonstrated that Ang-1 may also interact extracellular matrix and stimulate effects through a Tie-2 independent mechanism. This study is a study with us. Etoh and collegeas (31) suggested that the Ang/Tie-2 system play an important role in gastric carcinoma. This study high Ang-2 expression showed a significantly worse prognosis. But, were did not found important differences of the serum Ang-2 and Tie-2 levels in patients with gastric cancer. Also, we are not found our study these markers in the esophageal and pancreatic cancer, we investigated. However, we could not detect of the Angs expression and its receptor. It is explained as follows: Continuous growth of carcinoma induces hypoxia and necrosis in certain portion, thus directly up-regulating apopotosis. In the presence of Ang-1 and -2, tumor and invasion angiogenesis are aggravated.

It is notable that the elevated expression of Ang-1 and -2 and its receptor in gastric, colon and rectum carcinomas and the increased serum Angs levels in cancer patients did not show specification for a particular carcinomas, such as esophageal cancer and pancreatic cancer. The role of Angs in

carcinogenesis (particular carcinomas) and/or tumor progression and angiogenic activities are an important role.

We found that the expression of the serum Angs and its receptor were increased in the cancer patients than control groups, but, they are higher in particular carcinomas. These findings include that Ang-1 may be useful in detecting certain carcinomas and they could be further as a marker.

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Mid-trimester amniotic fluid levels of interleukin-6: predictor of preterm delivery

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Abstract

Objective: Preterm delivery develops in about 8% of pregnancies in Serbia. In this paper we tried to determine the value of amniotic fluid interleukin-6 (IL-6) in the prediction of preterm delivery (PTD).

Study design: Following genetic amniocentesis, a sample of amniotic fluid was sent for determination of IL-6 with commercial ELISA kits.

Results: Twenty five women who delivered preterm (<37 weeks) were matched with 50 controls. The amniotic fluid IL-6 concentrations in women with spontaneous PTDs were significantly higher than in those who delivered at term (IL-6: 202±79.1 pg/ml [40–340] vs. 73.4±33.5 pg/ml [5–183] Amniotic fluid IL-6 concentrations of >140 pg/ml had a sensitivity of 84% and a specificity of 92.8% for the prediction of spontaneous PTD.

Conclusions: Elevated mid-trimester amniotic fluid concentrations of IL-6 can identify women at risk for spontaneous PTD.

Key words: Interleukin 6, mid-trimester, spontaneous preterm delivery.

Introduction

Preterm delivery (PTD) remains one of the major problems in modern obstetrics. About 8% of pregnancies in Serbia ends with preterm delivery and this represents an important cause of perinatal morbidity and mortality. [1] It is now a well accepted fact that intrauterine infection causes a significant proportion of spontaneous PTDs, particularly earlier ones. Bacteria can ascend from the lower genital tract before or during pregnancy, infect the membranes and initiate an inflammatory response culminating in preterm delivery or preterm premature rupture of membranes (PPROM) [2]. The frequency of intra-amniotic infection diagnosed by clinical signs and symptoms has been calculated between 6% and 17% in women delivering preterm and between 1% and 11% in women delivering at term [3]. However, in many cases the infection remains sub-clinical, therefore it is difficult for an accurate diagnosis to be made early. An occult infection of the normally sterile amniotic cavity may activate the cascade of inflammatory mediators which stimulates prostaglandin synthesis and release, leading ultimately to uterine contractions and irreversible cervical changes [4]. The need to identify the microbial invasion of the amniotic cavity and thus manage pregnant women accordingly, has led to investigations where various cytokines have been used as markers for intrauterine infection, such as interleukin-6 (IL-6), IL-1β, IL-8, tumor necrosis factor (TNF-α), granulocyte stimulating factor [5-8]. Amniotic fluid IL-6 is consistently related with intra-amniotic infection, histologic chorioamnionitis, and the presence of bacteria in chorioamnion [9]. It is well known that a subclinical intrauterine inflammatory process may begin very early in pregnancy, in women who subsequently deliver preterm. We hypothesized that amniotic fluid IL-6 determination during the second trimester amniocentesis can potentially identify patients at risk for preterm delivery.

The aim of this study was to determine the value of amniotic fluid IL-6 in the prediction of preterm delivery in asymptomatic women undergoing mid-trimester genetic amniocentesis.

Materials and methods

This was a prospective study designed to examine the relationship between mid-trimester amniotic fluid levels of IL-6 and the occurence of spontaneous preterm delivery. Our subjects were 75 women,

who underwent mid-trimester genetic amniocentesis. The study had received the approval of the ethics Committee of the Clinical center of Vojvodina and the Medical faculty of Novi Sad. Written informed consent was provided from all the participants, in accordance with the Helsinki criteria. All amniocentesis were performed between 16 and 19 weeks of gestation. Each amniocentesis was preceded by a detailed ultrasound scan with a 3.75 MHz curvilinear transducer, to assess fetal anatomy and to determine the location of the placenta. The gestational age was assessed either by the last menstrual period or by an early ultrasound scan if there was a discrepancy of more than a week. Inclusion criteria included singleton pregnancy, normal pregnancy course prior to the procedure, maternal age > 18 years, intact fetal membranes, no signs of preterm labor or cervical dilatation at the time of amniocentesis and nondiagnosed fetal anomalies with an anatomy ultrasound scan. We excluded from the analysis cases with abnormal fetal karyotype, major fetal anomalies, and significant medical or obstetric complications such as preeclampsia, fetal growth restriction, gestational diabetes, poly- or oligohydramnios, placenta praevia leading to iatrogenic preterm delivery.

Pregnancy outcomes were obtained by accessing labor and delivery records in our hospital. All women who delivered preterm (<37 weeks of gestation) formed the study group. The control group consisted of the two subsequent women matched for maternal age, parity and indication for amniocentesis, who also underwent amniocentesis during the same time period and who delivered a neonate at term.

Samples

Amniotic fluid samples were collected during amniocentesis, in a polypropylene tubes. After centrifugation at 200 x g for 10 minutes, supernatant was collected and stored at -20^{o} C, within 1 h of the procedure.

Cytokines

Levels of IL-6 in amniotic fluid were measured with commercially available enzyme-linked immunosorbent assays (ELISA) (Immunotech SAS, Marseille, France). The ELISA was validated for amniotic fluid, and the samples were measured in duplicate with a microplate reader (Beckman Coulter). For amniotic fluid IL-6 the sensitivity of the assay was 3.0 pg/ml, intra-assay and inter-assay coefficients of variation were 6.8% and 7.9% respectively.

Statistical analysis

Student's t-tests were used and Mann–Whitney test otherwise. Chi-square and Fisher's exact tests were used for the comparison of proportions. The diagnostic ability of IL-6 for the prediction of preterm deliveries was evaluated using Receiver Operating Characteristic (ROC) curves. Sensitivity, specificity, negative and positive predictive values were calculated for optimal cut-offs. The area under the curve (AUC) was also calculated. Analyses were conducted using Statistical Package for Social Sciences (SPSS) for Windows.

Results

During the study period, 292 women were enrolled in the study. Among them, 8 were delivered <37 weeks for fetal or maternal indications. Abnormal second trimester ultrasound was found in 5 women. A chromosomal abnormality was found in 3 women. All the above cases underwent pregnancy termination. Four women were lost to follow-up, therefore were excluded from analysis. The remaining 272 women met the inclusion criteria. The prevalence of spontaneous preterm delivery before 37 weeks was 9,1% (25/272). The preterm delivery group consisted, therefore, of 25 cases, which were matched with 50 controls who delivered at term. *Table 1* presents the demographic and clinical characteristics of the two groups. Maternal age, the number of previous pregnancies, the gestational age at sampling and the number of previous preterm deliveries were not different between the two groups. As expected, birth weight and gestational age were significantly lower in the preterm delivery group compared to those who delivered at >37 weeks.

The preterm delivery group had significantly higher concentrations of IL-6. When ROC curves were constructed to evaluate the diagnostic performance of IL-6 *(Figure 1)* in the detection of preterm delivery it was found that it had significant discriminant ability. The area under the curve (AUC) for IL-6 was 0.882 (SE = 0.046, $p < 0.001$). The optimal cut-off value of IL-6 for the detection of preterm delivery was 140 pg/ml. Sensitivities,

Table 1. Demographic and clinical characteristics of women according to the gestational age of delivery

	Preterm delivery (PTD)		p (Mann-Whitney test)
	Yes (N=25)	No (N=50)	
Maternal age, mean±SD	36.2±3.2	35.7±3.6	0.263[a]
Previous PTDs; N (%)	2 (8)	7 (14)	0,221[b]
Birth weight, mean±SD	2765±668	3496±407	< 0.001
Gestational age at delivery, median (interquartile range)	35.2 (33.8-35.9)	38.6 (37.6-39.6)	< 0.001
IL-6, median (interquartile range) (pg/ml)	217 (66)	75 (50)	< 0.001

specificities and other diagnostic indices for the aforementioned cut-offs are presented in *Table 2*. Eighty four percent of the preterm delivery group had an IL-6 level over 140 pg/ml, compared to only 6% of the term delivery group. The risk for preterm delivery was 23.6 times greater for subjects with IL-6 level above 140 pg/ml.

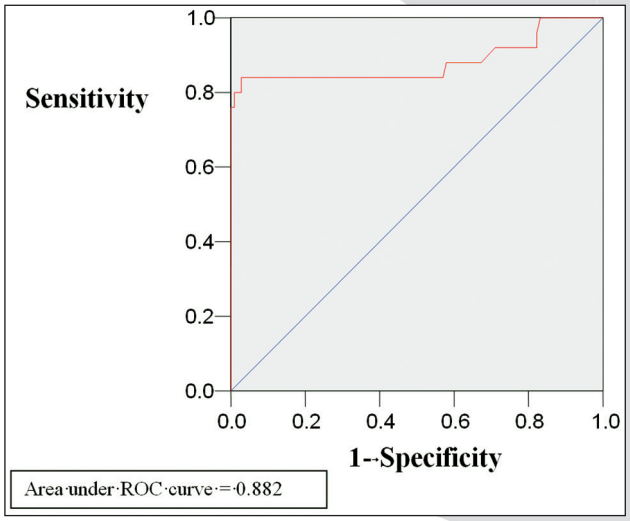

Figure 1. ROC curve of IL-6 for the prediction of preterm delivery. ROC – Receiver operating characteristics

Table 2. Diagnostic indices for interleukin-6 as predictor of delivery ≤ 37 weeks

	IL-6 > 140pg/ml
Sensitivity (%)	84.0
Specificity (%)	92.8
Positive predictive value (%)	87.5
Negative predictive value (%)	96.2
Relative risk (95% confidence interval)	23.6 (17.5-29.6)

Discussion

Interleukin 6 is a pleiotropic cytokine with a wide spectrum of biologic activities. Elevated amniotic fluid levels of IL-6 is considered a marker of intra-amniotic inflammation and they are often associated with the microbial infection of the amniotic cavity. [10] Microbiological studies suggest that infection may account for 25-40% of preterm birth. [11,12]. Infection is difficult to detect due to the limitations of standard microbiological techniques. It is noteworthy that only 1% of the whole microbial world can be detected by cultivation techniques. [13-15] Consequently, the frequency of microbial invasion of amniotic cavity (MIAC) reported previously in the literature, using standard cultivation techniques, represents minimum estimates. An alternative explanation is that the microorganism responsible for the intrauterine infection which will become clinically evident later, cannot be demonstrated and escape detection with the use of classic microbiologic techniques. [16] These figures are likely to change with the introduction of more sensitive methods for microbial recovery and identification, like polymerase chain reaction (PCR). [17] Intrauterine infection can also be present without a positive amniotic fluid culture for microorganisms or a positive PCR. If the infection is localized to the decidua or to the space between the amnion and the chorion, microorganisms may not be detected in the amniotic cavity. [18]

The results of our study demonstrate that a proportion of preterm deliveries could be the consequence of a chronic inflammatory process that begins early in gestation. It is obvious that this asymptomatic intra-amniotic inflammation which finally leads to preterm delivery might begin as early as, or even before, early second trimester. In

the amniotic fluid retrieved at a mid-trimester gestation during amniocentesis for standard genetic indications, we found that IL-6 levels were significantly higher in women who subsequently delivered preterm compared to those who delivered at term. The Relative Risk for preterm delivery in the presence of elevated amniotic fluid concentrations of IL-6 obtained at the time of genetic amniocentesis was 23.6. In our study population approximately 85% of the women who delivered preterm had IL-6 levels above the threshold which was determined by the ROC curve. In the studies of Romero [19] and Wenstrom [20], they recorded elevated mid-trimester amniotic fluid levels of IL-6 in pregnancies that ended with preterm delivery. Some other papers also pointed out the significance of mid-trimester amniotic fluid levels of IL-6 in the prediction of preterm delivery. [21,22]

In recent studies the association between inflammatory cytokine response in amniotic fluid obtained at the time of mid-trimester amniocentesis in asymptomatic women and the risk for subsequent preterm delivery has been investigated. The concentrations of MMP-8, IL-6 and TNF-α were significantly higher in patients who subsequently delivered preterm than in patients who delivered at term [22, 23].

High IL-6 concentrations in amniotic fluid are consisted a marker of intra-amniotic inflammation and are frequently associated with microbiological infection in the amniotic fluid. [10, 19-22, 24] The values of IL-6 in amniotic fluid are strongly associated with spontaneous PTD in asymptomatic women, suggesting that inflammation at the maternal-fetal interface, rather than systemic inflammation, may play a major role in the etiology of such spontaneous PTD [25]. The present study supports the hypothesis that a pathologic process such as intrauterine inflammation in the mid-trimester of pregnancy or even earlier, is a risk factor for preterm delivery. Our results demonstrated that measurement of amniotic fluid IL-6 levels can be used as markers for preterm delivery. Inter-relationship between antibiotic use and preterm labor is complex and at the moment there is no evidence that antibiotics alone can be of any help. It is possible that the introduction of Mitogen-activated protein (MAP) kinase inhibitors, Nuclear factor – kappa B (NFκB) inhibitors or some other biologics, in combination with antibiotics may delay delivery, in this group of patients. [26]

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The effect of protective sunglasses against heavy snowfall in age-related macular degeneration

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Abstract

Background: Age-related macular degeneration (AMD) is a major cause of blindness in the world.

Objective: Aim of this study is to describe the prevalence and the risk factors for the age related macular degeneration in people who no wearing protective sunglasses.

Methods: Total 60 participants were selected among the patients above the age of 60. All patient with complaints of near visual impairment were admitted to the clinic. All participants underwent a complete ophthalmic examination. Fundus photography were performed to all participants. Fundus images were graded as a Grade 0, grade, grade 2, grade 3, grade 4 and geographic atrophy.

Results: 75% of of the participants had grade 1 AMD. There was no difference between the men and the women ($P > 0.05$). 17% of of the participants had grade 2 AMD. There was no difference between the men and the women. The prevalence of Grade 3 or early AMD was 8%. There was no Grade 4 or late AMD in all participants.

Conclusions: The study sported that exposure to ultraviolet (UV) light from the sun as the result of heavy snowfall could increase the incidence of AMD in people who do not wear protective sunglasses.

Key words: Age-related macular degeneration, exposure to ultraviolet.

Introduction

Light interacts with tissues in three ways depending on the period of exposure and wavelength: thermal, mechanic and photochemical. Although much of the optic radiation spectrum is absorbed within the anterior structures of the eye, some part of the ultraviolet (UV)-A band penetrates into the retina[1]. Natural light sources like the sun give off UV beams for a relatively longer period of time. This energy is not held in the retina and accordingly, does not cause any thermal or mechanical damages. However, it can induce the development of photochemical damages [2, 3 4, 5]. Retinal photoreceptors turn the light into electric signals, and the signals are transferred to occipital cortex. Phospholipids of disc membranes have a unique fatty acid composition[6]. Exposure to light plays a role in inducing the lipid perocsidation because the light with proper wavelength might induce photooxidative reactions[7].

Age related macular degeneration (AMD) was widely investigated for a long time; however, its etiopatogenesis remains unclear. It is generally thought in the etiology that advanced age and some risk factors play a part. Age, race, gender, genetics, systemic hypertension, diabetes, socioeconomic and cardiovascular factors, hyperlipidemia, alcohol, diet, and UV beams increase the risk for AMD[8]. Changes seen for a long time in laboratory animals due to acute exposure are similar in patients with AMD, too. Light-dependent damage is induced with photochemical and photooxidative mechanisms. Although the lens filters most of the UV beams, the retina is more reactive to the damage done by the UV and blue light than the visible light with a longer wavelength[9, 10].

The first morphological finding in AMD is an abnormal accumulation of extracellular matter between cell plasma membrane of RPE as a basal laminar deposits [11]. These deposits cannot be followed oftalmoscopically but lead to functional failure of the retina and angyiographical changes in late phase. Accumulation of basal laminar and linear matter contributes to the soft drusen development. Drusen is the second finding of AMD and the first evidence of AMD oftalmoscopically[11,12]. Soft drusen has different varieties. The most common type arises in the form of local accumulation of basal linear matter[12]. Clinically, soft drusen are the lesions with yellow and grey colors, larger than hard drusen (>63μm), whose local lines are not clear under RPE. This type of drusen might bring about the RPE sero-

us decollement and plays a part in the development of CNVM[12]. Vingerling studied age-related macular prevalence in the elderly population. The prevalence of at least 63 microne or a wider prevalence of drusen was 40.8% among ages 55 – 64, and 52.6% among ages 85 and above[11]. According to Oshima' study, the prevalence of drusen rate was 9.6%. Prevalence of drusen increased with age ($P < 0.001$). 3.2% of patients had the hipo and/or hyperpigmentation of retina; 0.2% had geographic atrophy and 0.67% had age-related neovascular degeneration[13].

The previous study established that snow reflects back 75% of UV radiation from the sun. Therefore, it was observed that the severity and rate of sunburn increased in regions with heavy snowfall[12]. An other study it showed that fresh snow reflects 85% of UVR, the grass reflects only 1 – 2% of it. Thus, the effects of winter sun on the eye are greater than those of summer sun. Despite all these effects are temporary, the retina is more reactive to the damage done by the UV and blue light in age[14, 15, 16]. İn previous study was reported that eyeglasses were protective from UV light in acceptable limits[17].

The study compared the incidence of AMD with the literature in the region where wearing protective sunglasses is not common and where there is much prolonged exposure to UV beam in snowy weather.

Methods

Total 60 participants were randomly selected among the patients above the age of 60 who visited the mia clinic of the University of Atatürk. Healthy individuals with complaints of near visual impairment were admitted to the clinic. The patients selected for the study did not receive any treatment for AMD or any other clinical case. Patients with diseases such as exudative retinal diseases, retinal dystrophy, corneal opasite, cataract, glocom, PVR, retinal detachment, optic nerve diseases, inflammatory ocular diseases and high refractive defects were not included in the study. Further, the patients with systemic diseases such as cerebrovascular, hepatic, pulmonary, thyroid, immunosuppressive defects and diabetes were not included in the study, either. In addition, patients who have a history of alcoholism, mental disorders and are drug addicts and those who took corticosteroid, phenothiazine or antimalarial drugs during the study or one month before the study were not included in the study. Participants using sunglassesat were not included in the study. All participants have been living same region in all theri live. Study protocol was approved by the ethical committee of the Medical Faculty, University of Atatürk, and applied in conformity with the Helsinki declaration. Detailed medical and ophthalmologic history of the patients was received and the whole ophthalmologic examination was carried out. The investigation and all the assessments were carried out by the same doctors.

Fundus Grading

We determined AMD condition of samples by grading stereoscopic color fundus photographs using the the international classification system for Age-Related Maculopathy. Grade 0 (no early or late AMD), grade 1 (soft drusen (63 μm-125 μm) or only pigment disorder). Grade 2 (only uncertain soft (125 μm) or reticular drusen or soft drusen along with pigment disorder). Grade 3 or early AMD (soft or reticular drusen along with pigment disorder). Grade 4 or late AMD (both neovascular AMD serous or hemorrhagic retinal or retinal pigment epithelium detachment, subretinal neovascular membrane and periretinal fibrous scar presence) and geographic atrophy[18].

Results

60 participants was attended the ocular examination, All participants had at least one photograph taken in either eye. 75% of of the participants had grade 1 (soft drusen (63 μm-125 μm) or only pigment disorder) drusen. There were age trends with increased prevalence of soft drusen, but not with the presence of small hard drusen. Prevalence of soft drusen of both types increased with age ($P < 0.05$). There was no gender difference between the two types of soft drusens ($P > 0.05$). 17 of of the participants had grade 2 (only uncertain soft (125 μm) or reticular drusen or soft drusen along with pigment disorder) drusen. There was no difference between the men and the women ($P > 0.05$). Prevalence increased with age ($P = 0.022$). The prevalence of Grade 3 or early AMD was 8%. No participant has Grade 4 or late AMD.

Discussion

Pauleikhoff et al. argue that many medical or environmental risk factors cause for early and late age-related macular degeneration. İn addition, specific genetic, environmental, medical, and ocular characteristics are reason for progress of AMD[19].

Lifelong, constant exposure to light poses a serious risk for eyes as the result of oxidative mechanisms. Biochemical structure of the posterior segment is accepted as an important factor which makes the eyes more vulnerable to damage than the other organs. Despite the ability of the young to use normal photooxidative mechanisms, it might not be possible to maintain the retina structures with age, and thus irreversible damage occurs. In addition, the previous study found a significant reduced association of increasing ambient UV radiation with early AMD[20,21]. İn addition laboratory studies and the studies on animals showed that the blue light led to damage to the retinal pigment epithelium and coriocapillary, which might be a factor in AMD pathogenesis[22].

Cruickshanks et al.investigated that the relation of sunlight exposure with early age-related maculopathy (ARM). They Designed a longitudinal and population-based study for relation of sunlight exposure with early age-related maculopathy. All samples were reexamined in 5 years after the baseline examination in their study. At the end of study, stereoscopic color fundus photographs of samples were graded to determine the presence of ARM at the 5-year follow-up. Their results showew that exposure to sunlight may be a risk factor for early ARM[23]. At the same study, Tomany and all. examined the association of sunlight exposure with the 10-year incidence of age-related maculopathy. İn their study, all samples were reexamined. from 1988 to 1990, 10 years after the baseline examination. They found that significant associations between exposure to the summer sun and incidence of early ARM[24].

Roberts demonstrated a that exposure to the radiation can pose a hazard particularly if the recipient is over 40 years of age and this radiation can cause impaired vision. Therewithal UV radiation, blue visible light is a risk factor for the human retina. The dissipation of these ultraviolet radiations will reduce the risk of early retinal damage by wearing sunglasses that block short blue visible light. At the same time they explaned that wavelength of light wavelengths of light sunglasses to reduce the risk of age-related macular degeneration over 50 years old [10].

Otman et al. tested eye protection used for phototherapy patients. They surveyed the use of eye protection during phototherapy in 78 UK phototherapy units in this study. They tested sunglasses, small UV goggles and UV visors were between 270 and 420 nm. All samples Of 78 UK phototherapy units 33% use tinted goggles during UV exposures, two 3% use a visor only, 43% use both and nine 14%)use clear plastic (probably polycarbonate) goggles in their study. 28 (43%) use both and nine (14%) use clear plastic (probably polycarbonate) goggles. Result of this study conclused that All samples of glasses, goggles and visors are adequate protection in the UV range[25].

İn our study, continental climate is dominant in most of the region was carried out. Meteorological measures of the region have been taken since ١٩٢٩ for this region. İn this place, winters are long and harsh; summers are short and dry. The precipitation occurs in the form of snow. The most precipitation occurs in spring and summer. The city receives snow the least precipitation in winter. İt snow about ٥٠ days. It remains on the ground for 114 days. (General directorate of meteorology). The previous studies reported that fresh heavy snow reflects 75-85% of UVR. Retina is more reactive to the damage done by the UV and blue light which reflects by snow in winter sun[12].

For this, our study revealed that exposure to UV light from the sun as the result of heavy snowfall could increase the incidence of AMD in people who do not wear protective sunglasses.

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Citogenetic effects of Tridecactide

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Abstract

Introduction: Cytogenetic evaluation in peripheral blood lymphocytes (PBL) of patients with multiple sclerosis (MS) showed significantly higher frequency of chromosomal aberrations in MS patients, comparing to healthy subjects. Also, auto-reactive T lymphocytes are suggested to have the key role in the MS pathogenesis. The current study was designed to determine tridecactide effects on chromosomal aberrations number/type and lymphocyte-leukocyte ratio in the PBL cultures of female patients with relapsing remitting MS.

Materials and Methods: Whole blood was cultured based on the techniques of Moorhead et al. (1960), in the following manner: untreated culture (T0) and cultures incubated with tridecactide in concentrations 0,24 μg/ml (T1) and 24 μg/ml (T2). White blood cells count and standard analysis of chromosomal aberrations (200 mitoses/culture) were performed after standard Giemsa staining. The chromosomes in aberrations were identified by destaining and applying G-band technique.

Results: The treatment did not exert any statistically significant effect on the frequency of structural aberrations, mitotic index and lymphocyte/leukocyte ratio. However, chromosome fragmentation, ring chromosomes and dicentrics were present only in untreated cultures, while centric fragments were detected only after treatment. The chromosomes 1, 2 and 9 were most frequently engaged in aberrations, and chromosome 14 in translocations. Incubation with the lower concentration of tridecactide resulted in statistically significant increase in polyploidy and endoreduplications (p=0,047; p=0,033), and with higher concentration in aneuploidy (p=0,026).

Conclusions: Tridecactide didn't exert significant clastogenic effect, nor protective potential. Our results implicate tridecactide effect on the frequency of numeric aberrations, that needs further evaluation.

Key words: Tridecactide, α-corticotropine 1-13, α-melanocyte-stimulating hormone, chromosomal aberrations, multiple sclerosis.

Introduction

Tridecactide or α 1-13 corticotropin (α-ACTH 1-13), shares amino-acid sequence with α-melanocyte-stimulating hormone (α-MSH) and first 13 amino-acids sequence with adrenocorticotropine (ACTH 1-39). It is deacetylated on its N- and deamidated on its C- terminal fragment, and therefor differs from endogenous α-MSH. In combination with met-enkephalin, tridecactied is applied as immunomodulatory option in tretment of multiple sclerosis (MS).

The mechanism of action of tridecactide is unclear. However, two substances that are related to tridecacted are also used in MS tretment. Tetracosactide (ACTH 1-24) is indicated for treating MS and pulse corticosteroid treatment is routinely used for relapses. The principal effect of ACTH 1-39 is increasing production and release of corticosteroids (mostly cortisol). Also, α-MSH exhibits cytoprotective and antiinflammatory effects (1, 2, 3), that are mediated throught descending antiinflammatory pathways and melanocortin (MC) receptors on the cells in the perifery (4, 5). The α-MSH inhibits activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), trancription factor implicated in the activation of proinflamatory cytokine tumor necrosis factor-α (TNF-α) (6, 7). For α-MSH Adachi et al. registered biphasic effects (6).

MS is progresive demyelinizating dissease, with poligenetic predispozition, the most frequently presented in relapsing/remitting form (RRMS). The key role in its' patogenesis is attributed to autoreactive T lymphocites (myelin components are suggested

autoantigens). Cytogenetic evaluation in peripheral blood lymphocytes (PBL) showed significantly higher frequency of chromosomal aberrations in subjects with MS, comparing to healthy subjects (8, 9, 10), although some studies failed to detect significant difference (11). The current study was designed to determine tridecactide effects on chromosomal aberrations number/type and lymphocyte-leukocyte ratio in the PBL cultures of female patients with RRMS.

Patients, materials and methods

Test substance

Tridecactide was manufactured by Biotechnology Laboratories, Richmond, USA, and delivered as lyophilized powder in closed flacons. Test samples were dissolved in destilated water.

Cell culture

Blood samples were collected from nine female RRMS patients in relaps (Mc Donald diagnostic criteria, rev. 2005). Exclusion criteria were previous tretament with interferon and receiving corticosteroids last six months. Heparizied, whole blood was cultured based on the techniques of Moorhead et al. (1960), in the following manner: untreated culture (T0) and cultures incubated with tridecactide in concentrations 0,24 μg/ml (T1) and 24 μg/ml (T2). Cultures were incubated for 72 hours.

Slide analysis and statistical analysis

White blood cells count (performed in samples of all nine patients) and standard chromosomal aberrations analysis (200 mitoses/culture, in cultures of seven patiens) were performed after standard Giemsa staining. Mitotic index was calculated as a percent of lymphocytes in mitosis (M1 + M2) on 300 lymphocytes. The chromosomes in detected aberrations were identified by destaining and application of G-band technique. Wilcoxon Signed Ranks Test, nonparametric test for paired samples, was used for statistical testing.

Results

Patients' age and dissease duration are presented in table 1. Mitotic index, median and range of chromosomes engaged in structural aberrations are presented in the table 2. No statistically significant difference was detected in the number of chromosomes engaged in structural aberrations (excluding gaps) following test substance application in different concentrations, compared with the untreated cultures. Engagement of two chromosomes was considered for dicentrics and translocations. Test substance did not exert statistically significant effects on the number of breaks, gaps and translocations/marker chromosomes, and mitotic index. Some of detected structural abbera-

Table 1. Patients' characteristics

	n	X ± SD	X_{min}	X_{max}	Range
Age	9	41,89 ± 9,17	34	60	26
MS duration/years.	9	9,64 ± 7,89	0,25	19	18,75
Number of hospitalization	9	2,67 ± 1,80	1	6	5

Table 2. Chromosomes engaged in structural abberations and mitotic index

Treatment	n	Chromosomes in abberations			Mitotic index		
		X_{min}	X_{max}	Median	X_{min}	X_{max}	Median
T0	7	4	12	5	0,33	7,00	2,00
T1	7	1	8	2	2,00	7,67	3,00
T2	7	1	8	5	2,00	5,67	3,00

Table 3. Lymphocites and leukocyte count

Treatment	n	Lymphocytes			Other blood white cells		
		X_{min}	X_{max}	Median	X_{min}	X_{max}	Median
T0	9	0,22	0,70	0,54	0,30	0,78	0,46
T1	9	0,22	0,67	0,60	0,27	0,78	0,40
T2	9	0,23	0,81	0,56	0,19	0,77	0,44

tions are illustrated in the figure 1. Test substance did not exert statistically significant effects on the lymphocyte leukocyte ratio (table 3).

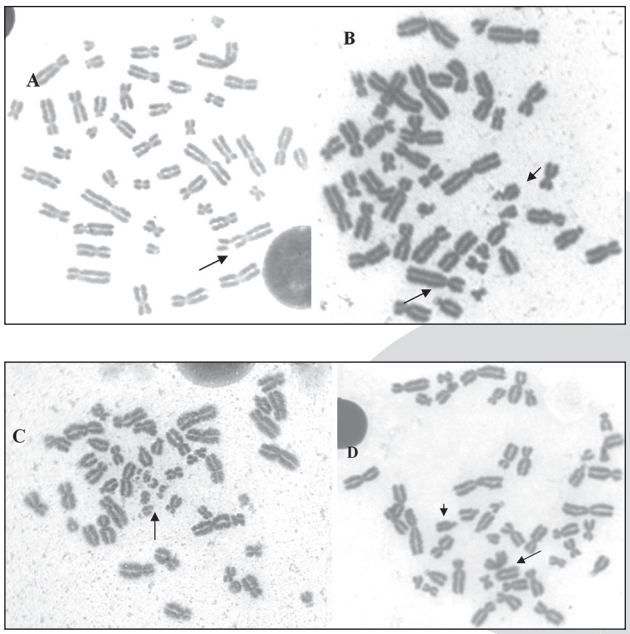

Figure 1. Detected structural abberations. Chromatide break and acentric fragment (A). Two marker chromosomes as the product of transocation (B). Chromosome fragmentation (C). Centric and acentric fragment (D)

The most frequently observed structural aberrations in untreated and treated cultures were breaks, gaps and marker chromosomes. Centric fragments were not detected in untreated cultures, while detected in cultures incubated with test substance. Three chromosome fragmentations were observed in one patients' untreated culture, and ring chromosome and dicentrics were observed in untreated cultures of four patients (figure 2). No fragmentations, ring chromosomes, or dicentrics were detected in treated cultures.

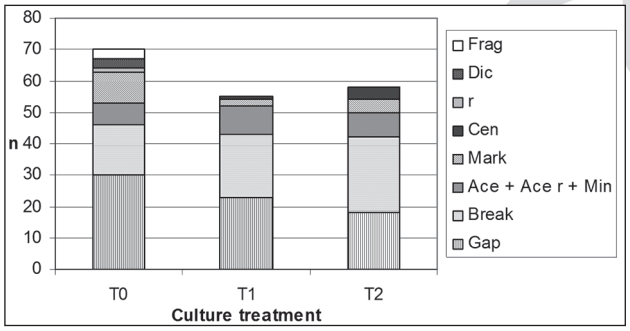

Figure 2. Structure of detected chromosomal abberration

Generally, the frequency of chromosomal engagement in the aberrations was decreasing from the chromosomal pair 1. to 22. Chromosome 1, 2 and 9 were the most frequently engaged in aberrations, and chromosome 14 was the most frequently observed in translocations. Two translocations between chromosomes 2 and 14 were identified in untreated cultures, and one translocation between chromosomes 7 and 14 in cultures treated with tridecactide lower concentration. Following the cultivation with higher tridecactide concentration, the translocation between chromosomes 4 i 12 and the translocation between chromosomes 6 and 13 were identified.

Endoreduplications were prevalent in the total number of numeric aberations (figure 4). Triploidy and tetraploidy (figure 3) were observed. Statistically significant increase (Wilcoxon Signed Ranks Test) in polyploidy (p=0,047) and endoreduplications (p=0,033) was noticed for the cultures treated with lower concentration of tridecactide, compared to the untreted cultures. Statistically significant increase in aneuploidy was detected for cultures treated with higher concentrations of tridecactide (p=0,038), while aneuploidy was mostly chromosome X selective.

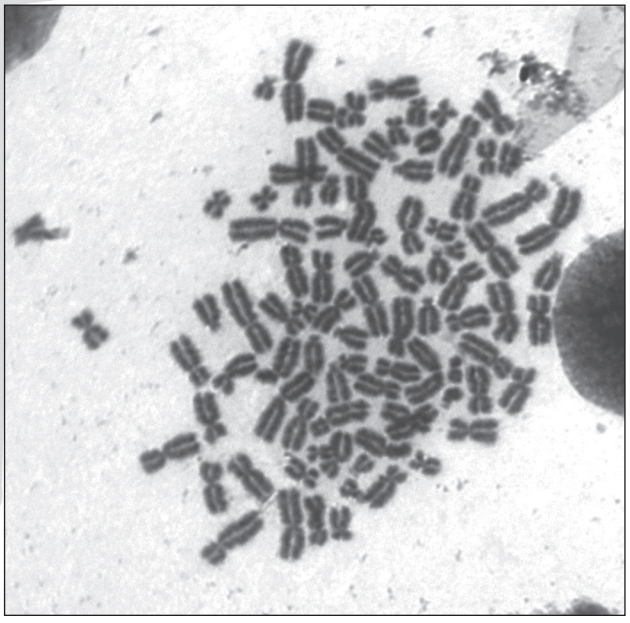

Figure 3. Tetraploidy

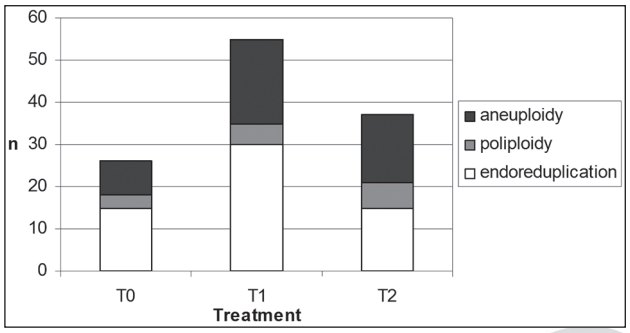

Figure 4. Structure of detected numeric abberrations

Discusion

Simmilar effects to dissapearance of the ring chromosomes, dicentrics and chromosome fragmentation detected Štambuk et al. with met-enkephalin (8). Met-enkephalin *in vitro* stimulation of PBL of subjects with immune-mediated diseases led to the significant reduction of chromosomally aberrant lymphocytes. Authors interpreted chromosome aberrations disappearance/reduction in the context of possible met-enkephalin role in the immunotherapy of diseases which involve chromosomal aberrations.

Individual chromosomes engagement in aberrations may provide useful information for future research. The expression of breaks on the fragile sites of proliferating lymphocites is suggested to be the consequence of coordinated gene expresion following the oncogene stress and functional caracterisation of the fragile sites' genes coordinated in their expression reveals significant number of genes signed with terms conected to the immune response (13). Modification of few genetic locuses in the animal model of experimental alergic encephalitis, results in eliminating of the risk of developing the disease (12).

Change in cells ploidy (specifically aneuploidy) is hypothesized as the chromosomal basis for the carcinogenesis (14). The potential of α-MSH to influence melanoma progression was suggested (17), but no malignant naevus transformation was detected following the neuropeptide high doses clinical application (18). According to the Mitelman database on chromosomal aberations in malignant disseases, trisomy X was the most frequent in acute limphoblastic leukaemia and limphomas (15). Nevertheless, the most common chromosome seggregation disorder in healthy females is X chromosome associated (16). Anyway, aneugen effects of other hormones were also experimentaly detected, including progesterone and folicule stimulating hormone (19, 20).

We can conclude that tridecactide didn't exert significant clastogenic effect, nor protective potential. Also, following tridecactide application no significant effect on the mitotic index and lymphocyte leukocyte ratio were detected. Our results implicate tridecactide effect on the frequency of numeric aberrations, that needs further evaluation.

Abbreviations

Ace - acentric fragment
Cen - centric fragment
Ace r - acentric ring
Min - minute
r - ring chromosome
Dic - dicentric
Break - chromosome and chromatide break
Gap - gap
Mark - marker chromosome
Frag - chromosome fragmentation

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The levels of prolactin and growth hormone in Multiple Sclerosis and the long-term effect of interferon β-1b in these hormones activity

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Abstract

Prolactin and growth hormones exert immunomodulatory actions and being involved in the development and progression of many autoimmune diseases such as multiple sclerosis. To address this question, we evaluated prolactin and growth hormones in male and female patients with definite multiple sclerosis during remission.

Interferon-beta therapy is widely used in patients suffering from relapsing remitting or secondary chronic progressive multiple sclerosis. To date, little is known about the changes in neuroendocrine system. Therefore, we compared these hormones level with the patients with using interferon therapy and not.

Prolactin and growth hormone levels were significantly higher in patients with multiple sclerosis than healthy subjects. However, we could not find any difference between multiple sclerosis patients with interferon-beta therapy and medical-free. No associations were found between these hormones and age, gender, disease duration or EDSS scores.

Larger studies in multiple sclerosis are clearly needed to reveal the neuroendocrine effects occur in response to interferon therapy.

Key words: Multiple sclerosis, prolactin, growth hormone, interferon beta-1b.

Introduction

Multiple Sclerosis (MS) is a common autoimmune inflammatory demyelinating disease of the central nervous system, which causes of chronic neurological disability in young population. It is characterized by multifocal areas of demiyelination, loss of oligodendrocytes, astroglial scarring, perivascular inflammation and axonal injury. To date, certain pathogenesis of MS is unknown, but the most accepted theory is that it is mediated by autoreactive lymphocytes following a combination of genetic, environmental and hormonal triggers.

Interferon-beta (IFNβ-1b) is now widely used in the treatment of MS patients, especially the relapsing remitting form. Several studies have demonstrated that IFNβ-1b therapy has interaction in the response of neuroendocrine and immune system (1,2). Thus, the aim of this study was to evaluate the plasma levels of prolactin (PRL) and growth hormone (GH) in healthy subjects and definite MS patients and also compare MS subgroups who use IFNβ-1b and have no treatment.

Materials and methods

Subjects

Fourthy patients (27 females and 13 males) satisfying the McDonald criteria 2001 for definite MS and healthy control group (n=20) were included in the present study. All the patients underwent a general history, complete physical and neurological examination. Thirty-three patients had relapsing-remitting (RR) MS and 7 had secondary progressive course. Patient age was in the range of 17-54 years and 18-54 years in the control group. In the patient group, while 20 patients were using IFNβ-1b, the others were not using any therapy. Disability was assessed using the Expanded Disability Status Scale (EDSS) separately in patients with beta-interferon and medication-free. Mean values of EDSS were found as 2 points in two patient groups (range of EDSS scores, 1-3.5 in patients with IFNβ-1b and 1-3 with patients with medical-free). All MS patients were in remission phase.

Informed consent was obtained from all subjects and study protocol was approved by the Hospital Ethics Committee. It was designed as case-control study.

Exclusion criteria

Patients with pituitary adenoma, hypophysectomy, major psychiatric illness, polycystic ovaries, hypothyroidism, hepatic, endocrine and renal disease, epilepsy, history of drug or alcohol abuse, different disease courses (i.e. fulminant, primary progressive and clinically-isolated syndromes) or other inflammatory and autoimmune diseases were excluded.

Endocrinological studies

Hormone profiles were performed in a stardardized way. Blood samples from patients and controls were taken from a cubital vein on the same day in the morning between 08.00 and 10.00 h, after 12 hours fast. PRL and GH levels were estimated in the plasma by using chemiluminescence immunoassay method (Chiron Diagnostic Corporation MA 02032, USA, ACS: 180 kits).

The average values of the PRL and GH levels were calculated by using two different values of these hormones which was obtained during an hour (two times in 30-min intervals) for all subjects.

Statistical analysis

Statistical analysis was performed using the computer statistical package SPSS/8.0 (SPSS, Chicago, IL, USA).

Data were presented as the mean ± SD. The significance of the difference in mean values between the study groups was tested with the t-test. Differences with the effects of interferon therapy were statistically tested for significance by analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

Results

The mean basal level of PRL for MS patients was significantly higher than the mean for normal controls (P=0.01). Although the mean for MS subjects with medical-free was higher than that for MS subjects with IFNβ-1b therapy, the difference between these two groups was not statistically significant (P=0.08).

The mean basal level of GH was significantly higher than the mean for normal controls (P=0.02) but the means MS subgroups (with IFNβ-1b and medical-free) did not differ significantly from each other ($p>0.05$). These findings were summarized in Table 1.

We also found no significant correlation between these two levels of hormone and age, gender, disease duration or EDSS scores.

Discussion

IFNβ-1b has multiple effects on the immun system and many cytokines which have been shown to activate or suppress the secretion of hormones. In addition, several studies have also found disturbances of the hypothalamo-pituitary axis in MS.

PRL is not only a hormone but also a potent immunomodulating molecule with the effects including regulation of the maturation of CD4-CD8- thymocytes into CD4+ CD8+T-cells , impairment of the autoreactive B cells negative selection, enhancement of the proliferative response to specific antigens and mitogens, increased immunoglobulin production, upregulation of Th1 cytokines and enhancement of interleukin-2 effects on lymphocytes (3).

Additionally, GH acts both on neurons and myelin-forming cells to promote myelination. The balance between oligodendrocyte progenitor proliferation and differentiation seems to be controlled by different types of growth factors (4).

According to our results, MS patients have mild chronic hyperprolactinaemia and increased GH secretion. Kira et al.(5) demonstrated that 30% MS patients had mild to moderate hyperprolactinaemia, with half of these showing prolactin rise during relapse and this finding was also supported

Table 1. Mean prolactin and growth hormone plasma concentrations of patients with/without using IFNβ-1b and control group. Concentrations are given in ng/ml

	Subjects		
Hormone	**Patients with IFNβ-1b n=20**	**Patients without IFNβ-1b n=20**	**Control group n=20**
Prolactin	13.04±1.09	14.24±1.72	9.14±0.67
Growth hormone	1.98±0.45	2.47±0.81	0.49±0.19

by our study in MS patients during remission phase too. However, we could not show that the usage of long-term IFNβ-1b may act directly on these hormones secretion.

In contrast, Goebel et al. (6) detected a rise in cortisol, GH and PRL at 4 and 8 hours after immediate injection of IFNβ-1b. Moreover, similar effects have also been demonstrated for IFNβ-1b in patients with chronic hepatitis C virus infection (7) and in patients treated with IFN-alfa or IFN-gamma for chronic viral hepatitis, haematological malignancies (8) and solids tumour (9).

In the present study, we did not analyse the short-term effects of IFNβ-1b. This can be one limitation of our study because transient changes of IFNβ-1b in endocrine systems could only detected by immediate usage. Besides, if we had analyzed acute effects of IFNβ-1b, we would have certainly found variant and interesting results.

Understanding of the relationship between the endocrine and immune system is very important for the clinical point of view. Since the action of IFNβ-1b therapy in MS is still clearly unknown and clinical data suggest that 30-50% of the patinets suffering from MS do not respond to this therapy (10). In the near future, it will be important to assess whether the efficiency of IFNβ-1b therapy in MS patients with an impaired neuroendocrine response is reduced.

Conclusions

The result of the present study supported that PRL and GH may play important role in the immunology of MS. However, further studies in MS patients after acute injection of IFNβ-1b are needed for revealing the impact of this therapy on neuroendocrine response.

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Tissue expansion for treatment of periorbital skin defects

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Abstract

Objective: To summarize the experiences in repairing periorbital skin defects with the tissue expansion.

Methods: The periorbital tissues are divided into five anatomic zones to help categorize lesions and create uniform terms for discussions on reconstruction. These zones include Zone I, upper eyelid; Zone II, lower eyelid; Zone III, medial canthus including the lacrimal drainage system; Zone IV, lateral canthus; and Zone V, contiguous structures including nasal, glabellar, brow, forehead, temple, malar, and nasojugal regions. According to different locations of periorbital skin defects secondary to the 28 cases of hemangioma, scar or nevus excision, one or two suitable size expanders were inserted in the adjacent or distant regions. After expansion, the expanded skin flaps were transposed as advancement flaps, transposition skin flaps or rotation flaps.

Results: In this series, we developed 21 expanded advancement skin flaps, 15 expanded transposition skin flaps and 7 expanded rotation flaps. No expanded skin flaps progressed to necrosis. Eyelid dysraphism, lower eyelid ectropion and eyebrow malposition occurred in two, one and one cases respectively. The outcomes were favorable in other cases.

Conclusions: Tissue expansion is an applicable method for treating periobtal skin defects. Caution should be taken to reconstruct the upper eyelid and lower eyelid. The satisfactory outcomes are based on the intimate preoperative design and understanding of the periobital anatomic complexities.

Key words: Soft tissue expansion, Periorbital skin defect, Expanded skin flap.

Introduction

Periorbital structures are very important and complicated and how to repair and reconstruct remains a challenging difficulty in the orthopedic field. Conventional treatments modalities have some disadvantages which limit its application in periorbital reparation. Tissue expansion [1-4] allows for the skin flap with the matched color, thickness and texture and minimizes the defect of the donor site. Since introduced, it has been is widely used in reparations of multiple tissues[5-21]. However, there is less reports on reparation for periorbital skin defect using the expander. This article mainly describes the application of the tissue expansion in the periorbital skin defect and summarizes the experiences on how to reduce complications and acquire aesthetic effects.

A total of 28 patients with periorbital scar, nevus or hemangioma were enrolled into this study. To facilitate discussion and analysis, the periorbital tissues were divided into five anatomic zones according to the Spinelli[22] method, zone I, upper eyelid; zone II, lower eyelid; zone III, medial canthus including the lacrimal drainage system; zone IV, lateral canthus; and zone V, contiguous structures, including the nasal, glabellar, brow, forehead, temple, malar, and nasojugal regions (Figure 1). We adopted different skin flap design according to different anatomic features and zones, which obtains a good result.

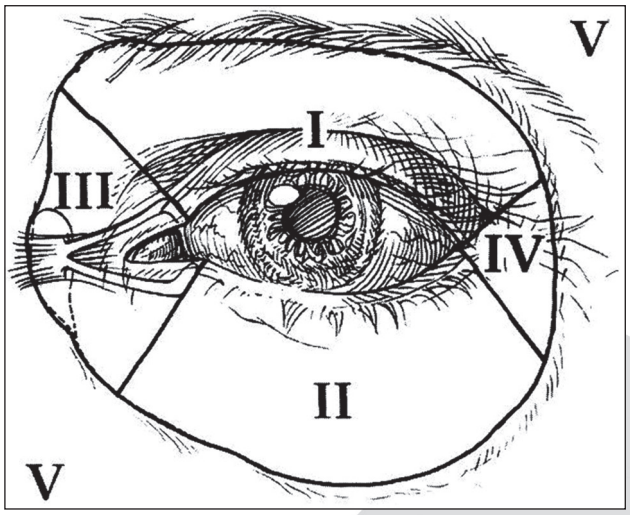

Figure 1. Zone I, upper eyelid; Zone II, lower eyelid; Zone III, medial canthus including the lacrimal drainage system; Zone IV, lateral canthus; and Zone V, contiguous structures including nasal, glabellar, brow, forehead, temple, malar, and nasojugal regions

Clinical data

General data

A total of 28 patients (6 males and 22 females) were recruited into this study with an average age of 17 years (range 8 to 26 years). Patients suffered from scar (n=11, 5 with posttraumatic scar, 4 with post-skin grafting scar and 2 following laser therapy), congenital nevus (n=9) or congenital hemangioma (n=8).Anatomic zones and number of patients were shown in Figure 1: zone I (n=4), zone II (n=5), zone III (n=3), zone IV (n=6) and zone V (n=10). The size of the defect area ranged from 4cm ×1cm to 1.5cm×2cm. The expanders were routinely implanted and expanded. After fully expanded and maintained for 3 to 4 weeks, expanders were removed and the expanded skin flaps were transposed as needed.The duration for expansion ranged from 2.5 to 3.5 months and the total hospital stay for the two stages of the surgery ranged from 6 to 17 days with an average of 11 days.

Positions of expander and incision

For the defect in the zone I, a 50ml or 80ml expander was selected was placed just above the homolateral eyebrow, slightly exceeding the median line with the long axis parallel to the eyebrow, or obliquely between the eyebrow and homolateral forehead with an included angle between the long axis and eyebrow. The incision was in the corresponding hair line.

To repair the defect in the zone II, an 80ml to 100ml expander was needed, which was placed in the malar, with a certain angle with the lower eyelid in the long axis. The incision was positioned in the juncture between the normal and abnormal skins.

For the zone III, 1 to 2 50ml-100ml expanders were selected according to the defect area. Expanders were implanted in the homolateral malar, or close to the media line of the forehead, or in both sites. The long axis of the expander was not parallel to the lower eyelid in the malar but was to eyebrow in the forehead. The incision was placed in the juncture between the normal and abnormal skins.

For defect in the zone IV, 2 expanders were required. If the defect was positioned medially of the os orbitale, a 30 to 50ml expander was placed superiorly and inferiorly of the defect in the temple and malar, respectively. If the defect was in the lateral edge of os orbitale, a 50 to 80ml expander was placed in the temprofrontal or tempromalar region adjacent to the defect, respectively. The long axis of the expander was determined in accordance with particular situations. The incision was in the hair line near the temple.

For the defect in the zone V, an 80ml expander was placed in the forehead adjacent to the defect in case of small defect and another expander was simultaneously implanted in adjacent forehead and temple according to the size of available normal skin. If the both sides of the defect had normal skins, expanders were placed in the both sides with the long axis parallel to that of the defect. For the skin defect in the juncture of pars zygomatica and nasojugal regions, a 50ml to 80ml expander was placed in adjacent parts. If the adjacent part had no normal skins, distal expanded skin flaps were used, generally from the front the homolateral anterior thorax or medial upper arm, and the expander was around 300 to 500ml.

Layers of expander implantation

The periorbital and eyelid skin were very thin, so only thin skin flaps met requirements. As a result, expanders in the forehead, temple and malar were implanted superficially in the subcutaneous tissue.

Transposition of expanded skin flaps

The expanded skin flap was generally transposition skin flaps. Stage II pedicle division was performed for the expanded skin flap above the brow but not required for the flaps in the glabella. The expanded skin flap to repair the defect in the zone II was designed as the advancement flap while the flap for the zone III was the transposition flap. Skin flaps were advancement or rotation flaps for the defect medially of the os orbitale in the zone IV but transposition flaps in the forehead and advancement flaps in the malar for defect laterally of the os orbitale. As for the zone V, advancement flaps were better for frontal defects and rotation flaps were more suitable for malar and nasal defect. In periorbital skin flap transposition, additional incisions were commonly designed in the upper edge of the eyebrow, hair line and nasolabial fold or in the front of the ear. A total of 43 expanded skin flaps were generated with the size of 5cm×3cm to 10cm×4cm.

Results

A total of 43 rectangular expanders were implanted in 28 patients, generating 43 expanded flaps, including 12 advancement flaps, 15 transposition flaps and 16 rotation flaps. Eight patients (28%) developed complications: eyelid dysraphism (n=2), eyebrow malposition (n=1), lower eyelid ectropion (n=1), flap blood flow obstacle (n=1), hematoma (n=2) and capsular contracture (n=1).

Eyelid dysraphism of the both patients was mild, caused by narrow expanded flaps in repairing the upper eyelid, and had little effects on eyelid functions. Patients refused further treatment.

Eyebrow malposition was resulted from contracture following skin flap expansion and local eyebrow position correction was operated 6 months later. Lower eyelid ectropion was induced by the relaxed supporting tissues of the lower eyelid during skin expansion due to the long-term dragging, resulting from the fact that the expander is far from the eyelid. This complication automatically resolved after removing the expander during surgery but appeared again 1 week later. Then, lateral canthoplasty was conducted and skin flap reparation was corrected.

Blood flow obstacle occurred in enhancement flaps, resulting in necrosis and abscission of 0.5cm distal epidermis. Local correction was performed with a good efficacy.

Hematoma was small and hemorrhage stopped automatically by proper pressure dressing without special treatments.

Capsular contracture was manifested as rigid skin flap on the surface of expanders, no loosening sensation between flaps and the expander, difficult water injection, significant pain during injection and elevated skin area less than the expander area. After slow water injection, the contractural capsule gradually braced and the finial outcome was not affected in spite of the long-term water injection.

A total of 19 patients were followed up for 3 to 13 months post surgery, including 7 via the telephone. The overall effect was satisfactory in terms of the facial symmetry, layophthalmos, eyelid shape and positions of hair line and eyebrow as well as scar shape(Figure 2-5).

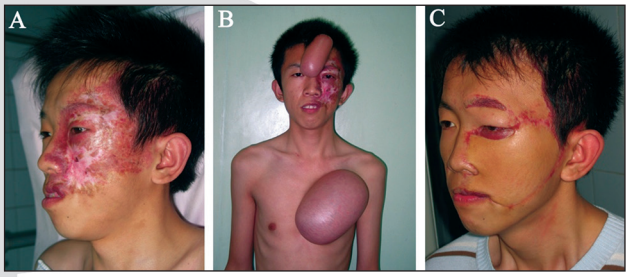

Figure 2. A. Preoperation; B. Expansion; C. Six months after operation

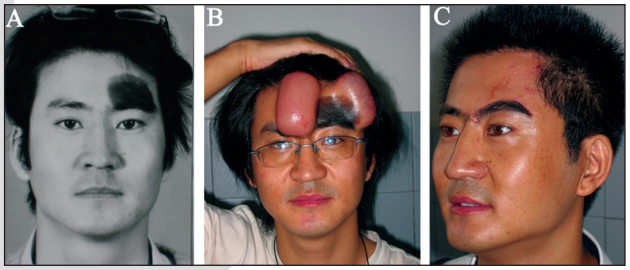

Figure 3. A. preoperation; B. expansion; C. six months after operation

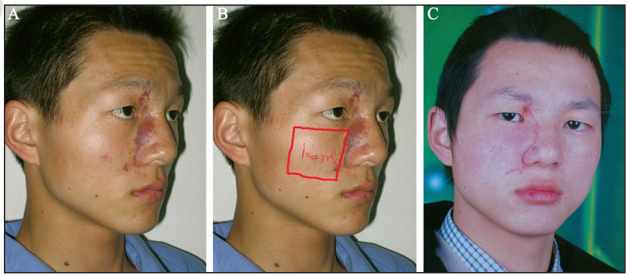

Figure 4. A. Preoperation; B. the diagram of the placement of expander; C. 3 months after operation

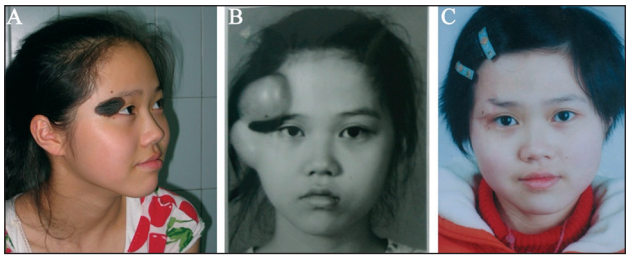

Figure 5. A. Preoperation; B. expansion; C. 9 months after operation

Discussion

Periorbital anatomic features

From the anatomic view, periorbital structures are characterized by many important structures, showing the highest density within the body. These structures are also important aesthetic markers on the face, including eyebrow, upper and lower eyelid, palpebral margin and medial and lateral canthus. Adjacent aesthetic markers include the hair line and temples. These aesthetic markers are required to be symmetric in both position and appearance. These periorbital structures determine the difficulty of reparation. Surgery should restore the best function and aesthetic effect[23].

Common reparation procedures

Common reparation procedures for periorbital skins include resection, suturing, free skin graft and local skin flap transposition.

Resection suturing is most suitable for the small periorbital skin defect but inapplicable for the defect of more than 1.5cm, for it can induce deformation of peripheral structures or secondary malformation.

Free skin graft is the most common method for defects in upper and lower eyelidand generally obtained from the opisthotic full thickness skin. The skin flap is similar in the thickness to the upper and lower eyelids. However, the donor region remains scar, patch shapes and local depressed malformation. In this study, 3 patients required further treatment for the unsatisfactory skin grafting efficacy of upper and lower eyelids. This procedure is gradually used less with enhancement of quality of life.

Local skin flap transposition is mainly used in the small skin defect in the upper eyelid. However, the donor skin flap is small and thick due to the new scar in the donor region.

Tissue expansion

Tissue expansion is a milestone in the orthopaedics, widely used since emergency. Adjacent expanded skin flaps fully meet the consistency requirement for skin defect reparation in terms of color, texture, hairs and thickness. There are reports on reparations of skin defects in forehead, temple and malar[24,25]. Harris et al.[26] deemed that not only the color was well matched and the dragging direction could be controlled for the skin flap reparation. Horizontal dragging of skin flaps can reduce the immediate ectropion following surgery and aids in resistance against traumatic basement contracture in the late stage. Conversely, the skin flap does not influence the position of eyelid during surgery, but inactively and uncontrollably promotes traumatic basement contracture in the late stage. As for other periorbital sites, the best choice is to acquire the homogeneous skin via tissue expansion.

Features of expanded flaps

Skin flaps can meet local requirements only in case of knowing features of various expanded flaps in detailed.

Advancement skin flap: It was previously paid many attentions for malar defects for advantages such as simple transposition and no additional incisions[10]. However, it also has some disadvantages [10,27]: not enough bilateral extension and low availability of expanded skins; all tension is bore directly by the endpoints, resulting in a wide scar following surgery.

Rotation (advancement) skin flap: it is most commonly used on the face. It needs less adjunct incisions and produces little distal tension in the skin flap. However, the expanded skin flap is hard to completely flatten, expanded tissues can not be effectively used, the cat-ear structure in the pedicle needs to be immediate or phase II trimming and the relationship between the advancement degree and pedicle width should be carefully weighted.

Transposition skin flaps: Previously, it is abandoned by most physicians due to complicated design. However, Joss[27] and Bauer[10] believed that the well-designed transposition flap had some advantages such as low deformation coefficient of anatomic unit, proper position of scar and low risk of scar contracture.

Features of reparation for periorbital skin defects via expanded skin flap

In managing reparation with the brow, the brow position, shape symmetry and motor function should be considered. The brow position may be changed during tissue expansion and post expanded skin flap transposition, mainly due to the close distance from the expander to brow. It is noticed that the brow is not affected when the brow is 0.5cm away from the site of dissecting the lacouna. Brow position change following expanded flap transposition is ascribed to expanded flap contracture and easily occurs in homeochronous reparation of skins upper and below the brow. For this, we should guarantee abundant skin in repairing defects upper and below the brow and superior and inferior edges of the brow are fixed with 5-0 absorbable sutures in corresponding periosteum. Expanded skin flaps are treated with the similar methods: dermis in the edges of skin flaps are fixed in the periosteum. The forehead wears the elastic sleeve for around 3 months post surgery and the efficacy is satisfactory. The good brow position represents the good shape. To protect the motor function of the brow is to avoid the damage of the frontal branch of facial nerve which is easily damaged in implanting the expanders in the frontal region. Generally, placement of the expander in the superficial frontal muscle will avoid this damage.

Two issues should be paid special attentions in repairing the upper eyelid using expanded flaps: the motor function of the upper eyelid should not be affected and the normal shape is returned as can as possible. The motor function of the upper eyelid is primarily related to the weight of the upper eyelid skin and may be influenced, accompanied with different degrees of ptosis, if the transposed expanded flap is too thick and heavy. Also, aponeurosis of levator palpebrae superioris must not be damaged in reparation. Therefore, in transposing expanded skin flaps, the skin flaps should be thinned and parts of orbicular muscles are removed for adhesion of expanded skin flaps and tarsal plate, which is beneficial for the motor driven by levator palpebrae superioris. To maintain the shape of upper eyelid, there are two important issues except for the thickness of expanded skin flaps: safeguard the integrity of the eyelid edge and guarantee enough expanded skin for reparation of upper eyelid.

The more important is to prevent lower eyelid ectropion except maintain a good shape in repairing the lower eyelid with tissue expansion. The procedures to maintain the shape are the same as that for the upper eyelid. The both ends of the expanded flap should exceed the level of the medial and lateral canthus and are fixed in the periosteum of the orbital medial margin to prevent lower eyelid ectropion due to skin flap ptosis or contracture. Lateral canthus anaplasty or lower eyelid constriction are more suitable for lower eyelid ectropion due to relaxed supporting tissues of the lower eyelid resulting from long-term dragging.

To acquire a good aesthetic effect, advancement flaps should be used as possible as can and incision is placed in the edge of the hair line or brow in repairing the frontal region; transposition flaps is used in repairing defects in the temple and the bilateral temples are not involved. In our opinion, tissue expansion is an alterative for the treatment of periorbital skin defect and the efficacy depends on the knowledge of periorbital anatomic structures and careful preoperative design.

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Is a Computed Tomography really harmful on children's health?

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Abstract

Aim: Computed tomography (CT) is one of the most frequently requested diagnostic tools. A rapid increase in its utilization has been noted in last two decades. But there are still many speculations on its usage whether it can harm to health. It is usually stated that the exposure to radiation during a CT may be detrimental to health via the oxidative stress. The aim of the current study is to investigate the effect of radiation taken by a single dose of CT on the total antioxidant capacity (TAC), total oxidant status (TOS) and the blood biochemistry.

Material and Methods: The study were randomly included 36 children being requested for a brain CT at Mustafa Kemal University Hospital. The gender were equal to half (18 male, 18 female) and age ranged from 1 to 14. The blood samples were gained before and after CT scan, and total oxidant status, total antioxidant capacity and biochemical tests (urea, creatinine, uric acid, AST, ALT, LDH) were evaluated in these samples.

Results: There was no statistically significant difference in terms of TOS, TAC, AST, ALT and LDH levels, but a meaningful difference on the levels of urea, creatinine and uric acid in the blood samples before and after CT scan.

Conclusion: There is a very widespread use of CT in medical practice. In the certain literature, in spite of many warnings to radiation-related injuries, it is found that there are no significant changes in TAC and TOS by taking a single dose of CT in the pediatric ages. But the influence of renal function should be taken into consideration.

Key words: Computed tomography, total antioxidant capacity, total oxidant status, oxidative stress, and children.

Introduction

Computed tomography (CT) scan is one of the frequently requested diagnostic tools. It is recently being utilized in the United States 20 fold and in England 12 fold more than last decade[1]. Particularly in pediatric patients, an increase was noted due to the advantage of the short scan time and the no need of anesthesia. Currently, the percentage of CT scan performed in pediatric population is reported in between 6% and 11%[2].

There is much more radiation exposure during CT scans in comparison to conventional radiographic examinations. For instance, organ dose is approximately 0.01 mSv during chest radiography, but 2 mSv in head and 3 mSv in thorax CT scan. According to the level of radiation and duration of exposure, CT scan generates ionizing radiation, and may increase the extent of oxidative stress[3]. Some studies showed that ROS may play an important role for the cellular damage produced by ionizing radiation[4]. Disturbance of the oxidant/antioxidant balance is considered to be a causative factor underlying the oxidative damage to cellular molecules such as DNA damage, peroxidation of membrane lipids and protein denaturation[5].

The purpose of this study is to investigate the effect of ionizing radiation during CT scan in terms of the TOS, TAC, biochemical parameters such as kidney and liver function in healthy children. The measurement of oxidants and antioxidants from different samples separately is a time-consuming procedure and not practical. Therefore, it is an easy, reliable and sensitive to measure TOS and TAC within one sample[6,7]. The measurements of these parameters are useful for the detection of oxidative status. It is revealed that radiation incre-

ases the reactive oxygen radicals. However, the changes of the oxidative status and biochemical parameters due to CT scan in pediatric healthy subjects have not been reported yet. In this study, our aim is to evaluate the biochemical and oxidative status in healthy children. In addition, we also assessed the effects of CT scan on liver and kidney functions by biochemical parameters. To the best of our knowledge, this is first clinical study investigating the effect of CT imaging in terms of oxidants and antioxidants on children's health.

Materials and methods

Thirty six volunteers were enrolled at the study. After the approval of the local Ethical Committee, all the participants were given informed consent. All subjects and their families had a standardized questionnaire including drug use, the history of chronic diseases, exposure to pollutants, and diagnostics and therapeutic radiological examinations during the last 3 months. The subjects who have any disorder affecting oxidative and biochemical status including autoimmune, kidney, liver or pulmonary disease, and acute or chronic inflammation were excluded. The individuals having any medication and drugs, were not also included. All the subjects were selected from the children who have normal radiological finding after the CT scan.

Same multi-slice CT device (Toshiba, Aquilion 64) was used for the present study. Heparinized blood samples were collected on the same day before and after 30 minutes during CT scan. Two milliliter of heparinized blood was pipetted into another tube immediately to measure for biochemical analysis. Remaining blood was centrifuged at 3000 rpm for 10 min to separate plasma. The plasma samples were stored at −70 °C until analysis of TAC and TOS.

Biochemical procedures

The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min and they were analyzed. Oxidants present in the sample oxidize the ferrous ioneo-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured by spectrophotometer, is related to the total amount of oxidant molecules present in the sample.

Renal function were assesed by serum urea, creatinine (Cr), uric acid levels, and the function of liver were with AST, ALT, LDL levels. The urea, Cr, uric acid, AST, ALT, LDH levels were assayed with an autoanalyzer (Beckmancoulter, Synchron Lx20) by using commercial Beckman Coulter diagnostic kits.

Measurement of total antioxidant capacity

Plasma TAC levels were determined using a novel automated measurement method, developed by Erel [6]. In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, mixing of ferrous ion solution and hydrogen peroxide solution produces the hydroxyl radical. In the most recently developed assay by Erel[6], same reaction is used. In the assay, ferrous ion solution, which is present in the Reagent 1, was prepared by dissolving 114 mg of xylenol orange (Merck 8677) and 8.18 g of NaCl (Riedel-de Haën 13423) in 900 mL of H_2SO4 solution (Merck 713), 25 mM. The pH value of the reagent was 1.75. It is mixed by hydrogen peroxide (Merck 8597), which is present in the Reagent 2. Reagent 2 was ferrous ion 5 mM and o- dianisidine 10 mM (Sigma D-3252) in 25 mM H_2SO4 solution. This method was applied to an automated analyzer, Aeroset (Abbott, USA) mean ± SD and based on linear type of calibration. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3%. The results are expressed as mmolTrolox equiv/l.

Measurement of total oxidant status

Plasma TOS levels were determined using a novel automated measurement method, developed by Erel[7]. In this method, oxidants present in the sample oxidize the ferrous ion-o- dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules (Carlo Erba

346165), which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter [μmol H_2O_2 equiv/L]. This method was applied to an automated analyzer, Aeroset (Abbott, USA).

Statistical analysis

The obtained data analysis was performed using statistical software package, which is "in SPSS 13.0 for Windows". Wilcoxon test to not normal distribution groups, and paired t test to those of the normal distribution was applied. Significance values (p) was determined and $p<0.05$ was accepted as a significant.

Results

The study is composed of 36 children (18 males, 18 females) with a normal CT report, and their ages were range of 1-14 years (8.9±4.3). Of these, 32 patients a cranial CT, one of them abdomen CT and 3 of them thorax CT were obtained, respectively. It was found statistically significant an increase between pre and post-CT scan in aspect of the serum levels of urea, creatinine and uric acid, and no significant difference on the levels of AST, ALT and LDH. However, it was detected an increase in terms of the TAC and TOS levels, compared to values before and after CT, but not statistically significant (Table 1).

Table 1. Pre-and post-CT scan urea, creatinine, uric acid, AST, ALT, LDH, and TAS, TOS levels. Results are presented as mean±SD

	Pre-CT	Post-CT	P value
Urea (mg/dl)	8.8 ± 2.5	9.3± 2.7	0.02
Creatinine (mg/dl)	0.4 ± 0.8	0.4 ± 0.09	0.002
UricAcid (mg/dl)	3.4±0.8	3.6±0.8	0.001
ALT(IU/L)	12.3±9.8	11±5.5	> 0.05
AST(IU/L)	20.5±7	19.7±5.8	> 0.05
LDH(IU/L)	188.1±42.2	194±56.9	> 0.05
TAC	1.25±0.13	1.28±0.14	> 0.05
TOS	13.3±7.2	18.6±23.1	> 0.05

Discussion

The effects of CT scan on children were investigated in terms of the total antioxidant capacity (TAC) total oxidant status (TOS) and biochemical parameters in in this study. However if there are some experimental study with high doses and long-term radiation exposure in the literature, to the best of our knowledge this is the first clinical study investigating CT effects on children.

The widespread use of CT represents one of the most important advances in the Radiology. However, as compared to plain radiography, CT involves much higher doses of radiation, resulting in a marked increase in the radiation exposure. The exposure to ionizing radiation results in the immediate formation of free radicals such as nitric oxide (NO), peroxy nitrites and hydroxyl radicals. The subsequent metabolic alterations in multiple intracellular processes are due to the initial oxidative damage caused by reactive oxygen species (ROS). They can bind covalently to proteins, lipids and enzymes, and may change enzyme activities, corrupt cell membranes and damage transport systems[8]. Erkal et al., demonstrated that a higher increase occurred in MDA levels within in the first few hours after the exposure at 20 Gy irradiation in the rats [9]. They also reported a significant reduction in the SOD, CAT and GPX activities within first few hours and declared that it occurred an oxidant injury within few first hours after brain irradiation. In the current study, no meaningful change occurred in TAC and TOS. To be compared with 20 Gy, we thought it was due to very low dose of radiation.

Clearly, radiation-induced production of ROS in the time of exposure contributes to oxidative damage as well as radiation response. Scavenging of these SOR with antioxidants can alleviate some of injury effects. Two pioneering studies implicating oxidative stress in radiation response were done by Oberley's and Petkau's groups and they showed that the alterations in antioxidant enzymes following radiation could result in radioprotection and implied that superoxide production following irradiation was participating in radiation-induced injury[10,11]. In these two studies, radiation doses given were rather higher than those given in our study. For this reason, we consider that no effect was observed on the oxidant system of the chil-

dren because of the low radiation dose spreading during CT scan in the study.

Biological systems have evolved an intricate network of defense mechanisms, which enable cells to cope with cytotoxicity involving ROS. Free radicals are eliminated by antioxidant systems. These defense mechanisms involve antioxidant enzymes, such as superoxide dismutase (SOD)[12], catalase, and peroxidases [13-15]. Measurement of total antioxidant capacity of plasma reflects the cumulative effect of antioxidants in the all body fluids. Thus, antioxidants do not have to be measured separately as the sum of all value[16]. Total oxidative status is determined by the balance between the oxidative stress and anti-oxidative status [17]. Therefore, the study is performed in the human plasma and refrained from invasive intervention.

In a study on survivors of atomic bomb explosions at Nagasaki and Hirosima, it was observed that leukemia incidence was increased in individuals exposed to radiation, when compared to general population[18]. Radiation-induced free-radicals may cause chromosome breaks for a long time[19]. Inano et al. showed that the NO is an important radical for the initiation of radiation-induced tumor genesis in rats [20]. Huang et al. proposed that there might be a relationship between the radiation dose of whole body PET/CT scan and cancer. Therefore, they recommend preferring as low as radiation doses, when it is clinically mandatory[21]. Current study aims that a CT scan in the daily routine impacts on whether the oxidant capacity and biochemical parameters on pediatric population and found no signicifant effect the parameters.

Consequently, it is suggested that there is a risk for development of radiation-related injury, although CT has important benefits at the diagnostic pattern. We found that the radiation spreading during CT scan causes no significant change in the oxidant status in children. Before establishing an indication, other imaging modalities should be used. In addition, patients with impaired renal function should be considered one more time before taking a CT.

Although some experimental studies with a high-dose radiation exposure in the literature, it is reported in harmful in a certain extent, the study shows that single-dose radiation exposure during CT scan do not cause measurable harm on the children's body via TAC, TOS. But, children with impaired renal function should be considered one more time before taking a CT.

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Prevalence of Trichophyton and Candida in erderly persons

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Abstract

Background: The prevalence of fungal infections is recently increasing, which may partly reflect the aging society.

Subjects and Methods: Thirty one college students (21.7 ± 1.0 years): Young adult group, 28 elderly subjects over 75 years from the outpatient department, who were keeping activities of daily living (**ADL**) (84.3 ± 4.5 years): Outpatient group, and 21 elderly subjects over 75 years from the inpatient department, who were bedridden and requiring hyperalimentation (IVH) (87.6 ± 6.0 years): Inpatient group, were enrolled. Oral health care was carried out in inpatient group.

Trichophyton infection in the toenails was diagnosed by a specific PCR method. *Candida* spp. were isolated from the tongue and identified by DNA sequencing.

Results: The prevalence of nail *Trichophyton* and total number of colonies of *Candida* spp. on the tongue were significantly higher in elderly groups than in the young adult group ($p < 0.01$ respectively). The number of colonies of *Candida albicans* (*C. albicans*) in the inpatient group was lower than in the outpatient group ($p=0.031$). This tendency was not observed for non-*C. albicans Candida* (NCAC) species.

Conclusions: Aging and neglected hygiene are considered to be risk factors for fungal overgrowth. The low colony number of *C. albicans* in inpatient group indicates the importance of keeping oral hygiene. The results that usual oral health care in our hospital did not decrease colony number of NCAC in inpatient group gives an insight into the recent increased prevalence of NCAC in hospitals.

Key words: Candida, elderly persons, non-C. albicans Candida (NCAC) species, Trichophyton.

Introduction

Over the last 30 years there has been a significant increase in the prevalence of fungal infections [1]. The social situation, for example, long-term care settings, nursing homes [2] and long-term treatment in hospital, plays a critical role in the development of mycoses in the elderly. NCAC are now frequently identified as common pathogens. This partly can be attributed to improved identification methods and also associated with the degree of diseases of the patients. NCAC is clinically emerging for its response to antifungal agent. There is still a lack of information about pathogenicity of NCAC [3].

We investigated current trends in commensal fungal colonization in later life [4] by determining the prevalence of *Trichophyton* and *Candida* spp. in elderly persons by using identification by fungal gene analysis, observed the involvement of long-term hospitalization with a bedridden state and the involvement of keeping hygiene in the growth of these fungi.

Subjects and Methods

Subjects

The study was carried out in randomly selected 31 college students (range 21-25 years, mean 21.7 ± 1.0; male 6, female 25): young adult group, 28 elderly subjects over 75 years from the outpatient department (range 75-95 years, mean 84.3 ± 4.5; male 9, female 19): outpatient group, and 21 elderly subjects over 75 years who were inpatients who were bedridden, demonstrated disuse atrophy, were not allowed oral intake and were under IVH management, and receiving broad-spectrum antibiotics (range 75-101 years, mean 87.6 ± 6.0, male 4, female 17): inpatient group.

Exclusion criteria included the presence of factors that may suppress immunity, such as usage

of continuous or temporal corticosteroids, immunosuppressants, or antineoplastic drugs. Administration of antibiotics was permitted. All samples were collected after obtaining informed consent.

In inpatient group, patients took bath by the assistance of automatic bath and shower bed system for bedridden patients once in 1 to 2 weeks or less. Nurses carried out oral health care by brushing and swabbing with wet gauze three times a day under the condition of removal of dentures.

Nineteen (90.5%) of the bedridden patients had been transferred and admitted to hospital from a nursing home, and the others had been admitted from their home. The period of an indwelling central venous catheter was 81.5 ± 33.4 (range 50 to 183) days, that of intravenous hyperalimentation was 78.5 ± 33.7 (range 44 to 182) days, and prohibition of food and drink was 78.3 ± 23.2 (range 52 to 144) days in the inpatient group. All of the patients had been administered antibiotics during their hospital stay. Ten of the admitted patients died, 5 were transferred to another hospital to continue management and therapy, and 6 were discharged to their nursing home.

Confirmation of *Tricophyton* infection in the toenails and fungal culture for tongue *Candida* infection were performed as follows.

Sampling of nail specimens and preparation of fungal DNA from nails and PCR-based Trichophyton identification

Specimens were collected from the toenails, after disinfection of the nail surface with 70% alcohol. Two or three pieces of each nail (each piece: about 1-2×4-7 mm) were obtained. Nail specimens were kept at –150℃, crushed with a mechanical crusher (Multi-Beads Shocker; Yasui Kikai, Osaka, Japan), and placed in lysis buffer (200 mmol/L Tris-HCl, pH 7.5, with 25 mmol/L EDTA, 0.5% w/v SDS, and 250 mmol/L NaCl). The samples were then incubated at 100℃ for 10 min and mixed with 150 μL of 3.0 M sodium acetate, kept at -20℃ for 10 min, and then centrifuged at 12 000 *g* for 10 min. The supernatants were extracted once with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v), and subsequently extracted once with chloroform. DNA was precipitated with an equal volume of isopropanol, washed with 150 μL of 70% ethanol, dried and suspended in 50 μL ultrapure water (Milli-Q Synthesis A10; Millipore). Aliquots of 2 μL of the resultant solutions were used as templates for conventional or real-time PCR methods with universal ITS1-specific primer pairs, *Trichophyton rubrum*-specific primers and *T. mentagrophytes*-specific primers, as reported previously, were used to identify the species of dermatophytes [5] [6].

Sampling of tongue specimens and isolation of Candida spp. from tongue specimens

After gargling with water or swabbing the oral cavity with gauze soaked with water in patients who could not gargle, specimens were collected with a sterilized polyurethane swab; BBL™ Culture Swab™ (Becton Dickinson and Company, NJ, USA), by rubbing five times in a reverse direction on the dorsum of the tongue with the swab. The swab-collected tongue specimen was kept at 4℃ and inoculated onto medium within 24 h. The specimen was suspended in 500 μl sterilized saline. Then 100 μl volumes of undiluted and 10^{-2}-diluted samples of the saline-contained clinical specimen were inoculated on CHROMagar *Candida* ™ (Kanto Chemical Co. Inc., Tokyo). After 48 h of incubation at 35℃, the number of colonies of each species of *Candida* spp. developed on the medium was counted separately by the colony color. All strains of *Candida* were identified by 28Sr DNA sequencing as follows.

The strains were sub-cultured on Sabouraud glucose agar at 35℃ for 2 days. DNA from these strains was rapidly prepared by the method described by the authors [5]. By polymerase chain reaction (PCR), the fragment of 28S rD/NA (D1/D2) was amplified with a specific primer-pair, 28SF1: 5'-AAGCATATCAATAAGCGGAGG-3' and 635: 5'-GGTCCGTGTTTCAAGACGG-3' [7]. Both strands of the PCR products were directly sequenced using a DNA sequencing kit (Applied Biosystems, CA, USA) with the respective primers, described above, and an automatic sequencer (Genetic Analyzer 310, Applied Biosystems, CA, USA) according to the manufacturer's instructions. The sequences were analyzed with Genetyx-Mac10 software (Software Development Co., Ltd., Tokyo, Japan) and searched on the DDBJ/EMBL/GenBank nucleotide database using BLAST programs [8].

Statistical analysis

Chi-squared test (Fisher's exact probability test) was used to analyze the results of the prevalence of *Trichophyton* spp. and *Candida* spp., and values of $p<0.01$ were regarded as statistically significant. Welch's test was used to analyze the results of *Candida* colony counts. Values less than 5 colony counts were taken as 1 for logarithmic transformation and statistical calculations. To normalize the data of colony count, the numbers were logarithmically transformed. Values of $p<0.05$ were regarded as statistically significant.

Results

Research on Tricophyton in toenails

Trichophyton genuses were not isolated in young adults, but were isolated in 44.9% (22/49) of elderly subjects. *Trichophyton rubrum* was the most frequently isolated species (13/49; 26.5%), followed by *T. mentagrophytes* (11/49; 22.4%). *T. rubrum* was more frequently isolated in the inpatient group than in the outpatient group, and *T. mentagrophytes* was frequently isolated in the outpatient group, but the difference was not statistically significant. There was a significant difference in prevalence of nail *Trichophyton* between the young adult group (0/31; 0.0%) and outpatient group (10/28: 35.7%, $p<0.01$) and inpatient group (12/21; 57.1%, $p<0.01$). In the elderly persons, there was a tendency for more frequent isolation of *Trichophyton* spp. in the inpatient group than in the outpatient group, but the difference was not statistcally significant. **(Table 1)**

Research on culture of Candida in tongue swabs

Candida genuses were isolated in 16.1% (5/31) of young adults, and in 61.2% (30/49) of elderly subjects. *Candida albicans* was the most frequently isolated species (21/49; 42.9%), followed by *C. glabrata* (13/49; 26.5%) and *C. tropicalis* (11/49; 22.4%). The prevalence of *Candida* spp. was significantly higher in the outpatient group (18/28: 64.3%) and inpatient group (12/21; 57.1%) than in the young adult group (5/31; 16.1%) ($p<0.01$, respectively). **(Table 2)**

Table 1. Prevalence of Trichophyton spp. in nail samples

	Young adults	Outpatients	Inpatients
	n=31	n=28	n=21
T. rubrum	0	3 (10.7%)	8 (38.1 %)
T. mentagrophytes	0	6 (21.4%)	3 (14.3 %)
T. rubrum + *T. mentagrophytes*	0	1 (3.5%)	1 (4.8%)
Total prevalence of nail *Trichophyton* spp.	0	10 (35.7%)*	12 (57.1%)**

The prevalence of nail Trichophyton in elderly persons, in both the outpatient group (*) and inpatient group (**), was high compared to that in the young adult group ($p<0.01$, Fisher's exact probability test).

Table 2. Prevalence of Candida spp. in tongue smear

	Young adults	Outpatients	Inpatients
	n=31	n=28	n=21
C. albicans	4 (12.9%)	3 (10.7%)	7 (33.3%)
C. tropicalis		1 (3.6%)	2 (9.5%)
C. glabrata		4 (14.3%)	2 (9.5%)
Candida spp.	1 (3.2%) ※		
C. albicans + *C. tropicallis*		3 (10.7%)	
C. albicans + *C. glabrata*		2 (7.1%)	1 (4.8%)
C. albicans + *C. tropicalis* + *C. glabrata*		3 (10.7%)	
C.albicans + *C. tropicalis* + *C. glabrata* + *C.orthopsilosis*		1 (3.6%)	
C. albicans + *C. tropicalis* + *Candida* spp.		1 (3.6%)	
Total prevalence of *Candida* spp.	5 (16.1%)	18 (64.3%)*	12 (57.1%)**

The prevalence of Candida spp. was significantly higher in the outpatient group (*) and inpatient group (**) than in the young adult group ($p<0.01$, Fisher's exact probability test). ※We could not identify this Candida spp., since sub-culture was not successfully completed.

The total colony number of *Candida* spp. on the tongue was significantly higher in elderly persons in the both the outpatient group and inpatient group than in the young adult group ($p<0.01$, respectively). **(Figure 1)** Although the colony number of *C. albicans* was significantly higher in the outpatient group than in the young adult group ($p<0.01$), it was lower in the inpatient group ($p=0.031$) than in the outpatient group. This tendency was not observed for NCAC species. **(Figure 2)**

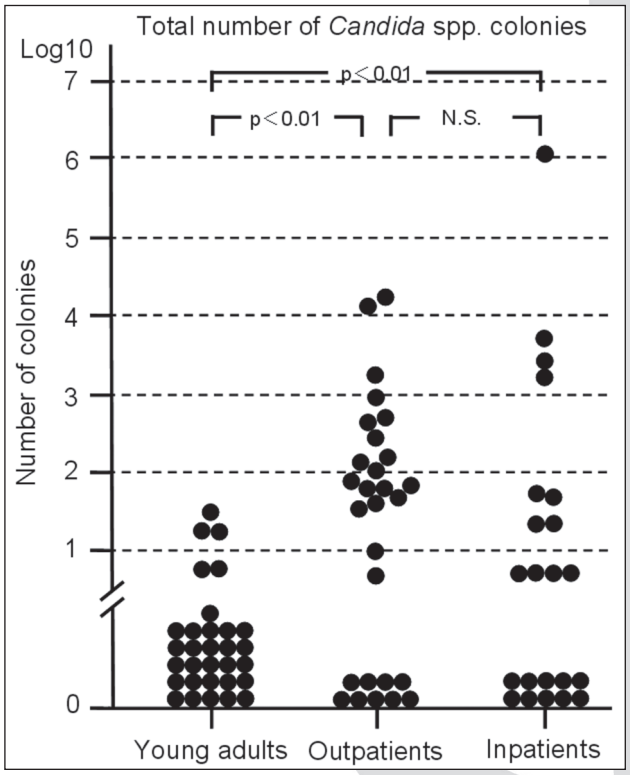
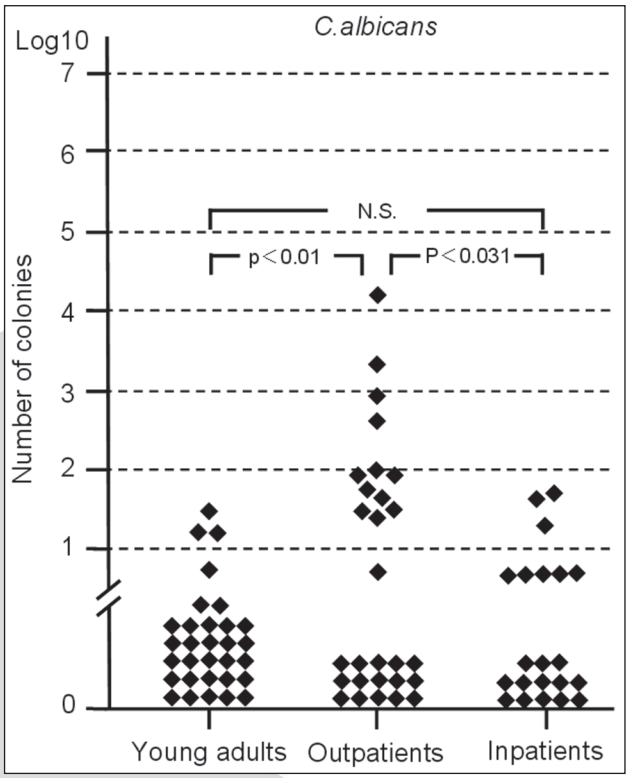
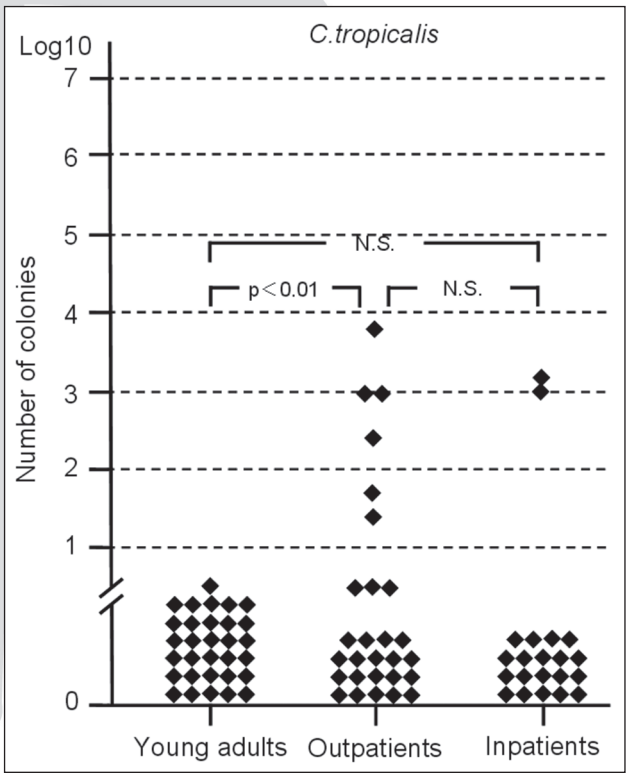


Figure. 1. Total number of Candida spp. colonies

The total colony number of *Candida* spp. on the tongue was higher in elderly persons in both the outpatient group and inpatient group than in the young adult group ($p<0.01$, Welch's test).

The colony number of *C. albicans* was significantly higher in the outpatient group than in the young adult group, and was lower in the inpatient group than in the outpatient group ($p<0.05$, Welch's test). This tendency was not observed for NCAC species.





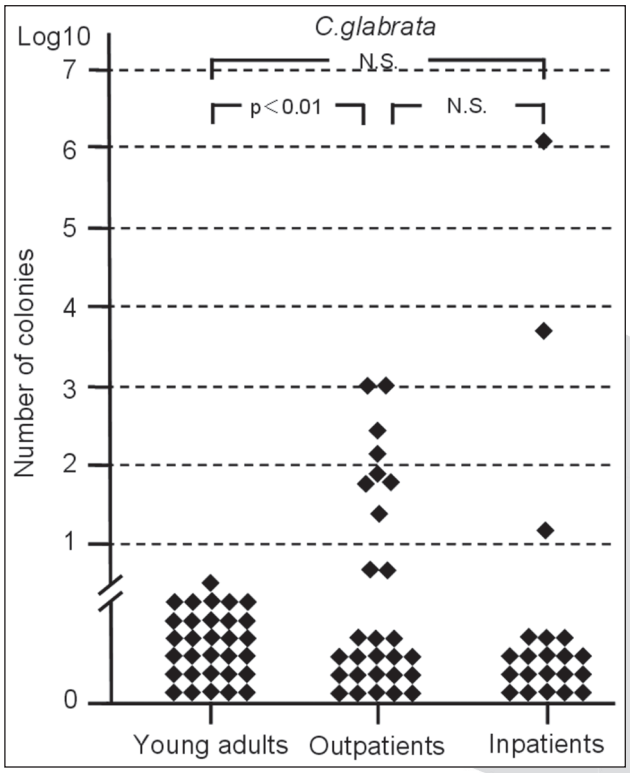


Figure 2. Number of C. albicans and NCAC colonies

Discussion

Immobilization, which is common in long-term care settings in nursing homes, is reported to play a critical role in fungal growth [2]. The status of the inpatients enrolled in this study was similar to that of nursing home care, and long-term hospitalization with a bedridden status is also considered to be a risk factor for fungal overgrowth.

An increased prevalence of infection of the skin and nails with fungi, dermatophytes, is observed in the elderly population [9]. Onychomycosis is especially common in elderly persons. *Trichophyton* is a commensal organism, and is well known as a major dermatophyte worldwide. Dermatophytosis occurring in later life manifests most frequently as *Trichophyton rubrum* infection of the toenails and plantar surfaces of the feet. Our data suggest that *Trichophyton* nail infections are frequent in elderly persons, in both inpatients and outpatients, showing the same tendency as that reported previously. Bed-ridden patients need to be bathed on a regular basis because their skin continues to produce oils and sweat. Superficial skin infections show a low tendency for self limitation, and neglected hygiene and difficulty keeping clean in a bedridden state may be involved in the prevalence. Furthermore, underlying risk factors, such as diabetes and reduced oxygen supply due to diminished blood circulation, in congestive heart failure, and arterial as well as venous alterations of vessels, may play a critical role.

Candida, which causes a variety of clinical entities, is a common and harmless commensal of the human skin, nasopharynx, oral and gastrointestinal mucosa, and vaginal mucosa of healthy subjects. Recently, the incidence of infections caused by *Candida* spp. has considerably increased. For example, the number of cases of sepsis caused by fungal organisms increased by 207 percent in the United States from 1979 through 2000 [10]. An increased aged population and higher number of immunocompromised patients may be involved in the incidence. Widespread use of antibiotics, which alter the physiological, competitive bacterial gut flora [11] [12], and invasive medical instrumentation, such as devices, long-term urinary catheters, and central venous catheters, have been implicated in the increased occurrence of fungal disease in the hospital environment [13] [14]. The use of hyperalimentation solutions is also a contributory factor, since yeast possesses a selective growth advantage in hyperalimentation solutions with high concentrations of glucose [15].

The source of *Candida* infections, especially as to whether skin or digestive tract, has been the subject of considerable debate. A recent review of published studies on potential sources of candidemia found support for a gastrointestinal origin of candidemia based on experimental, clinical, and molecular similarity studies [13] [16]. Great hope is that our observation of tongue candida may partly reflect the status of digestive tract.

In elderly individuals, decreased acid secretion, non-digested food stuffs, and partial lack of digestive enzymes are risk factors for candidasis in the digestive tract. The oral mucosa often becomes thin, smooth and dry, and an important risk factor in oral candidasis in the elderly may be hyposalivation [17]. It was reported that oral *Candida* spp. were observed significantly more frequently in elderly aged 56-70 (35%) and in an advanced age group 71-92 years (74%) [18]. Our result that colonies of *Candida* spp. were cultured from the tongue of 61.2% of both elderly outpatients and inpatients

supports this tendency for increased *Candida* spp. in elderly persons and the advanced age group.

C. albicans is the species most often associated with oral lesions, but other, less pathogenic NCAC such as *C. glabrata, C. tropicalis, C. parapsilosis*, and *C. krusei* are also occasionally but regularly isolated. A recent study that identified *Candida* isolates obtained from the oral cavity of elderly healthy individuals revealed a predominance of NCAC species (88.9%) rather than *C. albicans* (11%) [19]. The most frequent combination of mixed species infection by *Candida* species is *C. glabrata* and *C. albicans*, which was found in approximately 70% of patients with oral candidosis [20].

C. albicans is the main cause of candidosis; however, NCAC species such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis* are now frequently identified as human pathogens [14] [21], and the reasons for this might be related to improved diagnostic methods or altered medical practices. There are several specific risk factors for NCAC species [22]: urinary or vascular catheterization, hyperalimentation, and receiving broad-spectrum antibiotics [23] [24] [25] [26]. An association with patients with neutropenia and malignancy has also been reported [24].The mortality associated with *C. glabrata* [27] and *C. tropicalis* is reported to be high. The mortality of *C. tropicalis* may be associated with the virulence factors exhibited by this species such as biofilm formation, proteinase secretion and dimorphism [28].

Candida colonization of oral surfaces can serve as a reservoir for disseminated infections such as aspiration pneumonia and gastrointestinal infections [29]. More and better cleaning will reduce the prevalence of *Candida* spp. [30]. Professional oral health care (POHC) in elderly requiring daily nursing care reduced the cell numbers of potential respiratory pathogens and prevented respiratory infections [31].

Inappropriate denture hygiene, due to poor understanding and practice keeping their dentures clean, is often a problem in elderly persons [30]. *Candida* species adhere to and subsequently colonize the acrylic resin material of dentures. Fungi coexist by colonizing and forming biofilms on the denture. Although *C. albicans* was most frequently isolated, it tended to coexist with multiple and various kinds of NCAC in the outpatient group, reflecting the influence of attachment to dentures. According to our present results, the prevalence of *C. albicans* on the tongue in the inpatient group was lower than that in elderly persons in the outpatient group. Dentures were removed in all of the bedridden patients during IVH management, and nurses carried out oral health care by brushing and swabbing with wet gauze three times a day in our hospital, resulting in lower detection of *C. albicans*.

Interestingly, although the colony number of *C. albicans* was significantly lower in the inpatient group than in the outpatient group, this tendency was not observed for NCAC species, suggesting that our oral care was not a sufficient protective measure against NCAC colonization . The specific nature of those species, such as biofilm formation, proteinase secretion and dimorphism, and the involvement of therapy such as long-term administration of broad spectrum antibiotics in compromised bedridden elderly persons may be involved in the result. This phenomenon may partly explain the recent increased prevalence of NCAC in hospitals.

Conclusions

Fungi, such as *C. albicans*, are transmitted vertically, typically from mother to child around the time of birth. After years as commensals, the prevalence of these fungi such *Trichophyton* spp. and *Candida* spp. may increase with advanced age, as observed in our study.

Certain situations increase the risk of fungal colonization, such as aging, immobilization in bed, difficulty in maintaining hygiene, long-term administration of antibiotics, and long-term indwelling medical devices. Our observation that NCAC did not decrease after oral care gives an insight into the recent increased prevalence of NCAC in hospitals. More attention should be paid to this pathologic entity.

Conflict of Interest

One of the authors, K Makimura, received partial financial assistance by a Grant-in-aid for scientific research <KAKENHI> from the Japan Society for the Promotion of Science (JSPS).

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Dynamic of changes of oxidative stress during acute myocardial infarction depending on development phases of metabolic syndrome

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Abstract

Introduction: Oxidative stress is integrative part of pathophysiological processes in chronic course during atherogenesis, as well as in acute phases of ischemic heart disease, and is directly responsible for development of reperfusion phenomenons. Metabolic syndrome X also has a great aterogenic risk.

Aim: The aim of this study was to investigate dynamic of changes of oxidative stress during acute myocardial infarction depending on development phases of Metabolic syndrome X.

Method: This research included 29 patients; inclusion criteria were diagnosed Metabolic syndrome and acute myocardial infarction. According to the movements of glycemia and insulinemia all patients were divided in 4 development phases of Metabolic syndrome X. To evaluate oxidative status we determinated: lipid peroxids (malonyldialdehide), total antioxidative status, as well as oxidative stress coefficient.

Results: During hospitalisation in patients with acute myocardial infarction concentration of lipid peroxids increased, with maximum in the day 7th of hospitalisation, mostly in hyperinsulinemic phases of Metabolic syndrome. At the 1st day of myocardial infarction total antioxidant status increased, and decreased during next seven days. These changes are independent on the phase of Metabolic sindrome X. As result of inverse dynamic changes of these parametars, during period of exam came to progressive increase of oxidative stress coefficient, particularly in patients in hyperinsulinemic phase of Metabolic syndrome X.

Conclusion: During first seven days after acute myocardial infarction lipid peroxids concentration progressively increased, with decrease of total antioxidant status, that results in increase of oxidative stress. These changes are most distinctive in patients with hyperinsulinemic phases of Metabolic syndrome X.

Key words: Metabolic syndrome, oxidative stress, acute myocardial infarction.

Introduction

Metabolic syndrome X in natural course leads to one or few final disorders: type 2 diabetes mellitus, atherosclerosis, malignant, endocrine and immune diseases[1-3]. Acording to American Diabetes Association (ADA) criteria for Metabolic syndrome X are: central obesity, plus 2 of following disorders: arterial hypertension more than 130/85 mmHg, trygliceride concentration more than 1,7 mmol/L, HDL-cholesterol less than 1,03 mmol/L for woman, and 1,29 mmol/L for men, waist to hip more than 94 cm for men and more than 80 cm for women, fasting plasma glucosae more than 5,6 mmol/L or previous diagnose of Imapired glucose tolerance or type 2 diabetes mellitus and hyperuricemia[1]. In natural course of Metabolic syndrome X, depending on glucose tolerance and insulinemia level, we can set apart four phases: normal glucose tolerance and normoinsulinemia, normal glucose tolerance and hyperinsulinemia, glucose intolerance and hyperinsulinemia and glucose intolerance and normal/hypoinsulinemia[4].

Free radicals present high reactive compounds that originate under influence of physical and chemical agents, depending on defensive abilities organism may lead to cell damages and dominantly originate as result of reactivated oxygen form[5,6]. Lipid peroxyde may originate enzyme or nonenzyme way from polyunsaturated fatty acids, during this process malonyldialdehide (MDA) is originated. Atioxydant protective system puts aga-

inst free radicals effect. Increased oxydative stress originate becouse of out of proportion free radicals effects (increased intake and/or creation) and protective mechanism capacity, becouse of endothelial disfunction originate[7]. Except in atherogenesis, free radicals have role in changes during acute myocardial infarction (reperfusion arrythmias, "stunned myocard"). Free radicals, produced in great amount during myocardial ischemia and reperfusion, have role in degradation of celular and subcellular membrane structure. Source of free radicals in ischemic myocard are neutrophils who moved to necrotic tissue, as well as metabolic transformation hypoxantine and xantine in uric acid. Later reactions form lipid peroxides and cytotoxic products of oxidation, as MDA[8].

Lipid peroxydes presents free radicals level, it is well known that free radicals are important factor in endothelial disfunction, it is supposed that oxydative stress is one of pathophysiological connections between endothelial disfunction and dyslipidemia[11,12]. Stimulated polymorphonuclears during 30 minuter may increase MDA concentration for 25 %, some antioxydant enzymes may considerable decrease this concentration[9,10]. The importance of free radicals production during atherogenesis is considerable not only in chronic course condition (it is well known that atherosclerosis is followed by low inflamation level, one of main roles have mononuclears and polymorphonuclears), as well as acute vascular incidents (where inflammation presents part of physiological answer on damage originate).

There is balance between free radicals production and antioxydative protection mechanisms in organism. Increasing free radicals production and/or decrease activity of protective antioxydant system results balance disturbance between these physiological processes and «oxidative stress» commmencement[20,21].

The aim of this study was to investigate dynamic of changes of oxidative stress during acute myocardial infarction depending on development phases of Metabolic syndrome X (lipide peroxydmalonyldialdehid (MDA), total antioxidant status (TAS) and oxydative stress coefficient (KOS)).

Method

The study included 29 patients hospitalised in Coronary Unit, Center for cardiology in Clinical Center Kragujevac with acute myocardial infarction, with Metabolic syndrome X diagnosed at least 2 months after that. Group enclosed 23 men and 6 women, average 58,72+13,2 years old (from 33 to 85 years).

According to glycemia and insulinemia concentration during oral glucose tolerance test (OGTT) (done at least 2 months after acute myocardial infarction), patients were divided in four groups with Metabolic syndrome (first phase - normal glucose tolerance and normoinsulinemia (NTG-NI), second phase - normal glucose tolerance and hyperinsulinemia (NTG-HI), third phase - glucose intolerance and hyperinsulinemia (PTG-HI) and fourth phase - glucose intolerance and normal/hypoinsulinemia (PTG-NI). Inclusion criteria for patients were: negative biohumoral syndrome of inflammation, characteristic dynamic electrocardiographic changes, positive biohumoral syndrome of myocardial necrosis, without renal insufficiency, without diseases and/or medication that influence to studied parameters.

Diagnose of acute myocardial infarction is placed according to typical clinical picture, positive biohumoral syndrome of myocardial necrosis, dynamic electrocardiographic changes and echocardiography.

Glycemia during OGTT was determinated from whole blood sampling (serum was separated after 15-30 minutes on room temperature) in Center for medical biochemistry KC Kragujevac by enzyme color test (modified Trinder's method glycose-oxydase). Insulinemia during OGTT was determinated from serum samples (until analysing hold on -20 ^{o}C) in Department for *in vitro* diagnostic Center for Nuclear medicine KC Kragujevac RIA INSULIN (PEG) diagnostic package, INEP dijagnostic standardised to referent substrate WHO(66/304). Referent value for this diagnostic package is 1-20 mUI/l.

Parameters of oxidative status perceived determinating lipid peroxydes: MDA i TAS. According to that isolated observation of some oxydative status parameters we can not get complete picture, need imposed for few parameters integration in

matematical model. MDA and TAS have directly opposed effects, ratio of these two parameters was calculated, that value presents coeficient oxydativne stress - KOS.

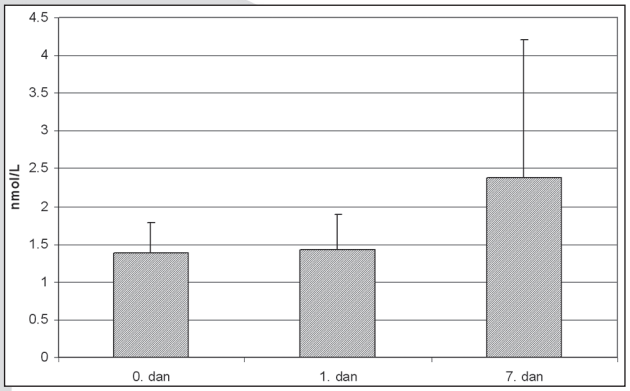
These parameters were determinated in Laboratory for experimental and clinical immunology Medicical faculty in Kragujevac, KC Kragujevac and Institute for patophysiology Medical faculty in Kragujevac, 0., 1st and 7th day of hospitalisation after acute myocardial infarction. Plasma concentration od lipid peroxydes determination is based on products of lipid peroxidation that react with tiobarbiturat acid[9,10]. Tiobarbiturat acid in this assay react with malonylaldehid who represents major product of lipid peroxidation. First was done lipid peroxydes extraction from plasma, and then malonyladehide level was determinated. MDA value was expressed in nmol/L. Total antioxydant status in plasma was determinated by original diagnostic sets Randox Laboratories Ltd. Assay principe based on reaction 2,2-Azino-di(3-ethylbenzthiazoline sulphonate) (ABTS) i H_2O_2 that produces releasing of ABTS+, that has relative stabile blue-green color that measure on 600 nm. Antioxidants added to sample supress production of this color who is proportional to anioxydative subsatces level. Average values of TAS for European working population is 1,30-1,77 mmol/L.

In statistical analysis we used descriptive methods and tests (χ^2 test and analiza varijanse). Probability was established on p-value < 0,05. The management was performed on personal computer using aplicative software (programme package SPSS 17.0).

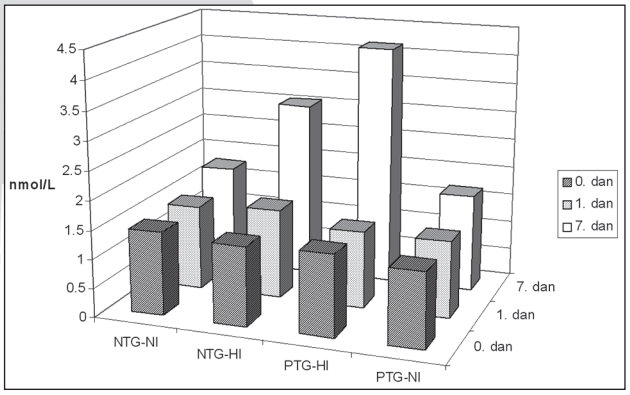
Results

Lipid peroxyd - Malonyldialdehide

MDA level during acute myocardial infarction grows. In the moment of admission to Coronary Unit (Day 0) MDA is 1,39±0,4nmol/L, in the day 1st 1,43±0,47nmol/L, in the day 7th reached 2,38±1,83 nmol/L (Picture 1). MDA level in the day 7th of acute myocardial infarction is statististical significantly higher than in the day 0 (p<0,001) and the day 1st (p<0,001). Using trofactor analysis varianse we examined influence of glucose tolerance, insulinemia level and time that elapsed from starting acute myocardial infarction on MDA level. The analysis showed statistical significance influence of these factors (p=0,003), dominant influence on differences between groups was insulinemia level (p=0,015) and time from starting acute myocardial infarction (p<0,001), and there is statistical significant interaction (p=0,006) (Picture 2). Analysing the differencies between groups showed that MDA level in the day 7th of acute myocardial infarction in subgroups patients with Metabolic syndrome in hyperinsulinemic phases (NTG-HI i PTG-HI) is statistical significant higher than in the day 0. i 1st, as well as in other phases, no matter the time from beggining of acute myocardial infarction. Between subgroup PTG-HI and other subgroups with Metabolic syndrome X there is high statistical significance (p<0,01), while between subgroup NTG-HI and other subgroups there is statistical significance (p<0,05) (Table 1).



Picture 1. Serum concentrations of MDA during acute myocardial infarction (p<0.001)



Picture 2. Serum concentrations of MDA during aute myocardial infarction depending on phase of Metabolic syndrome (p=0.003)

Table 1. MDA serum concetration (x±SD) (nmol/L) depending on phase of Metabolic syndrome and time (p=0.003).

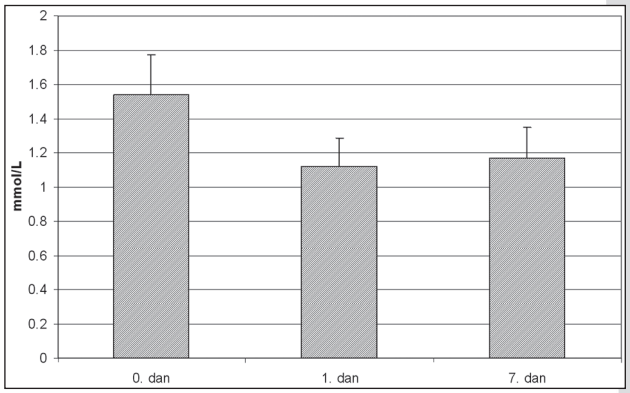
	NTG-NI	NTG-HI	PTG-HI	PTG-NI
Day 0	1.45 ±0.36	1.37 ±0.51	1.41 ±0.19	1.31 ±0.43
Day 1	1.46 ±0.33	1.55 ±0.43	1.33± 0.07	1.33 ±0.60
Day 7	1.76 ±0.42	3.04± 2.95	4.15 ±2.47	1.68± 0.53

Table 2. KOS (x±SD) depending on phase of Metabolic syndrome and time

	NTG-NI	NTG-HI	PTG-HI	PTG-NI
0.day	1.08 ±0.46	0.86 ±0.18	1.27 ±0.42	0.90 ±0.37
1.day	1.28 ±0.54	1.74 ±0.62	1.32 ±0.25	1.17 ±0.69
7.day	1.47 ±0.70	2.86 ±2.18	3.88 ±3.17	1.47 ±0.45

Total antioxidative status (TAS)

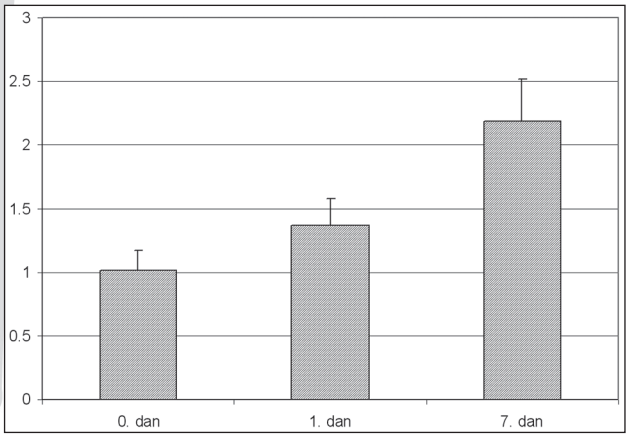
Dynamic changes of TAS levels in first seven days of acute myocardial infarction showed that during time this level statistical decrease (p<0,001) (Picture 3). It is shown that at the moment of acute myocardial infarction TAS level is statistical higher than in the day 1st (p<0.001) ant the day 7th (p<0.001). Using trofactor analysis of varianse the influence of glucose tolerance was examined, as well as insulinemia and time on TAS level, and showed no statistical significance (p>0,05).



Picture 3. Total antioxidant status (TAS) level during acute myocardial infarction (p<0,001).

Oxidative stress coeficient (KOS)

Observation total KOS changes depending on time of acute myocardial infarction, there is statistical significance (p<0,001). At the day 7th of acute myocardial infarction KOS levels are statistical significantly higher than at the day 0 (p<0,001) and the day 1st (p<0,001) (Picture 4). Trofactor analysis of variance found that insulin concentration influenced on KOS level in acute myocardial infarction (p=0,002) as well as the time from begining of acute myocardial infarction (p<0,001), but glocose tolerance not (p>0,05). It was found statistical significance of interaction factors: insulinemia level and time (p=0,006). KOS levels in subgroups NTG-HI i PTG-HI in the day 7th of acute myocardial infarction are statistical significantly higher, comparing to the day 0, and the day 1st, as well as other subgroups. In NTG-HI subgroup there is statistical significantly higher KOS level at the day 7th comparing to the day 0 (p<0,001) and the day 1 (p=0,046), as well as comparing to the day 1st and the day 7th in NTG-NI subgroup (p<0,001, p=0,005 i p=0,019), PTG-NI subgroup (p<0,001, p<0,001 i p=0,007) and NTG-HI (p=0,021, p=0,025 i p=0,002). Also, PTG-HI subgroup have statistical significantly higher levels than the day 0, the day 1st and the day 7th in NTG-HI and PTG-NI subgroup (p<0,001) (Table 2).



Picture 4. KOS level during acute myocardial infarction (p<0,001)

Discussion

With aim to evaluate oxydative status in patients with acute myocardial infarction, we followed lipid peroxyde levels (malonyldialdehide-MDA)

and total antioxydative status (TAS). We found increades MDA level in the day 7th comparing to the day 0 and the 1st. It is well known that in ischemic zones during acute myocardial infarction free radicals production is increased, starts 30 minutes after oclusion, and increase during reperfusion, catch maximum in first 16 hours of acute myocardial infarction[12]. MDA concentration increased from 30 minutes, and stays increased 72 hours after reperfusion. Increased free radicals production became more expressive after reperfusion, and the highest is 16 hours after oclusion[13-15]. Except this early change of MDA level, it is shown late increase of lipid peroxyde production during acute myocardial infarction, the highest expression in the day 7th, in accordance to results of other study[12]. Analysing phases of Metabolic syndrome X in patients where MDA level increase, it is shown that increase peroxyde levels originate in patients with hyperinsulinemia. It is shown that insulin may directly increase free radicals production (adipocyte culture increases H_2O_2 production in hyperinsulinemic conditions) [14]. Hyperinsulinemia leads to biochemical abnormalities and increased lipid peroxyde production[12].

Antioxydative protective system opposed to free radicals effects, composing of antioxydative enzymes and substances. Total activity of antioxydative protective system presents through total antioxydative status (TAS), that presents capability of plasma do decrease free radicals effects. It is shown TAS level change during acute myocardial infarction. At the moment of hospitalisation these patients had significantly higher TAS level compare to course of illness (the day 1st and the day 7th). Beside changes of oxydative stress parameters, during acute myocardial infarction antioxydative systems changes. Locally, in ischemic zone antioxydative substances level decrease in first 30 minutes after oclusion, and stays decreased for 72 hours[16,17]. Myeloperoxydases level progressive increase after reperfusion establishing, and catch maximum in 72nd hour. However, in systemic circulation, antioxydative substances level stay changeless in the day 1st during acute myocardial infarction, decrease until the day 7th, in the day 21st return to initial level, following inverse dynamic of lipid peroxydes. Based on these results some authors concluded that oxydative stress continue at least 72 hours from beggining of myocardial ischemia and reperfusion[18,19]. In this paper we got similar results. Initial increase of TAS in acute phase may explain with compensatory answer organism, with aim to oppose to increased free radicals production. In this way balance between these two oportune pathophysiological processes may be established on higher, supraphysiological level. Decreased TAS dynamic during acute myocardial infarction is similar to dynamic antioxydative substances decreasing. TAS decreasing may be understood as antioxydative capacities exhausting of organism, not as return to lower, physiological level, becouse in this phase free radicals production increase, that lead to unbalance. Comparing TAS levels, depending on Metabolic syndrome X phase between patients with acute myocardial infarction, there is no statistical significance, althought average values in hyperinsulinemic phases (NTG-HI i PTG-HI) are lower in the day 1st of ilness than in other two phases.

Parameters of oxydative status determinated in this research (MDA and TAS) come into correlative narrow interactions in biological systems. That results with appearance some typical forms their comportment (inverse interaction MDA and TAS). In consideration of correlative relation, it is clear that their separate analysis will not give complete picture, need imposed to integrate parameters in matematical model who present oxydative streess level. KOS increase beside compensatory TAS increasing in patient with coronary artery disease, conclusion inpose that beside rising to supraphysiological level, balance between free radicals production and antioxydative protective system to prevail to free radicals efects.

Analysing oxydative stress level during acute myocardial infarction showed growth tendency and that highest level is in day 7th. This is partly expected, on account of that inverse modality dinamics is registered between production free radicals process and antioxydative protection process. During acute myocardial infarction lipid peroxyde production increase, TAS level decrease, constantly make deeper discrepancies between increased needs for defence and organism possibility tomake sure adequate defence level from oxydative stress. Increased oxydative stress during acute oclusion coronary blood vessels is registered localy, in ischemic tissues, as well in systemic

circulation[22]. It is shown during study in patients with acute myocardial infarction that oxidative stress in systemic circulation continue longer, to the day 7th during hospitalisation. Following dynamic of changes of oxidative stress during acute myocardial infarction depending on development phases of Metabolic syndrome X, it is noticed that increased free radicals production following oxydative stress (perceived through the MDA and KOS level) the most distinctive in hyperinsulinemic phases ilnesses.

The fact that during acute myocardial infarction lead to amplification oxidative stress, especially in patients in hyperinsulinemic phases of Metabolic syndrome, may have great importance in undertaking preventive masures, as well as posibly therapy intervention.

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Prevalence and risk factors of cardiac arrythmias in patients with cardiomyopathy

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Abstract

Objective: To explore the prevalence and risk factors of cardiac arrythmias in patients with cardiomyopathy.

Methods: 622 patients with cardiomyopathy were enrolled in this study. Clinical features, laboratory results and electrocardiographic characteristics were retrospectively analysed. The relationship between ventricular morphology and cardiac arrhythmias was also investigated.

Results: The prevalence of ventricular arrhythmia in the patients with DCM and ARVC was the highest, while atrial arrhythmias was the most common in patients with HCM and RCM. Compared with ventricular centrality hypertrophy, the occurence of AF, ventricular arrhythmia and LBBB was higher in patients with ventricular eccentric hypertrophy (p<0.05). In addition, the patients with HCM had higher cholesterol level (p=0.006), and the patients with DCM higher UAC and glycosylated hemoglobin as well as lower HDL (p<0.05).

Conclusions: Cardiomyopathy was responsible for various cardiac arrhythmias, one of the main risk factors might be mechanical stretch. Moreover, higher cholesterol, UAC and glycosylated hemoglobin as well as lower HDL, might be other risk factors.

Key words: Cardiomyopathy, cardiac arrhythmia, risk factor, stretch-activated channel, ventricular remodeling.

Introduction

Cardiomyopathy is an anatomical and pathological diagnosis associated with myocardium and electrical activity of heart, causing a variety of arrhythmias.[1] The pathological characteristics are ventricular dilatation or hypertrophy. And the occurrence of arrhythmia in dilated cardiomyopathy (DCM) patients with congestive heart failure (CHF) was 5 to 10 times more than that in patients without heart failure.[1] According to the causes, cardiomyopathy can be divided into primary and secondary cardiomyopathy, with the main clinical manifestations of cardiac arrhythmias and heart failure. So far, the characteristic of cardiac arrhythmia in the patients with primary cardiomyopathy has been less reported, and the risk factors of cardiac arrythmias in patients with cardiomyopathy are not clear. In this study, clinical features, laboratory results, echocardiography and electrocardiographic characteristics were retrospectively analysed, to explore the prevalence and risk factors of cardiac arrythmias among all kinds of cardiomyopathies, and to provide theoretical basis for the clinical prevention in the patients with high risk factors.

Patients and Methods

Patients

According to the 2008 diagnostic criteria of the European Society of Cardiology, cardiomyopathy patients were consecutively admitted from January 2010 to October 2011 in Peking University People's Hospital, with the exclusion of a clear cause of systemic disease in patients with myocardial involvement. All the patients with cardiomyopathy were divided into primary cardiomyopathy group (n=534) and secondary cardiomyopathy group (n=88). Among them, 273 cases were DCM, 236 cases hypertrophic cardiomyopathy (HCM), 10 cases arrhythmogenic right ventricular cardiomyopathy (ARVC), 7 cases restrictive cardiomyopathy (RCM) and 8 cases unclassified cardiomyopathy.

Methods

The following data were collected at the time of registration: sex, age, duration and other basic information of the cardiomyopathy patients, and the concomitant diseases such as hypertension, diabetes, coronary atherosclerotic heart disease, cerebrovascular disease, gestosis and abnormal

thyroid disease. The laboratory data including levels of serum potassium, calcium, cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, uric acid, hemoglobin, c-reactive protein and blood glucose were collected. Various types of arrhythmias, ST segment changes, pathological Q wave, QRS wave duration and QTc interval were also investigated. In addition, ventricular configuration was divided into eccentric hypertrophy, centrality hypertrophy and concentric hypertrophy based on ventricular dilatation and hypertrophy. Left ventricular end-diastolic internal diameter ≥5.5cm in male was defined as left ventricular hypertrophy, while the limitation was 5.0cm in female. And the thickest part of interventricular septum was chosen as interventricular septum thickness.

Statistical Analyses

Measurement data of normal distribution described as mean ± standard deviation, were analysed by t test, while the count data by $\chi 2$ test. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). $P<0.05$ was considered statistically significant. $P<0.01$ was considered highly significant.

Results

Clinical features of primary cardiomyopathy and secondary cardiomyopathy

The male patients with primary cardiomyopathy and secondary cardiomyopathy were more than the female patients. And the prevalence of cardiomyopathy in patients over 50 years old was higher than the yonger. And both groups of patients could be coexisting with heart failure. Furthermore, the patients with secondary cardiomyopathy coexisted coronary heart disease and gestosis more than primary cardiomyopathy (Table 1). Finally, laboratory test results showed that there was no difference in the level of serum potassium, serum calcium, blood lipids, blood sugar and uric acid in both groups.

Comparison of the prevalence of primary cardiomyopathy and secondary cardiomyopathy patients with cardiac arrhythmias and other ECG manifestations

Both patients with primary or secondary cardiomyopathy suffered from various types of arrhythmia, ST segment changes, QTc interval prolongation, and pathologic Q waves. Among them，the prevalence of ventricular premature beat (VPB) in the patients with primary cardiomyopathy was higher (45.9%) , followed by atrial fibrillation (AF) /atrial flutter 30.0%, then atrial premature beats (APB) 29.4%. Compared with secondary cardiomyopathy, the occurrence of atrial arrhythmias and ventricular arrhythmias in patients with primary cardiomyopathy was higher ($P<0.05$), and ST-segment changes were more significant and QRS complex wave duration was longer ($P<0.05$) (Table 2).

Table 1. Clinical features of primary cardiomyopathy and secondary cardiomyopathy

		primary cardiomyopathy	secondary cardiomyopathy	Total	X^2	P
Sex	Male	356(66.7%)	49(55.7%)	405(65.1%)	4.01	0.045
	Female	178(33.3%)	39(44.9%)	217(34.9%)		
Age	< 50y	125(23.4%)	34(38.6%)	159(25.6%)	9.21	0.002
	≥ 50y	409(76.6%)	54(61.4%)	463(74.4%)		
coronary atherosclerotic heart disease		89(16. 7%)	30(34.1%)	119(19.1%)	14.83	0.000
hypertension		280(52.4%)	43(48.9%)	323(51.9%)	0.39	0.534
gestosis		5(0.9%)	6(6.8%)	11(1.8%)	11.85	0.001
diabetes		137(25.7%)	25(28.4%)	162(26.0%)	0.30	0.586
cerebrovascular disease		108(20.2%)	15(17.0%)	123(19.8%)	0.48	0.488
History of smoking		228(42.7%)	28(31.8%)	256(41.2%)	3.69	0.055
History of drinking		119(22.3%)	22(25%)	141(22.7%)	0.32	0.57
Family history		17(3.2%)	0(0%)	17(2.7%)	2.88	0.09

Abbreviation: y, years.

Table 2. Comparison of the prevalence of primary cardiomyopathy and secondary cardiomyopathy patients with cardiac arrhythmias and other ECG manifestations

	primary cardiomyopathy	secondary cardiomyopathy	X^2	p
sinus tachycardia	98(18.4%)	23(26.1%)	2.92	0.087
sinus bradycardia	122(22.8%)	12(13.6%)	3.79	0.087
atrial tachycardia	88(16.5%)	15(17.0%)	0.02	0.895
APB	157(29.4%)	16(18.2%)	4.74	0.030
VPB	245(45.9%)	30(34.1%)	4.26	0.039
atrial fibrillation	150(28.1%)	17(19.3%)	2.96	0.085
atrial fibrillation/ atrial flutter	160(30.0%)	19(21.6%)	2.58	0.108
ventricular tachycardia	81(15.2%)	7(8.0%)	3.24	0.072
atrial arrhythmias	301(56.4%)	37(42.0%)	6.25	0.012
VT	256(47.9%)	31(35.2%)	4.91	0.027
atrioventricular block	82(15.4%)	13(14.8%)	0.02	0.888
RBBB	39(7.3%)	5(5.7%)	0.30	0.583
LBBB	73(13.7%)	6(6.8%)	3.20	0.074
ST-segment changes	378(70.8%)	39(44.3%)	23.95	0.000
pathologic Q waves	121(22.7%)	17(19.3%)	0.49	0.485
QTc intervals(s)	0.45±0.50	0.46±0.49		0.417
QRS complex wave duration(s)	0.12±0.036	0.10±0.03		0.000

Abbreviation: APB, atrial premature beat; VPB, venticular premature beat; VT, ventricular tachycardia; RBBB, right bundle branch block; LBBB, left bundle branch block.

Table 3. The relationship between various types of primary cardiomyopathy and arrhythmia

	DCM	HCM	RCM	ARVC/D	p
sinus tachycardia	61(22.3%)	27(11.4%)	2(28.6%)	6(60%)	0.000
sinus bradycardia	39(14.3%)	77(32.6%)	0(0%)	6(60%)	0.000
atrial tachycardia	40(14.7%)	45(19.1%)	0(0%)	3(30%)	0.213
APB	67(24.5%)	82(34.7%)	0(0%)	6(60%)	0.003
VPB	154(56.4%)	76(32.2%)	2(28.6%)	6(60%)	0.000
atrial fibrillation	88(32.2%)	55(23.3%)	3(42.9%)	1(10%)	0.048
atrial fibrillation/ atrial flutter	93(34.1%)	59(25.0%)	3(42.9%)	1(10%)	0.047
atrial arrhythmias	158(57.9%)	128(54.2%)	3(42.9%)	7(70%)	0.584
VT	57(20.9%)	14(5.9%)	0(0%)	8(80%)	0.000
atrioventricular block	43(15.8%)	33(14.0%)	0(0%)	3(30%)	0.343
LBBB	66(24.2%)	5(2.1%)	0(0%)	0(0%)	0.000
ventricular arrhythmias	160(58.6%)	79(33.5%)	2(28.6%)	8(80%)	0.000
ST-segment depressed	168(61.5%)	172(72.9%)	6(85.7%)	6(60%)	0.028
ST-segment changes	172(63.0%)	187(79.2%)	6(85.7%)	6(60%)	0.001

Abbreviation: DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; RCM, restrictive cardiomyopathy; APB, atrial premature beat; VPB, venticular premature beat; VT, ventricular tachycardia; LBBB, left bundle branch block.

The prevalence and risk factors of arrhythmia in various primary cardiomyopathy

The relationship between various types of primary cardiomyopathy and arrhythmia

Ventricular arrhythmia was the most common (58.6%) in the patients with DCM, followed by AF (34.1%) , then LBBB (24.2%). By comparision, the incidence of atrial arrhythmia in the patients with HCM and RCM was higher (54.2% and 42.9%, respectively) , then ventricular arrhythmia (33.5%) and sinus bradycardia (32.6%).

In the present study, all the patients with ARVC coexisted with arrhythmias. Among them, ventricular tachycardia (VT) was the most common (80%) (75% of non-sustained VT) (Table 3).

Laboratory test results in various types of primary cardiomyopathy

In patients with various types of primary cardiomyopathy, serum potassium and calcium were all within the normal range. The cholesterol level in HCM patients was higher (p=0.006). Furthermore, in patients with DCM, the high-density lipoprotein (HDL) level was lower compared with HCM (p=0.002) , while the uric acid (UAC) level was higher than the patients with HCM and ARVC (p=0.000) (Table 4).

Cardiac geometry and arrhythmias

Compared with the centrality hypertrophy group, the incidence of AF, ventricular arrhythmias and left bundle branch block (LBBB) was higher in the eccentric hypertrophy group, while in the concentric hypertrophy group, sinus bradycardia and APB

Table 4. Laboratory test results in various types of primary cardiomyopathy

	DCM	HCM	RCM	ARVC/D	Unclassified	Total	F	p
serum potassium (mmol/L)	4.1±0.5	4.0±0.5	4.5±0.5	4.1±0.4	4.1±0.6	4.0±0.5	2.356	0.039
serum calcium (mmol/L)	2.2±0.2	2.2±0.2	2.2±0.1	2.1±0.1	2.2±0.1	2.2±0.2	1.134	0.341
TG (mmol/L)	1.4±1.0	1.5±0.9	1.1±0.5	1.8±1.0	0.8±0.3	1.5±0.9	1.834	0.105
TC (mmol/L)	4.0±1.0	4.3±1.0	3.5±0.6	3.8±0.5	3.3±0.7	4.1±1.0	3.406	0.005
HDL (mmol/L)	0.9±0.3	1.0±0.3	0.9±0.3	0.9±0.3	0.8±0.3	1.0±0.3	2.528	0.028
LDL (mmol/L)	2.5±0.7	2.6±0.8	2.2±0.8	2.2±0.5	2.0±0.7	2.5±0.7	1.726	0.127
creatinine (umol/L)	448.2±150.1	359.3±125.1	605.4±229.5	279.5±111.0	488.2±98.0	408.9±149.0	14.78	0.000
urea (mmol/L)	8.3±5.3	7.3±4.0	8.9±3.9	5.4±2.9	8.9±6.4	7.8±4.8	2.433	0.034
uric acid (umol/L)	448.2±150.1	259.3±125.1	605.4±229.5	279.5±111.0	488.3±98.0	408.9±149.0	18.43	0.000
albumin (g/L)	39.5±4.8	40.0±4.9	36.6±4.9	39.2±6.3	39.9±5.3	39.7±4.9	1.290	0.267
glucose (mmol/L)	5.9±2.1	5.4±1.4	5.1±1.5	5.0±1.2	4.7±0.7	5.5±1.8	0.810	0.543

Abbreviation: TG, total glycerin; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 5. The relationship between cardiac geometry and arrhythmias

		eccentric hypertrophy	centrality hypertrophy	concentric hypertrophy	X^2	P
sinus tachycardia		76(23.8%)	25(12.8%)	8(13.6%)	10.669	0.005
sinus bradycardia		50(15.6%)	68(29.7%)	20(33.9%)	19.045	0.000
atrial fibrillation/atrial flutter		102(31.9%)	46(23.6%)	18(30.5%)	4.127	0.127
atrial arrhythmias		181(56.6%)	102(52.3%)	35(59.3%)	1.297	0.523
VPB		176(55.0%)	57(29.2%)	26(44.1%)	32.523	0.000
VT		66(20.6%)	10(5.1%)	7(11.9%)	23.883	0.000
ventricular arrhythmias		184(57.5%)	60(30.8%)	26(44.1%)	34.985	0.000
VT	single	35(53.0%)	7(70.0%)	3(42.9%)	1.405	0.495
	multiple	31(47.0%)	3(30.0%)	4(57.1%)		
atrioventricular block		55(17.2%)	27(13.8%)	10(16.9%)	1.047	0.593
LBBB		71(22.2%)	4(2.1%)	3(5.1%)	45.889	0.000
RBBB		22(6.9%)	12(6.2%)	5(8.5%)	0.393	0.822
ST-segment depressed		194(60.6%)	142(72.8%)	37(62.7%)	8.068	0.018
ST-segment changes		200(62.5%)	155(79.5%)	40(67.8%)	16.325	0.000
pathologic Q waves		69(21.6%)	49(25.1%)	16(27.1%)	1.384	0.501

Abbreviation: VPB, venticular premature beat; VT, ventricular tachycardia; RBBB, right bundle branch block; LBBB, left bundle branch block.

was higher and the ST segment changes were more significantly (p<0.05). In addition, the occurrence of LBBB in patients with the eccentric hypertrophy was higher (p<0.05) compared with concentric hypertrophy. Moreover, the prevalence of VPB in patients with the concentric hypertrophy was higher than that of patients with the centrality hypertrophy. There was no difference in the incidence of other arrhythmias between two groups (Table 5).

Discussion

Cardiomyopathy is an anatomical and pathological diagnosis associated with myocardium and electrical activity of heart, causing a variety of arrhythmias.[1] The pathological characteristics of cardiomyopathy are ventricular dilatation or hypertrophy, and the main clinical manifestations are cardiac arrhythmias and heart failure. So far, the characteristics and risk factors of arrhythmia for both types of cardiomyopathy has not been known yet.

In the present study, the results showed that the prevalence of both primary cardiomyopathy and secondary cardiomyopathy in male was more than that in female. And the prevalence of cardiomyopathy in patients over 50 years old was higher than the yonger. Both the patients with primary and secondary cardiomyopathy suffered from a variety of arrhythmia, ST segment changes, QTc interval prolongation and pathologic Q waves. Furthermore, the occurrence of atrial arrhythmias and ventricular arrhythmias in patients with primary cardiomyopathy were higher (P<0.05) , and ST-segment changes and extension of QRS wave duration more significant (P<0.05) compared with secondary cardiomyopathy.

AF was very common in patients with primary cardiomyopathy. And the patients with new-onset AF during follow-up had poor prognosis.[2] Old age was the only important clinical factor that led to persistent AF in DCM patients.[3] Moreover, there was high prevalence of ventricular arrhythmias in DCM patients.[4] In addition, the patients who were suddenly dead due to VT accouned for about 50% to 80% of the total number of died patients with DCM.[5,6] This study showed that in the arrhythmia of patients with DCM, ventricular arrhythmia was up to 58.6%, which was consistent with the results of the literatures previously reported, then AF up to 34.1%, mostly occurring in the patients older than 50 years. In the present study, we also found that the incidence of LBBB was 24.2% in the patients with DCM patients, which was less previously reported. Some studies demonstrated that the level of c-creactive protein (CRP) in patients with AF was higher than the patients without AF,[7] suggesting that there was a relationship between AF and inflammatory response in the patients who had DCM.[2,7] Furthermore, in the sarcolemma of myocardial cells of DCM, the connection destruction of the cytoskeleton and sarcomere might cause the ion channel function abnormalities.[8,9] Moreover, McNair et al reported that SCN5A mutations occurred in a group of patients with familial DCM,[5] indicating that there was a relationship between the arrhythmia and sodium channel mutation in the patients with DCM.[6] In addition, Witteles RM speculated that in the patients with DCM, there was a pathological relation between left ventricular dysfunction and insulin resistance or impaired glucose metabolism.[10] Other studies also showed that the toxic effects of high glucose on myocardial function associated with microvascular dysfunction, caused progressive left ventricular remodeling.[8] Our data showed that the level of glycosylated hemoglobin in patients with DCM (7.23±1.47) was higher than other types of primary cardiomyopathy (6.51±1.20), which was consistent with the previous reports.[8,10]

Ventricular arrhythmias were also common in the patients with HCM, in which there was a relationship between non-sustained VT and the increase of the risk of sudden death.[9] In the present study, the results showed that in the HCM patients with cardiac arrhythmias, atrial arrhythmias was up to 54.2% (AF accounted for 25%) ; followed by ventricular arrhythmia (33.5%). which was inconsistent with the results of the previous reported literature,[11,12] so further research was needed. However, it had been shown that AF was the most common sustained arrhythmia in HCM patients, made adverse effects on heart function and prognosis.[11,13] In addition, Kochi RYOMA found that AF was a major risk factor for the death of these patients.[14] Recently, It was also demonstrated that the number of dead patients who had HCM with AF was 3 times more than the patients with sinus rhythm, and the risk of ischemic stroke in HCM patients with AF was increased by eight times.[9] However, the complications of AF reported for long-term prognosis were not consistent in the patients with HCM.[15]

It had been reported that the patients with RCM might have various types of arrhythmias, of which AF was the most common.[1] This study showed that the prevalence of AF was also the highest in the patients with RCM, accounted for 42.9%. The histopathologic features of RCM were endocardial scar and myocardial interstitial fibrosis, in some cases manifested as endocarditis,[16] and these factors might be one of the mechanisms of AF.

In the present study, all the patients with ARVC coexisted with arrhythmia. Among them, VT was the most common (80%) (non-sustained VT up to 75%). It has been shown that the level of CRP in the patients with ARVC was significantly increased compared with the patients with VT in right ventricular outflow tract,[11] and the level of CRP within 24 hours was significantly higher than that of 24 hours after the arrhythmias episode.[17] Some studies have demonstrated that ARVC was an inflammatory process, and there were T lymphocytes immersed in the biopsy tissue.[12]

This study also showed that the cholesterol level was higher in the patients with HCM, followed by DCM and RCM. Christ M et al[13] demonstrated that the cholesterol levels of primary DCM patients was negatively correlated with the severity of heart failure, which might be the result of severe metabolic disorders. However, cholesterol levels could not be independent predictors of poor prognosis of idiopathic DCM patients.[13]

It has been reported that acute ventricular dilatation can immediately lead to the occurrence of ventricular arrhythmias.[10,11] Mechanical stretch could also cause the changes of excitability and conduction velocity, and promote VPB to ventricular fibrillation (VF).[18]Furthermore, some studies on a single myocardial cell had shown that, VPB caused by mechanical stretch might be the results of mechanical sensitive channel activation,[16,17] inducing the membrane depolarization, and a reentrant tachycardia was easy to form.[19]

Recently, Chia-Ti Tsai et al. had demonstrated that mechanical stretch could significantly lower the threshold of action potential changes in atrial myocytes, reduce Ca^{2+} changes, and increase the inconsistency change in the spatial distribution of the atrial myocytes. Even in the complete atrium, mechanical stretch could also increase inconsistencies in the spatial distribution of action potential.[27] Our study also showed that there was a link between mechanical stretch of the myocytes and cardiac arrhythmias, especially ventricular arrhythmias. The origins of the VPB were often located in the left ventricular, which was related to mechanical stretch-induced membrane depolarization determined by the size and speed of the left ventricular volume expansion.[13] Moreover, the depolarization caused by mechanical stretch promoted the increase in selective or non-selective ion channel conductivity (conductance).[13] The reflection of stretch-activated channels in response to mechanical force was the tension on the membrane surface rather than pressure. Even if the short-term stretch, the beating heart could also cause cardiac electrophysiological response, that was late afterdepolarization.[28]

Recently, left atrial fibrosis was connected with left atrial function impairment, could be assessed through the left atrial tension and strain rate, both of which were related to atrial fibrillation risk.[24]Furthermore, the mechanical extension of the myocardial fibers promoted the threshold potential, increased interstitial myocardial cells and separated the cells from each other, which increased cardiac conduction resistance prone to cause reentry arrhythmia.[25] Moreover, the relationship between wall tension over physiological levels and left ventricular remodeling demonstrated that the changes of ventricular structure were the results of tension adjustment mechanism.[26]

In addition, the risk factors of arrhythmias in patients with secondary cardiomyopathy have been unknown yet. Perinatal cardiomyopathy may be associated with inflammatory response to abnormal immune response and cytokine-mediatede in process of pregnancy.[18] Alcoholic cardiomyopathy could affect the heart through the intervention of mitochondria, lipid, protein metabolism, and electrical-mechanical coupling.[19] Furthrmore, It was reported that the level of CRP in patients with ischemic cardiomyopathy was higher than that in patients with DCM. Moreover, ischemic cardiomyopathy was associated with left ventricular scar that led to the prevalence of arrhythmias.[20] These factors may be a part of the mechanisms of arrhythmia in different types of secondary cardiomyopathy.

In summary, cardiomyopathy was responsible for various cardiac arrhythmias, wherein ventri-

cular arrhythmia was the most common, then AF. The prevalence of cardiac arrhythmias was increased at the onset of cardiac morphologic changes in patients with cardiomyopathy, and one of the main risk factors for the arrhythmia might be mechanical stretch. Moreover, higher cholesterol, UAC and glycosylated hemoglobin as well as lower HDL, might be other parts of the risk factors for arrhythmia of cardiomyopathy.

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Research of some trace elements and E vitamin levels in some children with attention deficit disorder with hyperactivity

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Abstract

Objectives: The aim is to determine level of selenium, zinc and trace elements and antioxidant Vitamin E as factors playing role in the development and function of the brain in the children with attention deficit and hyperactivity (ADHD) and to compare such levels with the ones of the normal children.

Material and method: Levels of serum selenium, copper and zinc of 53 children with ADHD who are diagnosed with ADHD and of 53 normal children whose average of age is similar are measured with the atomic absorption spectrophometer, whereas the levels of serum Vitamin E are measured with spectroscopic method.

Findings: Average age in ADHd is 9,68±2,51, whereas it is 9,92±2,49 in the control group. Levels of serum, selenium, zinc, copper and vitamin E in the children with ADHD are found to be significantly lower than the healthy children ($p<0.001$).

Results: Lower levels in Vitamin E and trace elements under the structure of antioxidant enzyme defense systems make us to think to have caused some damage on the tissue of the brain depending on the free radicals on the children with ADHD. For this reason, it is necessary to research and determine the relations between trace elements, oxidative systems and free radicals in the ADHD.

Key words: Attention deficit hyperactivity disorder (ADHD), trace elements, Vitamin E.

Introduction

Attention deficit hyperactivity disorder (ADHD) characterized as problems in the fields of attention, concentration, activity and movement control is one of the most frequently observed psychiatrically disorder in the childhood period. Although some certain of recovery is achieved when treated, it may cause psychiatric and social problems when not recovered, and the recognition level of the illness has increased over the time, limited information on the etiology and patho-physiology increased the interest for the illness[1]. It is seen at the rate of 3-8% among the school age children[2,3]. It is more frequently seen in the boys tan the girls; the rate of boy/girl is reported to be between 3 and 5. It is considered that it is neglected or ignored in the girls because ADHD follows more in attention deficiency and cognitive disorders and the motivated and aggressive behavior problems are relatively less[1].

Although the etiology of the ADHD is not to be revealed yet fully, it is considered that genetic, biologic (neuro-anatomic and neuro-chemical) and environmental factors all play role[4,5]. However, the way how these factors play role in occurrence of disorder is not fully understood. Information related to ADHD supports the fact that the illness is of familial-transitional disorder and there is abnormal deviation in the structure, metabolism and information processing of the central nerve system (CNS)[3].

Up to now, no single mechanism which may explain all the subjects with ADHD has been revealed and the works for etiopathogenesis are still in process. In this context, one of the interest-arising research topics is the trace elements. Although there are limited number of studies about the role of these elements in ADHD, the obtained results require more studies to be carried out in this field. It is reported that the levels of some trace elements such as plasma zinc, iron, magnesium and calcium

in the children with ADHD are lower when compared to those of normal group[6].

It is proved that there are up to 60 elements in the tissue and body liquids of the human beings. Zinc, copper, cobalt, manganese, selenium, vanadium are among trace elements and these are known to be as trace elements because they are available at small amounts in the body[7].

For the first time Rotruck stated that selenium is a trace element and it is located in the active central of glutation peroxidasis enzyme in 1973[8]. Selenimum is obligator for glutation to be biologically active in the peroxidasis erythroside. In case of long-term selenium deficiency, there is decreased in the activity of glutation peroxidasis activity in all the tissues of the body[9]. Schweizer et. al. has emphasized that selenium is neuron protective and this effect is realized with the expression of selenoprotein[10]. Deficient brain selenium levels cause decrease in the brain functions, neuronal loss, trauma and malfunctions in other neurodegenerative cases.

Zinc is an important trace element in the normal function and development of the biological systems in the human beings[11,12]. It is important in metabolisms of zinc carbohydrates, lipid acids, proteins and nucleic acids[13]. It is included in structure of many metalloenzyme; it is likely to function as neurotransmitter or neuro-regulator in the central nerve system (CNS)[12,14]. It may affect the brain functions by affecting the content and receptor activity of the zinc and neurotransmitter[15]. Many of the cellular mechanisms are dependent on zinc. Zinc has important role in neurological functions and multiplication of the cells in the immune system and groth and development.

Cooper is necessary trace element for the human and animals. While the copper in the body varies in the forms of Cu^{+1} (cuprous) and Cu^{+2} (cupric), majority of the copper in the body is in the form of Cu^{+2}. As well as being important element because copper can receive and send electrons easily in the oxidation and reduction reactions, it also plays role in detaching the free radicals. Copper is included in the structure of some important enzymes[16,17].

The most important antioxidant among the lipid phased antioxidants is Vitamin E [18,19]. Its main function is that it is in-cellular antioxidant; moreover, it is determined to play role in immunity system[20].

Indeed, Vitamin E, which is a weak antioxidant out of the cell, is very effective when it is located in the cell membrane; it may protect the cell membrane and therefore cells against the oxidative damages. For this reason, Vitamin E ranks the firs among the plasma antioxidants. In the studies made, the effectiveness of Vitamin E, which is a strong antioxidant in preventing many chronic illnesses which are believed to occur as a result of the oxidative stress, has been reported. Effectiveness of Vitamin E in cases where many antioxidant defending elements are not sufficient and its effectiveness even in high concentrations of oxygen is an indicator that it is an important antioxidant for the brain which is highly populated with oxygen[21].

In this work, the aim is to compare plasma selenium, zinc, copper and Vitamin E levels between the patients with ADHD and healthy children and to determine the role of these elements and Vitamin E in the etiology of ADHD accordingly.

Materials and methods

Formation of Study Groups

53 children diagnosed with ADHD by using re-structured ADHD scale according to DSM-IV measurements of A. Turgay (average age: 9,68±2,51) and 53 healthy children (average age 9,92±2,49) are included. Study group is matched in terms of gender. In the patient group, there are 14 girls (26%) and 39 boys (74%), whereas there are 18 girls (34%) and 35 boys (66%) in the control group. The families of the children are informed of the same and their written permissions are obtained.

Collecting Blood Samples

Venous blood samples are taken into flat biochemical tubes between 9.00 and 10.00 in the morning after the 8-hours hunger and kept in the freeze at 4˚C. Then, it is centrifuged at 3000 rpm for 10 minutes and serum is extracted and it is kept at -80˚C until making analysis upon dividing it in various amounts. Serum zinc and copper levels are measured with fired atomic absorption spectrophotometer (GBC Avanta), whereas selenium levels are measured with graphite oven[22]. Serum E vitamin analysis is measured as spectroscopic[23].

Chemicals

All the chemicals have the analytical purity and have been provided from Sigma Chemical Company (St. Louis, USA).

Statistical Analysis

Statistical analyses are made in the package program of "SPSS (Statistical Package for Social Sciences) for Windows 17.0". For the gender comparisons, X^2 (chi square) is used, whereas t test is employed in comparing two groups in terms of age, serum selenium, zinc, copper and E vitamin levels. The level for the statistically significance is accepted as $p<0.05$.

Results

106 subjects, namely 3 patients (ADHD) and 53 persons as control group, are included in the study. 14 of the patient group is girls (26%) and 39 of them are boys (74%). On the other hand, in the control group, there are 18 girls (34%) and 35 boys (66%). No significant difference between two groups is found between two groups in term of gender distribution. (p=0,397; X^2 =0,716).

Age average of ADHD group is 9,68±2,51whereas it is 9,92±2,49 in the control group and age range between two groups is 6-15. No significant difference is determined between two groups in term of age distribution (p=0,614; t=-0,505). Average of the serum selenium levels is found to be 60,29±31,34 in ADHD group and 86,31±18,33 in the control group. Significant difference is found between these two groups (t=-5,22, p=0.01) (Table 1).

Table 1. The levels of serum selenium

	Average	SD	t	p
ADHD	60,29	31,34	-5,218	0,001
Control	86,31	18,33		

Level of zinc is found to (93,36±25,79) in the patient group, whereas as (109,38±23,36) in the control group. Significant difference is statistically determined between the groups in terms of the level of serum zinc (t=-3,35, p=0,001) (Table 2).

Table 2. The levels of serum zinc

	Average	SD	t	p
ADHD	93,36	25,79	-3,352	0,001
Control	109,38	23,36		

Level of serum copper is found to be (82,62±19,33) in the group of children with ADHD, whereas it is found to be (97,53±18,58) in control group. Significant difference is statistically determined between the groups in terms of the level of serum copper (t=-4,05, p=0,001) (Table 3).

Table 3. The levels of serum copper

	Average	SD	t	P
ADHD	82,62	19,33	-4,047	0,001
Control	97,53	18,58		

Analysis results of Vitamin E have been found to be lower in the group with ADHD (0,91±0,12) in consistency with the element-based analysis results than the normal children (1,29±0,22) and have been to be statistically significant (t=-11,28, p=0,001) (Table 4).

Table 4. The levels of vitamin E

	Average	SD	t	P
ADHD	0,91	0,12	-11,28	0,001
Control	1,29	0,22		

All the values of the patients are significantly lower than the children in the control group.

Discussion

Etiology of ADHD has not been still revealed completely; yet, it is considered that genetic, biological (neuro-anatomic and neuro-chemical) and environmental factors play role altogether[4]. In this context, what is interesting is the levels of trace elements among the researching issues. Because trace elements enter into antioxidant enzyme defending systems and they have important roles in the body functions, trace elements attract attention. This work has indicated that the levels of E vitamin and some serum trace elements in the children with ADHD are lower when compared to normal ones and the difference between them is statistically significant. These results appear to support the study results where plasma zinc and copper levels of children with ADHD have been previously researched[6].

The level of selenium, which is another trace element, is found to be lower in the patient group than control group. The selenium may play role in aggressive actions and anger bursts observed in the ADHD. Failures in activity of these enzymes are expected in the lack of the trace elements ente-

ring in the antioxidant enzymes which play important role in oxidative arrangement. Lack of selenium level causes decrease in the brain functions, neuronal loss and increase in malfunctions in cases of trauma and other neurodegenerative cases[10]. Lipid peroxidation occur with the decrease in the glutation peroxidase activity in lack of selenium and in the end, membrane damage and neuronal cell loss occur. In this work, levels of serum selenium and boron levels have been found to be significantly lower in ADHD group. In this studt, it is stated that there is decrease in the antioxidant enzyme defending system functions and therefore degradation may occur on the tissue of the brain depending on the freely accumulated radicals in the children with ADHD[24].

It is suggested that the lack of several lipid acids in ADHD may play role in formation of the symptoms of this disorder[25,26]. Essential lipid acids (EYA) are substrate of delta-6-desaturaz enzyme and arranges activity of cyclo-oxygenesis[27,28].

It is reported that there is decrease in the activity of delta-6-desaturaz enzyme in case of lack of zinc[29]. In this ay, the creation of many lipid mediator considered to play role in functions of cell membrane and signal transmission may therefore affect the function of the cell.

In this study, it is found that the serum copper level is lower than the controls in the children with ADHD. This result is in compliance with the studies in the literature[5,30]. Lower level of serum copper may be important in oxidative stress. It is because super oxide eliminating the super oxide radical gets into the structure of super oxide dismutosis enzyme and this is necessary for the function of this enzyme. Lower level of serum copper may cause malfunctions of the enzyme and thus the structure of the cell membrane and its function may be affected[31]. According to the studies carried out by Yorbik et. al, plasma copper and zinc have been found to be lower in the children with ADHD than controls[5].

There are also antioxidant effects of selenium and E Vitamin. The changes in the phospholipids structure in biologic membranes cause the structural destruction of the membrane and disintegration of the cell. Because the membranes carry lipid acids including multiple non-saturated bonds in their phospholipids, they are fragile particularly against lipid peroxidation. The number of couple bonds in the lipid acids in the phospholipids is direct related to the rate of peroxidation. Decreased selenium and E vitamin will be insufficient for protecting the cell membranes of the patients from oxidative damage. It is because it is reported that selenium and E vitamin obstacles lipid peroxidation in the mitochondria and microcosms in the tissue homogenates of diet resources[18].

Conclusion

These trace elements whose differences are different and essential in term of their biological functions and lower levels of E vitamin in the patients with ADHD have been found out in many studies. What is important is to research and reveal the reason for the decrease in these values. Are the decreased levels the reason or cause for ADHD For this reason, there are needs for researches and comparisons from different perspectives.

In future, more researches may be made on the differences in the levels of the trace elements included in the antioxidant enzyme defense system and psychiatric disorders which may arise depending on the free radicals in term of etiopathogenesis and treatment method.

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Magnesium and dyslipidemias, cardiovascular and other diseases

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Abstract

In many enzymatic reactions magnesium acts as a cofactor and also it is a physiological calcium antagonist. Magnesium participate in the regulation of many ion channels and phoshorylation reactions. Magnesium deficiency is a contributing risk factor in many diseases: osteoporosis, non-specific nerve hyperexcitability, pain, lethargy, mental confusion, preeclampsia, hypertension, atherosclerosis, stroke and myocardial infarction, diabetes mellitus, allergy, asthma and immune disorders. Hypomagnesemia is a common condition postoperatively in intensive care units. In aim to prevent increased morbidity and mortality in the critically ill patients, it is necessary to detect and correct magnesium levels in blood. Application of magnesium preparations at large doses are usually well tolerated. Concentrations of magnesium in plasma ranging from 2-3.5 mmol/L are considered to be safe in healthy patients.

Key words: Magnesium, Atherosclerosis, Diabetes Mellitus, Hypertension, Anesthesia.

Introduction

Magnesium (Mg) is an important intracellular cation and exists in several forms in the blood. It is distributed into three major compartments: mineral phase of the bones (65%), intracellular space (34%) and extracellular fluid (1%) (1). The most important fraction is ionized (free) magnesium (2). Magnesium is important in muscle contraction. Mg^{2+} acts as smooth muscle relaxant and Ca-channel blocker in vitro. Mg^{2+} is a co-factor in many enzyme systems, and is the second most abundant intracellular cation.

Mg deficiency is a contributing risk factor in many diseases: osteoporosis, non-specific nerve hyperexcitability, pain, lethargy, mental confusion, preeclampsia, hypertension, atherosclerosis, stroke and myocardial infarction, diabetes mellitus, allergy, asthma and immune disorders (3). In different metabolic diseases as well as diabetes mellitus, low Mg intake or abnormal Mg metabolism are associated with etiologic factors (4). Meta-analysis of 13 prospective cohort studies which included 536.318 participants and 24.516 cases, shows supporting the fact that magnesium intake is significantly inversely associated with risk of type 2 diabetes risk in a dose-response manner (5).

Hormones which regulates magnesium levels are calcitonin, parathyroid hormone and insulin. Administration of insulin and oral glucose load enhance the uptake of magnesium into cells via an ATPase pump. Mg as an integral part of activated MgATP complex regulating protein kinases. Magnesium is very important is in the control of glucose metabolism, peptide hormone receptor signal transduction, stimulus-secretion coupling and stimulus-contraction coupling. Magnesium deficiency in diabetes mellitus is reason of decrements in the enzymatic activities of several metabolic pathways (6).

Magnesium Metabolism

Magnesium is of vital importance to the human body. An average diet should provide sufficient magnesium intake. Magnesium absorption occurs primarily in the small intestine. Calcium, phosphorus, fats and higher environmental pH levels reduce magnesium absorption, while sodium, urea and sugars increase it. Approximately, 50-80% of magnesium excretion from the body is fecal, while the remaining amount is excreted in the urine. If the magnesium blood level increases rapidly (after parenteral magnesium treatment, for example) urinary magnesium excretion also increases, which indicates that

the kidney is an important organ involved in magnesium concentration regulation. Approximately 30% of magnesium in the blood plasma is bound to proteins, and 70% is therefore, filterable at the glomeruli. Diuretics increase magnesium excretion. It is believed that a human body, weighing approximately 70 kg, contains a total of 24 g of magnesium. The highest magnesium concentration is found in bones, muscles, liver, kidneys, spleen, brain, lungs and red blood cells. Magnesium metabolism is regulated by various hormones. Parathyroid hormone (PTH) acts to reduce intestinal magnesium absorption and enhances renal magnesium excretion. Renal excretion of magnesium is also enhanced under the influence of mineralcorticosteroids (aldosterone), thyroid gland hormones and insulin, while adrenalin increases plasma magnesium levels. Free, "ionized" serum magnesium is of utmost biological importance. It affects cell ion channels and transporter mechanisms. This role of magnesium is demonstrated through its control of calcium entry into cells, thus consequently controlling cardiac muscle tone and respectively, arterial smooth muscles (7).

Renal magnesium treshold is approximately 0.60 to 0.85 mmol/L. Since this value is close to normal serum magnesium levels, kidneys quickly excrete any excess magnesium from the serum. Since renal magnesium reabsorption competes with reabsorption of calcium in the thick ascending limb of the loop of Henle, calcium loss due to the use of diuretics, hypercalcemia and/or intravenous saline drips also lead to magnesium deficiency. When magnesium intake from food is reduced, the renal mechanisms aim to preserve magnesium levels so that the magnesium reserves are very slow to decrease (8).

Reference values for total serum magnesium concentration for adults range from 0.65 to 1.05 mmol/L (7). Magnesium supplementation is generally well tolerated, even when given in high dosages. Serum magnesium concentration ranging from 2 to 3.5 mmol/L is considered safe for healthy adults (9). Serum magnesium levels ranging from 4 to 6.5 mmol/L can cause nausea and vomiting, drowsiness, double vision, slurred speech and hyporeflexia. Magnesium blood levels ranging from 6.5 to 7.5 mmol/L cause muscular paralysis, respiratory depression, bradycardia, arrhythmia and hypotension. Plasma magnesium concentration over 10 mmol/L may lead to cardiac arrest (10).

Magnesium imbalance

Magnesium is necessary for normal functioning of bones, teeth, muscles and nerves. Magnesium helps prevent cardiovascular diseases and irregular heartbeat.

Hypomagnesemia

Magnesium deficiency have people who been fed intravenously for a long time, whose nutrition does not contain enough magnesium or who are can not absorb and excrete the mineral properly. Hypomagnesemia may cause excessive secreting of aldosterone, ADH or thyroid hormone. Loss of body fluids as a result of stomach suctioning or chronic diarrhea, long-term diuretic therapy, hypercalcemia, diabetic acidosis, complications of bowel surgery, chronic alcoholism, malnutrition and starvation can be associated with hypomagnesemia. Clinical signs of hypomagnesemia are: loss of weight and appetite, bloating and muscle pain, nausea, vomiting, muscle weakness, tremor, irregular heart beat, delusions and hallucinations, leg and foot cramps, muscle twitches, hypertension (3).

Hypermagnesemia

Hypermagnesemia appears in patients whose kidneys can not excrete the magnesium. In patients who take magnesium salts or in healthy people who use large quantities of magnesium-containing antacids, laxatives or analgesics, also can appears hypermagnesemia. In young people magnesium poisoning can cause severe diarrhea. In elderly people with renal failure, patients with intestinal disorders and kidney disfunction, people who use antihistamines, narcotics and muscle relaxants exists the high risk for complications of magnesium poisoning. Clinical symptoms and signs of hypermagnesemia can be: flush, drowsy, heavily perspiration, diarrhea, shallow breathing, disminish reflexes, muscle weakness and hallucinations. Coma and cardiac arrest also can appears as a results of magnesium overdose (3).

Magnesium-clinical effects

Cardiovascular effects and dyslipidemias

Magnesium is regulator of transmembrane and intracellular flows by calcium channels and

pumps. In myocardial contractility magnesium has an important role by inhibiting calcium uptake on the troponin C of myocytes. Rapid infusion of $MgS0_4$ have brought to reduction of systolic arterial pressure and to decreased systemic vascular resistance. Dysmagnesemia induced disturbances in cellular ionic movements and changes excitability of the heart cells of nodal tissue responsible for cardiac rhythm disorders (9).

Magnesium reduce oxidative stress and have anti-oxidant role in regulation of free radicals. In initial stadium of atherogenesis, magnesium deficiency contribute to reduction of arterial elasticity. Magnesium supplementation reduce triglyceride levels and change the ratio of LDL to HDL, which is very important in heart disease. Cholesterol levels will not reduce by magnesium supplementation, but it is maintains the elasticity of arteries and increase the amount of HDL. Reduction of LDL/HDL ratio decreases the risk of cardiovascular attacks. Magnesium prevents the deposition of calcium in arterial walls at place of micro-injury. This indicate that magnesium have a significant role in the prevention of atherosclerosis (11).

In conclusion, magnesium maintenance the healthy muscles of heart. The heart muscle have benefits from an adequate supply of magnesium (11).

Muscle and neuromuscular transmission

Magnesium and calcium have antagonistic action on muscles. Hypomagnesemia lead to muscle contraction and hypocalcemia lead to muscle relaxation. Neuromuscular transmission is changed by a presynaptic and a postsynaptic effect. Magnesium blocking the entry of calcium into presynaptic endings. That brings to reduction of presynaptic release of acetylcholine and altering neuromuscular transmission. Magnesium reduces the effects of acetylcholine on postsynaptic membrane and increase the threshold of axonal excitation (9).

Central nervous system

Magnesium have adjuvant effects in perioperative analgesia as an antagonist of N-methyl-D-aspartate receptors. This effects and increase production of prostaglandins have anticonvulsant function. Calcium inhibitory effect causes central arteriolar vasodilation and prevent vasospasms (9).

The onset of migraine headaches can be caused by magnesium deficiency. Patients with migraine need frequently calcium channel blockers for treatment (11).

Magnesium deficiency is involved in mental stress. In therapy of stress, lithium is often used. In process of neurotransmissions, magnesium have a crucial role in mediating calcium dependent synapse reactions (11).

Use of Magnesium in Anesthesiology

Magnesium sulfate has a depressant effect on the nervous system, thus reducing the need for anesthetics. It demonstrates antinociceptive effects as a competitive N-Methyl-d-aspartate (NMDA) receptors antagonist. In competition with calcium ions, magnesium inhibits the release of acetylcholine from the presynaptic membrane, thus potentiating the effect of neuromuscular blockers (12). Patients who had major surgery are at risk for hypomagnesemia during the early postoperative period, if they are not get magnesium (13). Magnesium is also used as an antiarrhythmic agent (14). Some *in vitro* studies demonstrate that magnesium inhibits catecholamine release from adrenal gland and adrenergic nerve endings and also causes coronary vasodilation (15). Ryu *et al.* demonstrated that the use of magnesium sulphate for achieving controlled hypotension in patients undergoing ear surgery, produces the same effects as the use of opioid analgesic Remifentanil (16). Magnesium sulfate can be used to treat hypocalcemia, asthma attacks and to protect the cardiac muscle after ischemia (17). It is belived that magnesium has anticonvulsant effects (9). Certain recent studies indicate that it also has anti-inflammatory effects (18). Magnesium sulfate is used as an anesthetic adjuvant therapy for patients with pheochromocytoma (19). Usmani *et al.* have been administering a bolus dose of 50 mg/kg of magnesium sulfate followed by continuos intravenuous infusion of 15 mg/kg/h for pain management (20). Cizmeci *et al.* have proved that continuous intravenous magnesium infusions of 8 mg/kg/h potentiate the effect of neuromuscular blockers (12). In a study conducted by Sevhan *et al.,* a 10 mg/kg/h magnesium sulfate continuous intravenous infusion was sufficient to significantly lower intraoperative doses of anesthetics and neuro-muscular blockers, as well as post-operative doses of analgesics (21). The use of

magnesium in anesthesiology is contraindicated by heart block, myastenia gravis and neuromuscular dystrophy (22).

Instead of a conclusion

Major electrolytes found in the human body are sodium, potassium, calcium, magnesium, chlorine and phosphate. Magnesium plays a very important role in bone calcium-binding and muscle relaxation. It also helps regulate the heart rate and lower cholesterol levels. Magnesium deficiency increases sensitivity to stress. High alcohol consumption, diabetes, diarrhea, use of diuretics or oral contraceptives facilitate its secretion into urine. Unbalanced diet causes deficiency in this oligo-element (23, 24).

Magnesium has an important role in osmolality control. Cell membrane is permeable to water and urea, but effectively impermeable to sodium, due to the continous action of the sodium-potassium pump. Intracellular osmolality is mainly determined by potassium and magnesium and their accompanying anions (inorganic phosphates, proteins and small quantities of bicarbonates). Plasma and interstitial osmolality is mainly determined by sodium concentration and its accompanying anions (chlorides and bicarbonates) (25).

The principal physiological function of magnesium is the maintanance of the phosphatase enzymatic systems necessary for cleaving phosphate bonds which serve as basic sources of energy. In critically ill patients recieving total parenteral nutrition, hypomagnesemia can occur due to administration of parenteral glucose and insulin solutions which affect magnesium redistribution, forcing magnesium to enter the cell. Clinical manifestations of hypomagnesemia are dominated by the signes of central nervous system excitation, tetany and convulsions. Typical changes occuring in the cardiovascular system are tachycardia, stenocardia, hypertension and vasospasm along with hypersensitivity to digitalis medications (26).

Magnesium, as a physiological calcium antagonist, plays a significant role in the treatment of endothelial dysfunction and atherosclerosis. Treatment of atherosclerosis and macroangiopathy, as well as of arterial hypertension, is especially important in patients with diabetes (27).

Perioperative use of magnesium sulphate can be useful. Magnesium has a vasodilator effect by enhancing prostacyclin synthesis, and is also an angiotensin converting enzyme inhibitor; it can, therefore, be used for inducing controlled hypotension during anesthesia. Antagonistic effects of magnesium on N-methyl-D-aspartate receptors contribute to its use in perioperative analgesia (28). Administration of magnesium before endotracheal intubation can attenuate hemodynamic responses to intubation better than Lidocaine (29).

Side effects of hypermagnesemia are very serious: respiratory muscle weakness, areflexia, bradycardia, cardiac arrest and central nervous system depression. Hypermagnesemia requires urgent correction since it is life-threatening to the patient. Firstly, the administration of magnesium is discontinued. The effect of magnesium is antagonized by administration of calcium chloride (CaCl). Magnesium levels can be lowered by stimulation of intracellular ion entry by applying intravenous glucose and insulin infusions. Hemodialysis is used in cases of reduced kidney function (30).

Despite all of the aforementioned, there is still an insufficient number of trials about influence of magnesium on the cardiovascular system, metabolic and endocrine diseases and the central nervous system. We hope this article will serve as a stimulus for future research that will provide explanations for many questions we still have no clear answers to.

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Knowledge and practice of nurses in Cyprus on subcutaneous heparin injection

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Abstract

Objectives: The aim of this desriptive study was to determine the knowledge and practice of nurses regarding subcutaneous heparin injection.

Material and Methods: The sample of the study comprised 154 nurses. As data collection form, question form aiming to determine demographic characteristices of nurses and their information and practice on subcutanesous heparin injection was used. Data were evaluated using One Way ANOVA, Tukey HSD and Pearson Correlation Coefficient tests.

Results: Knowledge scores of the nurses was found to be mean 76,8±11,3 out of 100 and their practice scores 81,8±8,2 out of 100. Highest scores of knowledge on heparin injection were obtained by nurses working in surgery clinics and highest scores of practice by nurses working in intensive care units. Knowledge and practice scores on heparin injection were found to be higher in those who received training on heparin injection during their inservice training ($p<0,01$). It was established that theoretical information of nurses on heparin is associated mostly with bleeding and their information on the selection of injection site and correct practice technique is inadequate.

Conclusions: It is recommended that practice standards and protocols be developed for special drugs, inservice training programs be developed and training booklets or manuals be prepared.

Key words: Heparin, drug administration, subcutaneous injection, nursing, knowledge, practices

Introduction

Heparin, which is one of the most beneficial drugs for prolonging life and increasing quality of life and also one of the most risky drugs due to its side effects, is the most commonly used of all anticoagulant drugs. Anticoagulants suppress coagulation by impairing the function and composition of coagulation factors and prevent the formation of thrombus (1,2, 3,4).

Heparin is used in general surgery especially in major operations leading to the formation of thrombus, in orthopedic surgery, and cardiovascular surgery, before and after neurosuergery operations, in cardiology for diseases of thrombus origin, and following neurological disorders (3,5).

Nurses administer some drugs to the loose connective tissue below dermis through subcutaneous injection. Drugs with small volume (0.5-1ml) and which can be dissolved in water can be administered by this route (2).

Since subcutanesous administration of large volume tissue damaging drugs may cause necrosis, pain, echymosis, hematoma and abscess in that region, nurses should exert caution during administration and should change the injection regions respectively at each administration (2,4,6,7,8).

Nurses should know the effects, side effects, dose, route of administration, and points to take care during administration and should perform the procedure accordingly. (2,3,4).

Side effects such as thrombocytopenia, hematoma, echymose and bleeding which may develop due to making subcutaneous injection to the wrong site, not rotating the site of injection, not entering the tissue at correct angle, should be known by the nurse and taken into consideration (2,3,4,6,7,8).

In the administration of drugs such as heparin, which have serious side effects as well as being life saving, correct technique and evaluation along with adequate information and experience of the nurse may decrease undue side effects and may even eliminate them completely (2,3,4).

Nurse is responsible for administration of drug in view of correct principles, holistic evaluation of the individual, monitorisation of the response

to the drug and for informing the individuals on drugs which they use and will use (2,3,9,10).

The nurse, who will administer heparin, should know the side effects of heparin, its routes of administration, regions where injections will be made and the rules of administration and should act according to this information during practice.

1. Methods

1.1. Participants

This study was carried out desciptively in a state hospital in Cyprus in order to evaluate the knowledge and practice of nurses regarding subcutaneous heparin injection. Hospital has 460 beds with 19 clinics, 1 surgical intensive care unit and 1 neurological intensive care unit. Overall 365 nurses work in the hospital. Heparin administration is made more commonly in orthopedics, cardiovascular surgery, general surgery, neurology, internal medicine, intensive care and dialysis-hemodialysis clinics. There is no protocol and booklet for nurses regarding heparin administration.

The sample of the investigation comprises 154 nurses who are willing to participate in the study and can be reached.

1.2. Intervention

In the collection of data, 'question form' prepared by the investigator in view of the literature in order to determine the socio demographic characteristics of the nurses and their information and practice regarding subcutaneous heparin injection was used (1,2,3,4,9,10).

Question form including two parts, i.e."demographic chraacteristics of the nurses' and "information and practice on heparin injection'was filled by face to face interview method at convenient times for the nurses. In order to prevent interaction between nurses, nurses working in the same clinic were interviewed separately within a day.

1.3. Ethical Considerations

The Institutional Review Board of Near East University approved the study. Informed consent was obtained from the nurses participating in the study. After forms were filled, questions asked by nurses on heparin injection were answered by the investigator.

1.4. Statistical Analyses

Obtained data was evaluated with Statistical Package For The Social Sciences (SPSS) for Windows 16.0 program using appropriate statistical methods.

Correct answers to questions asked to measure information level of nurses were scored as 1 and wrong answers as 0. For each nurse Heparin Injection Knowledge Score (HIKS) was calculated over 21 then by finding its equivalent over 100.

Correct answers given to questions aiming to evaluate heparin injection practice were scored as 1 and wrong answers as 0. For each nurse, Heparin Injection Practice Score' (HIPS) was calculated over 20, then by finding its equivalent over 100.

The effect of independent variables (sex, age, education status, clinic worked, duration of work etc) on both mean scores was evaluated using "One Way ANOVA" In order to determine the groups where the difference originated from, "Tukey HSD" test was employed. In addition, to determine the direction of the relation between scores, 'Pearson Correlation Coefficient' was calculated.

2. Results

96.1% of the nurses participating in the study knew that the main effect of heparin is prevention of the formation of thrombus and 94.2% knew that heparin should not be administered to patients with bleeding or potential of bleeding. In addition, 90.3% knew heparin should be stored under 25 degrees centigrade and 89% knew that the antidote of heparin is protamine sulphate while 75.3% knew heparin antidote is administered in case of bleeding. % 62.32 of the nurses knew that heparin should not be administered when potassium level in blood is high. 54.5% of the nurses knew correctly that heparin should be administered in abdominal region. 37.7% of the nurses knew correctly that heparin should not be administered to patients with chronic renal failure.

It was established that a large majortity of nurses washed their hands before administering the drug (%93.5), put on gloves before administration (%96.8) and gave information to the patiens before administration of the drug (%99.4). %65.6 of the nurses made the administration correctly without stretching injection area and %53.9 kept heparin antidote handy.

Table 1. The answers of nurses to information questions on subcutaneous heparin injection

Questions on Heparin Injection	Correct Answer*	The number of those who know	
		N	%
In patients treated with heparin, black stool, decrease in HTc values, and in blood pressure, and changes in mental status suggest bleeding	**Correct**	148	%96,1
The primary effect of heparin is the prevention of the formation of thrombus	**Correct**	148	%96,1
Patients with bleeding or potential for bleeding are administered heparin	**Wrong**	145	%94,2
Heparin should be stored under 25 degree.	**Correct**	139	%90,3
Subcutaneous heparin should be administered to legs first	**Wrong**	139	%90,3
Air in the injector containing drug is removed	**Wrong**	139	%90,3
The andidote of heparin is protamin sulphate.	**Correct**	137	%89,0
Heparin can be stored under all conditions	**Wrong**	137	%89,0
Injection site should not be massaged after injection	**Correct**	132	%85,7
Allergy should be considered when tremor, fever, and urticaria occur in patients treated with heparin	**Correct**	123	%79,9
Heparin should not be administered to patients with very high blood pressure.	**Correct**	120	%78,0
Heparin antidote is not administered in case of bleeding	**Wrong**	116	%75,3
Injection regions should be grasped with fingers in a way it will be raised for 2-3 cm.	**Correct**	115	%74,7
Tissue of the injection site should be stretched	**Wrong**	109	%70,8
Injector should enter the tissue at a degree of 20-30	**Wrong**	109	%70,8
Heparin can be administered to patients with active tuberculosis	**Wrong**	104	%67,5
Heparin is used when potassium level is high in the blood	**Wrong**	96	%62,3
In subcutaneous injections, a region should be completely used before transferring to another region	**Wrong**	90	%58,4
Subcutaneous heparin should be primarily administered to abdominal region	**Correct**	84	%54,5
Subcutaneous heparin should be administered to the arm first	**Wrong**	79	%51,3
Heparin should not be administered to patients with chronic renal failure	**Correct**	58	%37,7

** Answers that should be given to this question.*

Mean information score of the nurses on heparin injection was 76.8 (minimum 47.6, maximum 100). Practice scores of the nurses was minimum 55.0 maxium 95.0 and mean 81,8. There was significant positive correlation between knowledge and practice scores of the nurses ($p<0,01$). It was established that as knowledge scores on heparin injections increase, practice scores increase as well.

3. Discussion

The safe and accurate administration of medications is one of the most important responsibilities of nurses. Patient safety is among the most important components of basic nursing care as in all members of health care team. Before initiating treatment, nurse should know the mecahnism of action, dose, and factors which influence correct usage, modify its effect and lead to the deevlopment of side effects (10). When the theoretical knowledege of the nurses participating in the study on heparin treatment is considered, it can be seen that almost all (96%) know that the basic effect of heparin is the prevention of the formation of thrombus (Table 1).

However, the fact that heparin should not be adminsitered in patients with chronic renal failure was known by less than half of the nurses (%37.7). As Heparin is eliminated a from the kidneys, it should not be used in patients with chronic renal failure or should be used cautiously (3,10).

62.3% of the nurses knew correctly that heparin should not be used in case of high potassium levels in blood (Table 1). According to the literature, heparin should not be used in patients with high blood potassium levels since it increases potasssi-

Table 2. Answers of the nurses to practice questions on subcutaneous heparin injection

Practice Questions on Heparin Injection	Correct Answer*	Those who know	
		N	%
I give information to the patienst before administration	**Yes**	153	%99,4
I tell the patienst to inform me when there is echymosis or bleeding at injection region	**Yes**	152	%98,7
I change the site of injection at each administration	**Yes**	151	%98,1
I evaluate injection area in terms of echymosis and hematoma	**Yes**	152	%98,7
I clean injection area with alcohol soaked sponge from inner region to outside	**Yes**	152	%98,7
I evaluate the response to injection and bleeding status	**Yes**	149	%96,8
I put on gloves before administering the drug	**Yes**	149	%96,8
After making injection, I let go the tissue I grasped and withdraw the injector at the same direction I entered	**Yes**	145	%94,2
I wash my hands before administering the drug	**Yes**	144	%93,5
I massage injection area for 1-2 minutes	**No**	144	%93,5
I keep record of the area, time of the injection and response of the patient	**Yes**	142	%92,2
For subcutaneous heparin injection, I choose a region outside 5cm square area around umbilicus in lower abdominal region	**Yes**	143	%92,9
I tell the patient to take deep breath before sticking the injector	**Yes**	136	%88,3
I do not try to recapping the needle, I throw it into medical disposal box	**Yes**	124	%80,5
I stick the injector at 45- 90 degree angle	**Yes**	123	%79,9
I administer the drug rapidly	**No**	114	%74.0
I stretch the area where I will make injection	**No**	101	%65,6
I keep Heparin antidote handy	**Yes**	83	%53,9
After sticking injector, I withdraw piston	**No**	65	%42,2
I administer SC heparin primarily to arm	**No**	63	%40,9

** Answers that should be given to this question*

Table 3. Heparin Injection Information and Practice scores of nurses (N: 154)

Variable	N	Min.	Max.	Mean	Standart deviation	Those who scoe under mean (N)	Those whom score over mean (N)
Heparin Injection Information score	154	47,6	100,0	76,8	11,31	88	66
Heparin Injection Practice score	154	55,0	95,0	81,8	8,24	60	94

um level in the blood and causes hypercalcemia (3,11). Almost all of the nurses participating in the investigation (%94.2) knew that heparin should not be administered to patients with bleeding or bleeding potential (Table 1). A large majority of the nurses knew that (96.1%) in patients administered Heparin, decrease in hematocrit levels, fall of blood pressure and changes in mental status suggest bleeding (Table 1). This shows that most of the theoretical knowledege of nurses on heparins related to bleeding since heparin is a drug directly associated with blood. It was determined that conditions, which were not direcly related to bleeding, such as high blood pressure, active tuberculosis, high blood potassium levels, chronic renal failure and allergy are known at a lesser degree by the nurses (Table 1). In the study Bursalı (2006), it was established that theoretical knowledge of the nurses regarding heparin was mostly on bleeding and that nurses have less knowledge of conditions not related to bleeding (3). The results of our investigation are congruent with those of Bursalı.

It is stated in the literature that regions suitable for subcutaneous injection are external aspect of upper arms, anterior aspect of thigh, scapula and lower abdominal region (8,12). Heparin is suggested to be administered on primarily abdominal region among all of these regions. In abdominal region, deep fatty tissue is more common, which allows easier rotation of injections sites. Therefo-

re, it is thought that echymosis problem will occur more rarely in injections made to this region (5,8). %54.5 of the nurses stated in their answers that the region where heparin injection will be made should be primarily the abdominal region (Table 1). When it comes to practice, it is seen that 59.1% of the nurses prefer arm as injection region (Table 2). In the study of Şenturan et al (2008), it was established that 56.6% of the nurses preferred upper arm as injection region (7). In the study carried out by Rızalar et al (2007), it was established that echyomosis occurred at the highest degree in upper arm region (12). The question on whether injection region is raised and lifted for 2-3cm. by fingers was answered correctly by 74.7% of the nurses (Table 1). When practice was evaluated, %65.6 of the nurses stated in their anwer that they did not strectch injection area, but grasped the tissue (Table 2). It is seen here that nurses can not reflect their knowledge to practice completely.

In the literature, it is established that subcutaneous heparing administration should be made to site at least 50 mm far from the previous one, without injury and scar tissue (3,9). In subcutanous heparin injection, 58.4% of the nurses stated that injection region should be changed at every administration (Table 1). In practice, 98.1% of the nurses stated that they changed the place of injection at each administration (Table 2). It is seen that information of the nurses on this subject is inadequate but, in their practice they carry out the correct procedure, which indicates that practice of nurses depends on their experince rather than on their theoretical information.

It is stated that as Heparin is an anticoagulant drug, aspiration should not be made to control bleeding status during administration and injection region should not be massaged. (1,5,7). Although %94.2 of the nurses participating in the study grasp the tissue during practice and let go of the tissue after administration of drug, only 42.2% withdraw the piston after injection, which indicates that there is a large lack of information on this issue (Table 2). In the study of Şenturan et al (2008), although %70.7 of the nurses grasp the tissue during injection, only 50.4% aspirated the tissue?, which is similar to our results (7).

74% of the nurses particapating in the study gave the answer that they did not administered the drug rapidly (Table 2). In studies, it was established that longer duration of injection procedure decreased the formation of ecchymosis and pain (5,6,13,14). Although exertion of pressure to injection region after the administration of heparin is recommended, performing massage is not recommended at all. Exertion of pressure to place of injection after subcutanous injection of heparin is stated to prevent drain back of blood from the region and to decrease the development of ecchymosis (3,5,8,10). Majority of the nurses participating in the study (%93.5) stated that they do not massage injection area (Table 2).

As the age and duration of work increase, knowledge scores of the nurses also increase. However, there is no significant difference in their practice, suggesting that knowledge increases with experience and experience is influential.

It was established that nurses who obtained highest knowledge scores on heparin injection worked in surgery clinics and those who obtained lowes scores of information worked in internall medicine clinics. As to practice scores, the highest scores were obtained by nurses working in intensive care units while those obtaining lowest scores worked in internal medicine clinics.

It is thought that nurses working in surgery clinics obtained high scores due to the fact that they carry out high number of subcutaneous heparin administrations.

Those who received training on heparin during inservice training were found to have higher knowledge and practice scores, indicating how beneficial inservice training is. Professional information partly loses its validity in time, while new information emerges. Therefore, it has become imperative for health workers to receive inservice training constantly and to continue their personal development through such systematic training (15). Reinforcement of the information obtained in basic training via inservice training will eneble nurses to conduct their practice in view of this information.

4. Conclusion

As the results obtained from our study directly influence the quality of nursing care, the following recommendation are thought to be beneficial for increasing information and practice levels of nurses regarding injection of heparin:

1. administration standards and protocols should be developed in clinics,
2. inservice training programs should be developed and should incorporate practice training as well,
3. manuals or booklets for training should be prepared in clinics,
4. it can be recommended that this issue should be considered more thoroughly in the theorical/ practical training of nursing students.

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Value of plasma VEGF, TPS and TuM2-PK in evaluating the efficacy of bronchial arterial infusion in patients with lung cancer

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Abstract

Objective: To investigate the evaluation value of plasma VEGF, TPS and TuM2-PK in bronchial arterial infusion (BAI) of patients with lung cancer.

Methods: Plasma VEGF, TPS and TuM2-PK were detected by ELISA in patients with lung cancer at 1 day before treatment and after treatment for 1,3,7,14 and 28 days, respectively. All the indicators were also detected in 30 healthy controls.

Results: The levels of plasma VEGF, TPS and TuM2-PK in different phase of lung cancer were all significantly higher than those in healthy controls, and the level increased with the staging of lung cancer (P<0.05). After BAI treatment, the levels of plasma VEGF, TPS and TuM2-PK in lung cancer patients were significantly higher than those before treatment (P<0.05). After BAI treatment for 28 days, the ORR was 45.0%, and the level of plasma VEGF, TPS and TuM2-PK increased with the decrease of clinical effect (P<0.05).

Conclusions: Plasma VEGF, TPS and TuM2-PK in patients with lung cancer is over-expressed, and positively related with severity of the condition. Combined detection of plasma VEGF, TPS and TuM2-PK can be served as an evaluation indicator for BAI.

Key words: Bronchial arterial infusion, Tumor marker, VEGF, TPS, TuM2-PK.

Introduction

Lung cancer, as one of the most common malignant tumors in the world, is a serious threat to human health and life[1]. Because of the difficulty in early diagnosis of lung cancer, most cases of the disease cannot be diagnosed until it developed to an advanced stage. Only 20% of the patients are suitable for surgical treatment[2], so the prognosis is poor. Bronchial artery infusion (BAI) provides a new therapy for lung cancer patients who can not be treated with the surgical treatment, and BAI is now widely used in clinical practices. Imaging examination is applied to monitor the efficacy of interventional therapy for lung cancer, but the technique is limited by high costs, great radiation, poor specificity and non-quantification[3]. With the progress on tumor markers in recent years, the roles of vascular endothelial growth factor (VEGF), tissue polypeptide specific antigen (TPS) and tumor M2 pyruvate kinase (TUM2-PK) in evaluating the efficacy of interventional therapy for lung cancer become a research hotspot[4,5]. In this study, the feasibility of using the plasma levels of VEGF, TPS and TuM2-PK to assess the therapeutic efficacy of interventional therapy for lung cancer is investigated, aiming to provide a simple indicators for identifying the efficacy of interventional therapy for lung cancer, and further provide a theoretical basis for interventional therapy combined with other treatments (such as molecular targeted treatment).

Material and Methods

General information

Sixty patients underwent BAI in our hospital from July 2011 to June 2012. Inclusion criteria: (1) The patients had evaluable tumor lesions confirmed by bronchoscopic biopsy or pathological diagnosis; (2) The patients were 18-80 years of age, and they did not receive radiotherapy, chemotherapy or molecular targeted therapy in 3 weeks before treatment; (3) The patients with severe diseases of heart, liver and kidney and poor general conditions were excluded. There were 36 males and 24 females with the age from 42 to 74 years and an average of (48.3±5.7) years. TNM staging included: 20 patients of stage II, 12 patients of stage IIIa, 17 patient of stage IIIb and 11 patients of stage IV. Pathological types included:

34 patients of squamous cell carcinoma, 21 patients of adenocarcinoma and 5 patients of small cell lung cancer. Thirty healthy examinees in the hospital at the same period were selected as a control group. The two groups of patients had no statistically significant differences in age, sex, TNM staging and pathological types (P>0.05), and they were comparable.

Interventional therapy

Seldinger method was applied for catheterization via femoral artery to perform arteriography in the bronchial artery, subclavian artery or intercostal artery. The catheter was super-selectively inserted into the tumor feeding artery according to the results of angiography. The drugs for chemotherapy were selected from any three of MMC, DDP, 5-FU and THP, and the dosage of medication was DDP 80-100 mg, MMC 14-20 mg, 5-FU 750-1000 mg THP 40-60 mg according to the general conditions and body surface area of the patients. The pattern of interventional therapy was selected based on lesion characteristics, such as for those whose lung cancer with abundant blood supply or hemoptysis, the chemoembolization (BAE) could be performed after avoiding possible connected spinal arteries when the catheter was inserted into the feeding artery.

Sample collection

The peripheral blood (3 mL) was taken from each lung cancer patient who was fasting in the morning at different time including 1 day before the BAI and 1, 3, 7, 14 and 28 days after the BAI. Each blood sample was placed in each 3 mL tube containing 0.057 mL 15% ethylene diamine tetraacetic acid tripotassium (K3EDTA). The tubes were centrifuged at low temperature within 2 h (2000 r/min, 10min) to separate the plasma, which were stored at -70 ℃. Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of VEGF, TPS and TuM2-PK in all the sample plasma. The detection kit was provided by CanAg, Sweden, and the determination procedures were carried out strictly in accordance with the instructions. The expression levels of VEGF, TPS and TuM2-PK in the plasma of the 30 healthy examinees were also determined.

Clinical efficacy evaluation

The clinical efficacy was evaluated according to RECIST criteria including complete remission (CR), partial remission (PR), stable (SD) and progress (PD). The value of CR+PR was applied to calculate the efficiency (ORR).

Statistical analysis

SPSS 18.0 software was applied for statistical analysis. T-test or analysis of variance was applied for comparing measurement data and P<0.05 was considered to be statistically significant.

Results

Levels of tumor markers in patients with lung cancer at different stages

The plasma levels of VEGF, TPS and TuM2-PK on were significantly higher in the patient group than in the control group (P<0.05). As the lung cancer worsened, the plasma levels of VEGF, TPS and TuM2-PK increased successively, and the differences were statistically significant (P<0.05) (Table 1).

Plasma levels of VEGF, TPS and TuM2-PK in lung cancer patients before and after interventional treatment

After interventional treatment, the plasma levels of VEGF, TPS and TuM2-PK significantly decreased compared with those before treatment, and the differences were statistically significant (P <0.05) (Table 2, Figure 1).

Table 1. Comparison of tumor marker levels in patients with lung cancer at different stages ($\bar{x} \pm s$)

Stage	n	VEGF (pg/ml)	TPS (pg/ml)	TuM2-PK (U/ml)
Control	30	195.13±86.84	47.81±21.65	8.52±3.21
Stage II	22	208.14±89.21①	75.92±21.25①	14.34±5.27①
Stage III	29	305.21±124.23①②	204.25±75.93①②	38.22±13.38①②
Stage IV	11	326.18±138.95①②③	287.94±83.76①②③	53.22±21.54①②③

Compared with the control group, ①P<0.05; compared with the Stage III, ②P<0.05; compared with the Stage IV, ③P<0.05

Table 2. Plasma levels of VEGF, TPS and TuM2-PK in lung cancer patients and healthy controls before and after interventional treatment ($\bar{x}$ ±s)

	VEGF (pg/ml)	TPS (pg/ml)	TuM2-PK (U/ml)
Control group (n=30)	195.13±86.84	47.81±21.65	8.52±3.21
Treatment group (n=60)			
1 day before	314.22±136.23	267.25±81.14	49.25±21.53
1 day	281.89±115.54	238.54±71.62	41.32±18.97
3 day	262.72±116.36	208.45±66.54	37.23±20.64
7 day	253.12±119.87	167.81±54.61	29.84±14.95
14 day	235.49±106.17	108.22±42.14	20.27±10.63
28 day	215.13±97.57	57.34±26.78	13.25±7.53

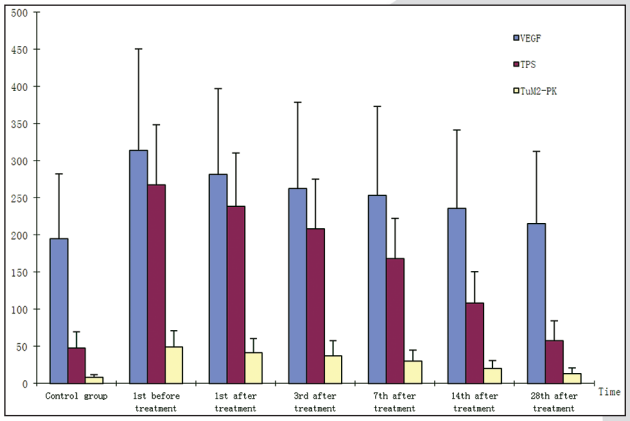

Figure 1. Comparison of plasma VEGF, TPS and TuM2-PK before and after treatment between control group and treatment group

Short-term efficacy

In the 60 patients receiving interventional treatment, there were no CR, PR in 27 patients, NC in 17 patients and PD in 6 patients, and the ORR was 45.0%. After the treatment for 28 days, the plasma levels of VEGF, TPS and TuM2-PK were significantly different among the patients with different efficacy (P<0.05), and along with the decline of the efficacy, the plasma levels of VEGF, TPS and TuM2-PK gradually increased (P<0.05) (Table 3).

Discussions

In recent years, with the continuous progress of interventional radiology development in the diagnosis and treatment of lung cancer and intervention techniques, the interventional therapy has gradually become one of the important means in treatment of patients with advanced lung cancer [6]. BAI can effectively improve local drug concentration in tumor, reduce or occlude tumor feeding artery, and exacerbate tumor necrosis [7], but the profile for evaluating BAI efficacy still needs further investigation. Until now, the efficacy of interventional therapy is mainly monitored by imaging examination, which has many shortcomings including high cost, ray contaction, non-quantification, unable to reflect tumor tissue necrosis, and without specific tumor markers. Therefore, exploring reproducible, quantifiable and simply detected parameters that can timely and effectively reflect BAI efficacy is significant in clinical work.

It has been widely accepted for the value of tumor markers for early diagnosis of lung cancer, but the options of tumor marker combinations and their role in evaluating the efficacy of BAI are still not clear. VEGF is known to be the strongest vascular endothelial growth factor in vivo, and tumor tissue

Table 3. Comparison of plasma levels of VEGF, TPS and TuM2-PK among the patients with different efficacy ($\bar{x}$ ±s)

Tumor markers	Efficacy		
	PR (n=27)	NC (n=17)	PD (n=6)
VEGF (pg/ml)	201.25±81.37	224.19±92.82①	258.97±94.72①②
TPS (pg/ml)	50.82±24.94	78.75±29.25①	109.34±36.28①②
TuM2-PK (U/ml)	10.22±6.14	17.28±7.23①	30.32±9.97①②

Compared with PR, ①P<0.05; compared with NC, ②P<0.05

ischemia and hypoxia can increase VEGF secretion. VEGF can promote tumor metastasis, and is highly expressed in advanced lung cancer, so it is used for clinical tumor diagnosis and differential diagnosis [8]. TPS is a tissue polypeptide antigen that is highly relevant to cytokeratin 18, and it is highly expressed in the different stages of tumors. TPS plasma level may reflect tumor cell division and proliferation activity, which is helpful for early detection of cancer and provides important clues for tumor relapse and efficacy monitoring [9, 10]. TuM2-PK is a newly discovered tumor markers, which is elevated in the plasma of patients with various types of tumors [11-14].

In this study, we detected the plasma levels of VEGF, TPS and TuM2-PK in lung cancer patients and their dynamic changes after interventional treatment. On the basis of the degree of tumor tissue hypoxia and necrosis, we further determined the efficacy of interventional therapy. The results showed the different stages of lung cancer patients whose plasma levels of VEGF, TPS and TuM2-PK were significantly higher than those of the control group ($P<0.05$), and the marker levels increased successively along with the lung cancer worsened ($P<0.05$), indicating the consistency between the marker levels and the degree of lung cancer development. The plasma levels of VEGF, TPS and TuM2-PK in lung cancer patients significantly reduced after BAI treatment than before treatment ($P<0.05$). When the patients' conditions was under control or relieved after BAI, the plasma levels of VEGF, TPS and TuM2-PK had varying degrees of decrease even close to the normal. The markers above have a high sensitivity to lung cancer, and the detection of their changes has a certain role in the efficacy evaluation of BAI. In this study, the ORR was 45.0% after BAI treatment for 28 days, and the plasma levels of VEGF, TPS and TuM2-PK gradually increased along with the efficacy decline ($P<0.05$). This result further confirms the plasma levels of VEGF, TPS and TuM2-PK can be applied as indicators for BAI efficacy assessment.

Taken together, the patients with lung cancer have high plasma levels of VEGF, TPS and TuM2-PK, and their expression is closely related to the progress of lung cancer. Combined detection of VEGF, TPS, and TuM2-PK plays an important role in dynamic monitoring of lung cancer and BAI efficacy evaluation.

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Autologous versus allogeneic transfusion in large-scale ortopedic surgery

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Abstract

Aim: The aim of this study was to prove efficiency, safety, cost-effectiveness, and (indirect) impact on the patients' outcome of autologous transfusion in patients who had the total hip replacement surgery.

Methods: During the controlled, prospective, randomised study we compared two groups of patients who had the total prosthesis implanted in their hip, in total there were 84 of them. The first group consisted of the patients who received the transfusion of other people's (allogeneic) blood (n=47) and the other one consisted of the patients whose blood was collected perioperatively (their own, autologous blood) (n=37). Transfusion trigger for both groups was haemoglobin level of 90 g/l.

Results: In the group of patients whose blood was collected perioperatively 32,43% received the transfusion of allogeneic blood, as opposed to the control group in which 95,74% received the transfusion of allogeneic blood ($p \leq 0,01$). For the group of patients whose blood was collected the number of days spent in hospital was 6,78, while for the control group it was 7,28 days.

Conclusion: Our conclusion is that during the total hip replacement surgery perioperative autologous blood collection is very effective measure in reducing consumption of allogeneic blood ,reduces likelihood of complications related to application of allogenic transfusion (indirect), and have positive effect on patient's overall clinical outcome.

Key words: Transfusion, allogeneic, autologous, blood, hip replacement.

Introduction

Total hip replacement (THR) surgery is one of the most frequent and extensive procedures in orthopaedic surgery, accompanied with some serious complications. Perioperative blood loss is one of the most serious losses, so it is vital to recognize and treat such losses properly. The timely and precise treatment of perioperative blood losses has an impact of the patients' outcome and the quality of postoperative recovery(1,2,3). The transfusion of allogeneic blood carries along certain risks, such as allergic reactions, anaphylaxis, hemolitic reactions, transmissive diseases, transfusion related lung injury (TRALI), graft-versus-host disease, etc. (4,5,6,9,11). In the past few decades a lot of effort has been made to find a solution to the problems connected with allogeneic transfusion. One of the alternatives is autologous blood transfusion, which is widely accepted as probably the only true alternative for the allogeneic blood. The justification for the use of this alternative method could be found in a certain level of morbidity and mortality which accompanies allogeneic blood transfusions. (7, 10, 12, 13, 17).

The autologous blood transfusion is a collection and re-infusion (transfusion) of the patient's own blood or blood components before, during or after surgical procedure. So the donor and recipient of the blood is the same person. Although not completely risk-free, autologous blood is the safest blood donation (8).

According to the American Association of Blood Banks the most important strategies for blood donation are**:** preoperative autologous donation, acute normovolemic hemodilution, and perioperative blood salvation. (15, 16).

Perioperative blood salvage is intraoperative collection by aspiration from the operative fields and postoperative collection of the blood from the wound drains (14).

Autologous transfusion is indicated in certain surgeries when we expect to have major blood losses. The prerequisite for this procedure is that there is no wound or systematic infection and normal hemoglobin levels in the patient's blood.

Total hip replacement surgery is one of the most serious operations in orthopaedic surgery. Frecvently accompanied with serious intraope-

rative and postoperative bleeding, so autologous blood salvation takes place from the collection by aspiration from the operative fields and from the wound drains in the perioperative period (8,10).

Our goal was to improve our everyday clinical practice, and this study will contribute to our better understanding of perioperative blood loss and its treatment in THR surgery with the ultimate goal to affect patients' outcome in a positive way.

The aim of this study was to prove efficiency, safety, cost – effectiveness, and impact on the patient's outcome of autologous transfusion.

Methods

This was a single-centre (Clinic for Orthopaedic Surgery and Traumatology-Clinical Center of Voivodina, Novi Sad), prospective, randomised, controlled study conducted on the patients undergoing THR surgery.

After review and approval by the Local Research Ethics Committee, we obtained informed consent and studied 84 patients, during the period of three months during 2010. Patients were randomely placed in two treatment groups, the first one receiving allogeneic blood (n=47), and the second receiving autologous blood (n=37).

The transfusion trigger for the group that received allogeneic blood was of 90g/l (1, 2, 3, and 4). We chose this value for the hemoglobin trigger because the majority of our patients are elderly people, usually with comorbidities. For the group that received autologous (their own) transfusion, blood was collected perioperatively, from the collection by aspiration from the operative filds (intraoperatively) and from the wound drains postoperatively during the period of four hours. The minimal amount of drained blood was ≥200 ml for the process to be successful (according to the manufacturer's manual and our experience). We used *Cell Saver* (*Haemonetics 5+,* USA) apparatus; the blood was collected, processed and re-infused to the patients. For this purpose we had allocated trained anesthesiology technician. One unit of autologous blood was 250 ml.

Hemoglobin levels were measured preoperatively and postoperatively after 6, 12 and 24 hours for all patients. Preoperatively, as well as 24 hours after the surgery, we measured APTT (activated partial thromboplastin time) and PT (prothrombin time).

The THR surgery was performed as a routine. The patients underwent general (balanced) anesthesia or spinal anesthesia, which have been standardized in terms of drugs and procedures.

Intraoperative blood losses were measured as losses in gauze and as losses in the hood.

Postoperative blood losses were measured as losses in wound drains during the period of first 48 hrs after the surgery for all patients, accompanied with the clinical examination of the patients.

We recorded the time when patients sat, stood, walked and had their meal for the first time after the surgery, by this we indirectly measured the quality of postoperative recovery. The length of staying in hospital was also recorded. The exclusion criteria for this study were: patients with septic complications, multiple fractures, malignancy, ASA physical status classification IV or more, hemiarthroplasty and all patients with incomplete data.

All data were analyzed in SPSS 16.0 software package. All frequencies, percentages, and median standard deviation were calculated. Binary variables were comparated by $\chi 2$ test, continuous variable were compared by Fisher's Exact test and the t-test. A statistically significant difference was defined as p value < 0, 05. All data (text, tables, and charts) were arranged by *Microsoft Word 2003 and Microsoft Excel 2003*.

Results

Out of 84 patients, 53 were women and 31 were men. For the purpose of this study we compared the age, gender, ASA status, comorbidities, chronic NSAID/aspirin use, anesthesia method, the type of prosthetic material, perioperative levels of hemoglobin, hematocrit, trombocite count, the mechanism of hemostasis, blood losses, the number of blood units of allogeneic and autologous blood per each patient, the time when patients sat, stood, walked and had their meals for the first time after the surgery, as well as the length of staying in hospital, in order to see how they affected the outcome of these patients.

In both groups the majority of patients were women 53 (63, 1%).

American Society of Anesthesiologists (ASA) status ASA III patients were most frequent in both groups – 45 patients in total (53, 6%). There were no ASA I patients.

Table 1. Patient's characteristics

	Autologous group	Allogeneic group	p value
Mean age	64,7(41-85)	63,0(39-80)	0,05
Gender			
male	12(32,4%)	19(40,4%)	
female	25(67,6%)	28(59,6%)	
Methods of anesthesia			
general	14(37,8%)	16(34,0%)	0,05
spinal	23(62,2%)	31(66,0%)	0,05
Type of kne prothesis			
cemented	27(73,0%)	19(40,4%)	0,05
cementless	10(27,0%)	28(59,6%)	

Table 2. Preoperative and postoperative values of haemoglobin, hematocrit, trombocytes, APTT and PT

	Autologous group	Allogeneic group	p value
Hemoglobin (g/l)			
preoperatively	134,24	134,43	>0,05
postoperatively:			
6 hrs	115,76	119,68	>0,05
24 hrs	106,24	112,74	>0,05
48 hrs	97,41	109,37	<0,01
Hematocrit (g/l)			
preoperativno	39,43	38,82	>0,05
postoperatively:			
6 hrs	33,81	34,76	>0,05
24 hrs	30,86	32,79	>0,05
48 hrs	28,31	31,41	<0,01
Trombocytes			
preoperatively	256,73	251,04	>0,05
postoperatively:			
6 hrs	191,24	199,66	>0,05
24 hrs	181,95	192,98	>0,05
48 hrs	164,81	191,89	>0,05
APTT*			
preoperatively	0,973(0,75-1,41)	0,932(0,79-1,15)	>0,05
after 24 hrs	0,959(0,57- 1,45)	0,965(0,83-1,26)	>0,05
PT *			
preoperatively	0,958(0,10-1,36)	0,991(0,83-1,17)	>0,05
after 24 hrs	1,119(0,89-1,52)	0,999(0,10-1,23)	>0,01
Blood losses			
intraoperatively	802,70(300- 1500)	800,00(400-1500)	>0,05
postoperatively:			
after 6 hrs	481,08(100-1100)	433,40(100-1050)	>0,05
after 24 hrs	264,71(50-700)	404,26(150-1250)	>0,01
after 48 hrs	170,83(50-400)	240,54(100-450)	>0,05

** APTT (activated partial thromboplastin time) and PT (prothrombin time)*

The majority of patients received spinal anesthesia - 54 patients (64,3%), and 30 patients (35,7%) received general anesthesia.

Comorbidities were very frequent, 64 patients (76,2%), in both groups, had some comorbidites, 20 patients (23, 8%) had none.

Almost all patients in both groups used some Aspirin or NSAIDs, only tree patient did not use any of these drugs. When we compared hemoglobin and hematocrit levels measured postoperatively, there was a significant difference after 48 hrs in favour of the allogeneic group.

Table 3. Number of blood units in autologous and allogeneic group

	Autologous group	Alogeneic group
Allogeneic blood (units)		
1 unit	7(58,3%)	2(4,4%)
2 units	4(33,3%)	31(68,9%)
> 3 units	1(8,3%)	12(26,7%)
Autologous blood (units)		
1 unit	13(35,1%)	0
2 units	21(56,8%)	0
3 units	2(5,4%)	0
4 units	1(2,7%)	0

Values of PT 24 hrs postoperatively were higher and showed a significant difference in the autologous group. That can be explained by blood processing in the autologous transfusion method. In this process only „washed" eritrocites are reinfused back to the patient, and the rest of plasma and coagulation factors, with cell detritus, anticouagulants, normal saline and bone micro fragments are disposed and wasted.

The transfusion triggers in both groups were hemoglobin levels of ≤90 g/l.

Out of 37 patients in the autologous group, 12 patients (32,44%) received the additional transfusion of allogeneic blood (n= 18 units of allogeneic blood), so that means that 25 patients (67,56%) received only their own (autologous) blood.

In the allogeneic (control) group 45 patients received allogeneic blood, 31 patients (68, 9%), patients received 2 units of allogeneic blood, 2 patients (4, 4%) received 1 unit of allogeneic blood, 12 patients (26,7%) received more than three units of allogeneic blood. Only two patient did not receive allogeneic blood.

In the autologous group the majority of patients received one or two units of autologous blood (n= 34 patients), two patients got 3 units of autologous transfusion and only one patients got 4 units of autologous transfusion.

The time when patients sat, stood, walked and had their meal for the first time after the surgery served as the indirect indicator of quality of postoperative recovery. In the autologous group patients sat, stood and walked earlier (p<0,001) than in the allogenic group. In this group, patients were able to eat (their first meal) 17,45 hrs earlier than in the allogeneic (control) group (p<0,001).

An average hospital stay in the autologous group was 6,78 days, and in the allogeneic (control group) 7,28 days. The final decision about when the patient was going to be discharged from hospital was on attending the surgeon, and our team, being researchers, had no impact on it.

We analysed deep venous thrombosis (DVT), pulmonary tromboembolic complications, sepsis, wound infection and major cardiovascular complications. Perioperative complications were rare and there were only two cases in the allogeneic (control) group. One was the wound infection and other was chest pain with no major morbidity. In the autologous group the were no complications .

Discussion

The average patient in our study was female, 66.5 years old, ASA III status with comorbidities and chronic usage of NSAID's or Aspirin. These data are consistent with the fact that indications for THR surgery are degenerative diseases of the hip, which are painful states, more frequent in elderly females(18,19).

We think that the most important result of our study is one showing that autologous transfusion is very effective measure in reducing consumption of allogeneic blood. In the allogeneic (control) group 95.74% of patients postoperatively received allogeneic blood transfusion. In the au-

Table 4. Postoperative recovery

	Autologous group	Allogenicgroup	p value
Postoperative recovery (hrs)			
sitting	10,22(8-24)	24,00(24-24)	<0,01
stending	17,24(12-24)	24,26(24-36)	<0,01
walking	18,86(12-48)	27,13(24-48)	<0,01
eating	9,68(4-24)	27,13(24-48)	<0,01
Hospital stay (days)	6,78(3-13)	7,28(1-23)	>0,05

tologous group of patients 32,43% received allogeneic blood transfusion, which is almost tri times reduction in the consumption of this type of blood (12, 20, 21) .

There is a shortage of blood everywhere in the world, the same situation is in Serbia, so every method that reduces consumption of allogeneic blood is of vital importance. In our country blood donation is voluntary, which leads to a wrong conclusion that blood itself is free of charge. The process of making blood and blood components safe is an expensive part of blood production. The analysis of cost-effectiveness of autologous transfusion was not the aim of this study, and it is very hard to analyse it because there is no official data about the cost of blood in our country. Today, blood and blood products are safer than ever, but still there are some known morbidities and mortalities connected to allogeneic transfusion, so by reducing its consumption we reduce those risks. Autologous transfusion has its own costs, but an additional justification for its usage can be found in the improved safety of this method.

The patients in the autologous group lost significantly less blood in general. There is also a significant difference in the postoperative blood loss between two groups 24 and 48 hrs after the surgery; the patients in autologous group lost less blood too.

Postoperative recovery after THR surgery is multifactorial, but there is a statistically significant difference in the speed and quality of postoperative recovery in the autologous group, accompanied with fewer complications (22). Unfortunately, we were not able to follow the patients and complication rate after hospital dismissal, and we know that such data (collected for the first six months postoperatively at least) could be very important for the introspection of this method itself. Staying in hospital is reduced in this group of patients.

However, this study has some limitations. In spite of the local study recommendations for the transfusion trigger of 90g/l, sometimes this strict protocol was not followed and some surgeons are still reluctant to apply these recommendations since they consider them too low. This fact maybe explains higher hemoglobin levels in the allogeneic (control) group, because there is a strict protocol appliance in the autologous group controlled by the anesthesia technician and anesthesiologist.

The length of staying in hospital is reduced in the autologous group of patients. There is no consensus in our clinic about how long patients should be hospitalized after THR surgery, so this decision was made by consulting the surgeon as well.

Conclusion

Autologous blood transfusion is a effective method for reducing allogenic blood use, as well as for preventing of allogenic blood induced side effects and complications. This research confirms clear benefit of this particular method.

When we compare our results with recent studies, we can conclude that they are consistent.

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Does N-acetyl cysteine prevent the changes in kidney functions caused by Sevoflurane?

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Abstract

Objectives: Sevoflurane is preferred for both induction and maintenance of anesthesia. With the studies previously carried out, it has been demonstrated that sevoflurane may lead to nephrotoxicity. However, in the literature, there is no study investigating whether or not N-acetyl cysteine prevents the changes in kidney functions, which are caused by sevoflurane. In this study, we aimed to ascertain whether sevoflurane-induced nephrotoxicity can be hindered by N-acetyl cysteine.

Materials and Methods: Sixty patients to be performed surgical operations under sevoflurane anesthesia were divided into two groups. The patients in one of these groups (Group 1) were given intravenously 20 mg/kg of N-acetyl cysteine 30 minutes prior to the induction of general anesthesia, those in the other group (Group 2) were given isotonic solution. Preoperative and postoperative 24-hour blood samples were analyzed for BUN, creatinine, Na, K and GGT; the urine samples were tested for N-acetyl-β-D glucosaminidase (NAG), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), beta 2 microglobulin (β2-μG), microalbumin and protein.

Results: The blood BUN, creatinine and the urine creatinine levels obtained at 24. hour were elevated within physiological ranges in comparison with preoperative values in both groups, but this was not significant. The urine LDH and protein values at postoperative 24 hour were elevated in physiological ranges in both groups, however, this increase was statistically significant only in the group 2.The urine β2 microglobulin and N-acetyl-β-D glucosaminidase values measured at postoperative 24 hour were significantly elevated in physiological ranges in both groups ($p<0.05$). The urine micro albumin values measured at postoperative 24 hour were significantly elevated in both groups, but this increase was statistically significant only in the group 1 ($p<0.05$).

Conclusions: We think that sevoflurane effects particularly renal tubular functions and N-acetyl cysteine cannot prevent this effect.

Key words: Sevoflurane, renal function tests, N-acetyl cysteine.

Introduction

Sevoflurane is one of the halogenated inhaled anesthetics, which is a fluorinated derivative of methyl isopropyl ether (1). Sevoflurane is metabolized to inorganic fluorine and hexafluoroisopropanol in *in vivo* conditions, whilst it produces in vitro 5 distinct compounds (compound A, B, C, D, and E) interacting with bara-lime and soda-lime. Until recently, inorganic fluoride has been thought to be the aetiological agent responsible for fluorinated anaesthetic nephrotoxicity, with a toxic concentration threshold of 50 μmol/L in serum. However, studies of sevoflurane administration in animals and humans have not shown evidence of fluoride-induced nephrotoxicity, despite serum fluoride concentrations in this range. Compound A (fluoromethyl-2,2-difluoro-1-[trifluoromethyl] vinyl ether) is a breakdown product of sevoflurane produced by its interaction with carbon dioxide absorbents in the anaesthesia machine. Compound A produces evidence of transient renal injury in rats. The mechanism of compound A renal toxicity is controversial, with the debate focused on the role of the renal cysteine conjugate β-lyase pathway in the biotransformation of compound A. The significance of this debate centres on the fact that the β-lyase pathway is 10- to 30-fold less active in humans than in rats. Therefore, if biotransformation by this pathway is responsible for the production of nephrotoxic metabolites of compound A, humans may be less susceptible to compound A renal toxicity than are rats (2, 3, 4, 5).

The compound A generates glutathione S conjugates reacting with liver-origin glutathione. Both

compound A and other S conjugates cause renal necrosis, albuminuria, glycosuria and enzymuria (6).

N-acetyl cysteine is an effective antioxidant agent. It leads to intracellular accumulation of sulfhydryl, plays a role as a precursor of reduced glutathione, augments the activity of superoxide dismutase, and prevents autocatalytic lipid peroxidation (7). Previous studies have shown that sevoflurane causes impairments in kidney functions. However, in the literature, there is no study investigating whether N-acetyl cysteine hinders the changes in kidney functions, which are caused by sevoflurane. In the present trial, we aimed to study whether N-acetyl cysteine prevents the sevoflurane-induced changes in kidney functions.

Materials and methods

This study, which was supported by Scientific Research Projects Management and Coordination Unit at Yuzuncu Yil University as the project no.TFU-132, was conducted in the operating room after receiving the approval of Yuzuncu Yil University Ethics Committee and the written consents of the patients.

The study included 60 patients who were aged between 20-60 years, undergoing elective surgeries lasting more than 2 hours, and were provided general anesthesia with sevoflurane. The patients having any cardiac disorder, hypertension, history of goiter, undergone surgery in the past 6 months and kidney or liver diseases were excluded from the study. Vascular access was provided with a 22 G cannula and 5-10 ml/kg/h infusion of 0.9% sodium chloride (NaCl) was started. The patients were randomly divided into two groups. Thirty minutes before the induction of anesthesia, the patients in one of these groups were given intravenously 20 mg/kg of N-acetyl cysteine (Group 1) and those in the other group were given intravenously isotonic solution (Group 2).

The patients were positioned on the operating table and performed routine anesthesia monitoring, electrocardiography (ECG), pulse oximetry (SpO2), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and BIS monitoring. The induction of anesthesia was achieved with 2 μgr/kg of fentanyl, 2 mg/kg of propofol, 0.1 mg/kg of vecuronium. The orotracheal intubation was done without any problem. For the maintenance of anesthesia, it was used 60% N2O + 40% O2 4-6 L/min, 1-2% sevoflurane and in every 45 minutes 0,7 μgr/kg of fentanyl, 0,03 mg/kg of vecuronium, sustaining BIS value in a range from 40 to 60.

Totally two blood and urine samples were collected from the patients in the preoperative period and 24 hours after the induction of anesthesia. The blood samples collected into biochemistry tubes were centrifuged at 3500 rpm for 10 minutes; the serum specimens separated and the urine samples were stored at -20°C. The samples collected were analyzed for blood BUN, creatinine, Na, K, GGT and urine N-acetyl-β-D glucosaminidase (NAG), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), beta 2 microglobulin (β۲-μG), micro albumin and protein.

The urine β2-μG levels were analyzed on Immulite 2000 (USA) analyzer using chemiluminescence technique with commercially available kits, NAG was tested manually and calculate by proportioning to the urine creatinine level. The levels of Na, K, GGT, BUN, and creatinine was measured on Roche Cobalt Integra 800 Modular PP auto-analyzer using commercially available kits.

Statistical analysis

The descriptive statistics for continuous variables were presented as mean, standard deviation, minimum and maximum values; whilst categorical variables were presented as number and percentage. To determine if there was a difference between the drugs and the times of measurements for continuous variables, it was done two-factor repeated measures analysis of variance, in which one of the factors is repetitive. After the analysis of variance, Tukey's multiple comparison test was used to determine different measurement times. p value <0.05 was accepted to be statistically significant.

Results

There was no statistically significant difference between the groups with regard to demographic data. The demographic data of the patients and surgical procedures performed are shown in Table 1.

In comparisons between the groups, there was no significant difference with regard to SBP, DBP,

MAP, KAH and BIS values.The values of preoperative and postoperative 24. hour blood parameters of the patients are shown in Table 2.

Table 1. Demographic data of patients

	Group 1 (n=30)	Group 2 (n=30)
Age(year)	36.7 ± 13.42	33.4 ± 10.36
Gender (M/F)	15/15	15/15
ASA I/II	13/17	12/18

ASA: American Society of Anesthesiologists classification

There was no significant difference between the groups with regard to BUN values. In in-group comparison, BUN values were elevated within physiologic ranges at postoperative 24. hour in comparison with preoperative values in both groups. In comparisons between the groups, preoperative blood creatinine levels, although within physiological ranges, were significantly higher in the group 1 than the group 2. In in-group comparison, the blood creatinine levels obtained at 24. Hour were elevated within physiological ranges in comparison with preoperative values in both groups, but this was not significant. In comparisons between and intra the groups, there was no significant difference between preoperative and postoperative 24. hour levels with regard to blood sodium (Na^{++}) values. There was no significant difference between the groups with regard to blood potassium (K^{+}) values. In in-group comparison, the blood K^{+} values measured at postoperative 24. hour were elevated in physiological ranges in comparison with preoperative levels in both groups, however, this condition was significant only in the group 2 ($p<0.05$).

Table 3 shows the values of preoperative and postoperative 24-hour urine parameters of the patients from both groups.

There was no significant difference between the groups with regard to the urine creatinine levels. In in-group comparison, the urine creatinine levels measured at postoperative 24 hour were elevated in physiological ranges in comparison with preoperative levels in both groups, however, this condition was not statistically significant.

There was no significant difference between the groups with regard to urine LDH values. In in-group comparison, the urine LDH values measured

Table 2. The values of preoperative and postoperative 24.hour blood parameters of patients

	Group 1 (n=30)		Group 2 (n=30)	
	Preoperative blood	Postoperative blood (24. hour)	Peoperative blood	Postoperative blood (24.hour)
BUN(mg/dL)	12,02 ± 4,3	Δ 15,4 ± 6,3	11,5 ± 2,8	# 13,3 ± 4,0
Creatinine(mg/dL)	Δ 0.77 ± 0.3	0.86 ± 0.4	0.62 ± 0.2	0.87 ± 0.8
Sodium (mmol/L)	137.7 ± 6.5	140.6 ± 6.6	136.8 ± 6.7	137.9 ± 6.2
Potassium(mmol/L)	3.7 ± 0.4	3.9 ± 0.6	3.6 ± 0.4	#3.9 ± 0.4

Δ: In in-group 1 comparison p<0.05
#: In in-group 2 comparison p<0.05
**: Comparison between the groups p<0.05*

Table 3. The values of preoperative and postoperative 24. hour urine parameters of patients

	Group 1		Group 2	
	Preoperative Urine	Postoperative Urine (24. hour)	Preoperative Urine	Postoperative Urine (24. hour)
Creatinine	95.8 ± 63.0	116.7 ± 98.2	90.0 ± 63.5	111.8 ± 86.7
Protein	82.8 ± 76.5	Δ 126.2 ± 123	99.0 ± 86.5	106.1 ± 76.7
LDH (U/L)	6.0 ± 9.1	8.7 ± 13.2	7.03 ± 9.2	#11.6 ± 13.9
β2-μG (U/L)	54.7 ± 49.6	Δ 129.7± 213.5	77.4 ± 125.8	#165.3 ± 220.1
NAG (mg/dL)	5,00 ± 3,2	Δ 6,5 ± 3,2	4,8 ± 3,3	#6,2± 2,9
μA (mg/dL)	17.8 ± 30.6	Δ 31.3 ± 46.5	16.2 ± 27.6	21.5 ± 28.8

Δ: In in-group 1 comparison p<0.05
#: In in-group 2 comparison p<0.05
**: Comparison between the groups p<0.05*

at postoperative 24 hour were elevated in physiological ranges in comparison with preoperative levels in both groups, however, this increase was statistically significant only in the group 2 ($p<0.05$).

There was no significant difference between the groups with regard to the urine β2 microglobulin values. In in-group comparison, the urine β2 microglobulin values measured at postoperative 24 hour were significantly elevated in physiological ranges in comparison with preoperative levels in both groups ($p<0.05$).

There was no significant difference between the groups with regard to the urine N-acetyl-β-D glucosaminidase (NAG) values. In in-group comparison, the urine N-acetyl-β-D glucosaminidase (NAG) values measured at postoperative 24 hour were significantly elevated in physiological ranges in comparison with preoperative levels in both groups ($p<0.05$).

There was no significant difference between the groups with regard to the urine micro albumin values. In in-group comparison, the urine micro albumin values measured at postoperative 24 hour were significantly elevated in physiological ranges in comparison with preoperative levels in both groups, but this increase was statistically significant only in the group 1 ($p<0.05$).

There was no significant difference between the groups with regard to the urine protein values. In in-group comparison, the urine protein values measured at postoperative 24 hour were significantly elevated in physiological ranges in comparison with preoperative levels in both groups, but this increase was statistically significant only in the group 1 ($p<0.05$).

Discussion

The tests representing renal functions should be interpreted properly and accurately. A transient impairment in renal function tests even in the absence of any histopathologic damage may lead to physiological conditions that can be presumed as renal injury or toxicity (8). Sevoflurane produces its effects on the kidney via the level of fluorine and the compound A. However, in our study, the levels of fluorine and compound A could not have been analyzed. Instead, our study was designed to being based on the parameters reflecting renal functions.

The first step of metabolic pathway of haloalkanes is identical in humans and rats; the glutathione mechanism occurring in the liver is crucial for renal damage. The nephrotoxic effect of the compound A is reversible and can be returned to normal levels and morphology 4-5 days after the administration (9,10).

NAC is the precursor of glutathione (γ-glutamyl-cysteinyl-glycine) (GSH), an intracellular tripeptide, and markedly augments the activity of glutathione S-transferase in the liver. This activity is fundamental for antioxidant, anti-carcinogenic and anti-mutagenic effects of this agent (11). The clinical studies carried out have demonstrated that it is a very safe drug and plays a preventive role in several experimental renal failure models. We aimed in our study to utilize potentially beneficial effects of NAC against possible kidney injury that can occur with sevoflurane anesthesia.

BUN is a simple and significant monitoring mean in renal dysfunction and used together with serum creatinine concentration. Creatinine is the standard laboratory test to recognize kidney diseases. As GFR reduces, serum creatinine concentration increases (12). There are authors asserting that BUN and creatinine are not necessarily increased in sevoflurane toxicity (13), on the other hand, there are authors claiming that renal damage does not occur as long as these parameters do not alter (14). Mazze et al. asserted that increased BUN and creatinine was an important criterion reflecting preoperative and postoperative renal functions, however, the levels of BUN ad creatinine were not correlated with renal toxicity due to sevoflurane anesthesia (15).

Keller et al. reported that after nasal administration of 0, 30, 61, 114 or 202 ppm of compound A with 3-hour intervals to the rats, BUN and creatinine levels were increased by only high doses (202 ppm) (10). In a study, Kumano et al. (16) investigated the effects of the administration of 2.17 and 1.29 MAC sevoflurane for less than 4 hour on the renal tubular functions and the authors did not find any significant changes in BUN and creatinine levels. Neither Guler et al. (17), in a study evaluating the kidney functions on the days 1, 3, and 7 using 1-2,5% sevoflurane, nor Obata et al. (14), in a study evaluating kidney functions after the use of low- and high-flow sevoflurane (1 and

6-10 L/ minute, respectively) in surgical operations lasting over 10 hours, did find any alterations in BUN, creatinine concentrations and in creatinine clearance.

In a study, Lawrence et al. gave sevoflurane to monkeys respiring spontaneously for 3 hours a day 3 times a week throughout 8 weeks and they did not find any increase in urea and creatinine levels (18). In our study, although there was no difference between the groups with regard to BUN levels, it was found that BUN values measured at postoperative 24. hour were significantly elevated within physiologic ranges in comparison with preoperative values in both groups. There were no differences between the groups with regard to plasma and urine creatinine levels. It was likely to be due to that the amount of standard fluid remained insufficient since the duration of fasting varied among the patients.

An increased urine level of β۲-μG is an early indicator of proximal tubule functions and minimal injury (18,19,20). In a study, Sekeroğlu et al. investigated the effects of sevoflurane with 6 lt/ min flow rate and 2% MAC and found that the level of β2-μG did not change at the first, second, and eighth hours. They denoted that sevoflurane did not have any significant effect on biochemical parameters and renal tubular damage since this increase returned to normal during the late period of anesthesia (22). Nishiyama et al. did not find any significant increase in urine β2-μG levels in patients exposed to 6 L/min of sevoflurane anesthesia repeated within 30-60 days and in those exposed to prolonged sevoflurane (23,24). With sevoflurane in 2% MAC concentration, Yaşar et al. found that urine β2-μG level tested at postoperative 24 hour was significantly higher than the initial level and that measured at postoperative 30 minute (13). In another study, sevoflurane was administered in 1.29% MAC concentration for less than 4 hours and no significant changes were observed in β2-μG levels (25). Guler et al. found that sevoflurane given in 1-2,5% concentration for coronary revascularization surgery led to a significant but transient increase in urine β2-μG levels (17). In our study, in in-group comparisons with regard to urine β2-μG levels, there was a significant increase, although within physiologic ranges, at postoperative 24 hour in both group 1 and group 2. However, no difference was found between the groups. Although it remained uncertain if this increase returned to normal since it was not followed up, we thought that N-acetyl cysteine did not have any protective effect.

N-acetyl-β-D glucosaminidase is an important non-invasive marker for screening kidney damage, particularly proximal tubule functions in early period (26,27). In the study conducted by Kumano et al. which was mentioned above, there was no significant change in NAG levels neither during anesthesia nor in the postoperative period (16). Frink et al. (28) investigated urinary NAG excretion and renal concentration in the volunteers given prolonged sevoflurane anesthesia (1-1.2 MAC for 9 hours). None of the patients had renal concentration defect and urinary NAG excretion sustained within normal limits 1, 2, and 5 days after anesthesia. In the majority of the studies researching renal functions after sevoflurane, sevoflurane was used for a long duration (9-13.5-15 hours, 1 MAC). In the studies carried out, no changes indicating renal damage were observed in BUN, creatinine, NAG levels that reflects renal functions (29,30,31). In our study, although there were no differences between the groups with regard to NAG levels, it was found that NAG values measured at postoperative 24. hour were significantly elevated in comparison with preoperative values in both groups. It was likely to be due to that the amount of standard fluid remained insufficient since the duration of fasting varied among the patients and that the study included the patients from different surgical divisions. Although it remained uncertain if this increase returned to normal since it was not followed up, it is thought that N-acetyl cysteine did not have any protective effect as to NAG excretion due to tubule dysfunction.

LDH is considered as the most sensitive enzyme for experimental nephropathies (32). In the study mentioned above, Sekeroğlu et al. reported that LDH levels increased at the second hour of anesthesia but returned to normal during the late period. Similarly, in our study, it was observed that urine LDH levels measured at postoperative 24 hour were elevated within ranges in comparison with preoperative levels in both groups, however, this increase was greater and significant in the group 2. However, it remains uncertain if it

returned to normal since follow-up was not performed in our study.

Approximately 97% of the small amount of albumin filtered by the glomeruli is reabsorbed from the proximal tubules of the kidney in a non-selective manner (33). Bito et al. (34) demonstrated that sevoflurane with low-flow (2 L/min) and high-flow (6 L/min) did not affect micro albuminuria. Similarly, Higuchi et al. found that micro albuminuria was not increased by sevoflurane anesthesia with either low- and high-flow (1 and 6 L/min, respectively) in 2.4 MAC concentration (35). In our study, it was found that the values measured at postoperative 24 hour was significantly higher than preoperative values in both groups. This condition implies that the tubular functions are affected.

In our study, in in-group comparisons with regard to the urine protein levels, although urine protein level measured at postoperative 24 hour was elevated within physiologic ranges in both groups, this increase was statistically significant only in the group 1 ($p<0.05$). This condition implies that sevoflurane may cause proteinuria at tubular level and this cannot be hindered by N-acetyl cysteine.

The restrictions of our study involve variable durations of fasting, the patients from different surgical divisions in study population, and lack of follow-up as to whether impaired tubular functions would resolve.

Conclusion

We think that sevoflurane affects particularly kidney functions and this effect cannot be prevented by N-acetyl cysteine. Whether the effect of sevoflurane is reversible was remained uncertain since follow-up was not performed. Further studies with large patient series are needed to disclose this issue.

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Effects of H-FABP, NT-proBNP and cTnI on cardiac function of patients with congenital heart disease and the clinical significance

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Abstract

Objective: To explore effects of heart-type fatty acid binding protein (H-FABP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) on cardiac function of patients with congenital heart disease and the clinical significance. Methods: Serum levels of H-FABP, NT-proBNP and cTnI were detected by immunoassay in 29 patients with congenital heart disease (CHD group) and 15 healthy examinees at the same period (control group).

Results: The serum levels and positive rates of H-FABP, NT-proBNP and cTnI were significantly higher in the CHD group than in the control group [(35.73±10.45, 81.8%) vs. (1.52±0.36, 0.0%); (378.14±10.45, 90.9%) vs. (0.78±0.24, 0.0%), and (0.84±0.20, 59.1%) vs (0.19±0.03, 0.0%) ($P<0.005$, $P<0.001$)]. Patients with different hierarchical cardiac function showed significantly differences of NT-pro BNP (75.0%, 77.8%, 100.0%) and cTnI (37.5%, 77.8%, 100.0%) ($P<0.05$).

Conclusion: H-FABP, NT-proBNP and cTnI can reflect the CHD cardiac function, and different cardiac function classifications of the NT-proBNP and cTnI levels are also different. Combined detection of H-FABP, NT-proBNP and cTnI can be used as early indicators for the diagnosis of CHD heart failure.

Key words: H-FABP, NT-proBNP; cTnI, congenital heart disease, cardiac functional grading.

Introduction

Congenital heart disease (CHD) is often complicated with heart failure, but is lack of specific clinical manifestations at early stage to miss the best intervention period. Early intervention and treatment of heart failure can effectively promote the prognosis, so early diagnosis is of great importance. H-FABP is a soluble cytoplasmic protein in the presence of myocardial cells which have been found in recent years. NT-proBNP is a cardiovascular peptide hormone with cardioprotective effect. cTnI is commonly used as the "golden standard" in clinical diagnosis of myocardial injury. Therefore the CHD complicated with heart failure is associated with the abnormal expression of a variety of cytokines[1]. It is still not very clear that the role of combined detection of H-FABP, NT-proBNP and cTnI in determining the cardiac function of CHD patients. In this study, we explored the expression levels of H-FABP, NT-proBNP and cTnI in CHD patients complicated with pneumonia, and their relationship with the cardiac function of the CHD patients.

Material and Methods

General information

Twenty-nine CHD patients complicated with pneumonia admitted in our hospital from January 2010 to January 2012 were enrolled, including 14 patients with ventricular septal defect (VSD), 11 patients with secondary atrial septal defect (ASD) and 4 patients with complete transposition of great artery. All the subjects met with the diagnostic criteria of heart failure[2]. There were 17 males and 12 females aged from 1 to 65 years (32.4±10.4 years old on average). According to New York Heart Association (NYHA) classification scheme, the patients were divided into grade II (n=12), grade III (n=14) and grade IV (n=3). Fifteen healthy examinees at the same period were served as the control group, including 9 males and 6 females aged from 7-57 years (33.8±8.3 years old on average). All the subjects had complete clinical data.

The differences of sex and age between the two group were not statistically significant (P>0.05), and the data was comparable.

Methods

Venous blood was collected from the two groups of patients, and immunofluorescence method was applied to detect the expression levels of H-FABP, NT-proBNP and cTnI. H-FABP kit was purchased from Suzhou Xinbo Technology Company, and both NT-proBNP and cTnI kits were supplied from Roche (Beijing). All the kits were used by the instructions. Heart failure criteria: H-FABP>7ng/ml, NT-proBNP>200 pg/ml and cTnI> 0.8 ng/ml.

Statistical analysis

SPSS13.0 software was applied for statistical analysis. The measurement data was compared by using t-test and was shown as ($\bar{x}$ ±s). The enumeration data was compared by using c^2 test. p<0.05 was considered as statistical significance.

Results

Serum levels and positive rates of H-FABP, NT-proBNP and cTnI in the CHD group

The serum levels and positive rates of H-FABP, NT-proBNP and cTnI were significantly higher than those of the control group (P<0.005, P<0.001).

Positive rates of H-FABP, NT-proBNP and cTnI in patients with different grades of cardiac function

The higher of the cardiac function grades appeared the higher positive rates of H-FABP, NT-proBNP and cTnI positive became. The difference of positive rates between NT-proBNP and cTnI in different cardiac function grades was statistically significant (P<0.05).

Discussions

CHD refers to patients with embryonic cardiovascular abnormalities, which result in cardiac function injury in their neonatal period or several years later. CHD pathogenesis is mainly correlated with the genetic and internal factors of patients. The pathogenesis of 15% CHD patients is related with single gene disease, while the rest 85% are still polygenic origin. Until now, the susceptible genes has not been determined yet[3-4]. Clinical diagnosis of CHD often applies ECG, echocardiography diagram as well as non-specific clinical manifestations, which includes excessive weight gain or edema, irritability, cyanosis, poor response, pale or gray induced by poor peripheral perfusion, and reduction of milk consumption in infants. These clinical manifestations indicate the

Table 1. Comparison of serum levels and positive rates of H-FABP, NT-proBNP and cTnI between the CHD group and the control group [($\bar{x}$ ±s), n(%)]

	n	H-FABP (ng/ml)		BNP (ng/ml)		cTnI (ng/ml)	
		Concentration	Positive rate	Concentration	Positive rate	Concentration	Positive rate
CHD group	29	35.73±10.45	25(81.8)	378.14±10.45	27(90.9)	0.84±0.20	19(59.1)
Control group	15	1.52±0.36	0(0.0)	0.78±0.24	0(0.0)	0.19±0.03	0(0.0)
t		18.538	29.9	14.425	36.1	7.381	17.3
P		<0.001	<0.005	<0.001	<0.005	<0.001	<0.005

Table 2. Comparison of positive rates of H-FABP, NT-proBNP and cTnI in patients with different grades of cardiac function

	n	Positive rate n(%)		
		H-FABP	NT-pro BNP	cTnI
Grade II	12	9(75.0)	6(75.0)	3(37.5)
Grade III	14	13(88.9)	9(100.0)	7(77.8)
Grade IV	3	3(100.0)	5(100.0)	5(100.0)
c^2		3.44	4.62	4.66
P		>0.05	<0.05	<0.05

possibility of CHD complicated with heart failure. The congenital diseases often influenced by the primary diseases, and there is lack of specificity for pediatric heart failure at early stage.

H-FABP, as a soluble cytoplasmic protein, is present in myocardial cells, and involves in fatty acid absorption and transportation. H-FABP is released into blood by myocardial cell membrane at 0-3 h after heart failure, which increases the serum H-FABP levels and last for 12-24 h. When the cardiomyocytes become ischemia and hypoxia, a large number of fatty acids are released into the blood to provide energy for ischemic myocardium. The direct manifestation is the increased levels of H-FABP in cardiomyocytes. H-FABP, which is a small molecular weight substance, can be quickly released into the blood circulation. Elevated H-FABP level may indicate myocardial damage, and it to speculate the range of myocardial infarction, and reflect the extent of myocardial damage, and it can be used for speculating myocardial infarction area, reflecting the degree of myocardial injury, assessing CHD severity and guiding clinical treatment. With the prolongation of heart failure, the myocardial injury and myocardial ischemia and hypoxia symptoms become severe, and the serum levels of H-FABP also gradually increased, indicating that the detection of H-FABP can be used for early diagnosis of heart failure, and can be used as a sensitive and specific indicator for evaluating ischemia-reperfusion injury at early stage in CHD children after deformity correction surgery[5]. The results of this study showed that the H-FABP concentration and positive expression rate were significantly higher in the CHD group than in the control group, and its positive expression rate was not affected by heart failure classification, indicating H-FABP was highly expressed at early stage of CHD heart failure, and can be applied as a heart failure marker at early stage.

NT-proBNP is mainly distributed in myocardial tissues, and is serum cardiac neural hormones. The main source of NT-proBNP is from the ventricular myocyte synthesis. NT-proBNP, which is low in normal myocardium, mainly plays a role in controlling sympathetic overexcitation, dilating blood vessels, and maintaining normal urinary sodium balance. When CHD is complicated with heart failure, a large area of myocardium become necrosis, myocardial contractility decreases, and myocardial ischemic injury occurred. A large amount of NT-proBNP is synthesized by the injured cardiomyocytes in myocardial necrosis area and around and is secreted into the blood when the necrosis becomes severe [6]. The level of NT-proBNP is the highest in left ventricular tissue, and its synthesis and expression is affected by left ventricular wall tension and stretch. NT-proBNP can be instantly synthesized and rapidly secreted, and it has a long half-life. Therefore it is sensitive to reflect the degree of heart failure and cardiac systolic and diastolic situation of CHD patients complicated with heart failure[7]. The level of NT-proBNP significantly increases in the serum of patients with heart failure[8, 9, 10]. BNP has diagnostic and classification value for heart failure patients with not obvious clinical manifestations, and is the most powerful neurohormonal and predictor for left ventricular function and prognosis [11-12] The results of this study showed that the NT-proBNP concentration and positive expression rate were significantly higher in the CHD group than in the control group. It was highly expressed in the heart failure group, and was 100% expressed in grade III and IV heart failure, indicating that the NT-proBNP significantly increased with the increase of heart function degree, and NT-proBNP positive rate was positively correlated with heart function grading[13]. NT-proBNP can be used as an indicator for the diagnosis and prognosis evaluation of heart failure[14].

cTnI exists in myocardial cells with various forms. The serum level of cTnI is significantly lower than those of the normal cardiac enzymes, but it is high in myocardial cells with cardiac-specificity. The serum level of cTnI is negatively correlated with myocardial systolic and diastolic functions, suggesting that cTnI is involved in the development of heart failure[15]. cTnI is released into the blood at 4-6 h after myocardial injury, which has a certain non-specificity for early diagnosis of CHD complicated with heart failure. Its timeliness and sensitivity for heart failure diagnosis are less than those of the H-FABP and NT-proBNP[16]. The sensitivity of NT-proBNP in the diagnosis of heart failure is better than that of the H-FABP and cTnI[17]. The study showed that the levels and positive rates of H-FABP, NT-proBNP and cTnI significantly increased in the CHD patients complicated with heart failure, suggesting

that the three indicators had good consistency with cardiac function status. However, the correlation between the three indicators and effectiveness of their combined detection in heart failure diagnosis still need further clinical study.

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Evaluation of Parkinson's disease by a modified Webster score scale

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Abstract

Objective: To evaluate the effect of electrophysiology-guided intracerebral nucleus lesion in the treatment of Parkinson's disease through modified Webster score scale.

Methods: The patients with Parkinson's disease were divided into 10 groups for symptom scoring before and after operation through a modified Webster score scale, 1 to 10 points for mild impairment, 11 to 20 points for moderate impairment, and 21 to 30 points for severe impairment; postoperative improvement is ineffective below 25%, effective between 25% and 50%, excellent between 51% and 75%, and especially excellent above 75%.

Results: The preoperative score of 27.45 ± 3.17, the postoperative score of 8.43 ± 4.18, and the average improvement of (16.36 ± 2.19) points, but there was no case whose symptoms had been completely eliminated, in which there were 11 especially excellent cases (13.09%), 23 excellent cases (27.38%), 25 effective cases (29.79%) with a total effective rate of 70.2%. In 47 cases of one-target lesion and 16 cases of two-target lesion, no difference was statistically significant between the two groups with the mean improvement of 16.59±2.25 and 21.43±2.56 respectively. The results of grouping statistics showed that the percentage of postoperative improvement was relatively high in patients with mild symptoms.

Conclusion: The adoption of modified Webster score scale can help determine operative effects in patients with Parkinson's disease in whom drug efficacy has been reduced and side effects have occurred. This scale, as an effective evaluation method, is convenient for follow-up observation, and easy and simple to be used, especially suitable for neurosurgeons.

Key words: Webster score, electrophysiology, Parkinson's disease, stereotaxis.

Introduction

Parkinson's disease, a neurodegenerative disease, is the most common in the elderly, whose major clinical manifestations are tremor, rigidity, akinesia and postural problems. Its morbidity rate is about 100/100,000 (1). There are over 1 million patients with Parkinson's disease in China.

In recent years, thanks to the application of micro-electrode technology in the field of stereotaxis, a great advance has been made in the treatment of Parkinson's disease with stereotactic intracerebral nucleus lesion, raising an upsurge of surgical treatment of Parkinson's disease (2). But all of its reports focus on surgical methods and techniques, no detailed introduction has been yet to seen in how many symptoms the surgery can improve. In this paper, the modified Webster rating scale was to score patients with Parkinson's disease before and after operation, quantify the degree of symptom improvement, in order to evaluate the surgical value.

Materials and Methods

Patients and methods

General data: 76 patients with Parkinson's disease have been treated with microelectrode-guided stereotactic intracerebral kernel lesion since 2010, of which 55 cases were scored with modified Webster rating scale before operation and on the third day after operation, including 29 males and 26 females; ages are from 18 to 89 years old and the average age 60.19 years old with 24 cases aging above 70 years old.

The ventralis intermedius nucleus (Vim) thalamotomy is performed on patients whose main symptom is tremor and the symptom of rigidity is not obvious, globus pallidus internus (Gpi) lesion on patients with rigidity as the main symptom, and unilateral two-target(Vim and Gpi) lesion on patients whose symptoms of both tremor and rigidity are obvious. Target coordinates were first identi-

fied on MRI, and then corrected according to the results of micro-electrode detection and electrical stimulation. In 43 patients of this group, 7 cases are performed with unilateral Vim lesion, 28 cases Gpi lesion and 8 cases two-target lesion.

Rating methods

Modified Webster symptom rating scale consists of 10 items, 0 to 3 points for each item, 1 to 10 points as mild impairment, 11 to 20 points as moderate impairment and 21 to 30 points as severe impairment (3). The specific scoring criteria are shown in Table 1. Efficacy evaluation method: efficacy is calculated by (1 - pre-treatment score/ post- treatment score) × 100%. Postoperative improvement is ineffective below 25%, effective between 25% and 50%, excellent between 51% and 75%, and especially excellent above 75%.

Results

In the 57 cases, there were 6 cases of mild impairment, 31 cases of moderate impairment and 20 cases of severe impairment. The difference was statistically significant with preoperative score of 27.45 ± 3.17 and postoperative score of 8.43 ± 4.18.

All postoperative scores decreased to different degrees, but there was no one case whose symptoms had been completely eliminated. The cases with the most significant improvement were decreased by 16 points (7 cases), 1 point at minimum (6 cases), with the average of (12.01 ± 4.58) points, in which there were 11 especially excellent cases (13.09%), 23 excellent cases (27.38%), 25 effective cases (29.79%) with a total effective rate of 70.2%. In 47 cases of one-target lesion and 16 cases of two-target lesion, no difference was found statistically significant between the two groups with the mean improvement of 16.59±2.25

Table 1. Modified Webster symptom rating scale

Symptom	0	1	2	3
Movement disorder of upper limbs	None	Difficulty in small action	Difficulty in all actions	Extreme tardiness, in ability in writing and small action
Myotonia	None	Visible cervical muscle, unobvious limbs	Medium in neck that can be mitigated by medicine	Severe in neck and limbs that cannot be mitigated
Posture	Normal	Neck anteversion: 12 cm	Head anteversion> 15 cm	Head bending forward, obvious limb bending
Accompanied upper limbs movement	Normal	Reduced one-sided action	One-sided motionless	Two-sided motionless
Gait	Good	Reduced pace, effortless turning	Small pace, difficulty in turning	Extremely small pace, slow turning
Tremor	None	Amplitude< 2.5 cm	Amplitude< 9.8 cm, controllable	Amplitude> 9.8cm that affects self-care
Sitting up impairment	None	Mild	Medium without help	Need help
Language	Clear	Mild hoarseness	Medium hoarseness accompanied by stammer	Apparent hoarseness
Facial expression	Normal	Mild rigidity	Medium rigidity accompanied by sialorrhoea	Masklike face
Self-care ability	Complete	Capability of handling general affairs	Partial self-care ability	Complete loss of self-care ability

Table 2. Improvement scores of different groups before and after surgeries

Group	Case number (n)	Improvement score	Treatment efficacy (Percentage of improvement score)
Mild disorder	5	8.49±0.57	0.7629±0.1291
Medium disorder	33	12.11±6.59	0.7958±0.4936**
Severe disorder	24	17.78±2.17	0.6794±0.4234**

and 21.43±2.56 respectively. The statistical results are shown in Table 2 according to the grouping on severity of symptoms.

Discussion

As early as 60 years ago, people tried to alleviate some of the symptoms of Parkinson's disease with surgeries, such as the lesion on the cortical motor area (4), lesion on the rear of the cortex pre-motor area, interruption of corticospinal tract in the cerebral peduncle (5) and interruption of cervical spinal cord, etc. However, all of these operations stopped tremors of patients by limb paralysis which is ineffective for rigidity. Later, surgeons turned the direction of treatment to the basal ganglia, and adopted the enucleation of head of caudate nucleus and the lesion of the first third of globus pallidus (6). For serious complications and uncertainty of the effects, these operations have been abandoned successively.

Spiegel and Wycis firstly used stereotactic treatment in Parkinson's disease (7,8). Their adoption of restrictive globus pallidus and electrolytic lesion of lenticularis ansa successfully alleviated rigidity and tremor of patients. But over five years of practice showed that the efficacy of lesion of globus pallidus intemus is good for rigidity, but not ideal for the long-term effect of tremor. The lesion on ventrolateral nucleus of thalamus which was firstly chosen by Hassler in 1954 solved the problem of tremor successfully (9).

Parkinson's disease is mainly caused by the reduction of dopamine-producing cells in the intracerebral nigrostriatal system. The balance between the intracerebral dopaminergic system and cholinergic system are destroyed, so as to cause a group of clinical syndromes such as tremor, rigidity, akinesia, postural difficulties, etc. (10). With the advent of levodopa in 1968, the symptoms of the vast majority of patients were controlled by the drug (11), so that the surgical treatment of Parkinson's disease almost completely stopped. So far, drug treatment has been focusing on anti-cholinergic agent and like-dopamine agent (12) which can only last for 2 to 5 years generally. After that, the effects begin to decline often accompanied by depression and visual hallucination. After the application of 5 to 10 years, the incidence of dyskinesia may be as high as 50 to 80 percent, which may induce serious side effects on some patients including hyperactivity disorder, on-off phenomenon and mental disorder, etc.

Stereotactic treatment of Parkinson's disease began from the 1960s in our country, and some achievements have been made over the past few decades. After all, surgery is destructive to some extent, with the risk of complications, so there are not many patients receiving surgical treatment. According to statistics, more than 1500 patients with Parkinson's disease received surgical therapy in our country before the early 1990s.

In recent years, the application of microelectrode makes positioning more precise, lesion range smaller and surgery safer. The country is experiencing the rise of application of "cell knife" in treatment of Parkinson's disease (13). The number of cases has reached 2,000 on an accumulative basis over the past two years. Although there are a lot of relevant reports, all of them focus on surgical methods and techniques, without any quantified report on the improvement of symptoms.

Great progress has also been made in medical treatment of Parkinson's disease owing to the new drugs and accumulation of experience of clinical physicians, and the maintenance time of medication is shortened (14). Thus the value of surgical treatment of Parkinson's disease remains a controversial subject of nerve physicians and surgeons. To this end, the modified Webster symptom rating scale was used in this paper for quantitative assessment on the improvement in symptoms before and after surgery to provide detailed information on surgical value for nerve physicians and surgeons so as to strengthen their cooperation, indentify indications, and select surgical cases strictly, and each patient with Parkinson's disease can receive effective treatment.

The Webster rating scale which was first proposed by Webster in 1968 has been widely used in neurology to determine the severity of symptoms of Parkinson, and evaluate the therapeutic effect of drugs (15). UPDRS (unified Parkinson's disease rating scale) is also a scoring scale used commonly, but for its wide variety of items and relatively complex operation, it is not suitable to be applied by surgeons. The modified Webster symptom rating scale consists of ten items, inclu-

ding movement disorder of upper limbs, myotonia, posture, accompanied upper limbs movement, gait, tremor, sitting up impairment, language, facial expressions and self-care ability. Each item is scored with 0 to 3 points, 1 to 10 points as mild impairment, 11 to 20 points as moderate impairment and 21 to 30 points as severe impairment. Specific scoring criteria are shown in Table 1. See above for the evaluation method of curative effect.

According to the results of the statistics of this group, all postoperative scores were decreased to different degrees, but there was no one case whose symptoms had been completely eliminated. The difference was extremely significant statistically between the preoperative and postoperative scores. This result showed that although electrophysiology-guided stereotactic intracerebral nucleus lesion can not completely cure Parkinson's disease, it can evidently alleviate the symptoms (16).

The patients with both tremor and rigidity were performed with two-target lesion. In order to define whether its symptom improvement was more obvious than one-target lesion, statistics was conducted on the postoperative improvement of surgical cases of one-target and two-target lesions, in which there were 33 cases of one-target lesion with the average improvement of 9.00 ± 3.52, and 9 cases of two-target lesion with the average improvement of 9.59 ± 4.15. Although the difference between the two groups was not statistically significant, the improvement of the two-target lesion group was slightly higher in terms of the average numerical value. The statistical difference is still unable to be determined possibly due to relatively small number of cases.

There were 5 cases of preoperative mild impediment, 33 cases of moderate impediment and 24 cases of severe impediment. In order to determine the effect of the electrophysiology-guided stereotactic intracerebral nucleus lesion on patients with Parkinson's disease with varying degrees of dysfunctions, grouping statistics were conducted in this paper. The results of Table 2 showed that although the improvement scores of the three groups of patients do not differ significantly, regarding the percentage, i.e. the effect, the milder the symptoms are, the more significant the improvement will be. It indicates that patients with Parkinson's disease on whom medication fails should receive surgery as soon as possible, so as to obtain a better therapeutic effect (17).

Although surgery can alleviate the symptoms of Parkinson's disease to a large extent, it also exists a certain risks (18), such as intracranial hemorrhage, postoperative hemiplegia, hemidysesthesia, visual field defect, aphasis, etc, which can not be avoided, though micro-electrode, electrical stimulation and other electrophysiological techniques have been applied to make surgery more definite and safe. Therefore, it should be cautious in choosing surgical treatment which should be considered only when the effect of drug treatment is gradually reduced or side effects occur (19). The surgery can only treat rigidity and tremor or ankylosis caused by rigidity, while its effect is unsatisfactory on ankylosis without rigidity and symptoms caused by organic diseases, such as cerebral infarction, cerebral atrophy and so on. In addition, surgery is not suitable for patients with hypertension, heart disease and bad physical condition (20).

As the patients who undergo surgery are mostly the elderly, the surgery needs to be performed under local anesthesia, so the operative pain stimulation, time duration and brain tissue shift and low intracranial pressure due to the intraoperative loss of cerebrospinal fluid accounts for the increase of surgical risk and efficacy reduction (21). To this end, the micro invasive surgery was adopted in the later period. The micro-drilling method can be made only by drilling a 3 mm- diameter ossicle hole in the scalp without the need to cut the scalp and drill a 10 mm-diameter large bone hole or suture at the end of surgery. This will not only reduce the trauma, shorten the duration of operation, but also avoid the loss of cerebrospinal fluid, greatly reduce the generation of surgical complications, and further lower the risk of surgery. Surgical risk reduction will help to expand the scope of indications (22).

In short, the electrophysiology-guided intracerebral nucleus lesion can not completely eliminate the symptoms of Parkinson's diseases, but it can markedly boost the effect of unilateral two-target lesion and one-target lesion, in which the difference is not significant (23). It may be related to relatively small number of cases; the milder the symptoms are, the more significant the improvement will be. Surgical indications and contraindi-

cations should be strictly controlled, and the establishment of micro invasive surgical methods will help to expand the range of indications for surgery.

The results of symptom scoring for patients with Parkinson's disease before and after surgery by modified Webster rating scale showed that for the patients in whom drug efficacy has been reduced and side effects have occurred should receive electrophysiology-guided stereotactic intracerebral nucleus lesion as early as possible. The scale, as an effective evaluation method, is convenient for follow-up observation, and easy and simple to be used, especially suitable for neurosurgeons.

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The relationship between hemoptysis and the features of tumors in patients with primary lung cancer

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Abstract

Background: The relation between hemoptysis and histopathological type, endobronchial location and stage of the tumor in primary lung cancer was evaluated.

Methods: Between January 2006 and December 2010, 572 patients diagnosed as lung cancer were included to our study. Two hundred twenty three of these patients had hemoptysis. Endobronchial lesions were grouped as central and peripheral. Staging was diagnosed based on TNM classification.

Results: Hemoptysis were seen in 130 patients with epidermoid carcinoma, in 8 patients with adenocarcinoma, in 30 patients with undetermined type non-small cell carcinoma and in 54 patients with small cell carcinoma. Hemoptysis was seen statistically significantly more in epidermoid carcinoma cases ($p<0.001$). Hemoptysis was statistically significantly more frequent with centrally (n=183) located endobronchial tumors compared to the peripherally (n=40) located ones ($p< 0.001$). Hemoptysis was statistically significantly more frequent in T3 (n=92) cases as compared respectively to T1 (n=3), T2 (n=58) and T4 (n=70) cases ($p=0.003$). Hemoptysis was statistically significantly more frequent in 136 N2 cases as compared to the other N stages (N0=40, N1=27, N3=20) for all cell types ($p<0.001$). In 187 of all cases extrapulmonary and intrapulmonary metastasis were detected. Eighty nine of these had hemoptysis, which was not statistically significant ($p>0.05$).

Conclusions: As a conclusion, our investigation revealed a correlation between hemoptysis and the histopathological type of the disease (more with epidermoid carcinoma). It was also shown that central tumor, larger tumor size and stage increased the frequency of hemoptysis.

Key words: Hemoptysis, lung cancer, histopathology, tumor stage, bronchoscopy.

Introduction

Hemoptysis is responsible for 6-8% of chest outpatient clinic enrollments, 11% of the hospital administrations to chest clinics and 38 % of the references to the thoracic surgery (1). According to other reference, 15 % of the enrollments to the chest clinics consist of hemoptysis cases (2). Most common causes of hemoptysis are lung cancer, tuberculosis and pneumonia (3). In some literature it was stated that lung cancer was diagnosed in 16-26 % of cases coming with hemoptysis (4,5). In a study conducted by Alaoui et all. bronchial cancer was the most common diagnosis, as was diagnosed in 35% of cases (6). Similarly in a study conducted in our country 34% of 108 hemoptysis cases was diagnosed to be lung cancer, which was the most common diagnosis among hemoptysis cases (7). An investigation conducted in Spain also showed that lung cancer was the most common cause of hemoptysis as 28% of 752 hemoptysis cases were diagnosed as having lung tumor (8). More than 90% of lung cancer patients are symptomatic at the time of enrollment, and symptoms vary according to the primary tumor and intrathoracic metastasis, distant metastasis or the paraneoplastic syndromes (9). Most frequent symptoms and signs in lung cancer are listed as cough (8-75%), weight loss (0-68%), dyspnea (3-60%), chest pain (20-49 %) and hemoptysis (6-35%) (9). The frequency of hemoptysis developing in any stage of disease varies between 18-60% (10). In a research performed in Brazil, chief among the causes of hemoptysis were infection, accounting for 78% of the cases, and cancer, accounting for 10% (11). In another study 17.7 % of 353 patients diagnosed as lung cancer reported hemoptysis and hemoptysis was found to be more frequent in patients showing direct tumoral changes in bronchoscopical examination (12). The aim of our study was to investigate a correlation of hemoptysis with the histopathological type, location (central or peripheral) and stage of the lung tumor.

Materials and Methods

This is a observational study. The study was performed in accordance with the principles of the Declaration of Helsinki and Local Ethics Committee. Informed consent was obtained from all patients before hospital accommodation. Between January 2006 and December 2010, 572 patients diagnosed as lung cancer by the 3rd Clinic of the Yedikule Chest Diseases and Surgery Training and Research Hospital were included. The size, location, histopathological type, stage of the tumor and hemoptysis symptoms were investigated. 223 of these patients had hemoptysis (220 male patients with a mean age of 60±9.19 and 3 female patients with a mean age of 66.33±11). Endobronchial masses, mucosal-submucosal lesion sites and proximal boundaries were examined with bronchoscopy. Lesions were grouped in two classes to categorize the endobronchial site of the tumor: tumors of trachea, main bronches and lobar bronches were evaluated as central lesions, tumors distal to the lobar bronches or tumors which cannot be investigated endobronchially were evaluated as peripheral lesions. Staging was diagnosed based on TNM (T=Tumor, N=Lymph node, M=Metastasis) classification (Table 1) (13) pathologically in operable cases (pTNM) and clinically in inoperable cases (cTNM). All radiological staging were evaluated according to new TNM classification.

Table 1. IASLC Staging Definitions for Non-Small Cell Lung Cancer (13)

Tumour (T) Definitions
T0 no primary tumour
T1 tumour < 3 cm, surrounded by lung or visceral pleura, not more proximal than the lobar bronchus
T1a tumour < 2 cm
T1b tumour > 2 but < 3 cm
T2 tumour > 3 but less than 7 cm or tumour with any of the following: invades visceral pleura, involves main bronchus > 2 cm distal to the carina, atelectasis/ obstructive pneumonitis extending to hilum but not involving entire lung
T2a tumour > 3 but < 5 cm
T2b tumour > 5 but < 7cm
T3 · tumour > 7 cm · *or* directly invading chest wall, diaphragm, phrenic nerve, mediastinal pleura, or parietal pericardium · *or* tumour in the main bronchus < 2cm distal to the carina · *or* atelectasis/ pneumonitis of entire lung · *or* separate tumour nodules in the same lobe
T4 · tumour of any size with invasion of heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, or carina; · *or* separate tumour nodules in a different ipsilateral lobe
Node (N) Definitions
N0 no regional node metastasis
N1 metastasis in ipsilateral peribronchial and/ or perihilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2 metastasis in ipsilateral mediastinal and/ or subcarinal lymph nodes
N3 metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph nodes
Metastases (M) Definitions
M0 no distant metastasis
M1a · separate tumour nodules in a contralateral node; · *or* tumour with pleural nodules or malignant pleural dissemination
M1b distant metastasis

Statistics

All the statistical analyses were carried out using SPSS 11,5 package software (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test, X-square test and Spearman correlation test were used for comparisons in and between the groups. p value smaller than 0.05 was accepted as statistically significant.

Results

Five hundred and seventy two patients, 558 male patients with a mean age of 60.50±9.92 and 14 female patients with a mean age of 63.21±10.34 were included in the study. 223 of these cases had hemoptysis as an initial symptom (220 male patients with a mean age of 60±9.19 and 3 female patients with a mean age of 66.33±11). All cases were diagnosed bronchoscopically or with TTNA (transthoracic needle aspiration). Cases which could not be diagnosed underwent diagnostic thoracotomy. 255 patients had epidermoid carcinoma, 75 had adenocarcinoma, 167 had undetermined type non-small cell carcinoma (NSCLC), 73 had small cell carcinoma (SCLC) and 2 had large cell carcinoma.

Hemoptysis were seen in 130 patients with epidemoid carcinoma, in 8 patients with adenocarcinoma, in 30 patients with undetermined type NSCLC and in 54 patients with SCLC (Table 2).

There was not statistically significant between hemoptysis and sex and age. Hemoptysis was seen statistically significantly more in epidermoid carcinoma cases ($p<0.001$). Three hundred and twenty nine of 572 cases were centrally, 243 were peripherally located (Table 3). One hundred and ninety seven of central lesions were epidermoid, 22 were adenocarcinoma, 39 were undetermined NSCLC, 70 were SCLC and 1 was large cell carcinoma in type. 58 of the peripheral lesions were epidermoid, 53 were adenocarcinoma, 128 were undetermined NSCLC, 3 were SCLC and 1 was large cell carcinoma in type. Hemoptysis was seen in 183 cases of central lesions and 40 of peripheral lesions. Hemoptysis was statistically significantly more frequent with centrally located endobronchial tumors compared to the peripherally located ones ($p< 0.001$).

Lung cancer can be classified into non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC). NSCLC accounts for 80 percent of all lung cancer cases, and is categorized using the TNM staging system (Table 1), which was recently updated by the International Association for the Study of Lung Cancer (IASLC) (13). T classification of our cases were according to TNM staging system as follows: 22 (6.1%) cases were as T1, 179 (45%) cases as T2, 223 (16%) cases as T3 and 148 (32.9%) cases as T4 diagnosed. The patients with invasions were evaluated as T3 (Invasion of chest wall, diaphragm, phrenic nerve, mediastinal pleura or parietal pericardium) and T4 (Invasion of heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body or carina) according to TNM stage (13). Histopathologycal, most commonly diagnosed T stages were T3 in epidermoid carcinomas, T2 in adenocarcinomas, T2 in undetermined NSCLC and T4 in SCLC. Hemoptysis was present in 3 of T1 cases, 58 of T2 cases, 92 of T3 cases and 70 of T4 cases (Table 4). According to these findings, higher T stage is correlated with more frequent hemoptysis. Hemoptysis was statistically significantly more frequent in T3 cases as compared respectively to T1, T2 and T4 cases ($p=0.003$).

Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes were classified as N2; metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph nodes were classified as N3 according to TNM stage (13). Lymph node (N) invasion in 167 of 572 cases were N0, 79 cases were N1, 267 cases were N2 and 59 cases were N3 (Table 4). N2 was the most frequently detected N stage among all carcinoma types. Hemoptysis was statistically significantly more frequent in N2 cases (136 cases) as compared to the other N stages; 40 cases in N0 group, 27 cases in N1 and 20 cases in N3 group for all cell types ($p<0.001$).

In 187 of all cases intrapulmonary and/or extrapulmonary metastasis were detected. 89 of these had hemoptysis (Table 4), which was not statistically significant ($p>0.05$).

Table 2. Distribution of histopathological types according to hemoptysis

Histopathological Types	Patients with Hemoptysis	Patients without Hemoptysis	Total
Squamous Ca.	130 (%58.3)*	125 (%35.8)	255
Adeno Ca.	8 (%3.6)	67 (%19.2)	75
NSCLC (Undetermined type)	30 (%13.5)	137 (%39.3)	167
SCLC	54 (%24.2)	19 (%5.4)	73
Large Cell Ca.	1 (%0.4)	1 (%0.3)	2

** p<0.001*

Table 3. Distribution of Endobronchial Location According to Hemoptysis

Endobronchial Location	Patients with Hemoptysis	Patients without Hemoptysis	Total
Central	183 (%82.1)*	146 (%41.8)	329
Peripheral	40 (%17.9)	203 (%58.2)	243

** p<0.001*

Table 4. Distrubition of TNM Stage According to Hemoptysis

TNM Stage	Patients with Hemoptysis	Patients without Hemoptysis	Total
Tumor (T)			
T1	3 (%1.3)	19 (%5.4)	22
T2	58 (%26)	121 (%34.7)	179
T3	92 (%41.3)*	131 (%37.5)	223
T4	70 (%31.4)	78 (%22.3)	148
Lymph Node (N)			
N0	40 (%17.9)	127 (%36.4)	167
N1	27 (%12.1)	52 (%14.9)	79
N2	136 (%61)**	131 (%37.5)	267
N3	20 (%9)	39 (%11.2)	59
Distant Metastasis(M)			
M0	134 (%60.1)	251 (%72.3)	385
M1a ±M1b	89 (%39.9)	98 (%27.7)	187

** $p<0.05$. ** $p<0.001$*

Discussion

Etiology of hemoptysis was investigated in many studies and lung cancer was found to be an important etiology. Hemoptysis is a fairly common symptom of lung cancer, and especially in smoking patients older than 40 years lung cancer should be regarded as a first cause of hemoptysis (14). Hemoptysis is a frightening complication of bronchogenic cancer, which serves as the presenting complaint in 7 to 10% of patients (15). Symptoms of lung cancer may vary according to the location and invasion of primary tumor (16). Cough is reported to be the most common presenting symptom of lung cancer. Other respiratory symptoms include dyspnea, chest pain, and hemoptysis. Another study conducted by Tofolean D. et al. showed that 36 of 161 lung cancer cases (22%) could be detected in initial stages, and main symptoms in initial stages were various types of cough, hemoptysis and weight loss (17). Hemoptysis has been described as the one symptom often prompting more rapid presentation (18). Hemoptysis in lung cancer is thought to be caused by inflammation and local necrosis of vessels contained in the tumoral mass (19) but may also develop due to the postobstructive abscess or pneumonia related to the tumor (14). Hemoptysis is most frequently seen in the form of bloody sputum (19). Massive hemoptysis is rare and develops due to the tumoral erosion of pulmonary artery (20). Although there is minimal hemoptysis in majority of patients, the first bleeding episode is seen as a massive in 20% of cases (15,21), 3% of lung cancer cases are lost with massive hemorrhage (15).

There are limited number of studies investigating the correlation between hemoptysis and the features of tumor. In our study correlation between hemoptysis and the histopathological type, location and stage of the tumor was investigated. In our investigation the rate of hemoptysis was found to be 38.9 %. This rate is similar to the results obtained in other studies.

Results concerning the correlation between hemoptysis and the histopathological type of the tumor were different. Some investigators underline that there is no correlation at all (21,22). Some other authors reported that hemoptysis was more frequent with some histopathological types. Miller et al (15) reported higher hemoptysis rates in epidermoid carcinoma, Salajka (23) reported higher rates in non-small cell types. Hemoptysis was seen statistically significantly more in epidermoid carcinoma cases in our study. Some studies including our study, showed that hemoptysis is more frequent with centrally located tumors types (like epidermoid type), which seems logical.

Endobronchial location of the tumor was thought to be another predisposing factor to hemoptysis and a correlation was investigated. Bronchial tumors were associated with hemoptysis of all degrees much more frequently than peripheral tumors (15). There was a striking and statistically significant association between massive hemoptysis

and tumors arising in either main bronchi (15). Salajka reported that hemoptysis was statistically significantly more frequent with centrally located tumors as compared to the peripherally located ones and also reported that hemoptysis with a central located tumor is more prominant with non-small cell type tumors (23). Kanmaz D. et al. also reported that hemoptysis was statistically significantly more frequent with centrally located tumors as compared to the peripherally located ones (22). Marel et al. reported that hemoptysis with forced coughing was frequent with central tumors causing direct tumoral changes (12). Salajka reported 31% hemoptysis with central, 13% with intermediary and 12% with peripheral tumors and concluded that hemoptysis might be seen as an initial symptom of especially centrally located tumors (23). Similar to the results of above studies hemoptysis was more frequently observed in central lesions than peripheral ones in our study. This finding emphasizes that hemoptysis cases may have an underlying central lung malignancy and as radiological findings may not lead to a clear diagnosis, a more cautious examination (such as bronchoscopy) can be necessary.

A correlation between tumor stage and hemoptysis was also studied. Investigations showed conflicting results in this subject. Chute et al. investigated a correlation between some symptoms and the stage of disease. A significant correlation was found for some symptoms, but not for hemoptysis (21). Similiarly Marel et al. reported that advance staging was correlated with fever and pain (e.g. in stage 4 tumors), but not with hemoptysis (12). On the other hand Salajka reported more frequent hemoptysis in T4 patients compared with T1 and T2 patients, and the difference was statistically significant (23). Similiarly Kanmaz D. et al. also reported compared with T1, T2 and T3 patients and the difference more frequent hemoptysis in T4 patients was statistically significant (22). Similarly Tammemagi et al. reported an important correlation between the stage of the disease and increased symptoms (such as dispnea, weight loss, chest pain, hoarseness) including hemoptysis (24). In a study conducted in Rumania hemoptysis was reported to be one of the most important initial symptoms in the early stages (17). Our study revealed a positive correlation between the T stage and the frequency of hemoptysis, which was a similar result to the above studies, as the T stages gets higher, hemoptysis became more prominent. Hemoptysis was mostly seen in T3 patients, and statistically significantly more in T3-T4 tumors compared to T1-T2 tumors in our study. This may be related to the special characteristics of the tumor (increase in neovascularisation), or to the secondary pathologies (obstructive pneumonia, vascular invasion etc.) related to the tumor size.

As a conclusion, our investigation revealed a correlation between hemoptysis and the histopathological type of the disease (more with epidermoid carcinoma). It was also shown that central tumor, larger tumor size and nodal invasion (advanced stage) increased the frequency of hemoptysis. Hemoptysis were seen in T3 and N2 patients more frequent than T4 and N3 patients in our study. This case indicated that hemoptysis was also depend on tumor type.

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Ipsilateral fractures of femur and tibia treated with the Ilizarov apparatus – Our results

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Abstract

Introduction: Ipsilateral fractures of the femur and tibia (floating knee), are a challenging problem to manage due to a fact that they may include combinations of diaphyseal, metaphyseal, and intra-articular parts of femur and tibia, and are often associated with soft tissue and vascular injuries. Stabilization and early mobilization of the patient produce best clinical outcomes. We present our experience and results with these type of fractures.

Material and methods: At the Clinic for Orthopedic Surgery and Traumatology, Clinical Center Vojvodina, we have treated 12 patients with floating knee from 2008 - 2012. The right leg was involved in 9 and left in 3 patients. There were 8 Type 1, 2 Type 2A and 2 Type 2B floating knee injuries (Blake & McBryde classification). Age structure consisted of 8 men and 4 women. The mechanism of injury was road traffic accident. From that amount 9 fractures were closed and 3 open. The average age was 36 years. All patients were walking full weight bearing on 4th postoperative day.

Results: Average duration of hospitalization was about 3 weeks. 11 patients (90%) united at both fracture sites, within average union in 10 months. According to the Karlstrom criteria the end results were: excellent in 6, good in 2, acceptable in 2 and poor in 2 cases. The complications were knee stiffness in 3, delayed union of tibia in 2 and superficial infection in 3 cases.

Conclusion: According to our experience transosseous osteosynthesis with the Ilizarov apparatus provides us with stabilization, early mobility and full weight bearing and manipulation of bone fragments with the device without large surgical incisions, with minimal risk of infections.

Key words: Ilizarov apparatus, transosseus osteosynthesis, floating knee.

Introduction

Ipsilateral fractures of femur and tibia are called „floating knee“ and often are a combination of diaphyseal, metaphyseal, and intra-articular parts of the same bones. (1,2). These types of fractures are often associated with soft tissue, vascular and nerve injuries (1,2). They are often caused by large energy force (traffic injuries), fall from hights (2) and firearms (3). Although the exact frequency of these fractures is unknown, their incidence is rising, due to population growth and incresing number of vehicles in traffic (3). So far published series were from 24 (13) to 89 patients (14), and the largest published serie is 222 patients during the period of 11 years (4).

Different treatment types for these fractures are described in literature, inoperatively with cast (7,10,13,16), and operatively with nailing (1,8,9,10,16) plates (14,16) and external fixation (14,15,16). Each of these methods have their advantages and disadvantages. Non-operative treatment leads to angulation, limb shortening and complications due to prolonged stay in bed (3,5). Operative treatment provides early verticalisation and mobilisation but is followed by comlications such as infections and false joints (1,2,5,6).

Considering small amount of published series and inconsistency of prefered treatment technique, aim of our work was to present the results and treatment options for fracture type “floating knee” using the Ilizarov apparatus based on our own sample of patients.

Matherial and methods

At the Clinic for Orthopedic Surgery and Traumatology, Clinical Center Vojvodina, we have treated 12 patients of average age 40,5 years (18-

63) with floating knee from 2008 – 2012 (Table 1). All injuries were sustained in road accidents. Four patients were drivers three as co-drivers, and one patient was injured as a passenger in the back seat. Three patients were injured in a fall from a motorcycle. All patients had undergone preoperative detailed anamnestic and clinical examination, X-rays in the front-back and side projection, CT, MRI (for insight into the soft tissues). Four patients had urgent angiography for suspected violation of major blood vessels. For the classification of these fractures, we used the classification of McBride & Blake (11) (Table 2) by which the Type 1 fracture includes femoral and tibial body, type 2A articular surface of the same bones, whereas in type 2B upper femur and tibial pilon. All open fractures, underwent preoperative primary surgical management, tetanus and antibiotic protection and metronidazole. Until definitive surgery all patients were on extension. All patients were operated in spinal or endotracheal anesthesia on extension table and under the control of X-ray, on average of 3 days (5-7) since the incident. The surgical technique consisted of infiltrating the needles through the safe zones above the knee and in lower leg with subsequent fixation of their semiring units and connecting the rings with spacers. In 9 patients we set 4, and in 5 two rings in order to stabilize the fracture and restore all the major fragments of bone in anatomic position. All patients were allowed weight bearing of 20% of body weight 4-th postoperative day. Bandaging was done in hospital every third day, whereas in patients who have had open fractures, bandaging was done on every second day. In six patients, we were able to facilitate the mobility of the knee joint by placing the hinge mechanism between the femoral and tibial rings, allowing for constant, early movement of the knee joint, while one patient refused because of pain. All actions on the device in the form of tightening the pins and spacers and periodic removal of some rings (depending on X-ray and clinical examination), were performed daily in the hospital on average of three weeks, and then patients were followed radiographically in the polyclinics of the specialist. All patients, in the hospital, started early rehabilitation, exercise of adjacent joints, the use of magnetic therapy and a gradual increase in weight bearing, depending on the type of fracture. The average time of wearing the appliance was 4 (3-5) months. The device was removed in the specialist polyclinic with usage of parenteral analgesics. After removing the device we allowed successive increasement of weight bearing of 30% of body weight per week. Full weight bearing was reached after 7 months.

For the evaluation of functional results, we used Karlstrom criteria (12) (Table 3).

Table 1. Matherials and methods

Number of patients	Sex structure	Age groups	Extremities	Fracture classification	Blake-McBryde classification
12	8 males	18 do 30 3 patients (25%)	Right leg - 9 pac.	9 closed fractures	8 Type 1
	4 females	30 do 40 4 patients (33%)	Left leg - 3 pac.	3 open fractures	2 Type 2A
		40 do 50 2 patients (17%)			2 Type 2B
		50 do 60 2 patients (17%)			
		Over 60 years 1 patient(8%)			

Table 2. Blake and McBryde classification for Floating Knee injuries

Type 1 – True floating knee	Fractures of femoral and tibial diaphysis
Type 2 – Variants of floating knee	Involves one or more joints
Type 2A	The knee joint alone is involved
Type 2B	Involves the hip or ankle joints

Table 3. Karlstrom criteria for functional assessment after management of floating knee injuries

Criterion	Excellent	Good	Acceptable	Poor
Symptoms from thigh or leg	None	Intermittent slight symptoms	More severe symptom impairing function	Considerable functional impairment: pain at rest
Symptoms from knee or ankle joint	None	Same as above	Same as above	Same as above
Walking ability	Unimpaired	Same as above	Walking distance restricted	Uses cane, crutch or other support
Work and sports	Same as before	Given up sport; work same as before	Change to less strenuous work	Permanent disability
Angulation, rotational deformity or both	0	< 10 degrees	10 – 20 degrees	> 20 degrees
Shortening	0	< 1 centimetre	1 – 3 centimetres	> 3 centimetres
Restricted joint mobility	0	< 10 degrees at ankle; < 20 degrees at hip, knee or both	10 – 20 degrees at ankle; 20 – 40 degrees at hip, knee or both	>20 degrees at ankle; >40 degrees at hip, knee or both

Results

11 patients (90%) had full sanation of both fracture sites with an average sanation time of approximately 6 months, while in one case (10%) we had pseudoarthrosis of the proximal part of tibia. According to Karlstrom criteria the final results were: excellent in 6, good in 2, acceptable in 2 and poor in 2 cases (Chart 1). Following complications were noted: limitation of knee bending in 3 cases (in one patient to 20 degrees in 2 to 23 degrees), extended sanation in 2 and superficial infection in 3 cases (Chart 2).

Discussion

So far published series were from 24 (13) to 89 patients (14), and the largest published serie is 222 patients during the period of 11 years (4).

Our serie was consisted of 12 patients with average age of 40,5 years (18-63). Huseyin Arslan et al. (13) treated 24 patients of average age 38 years (17-75). Hwan Tak Heei et al. (14) published serie consisted of 89 patients of average age 42,5 (15-70) years. Anoop Kumar et al. (15) described 42 patients of average age 22,5 years (15-30). R. D. Fraser et al. (16) published largest study of treatment and followup of 222 patients of average age 52 years (14-90).

Sex structure of or patients was made of 8 males and 4 females. Huseyin Arslan et al. (13) had 22 males and 2 females, Hwan Tak Heei et al. (14) 80 male i 9 female patients, Anoop Kumar et al. (15) 30 male and 12 female patients, R. D. Fraser et al. (16) 190 males and 32 females.

All injuries within our patients were sustained in road accidents. Four patients were drivers three as co-drivers, and one patient was injured as a passenger in the back seat. Three patients were injured in a fall from a motorcycle. Huseyin Arslan et al. (13) did not specify the mechanism of injury of their patients. Hwan Tak Heei et al. (14) listed 57 patients as injured drivers and 32 patients injured in a fall from a motorcycle. Anoop Kumar et al. (15) listed 30 injured as drivers, 5 as passengers, 2 were hit while crossing the road outside of a pedestrian crossing and 5 patients injured in falls from motorcycles. R. D. Fraser a et al. (16) described 78 injured drivers, 70 pedestrians and 74 motorcyclists.

We had 9 closed and 3 opened fractures, Huseyin Arslan et al. (13) 21 closed and 3 opened fractures, Hwan Tak Heei et al. (14) 34 closed and 55 opened fractures, Anoop Kumar et al. (15) 28 closed and 14 opened fractures, R. D. Fraser et al. (16) 160 closed and 62 opened fractures.

Within 4 patients we had lesions of the blood vessels and nerves, Huseyin Arslan et al. (13) 10, Hwan Tak Heei et al. (14) 25, Anoop Kumar et al. (15) 16, R. D. Fraser et al. (16) 80.

Huseyin Arslan et al (13) and Hwan Tak Heei et al. (14) had not listed cases of amputation, Anoop Kumar et al. (15) had 13 cases, R. D. Fraser et al. (16) listed 7. We did not have any cases of amputations.

In functional results according to the Kallstrom criteria (12) we had: 6 excellent, 2 good, 2 acceptable and 2 bad results. Huseyin Arslan et al. (13) 3 excellent, 9 good, 5 acceptable and 6 bad results. Hwan Tak Heei et al. (14): 12 excellent, 20 good, 28 acceptable and 29 bad results. Anoop Kumar et al. (15): 7 excellent, 14 good, 14 acceptable i 7 bad results. R. D. Fraser et al. (16): 3 excellent, 15 good, 30 acceptable i 15 bad results.

Surgical treatment is the method of choice in the treatment of these fractures (5). Internal fixation compared to conservative treatment has the advantage because it rarely leads to stiffening of the knee and limb shortening, and patients spend less time in the hospital and are less time off from work (6). Omer et al. treated these fractures conservatively and and surgically and found that the time required for the splicing was 8 weeks shorter when the fractures were treated surgically (7). Behr et al. in the treatment of these fractures used intramedullary nailing, with average time required for healing of the femur of 10.5 weeks and 18 weeks for tibia (8). Ostrum et al treated patients with retrograde nailing with an average healing time for femur of 14.7 weeks and of 23 weeks for tibia (9). Dwyer et al. concluded that surgical or conservative treatment of the tibia does not significantly affect the subsequent movement of the knee joint later on (10). Lundy and Johnson claim that surgical stabilization of these fractures prerequisites for early mobilization of patients thus giving the best results (1).

In the treatment of these fractures using Ilizarov apparatus we kept strictly to the following rules: accurate repositioning of fracture fragments, stable and adequate immobilization of the fracture, not causing any additional trauma in the pathological focus, preservation of vascularization and sources for reparative regeneration of bone tissue, gradually performing all manipulations, achieving a permanent immobilization through strained needles, early dosed limb load.

Using the Ilizarov apparatus provides optimal conditions for the formation of bone tissue, and establishment of the anatomical structure and functioning of extremities.

Conslusion

Good treatment tactic, stabilisation and early mobilisation of patients have the best clinical outcomes in treatment of „floating knee“ type of fractures. Transosseus osteosynthesis with the Ilizarov apparatus, provides us with stabilisation, early mobilisations and full weight bearing, as well as manipulation of bone fragments with the apparatus without making large surgical incisions with minimal risk of infection, which is why we consider it a method of choice in the treatment of these types of fractures.

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Surgical timing of decompressive hemicraniectomy for malignant middle cerebral artery infarction

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Abstract

Objective: To explore the effects of surgical timing on the mortality rate and functional recovery of malignant middle cerebral artery (MCA) infarction.

Methods: The effects of surgical timing on the postoperative 6th month mortality rates and functional recoveries of 78 cases of massive brain infarction patients were retrospectively analyzed.

Results: The mortality rates of the patients undergoing surgeries prior to brain herniation were lower than those after brain herniation with a significant difference (P<0.05); the mortality rates of the patients receiving surgeries at different time (<24 h, 24-48 h and >48 h) after the onset of infarction did not differ significantly (P>0.05). The patients undergoing surgeries before brain herniation recovered better than those after brain herniation with a significant difference (P<0.05); the functional recoveries of the patients receiving surgeries at different time (<24 h, 24-48 h and >48 h) after the onset of infarction did not differ significantly (P>0.05).

Conclusion: Performing surgery prior to brain herniation can significantly reduce the mortality rate and improve the functional recovery of malignant MCA infarction.

Key words: Middle cerebral artery, cerebral infarction, decompressive hemicraniectomy, surgical timing.

Introduction

Middle cerebral artery (MCA) infarction, which is named according to the blood vessel that has lesions, has attracted particular attention from experts and clinical physicians due to the high mortality rate (80%) and disabling rate (1). As a so-called "fatal" infarction, MCA infarction accounts for approximately 15% of the ischemic stroke. Massive brain infarction, which refers to an obvious space-occupying effect owing to the severe cerebral edema after infarction (2), can be clinically divided into cerebral and cerebellar infarctions. Massive brain infarction is accompanied by progressive aggravation. Decompressive hemicraniectomy (DH) besides internal medical treatment is in need for the majority of such patients. Although DH can reduce the mortality rate (3) and improve the prognosis of malignant MCA infarction (4,5), the surgical timing is still controversial (6,7). The clinical data of 78 cases of massive brain infarction patients who received DH were retrospectively analyzed.

Materials and Methods

General information

78 out of 103 cases of massive brain infarction patients enrolled in ***** Hospital from January 2002 to January 2012 (male: 49 cases, female: 29 cases; age: 38-70, averaged 56.80±12.13) were included in this study.

Inclusion and exclusion criteria

Inclusion criterion: 1) diagnosis criterion of Hacke et al. (1); 2) completed medical records; 3) follow-up time≥6 months.

Exclusion criterion: 1) incomplete medical records; 2) contact lost within 6 months.

Grouping

The patients were grouped by two ways: 1) before (32 cases) and after (41 cases) brain herniation; 2) at different time after the onset of infarction (<24 h: 15 cases, 24-48 h: 37 cases, >48 h: 22 cases).

Treatment method

All patients were subjected to extended pterional craniotomy with question mark-shaped incision on the frontotemporal top (bone window diameter: 11-13 cm, bone flap diameter: at least 12cm). The incision was located from the centerline within the hairline to the parietal tuber (from front to back).

The incision was also extended to the central basicranial area. The middle cranial fossa was fully exposed by removing the bone flap, forceping the temporal bone until the temporal fossa and part of the squama occipitalis (8). Epidural hematoma was prevented by suspending the dura mater along the margin of bone window. The dura mater was repaired by synthetic dural substitute or autologous fascia after it had been cut radially and stanched strictly. External rather than internal DH was utilized because the boundaries of infarcted brain tissues could hardly be defined and necrosized brain tissues and ischemic penumbra could not be easily distinguished during the surgery.

Evaluation of treatment efficacy

1) Mortality rates 6 months after surgery; 2) BI index was used to evaluate the prognosis (>60: good functional recovery, ≤60: poor functional recovery).

Statistical analysis

All the data were analyzed by SPSS16.0, the measurement data and numeration data were analyzed by t test and x^2 test ($P < 0.05$).

Results

Comparison between the postoperative 6th month mortality rates

The mortality rates of the patients undergoing surgeries prior to brain herniation were lower than those after brain herniation with a significant difference (Table 1); the mortality rates of the patients receiving surgeries at different time (<24 h, 24-48 h and >48 h) after the onset of infarction did not differ significantly (Table 2).

Comparison between the postoperative 6th month functional recoveries

The patients undergoing surgeries before brain herniation recovered better than those after brain herniation with a significant difference (Table 3); the functional recoveries of the patients receiving surgeries at different time (<24 h, 24-48 h and >48 h) after the onset of infarction did not differ significantly (Table 4).

Table 1. Mortality rates of the patients undergoing surgeries before and after brain herniation: case (%)

Surgical timing	Number of cases	Mortality	Survival	x^2	P value
Before brain herniation	33	7 (21.2%)	26 (79.8%)	4.541	0.039
After brain herniation	45	20 (40.4%)	25 (59.6%)		

Table 2. Mortality rates of the patients receiving surgeries at different time after the onset of infarction: case (%)

Surgical timing	Number of cases	Mortality	Survival	x^2	P value
<24 h	18	6 (33.3%)	12 (66.7%)		
24-48 h	37	11 (29.7%)	26 (71.3%)	1.201	0.431
>48 h	23	10 (43.5%)	13 (56.5%)		

Table 3. Functional recoveries of the patients undergoing surgeries before and after brain herniation: case (%)

Surgical timing	Number of cases	Good	Poor	x^2	P value
Before brain herniation	24	19 (79.2%)	5 (20.8%)	7.725	0.006
After brain herniation	27	11 (40.7%)	16 (59.3%)		

Table 4. Functional recoveries of the patients receiving surgeries at different time after the onset of infarction: case (%)

Surgical timing	Number of cases	Good	Poor	x^2	P value
<24 h	12	10 (83.3%)	2 (16.7%)		
24-48 h	29	16 (55.2%)	13 (44.8%)	2.325	0.314
>48 h	10	4 (40.0%)	6 (60.0%)		

Discussion

Malignant MCA infarction leads to fatal space-occupying brain edema, which is cytotoxic initially without destroying the blood-brain barrier obviously. The area of infarction cannot be easily determined and typical brain infarction is hardly shown in CT at this stage. Thereafter, the blood-brain barrier is destroyed that induces vasogenic brain edema, leading to typical massive cerebral edema after brain infarction. Generally, the edema of infarcted brain tissues has become prominent, reaches maximum and recedes 1 day, 3-5 days and 2 weeks after the onset, respectively. Patients get worse or even die of the high intracranial pressure induced by brain edema, the shift of brain tissues, enlarged infarction area and brain herniation. Malignant MCA infarction accounts for 10%-15% of brain infarction. The survival rate at 20% after conservative treatment can be increased to 67%-84% after surgical treatment (9). DH treatment can reduce the intracranial pressure, raise the perfusion pressure, facilitate the reflux of pial collateral vessels and increase the blood supply in the branch of MCA. Therefore, the blood flow in the ischemic penumbra is increased, the necrosis of brain tissues is alleviated, and the brain shift is corrected.

The effects of the surgical timing of DH on the survival and functional recovery are still controversial (10). Previous animal studies have shown that DH performed 1-24 h after the onset of malignant MCA infarction could reduce the mortality rate and improve the neurological function. The postoperative 2nd week infarct volume and the neurological function which obtained 1-12 h after infarction outweighed those obtained 24 h after infarction (11). Besides, it has been reported that the postoperative 1th month and 6th month mortality rates of the patients who received DH after deterioration were 19.1% and 27.6%, respectively. However, the values drastically reduced down to 4.8% and 17.2% when they received DH as soon as MCA infarction was diagnosed. However, the functional recoveries of the two groups did not differ significantly (12). Schwab et al. also reported that early surgery (<24h) could significantly reduce the mortality rates of massive brain infarction patients and promote the functional recovery (13,14). Nevertheless, it has also been reported that mortality rate and functional recovery are irrelevant to surgical timing. From the perspective of evidence-based medicine, two recently published Meta analyses also failed to confirm the advantages of early surgery.

Whether DH should be performed according to the onset time or the disease progression is also controversial (15). The patients who can be cured by conservative internal treatment do not need the unnecessary invasive DH, whereas delayed DH may result in irreversible brain stem damage due to brain herniation that affects prognosis. Although the morbidity rates and the functional recoveries of the patients receiving surgeries at different time (<24 h, 24-48 h and >48 h) after the onset of infarction did not differ significantly, the mortality rates and the prognosis of the patients undergoing surgeries prior to brain herniation were significantly lowered and improved, respectively. The results indicate that DH surgical timing should be determined on the basis of disease progression rather than onset time. DH should be performed immediately confronting one of the following cases before mydriasis:

1) somnolence, drowsiness or progressively deteriorated consciousness;
2) clear brain edema with the midline shift higher than 5 mm (CT);
3) completed infarction of MCA or internal carotid artery (DWI magnetic resonance imaging).

As a retrospective study, there are some limitations, such as the small sample size, which is insufficient for hierarchical analysis. Therefore, a random large-scale sample control experiment will be required to evaluate the effects of DH on the functional recovery of malignant MCA infarction objectively and reliably.

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The investigation of lipid levels in patients with ischemic stroke and vascular dementia

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Abstract

Background: The study was to determined concentrations of cholesterol, triglycerides HDL and LDL cholesterol in patient's serum after stroke.

Methods: Our study included 600 subjects, 200 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase (initial 24-48 hospitalization hours), 200 patients diagnosed with vascular dementia and 200 healthy subjects. Lipids were determined using DIMENSION LxR automatic analyser of DADE BEHRING.

Results: Our results show that the concentration of HDL cholesterol was significantly lower in the group with ischemic stroke and vascular dementia than in the control group. Average concentrations of cholesterol and LDL cholesterol were significantly higher in the group with ischemic stroke and vascular dementia than in the control group. In the group with ischemic stroke, the concentration of LDL cholesterol was higher than 4.3 mmol / L in about 57% of patients, whereas in the group with vascular dementia, the concentration of LDL cholesterol was higher than the upper reference value in about 49% of patients. The mean concentration of triglyceride was significantly higher in the stroke group.

Conclusions: Increasing concentrations of LDL and c/HDL (AHDL Risk factor) values in the serum of patients with ischemic stroke affects the possible further development of atherosclerosis development of new stroke and vascular dementia.

Key words: Lipids, ischemic stroke and vascular dementia.

Introduction

Atherosclerotic vascular changes are responsible for cardiovascular events such as myocardial infarction and stroke (1). Atherosclerosis is characterized by the thickening of coronary artery wall from the deposits of plaques, resulting in reduced blood flow. The risk factors include hypercholesteremia, hypertension, smoking, gender and diabetes mellitus (2).

Stroke was defined as clinical syndrome characterized by rapidly developing clinical symptoms and or/ signs of focal and at times global loss of brean function, leading to early death, and with no apparent cause of other than of vascular orgin. Approximately, 25 % of men and 20 % women can expect to suffer a stoke if they live to reach 85 years. (3,4).

The term "dementia" refers to a group of disorders that cognitive decline as a result of death or damage to brain cells. By definition, dementia causes a decline in at least two of four essential cognitive functions: (1) memory; (2) ability to speak or understand language; (3) capacity to plan, make sound judgments, and carry out complex tasks; (4) ability to process and interpret visual information. The vascular dementia will occur in a patient who has several times getting over a stroke (mean damage to the blood vessels of the brain and brain stem damage that may be of different scope (5, 6). The elevated concentration of cholesterol (cholesterol> 5.2) in blood is an increased risk for ischemic stroke accident. There was a connection between the risk for ischemic stroke and increased concentrations of total cholesterol, LDL-cholesterol (low density lipoprotein cholesterol) and relationships cholesterol / HDL-cholesterol. Clinical studies of patients with cardiovascular disease (CVD) found increased mortality in patients with decreased concentration of HDL cholesterol (high density lipoprotein cholesterol). Plasma low concentration of HDL cholesterol is an independent risk factor for heart disease and stroke. The every rice of HDL cholesterol for 0.025 mmol/L will decrease

risk for cardiovascular events by 2 % in men and 3 % in women. This relationship is independent of total cholesterol concentration. LDL is the major atherogenic lipoprotein and has long been identified by as the primary target of cholesterol-lowering therapy. The positive relationship between serum cholesterol levels and the development of CVD observed over a broad range of LDL cholesterol levels if it is a higher level it is a greater risk. Recent clinical trials indicate that 1% decrease in LDL cholesterol reduces risk of developing CVD by about 1 %. Recommendations for reduced risk of CVD are that patients with CVD or other atherosclerotic disease should have LDL concentration consistently below 3.0 mmol/L (7-9). At our study we have determined concentrations of cholesterol, triglycerides HDL and LDL cholesterol in patient's serum after stroke and vascular dementia.

Material and methods

Patients

The studie included 600 patients, 200 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase (initial 24-48 hospitalization hours), 200 patients diagnosed with vascular dementia and 200 healthy subjects. The 600 blood samples were collected during 2009 - 2011. All investigation was done respecting ethical standards by the Helsinki Declaration. The patients were hospitalized at Clinic of Neurology, Clinical Centre University of Sarajevo, Old home "Nedzarici, and Old home "Ernest Grin". The control group included 200 healthy subjects who were protégé at Old home "Nedzarici". The study criteria for entry in study were: neurological examination and neuroimaging methods, diagnosis of ischemic stroke was strictly verified, no disorders related to hepatic, renal and endocrinologic functions, no systemic malignancy (10-14).

Criteria for inclusion of patients with stroke and vascular dementia were: diagnosis of first ischemic stroke determined by computerized tomography (CT), presence of vascular dementia identified using computerized tomography (CT) and nuclear magnetic resonance (NMR), "*Hachinski ischemic score*" grater or equal to 7 for patients with vascular dementia, age over 65 years for both sexes, two weeks treatment in hospital for patients with ischemic stroke, stroke in patients with vascular dementia in last three to six years. The study inclusion criteria for control group were: excluded ischemic stroke or vascular dementia by computed tomography (CT) and nuclear magnetic resonance (NMR), age over 65 years for all controls.

The blood samples were collected at the mornings before the first meal and after 12 hours of fasting. For the group of 200 patients diagnosed with the first ischemic brain stroke, blood samples were taken during the acute phase (initial 24-48 hospitalization hours). For the group of 200 patients diagnosed with vascular dementia developed as a consequence of ischemic brain stroke, i.e. of many small ischemic focus of various age. The study included 108 men and 92 women in control group. There were 108 males and 92 females in the group with ischemic stroke and 110 men and women 90 in the group with vascular dementia. By analyzing the history of disease we collected data about radiological examinations of brain computed tomography (CT) and brain nuclear magnetic resonance (NMR). 92% (184) of patients suffering from ischemic stroke had the diagnosis *ICV per trombosim* and at 8% (16) patients the diagnosis was *ICV per emboliam*.

Sample preparation

The patient samples of blood were collected in serum separation Vacutainer test tubes (Becton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL. We have used tubes with gel for blood collection. After collection, blood samples were obtained by centrifugation at 3000 rpm using centrifuge (Sigma 4-10).

Study design

Lipids were determined using DIMENSION LxR automatic analyser of DADE BEHRING.

Measurement of cholesterol

The cholesterol method is based on quantitative determination of total cholesterol in human serum. The cholesterol esterase catalyzes the hydrolysis of cholesterol esters to produce free cholesterol which, along with pre-existing free cholesterol is oxidized in a reaction catalyzed by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase, the hydrogen peroxide formed is used to ox-

idase N,N-diethylaniline-HCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm.

Measurement of triglycerides

The triglycerides method is based on an enzymatic procedure in which different enzymes are employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5-triphosate (ATP) to glycerol-3-phosphate. Glycerol-3-phosphate-oxidase oxidases glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2) and catalytic action peroxidase (POD) forms quinoemine from H_2O_2, aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinonemine is directly proportional to the total amount of glycerol.

Measurement of HDL cholesterol

The method for HDL cholesterol consisted of two form of reagent and depends on the properties of a unique detergent and it is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectivity using specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a color less product. The second reagent consists of detergent capable of solubilization HDL specifically cholesterol esterase (CE) and chromagenic coupler to develop color for the quantitative determination of HDL cholesterol.

Measurement of LDL cholesterol

It was use a detergent that dissolves only non-LDL particles, free cholesterol reacts with cholesterol oxidase and esterase and gives unpainted product (15-16).

The referentne value are : cholesterol 3.1-5.2 mmol/L, tryglicerides 0.11-1.70 mmol/L HDL-a 1.06-1.94 mmol/L and LDL 2.00-4.30 mmol/L, c/HDL (AHDL Risk factor) 0.0-5.0, Atero index (LDL/HDL) 1.2-4.0.

Results

At out study mean age of the patient group with ischemic stroke was within the range of 70.12 ± 7.52, mean age of the patient group with vascular dementia was within the range of 73.74 ± 4.45 and the mean age of the control group was within the range of 69.34 ± 4.45. In respect of the risk factors, Diabetes Mellitus, hypertension, smoking, alcohol, displayed significantly higher rates of prevalence in the patient population then in control group. The hypertension and smoking was found to be significantly higher in group with ischemic stroke than in group with vascular dementia and control group. Without regular antihypertensive and hyperlipidaemia treatment were all 200 patients in group with ischemic stroke. The 110 patients (55%) with vascular dementia take antihypertensive drugs and statins. All patients in control group have been used antihypertensive drugs and statins. The results of risk factors are shown in Table 1.

In the ischemic stroke group the mean concentration of total cholesterol, LDL cholesterol and triglycerides were significantly higher then in group with vascular dementia and control group. The mean concentration HDL cholesterol was si-

Table 1. Characteristiic of patients and control subjects

Comparison groups		Diabetes Mellitus	Hypertension	Smoking	Alcohol
Ischemic brain stroke after 24-48 hours (N=200)	N	72	168	74	16
	%	36	84	37	8
Mean Age ± SD	70.12 ± 7.52				
Vascular dementia (N=200)	N	74	120	50	8
	%	37	60	25	4
Mean Age ± SD	73.74 ± 4.45				
Control group (N=200)	N	5	50	10	2
	%	2.5	25	5	1
Mean Age ± SD	69.34 ± 4.45				

gnificantly lower in group with vascular dementia then in patients with ischemic stroke and in control group. The LDL/HDL ration (aterogenic index) and total cholesterol/HDL cholesterol ration (AHDL Risk factor) were higher in patient group then in control group. The patients with ischemic stroke have LDL cholesterol concentration higher than 4.3 mmol/L in about 57% of patients, whereas in the group with vascular dementia, the concentration of LDL cholesterol was higher than the upper reference value in about 49% of patients. The mean concentration, standard deviation of total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C, aterogenic index and c/HDL in patients and control group are summarized in Table 2.

We have make comparison of lipid parameter in patients groups with control group using ANOVA test for statistical difference for $P<0.05$. There was statistical difference in average concentration of total cholesterol, LDL cholesterol and LDL/HDL ratio between the two patients groups and control group. It was statistical difference in average concentration of triglyceride between ischemic brain stroke and control group but it was not statistical difference between vascular dementia and ischemic brain stroke group or with control group. A comparing the group with ischemic stroke and vascular dementia with control group it was found statistic difference in average concentration of HDL cholesterol and total cholesterol/HDL cholesterol ratio. It was not found statistical difference in average concentration of HDL cholesterol and total cholesterol/HDL cholesterol ratio between vascular dementia and ischemic brain stroke group. The results of comparison between the groups are shown in Table 3.

Discussion

The incidence of stroke and dementia rises exponentially for patients with cerebrovascular risk factors such as hypertension, cardiac disease, diabetes, smoking, alcoholism and hyperlipidemia. Risk of vascular disease, stroke and vascular dementia increases significantly with age and people over 65 are at greatest risk, with risk doubling

Table 2. The mean concentration of lipid levels at risk groups and control group

Comparison groups		Cholesterol mmol/L	triglyceride mmol/L	c/HDL	HDL mmol/L	LDL mmol/L	Atero index
Ischemic brain stroke after 24-48 hours (N=200)	X_{sr}	6.31	1.74	5.64	1.15	4.36	3.93
	S.D.	1.29	0.78	1.45	0.26	1.15	1.21
	S.E.	0.18	0.10	0.20	0.25	0.16	0.17
Vascular dementia (N=200)	X_{sr}	5.59	1.64	5.19	1.08	3.78	3.71
	S.D.	0.92	0.71	1.23	0.24	0.84	1.21
	S.E.	0.92	010	0.17	0.33	0.83	0.17
Control group (N=200)	X_{sr}	4.96	1.39	3.76	1.39	2.93	2.2
	S.D.	0.63	0.54	0.94	0.27	0.56	0.64
	S.E.	0.90	0.76	0.13	0.03	0.07	0.90

Table 3. Comparison of serum lipids concentration in risk groups of patients with control group

Comparison groups		Cholesterol mmol/L	triglyceride mmol/L	c/HDL	HDL mmol/L	LDL mmol/L	Atero index
ischemic brain stroke *with* control group	Mean difference	1.3460	0.34380	1.8780	-0.24920	1.42100	1.3460
	S.E.	0.1979	0.13693	0.2454	0.05140	0.17720	0.1979
	p	0.000*	0.039*	0.000*	0.000*	0.000*	0.000*
ischemic brain stroke *with* vascular dementia	Mean difference	0.7180	0.09660	0.4456	0.07300	0.57980	0.7180
	S.E.	0.1979	0.13693	0.2454	0.05140	0.17720	0.1979
	p	0.001*	1.000	0.214	0.473	0.004*	0.001*
vascular dementia *with* control group	Mean difference	0.6280	0.24720	1.4324	0.32220	0.84120	0.6280
	S.E.	0.1979	0.13693	0.2454	0.05140	017720	0.1979
	p	0.006*	0.219	0.000*	0.000*	0.000*	0.006*

** P < 0.05 (ANOVA - test)*

for every five years after 65. Vascular dementia is the third most common brain disorder and incidence of vascular dementia ranges from 1-4% of the total population. The prevalence is seen to be more common in men than women. (17,18). In our studie we have 56% of mens and 44 % of womens in group with ischemic stroke and 55 % of mens anf 55 % of womens in group with vascular dementia. Our study have confirmed that vascular dementia is more offen in mens then in womens. The patient with ischemic stroke come from a families with very low socio-economic status. The risk factors can be caused by socio-economic risk factors such as poverty/low income and/or discrimination related to factors such as disability, ethnicity or gender (19). We have found diabetes in 36 % of patients with ischemic stroke and in 37 % patients with vascular dementia. The cognitive function could decline with hypertension and diabetes as one of the case of cardiovascular diseases. It is find influence of insulin and insulin-degradin enzyme on dementia patology (20). However, our study have not association of cognitive disfunction in hypertensive subjets with diabetes mellitus. Arterial hypertension was definined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher. Research data has estimated that about 50 percent of vascular dementia results from high blood pressure (19,21). In our study 84 % with ischemic brain stroke and and 60% vascular dementia have arterial hypertension, the results are shown in Table 1. Mainly patients with ischemic brain stroke have not take regular antihypertension therapy and statins. It was previously described that arterial hypertension may be a a certain risk facror for cognitive dysfunction in the eldery. It is described that cognitive impairment proceded by high diastolic blood pressure, but blood pressure declined during the years prior to dementia onset. The positive effect of higher blood pressure in patients with vascular dementia was high significant for heavy smokers (18,21). It could be an explanation of lack association between blood pressure and cognition in our study. Results of our study indicate smokers in group with ischemic stroke in 37 % and vascular dementia in 25 % of patients. Our results indicate the possible lower congitive abilities in patients who were heavy smokers. In the early 1990s, smoking habits have association with higter scores of global cognition. Accoding to these findings it is find at patients with vascular dementia a number of neuritic plaques increased in patients who were smokers. Lifestyle risk factors like being overweight or obese, and smoking can contribute to vascular dementia (22- 24).

The results of our study have we find drinking of alcohol in 8 % with ischemic stroke and in 4 % patients with vascular dementia. One of the risk factors associated with ischemic stroke and vascular dementia is excessive drinking of alcohol (19). In this study, we evaluated serum lipid concentration in patients with ischemic stroke and vascular dementia. Hyperlipidemia and high concentrations of cholesterol and LDL cholesterol are important risk factors for the development of arteriosclerosis. Our patent with ischemic stroke and vascular dementia have higher concentration of cholesterol and LDL holesterol than control group. The mean diference between ischemic brain stroke and control group was 1.3460, $P < 0.05$ using ANOVA – test. In our study mean diference for cholesterol between ischemic brain stroke and vascular dementia was 0.7180, between vascular dementia and control group was 0.6280, $P < 0.05$ using ANOVA – test. The concentration of cholesterol is significantly different between groups with ischemic stroke, the group with vascular dementia and control groups for $P < 0.05$. The group with ischemic stroke had significantly higher cholesterol concentrations than the other two groups (Table 2). According to our results in 90% of patients with ischemic stroke and 80% of patients with vascular dementia, cholesterol concentration was above the upper reference value that is greater than 5.2 mmol/L. Results of Adachi and associates (25) showed that the increased risk of ischemic stroke increases with the increase of cholesterol, the increase of cholesterol by 1 mmol/L increases risk by 1.41 times. When the cholesterol concentration of greater than 4.14 mmol/L was significant increase in risk of ischemic stroke. According to the Asia-Pacific Women and the Poopling study for every 1 mmol/L increase in total cholesterol increases the risk of stroke by 23-25%. At same study, ischemic group had a higher concentration of total cholesterol by 6.21 mmol/L and LDL cholesterol greater than 4.14 mmol/L (26). The concentrati-

on of triglycerides were significantly higher in the group with ischemic stroke compared to controls (P <0.05). In other studies it was confirm that triglycerides concentration was significantly higher in patients with ischemic stroke (27). The potential biological mechanism responsible for association between triglycerides level and stroke severity is unknown. The triglycerides level can reflect nutritional status before the patients have got ischemic stroke. It is possible significant changes in triglycerides concentrations during first days after stroke, so we cannot exclude that acute phase reaction accompanying stroke can at some degree influence triglycerides level. The patients with ischemic stroke have not regular hyperlipidaemia treatment, therefore elevated triglyceride level, which affects blood vessels in the brain and produce ischemic stroke. We have not found significant mean difference for triglycerides between vascular dementia and control group or ischemic stroke group. It could be explained that patients with vascular dementia and control group have mainly treatment with statins. In prospective investigation, elevated LDL cholesterol was an independent risk factor for development of dementia with stroke in elderly patients. The high level of cholesterol later produces atherosclerosis (2,28). According to our results, the concentration of cholesterol in the group with ischemic stroke was 6.31 mmol/L, LDL 4.36 mmol/L, HDL 1.15 mmol/L. The Fischer and investigator results show in group of patients with ischemic stroke concentration of total cholesterol higher than 6.21 mmol/L and LDL cholesterol concentration greater more than 4.14 mmol/L. The risk of ischemic stroke increases by 14% if the concentration of LDL cholesterol increases for every 1.03 mmol/L (1). The concentration of LDL cholesterol was significantly higher in the group with vascular dementia and ischemic stroke compared to the control group (P <0.05), the results are shown in Table 3. The our results have show significant mean difference 0.57980 between ischemic brain stroke and vascular dementia for P <0.05. In the group with ischemic stroke LDL cholesterol concentration was higher than 4.3 mmol/L in 57% of patients, whereas in the group with vascular dementia, the concentration of LDL cholesterol was higher than the upper reference value in 49% of patients.

HDL cholesterol is responsible for cholesterol transport to liver for his degradation or recycling. In a large prospective study of the male population found that the overall increase in HDL cholesterol for 1.33 mmol/L reduces the risk of fatal stroke by 40% (1). The concentration of HDL in the group with ischemic stroke and vascular dementia was significantly lower than in the control group. The mean difference was significant between ischemic stroke and control group P <0.05. According to our results the mean difference 0.84120 was significant between vascular dementia and control group P <0.05. Among the group with vascular dementia, and the group with ischemic stroke was not significant difference in the level of significance of P <0.05 (Table 3). Mean values of HDL concentration in patients groups were not different, the concentration of HDL remained lower in patients with ischemic stroke and is one of the prerequisites for further development of cardiovascular disease and vascular dementia. A prospective study Haralamposa and associates (2005) showed that the decrease in the concentration of HDL cholesterol associated with increased risk for new stroke (28). Our data are in good agreement the findings of other authors. Kurijama et al. reported lower HDL-C levels in patients with vascular dementia compared with controls (29), while Katzman et al. found lower HDL-C levels in older men with dementia "with a vascular component"(30). Possible reason is that low HDL-C levels might be involved in the pathogenesis of vascular dementia (31). Interestingly, Bonarek et al. have found that, in a case-control study, elevated HDL-C levels were associated with a decreased risk of dementia at multivariate analysis (32). Atherogenic index LDL / HDL cholesterol was significantly elevated in the group with ischemic stroke and vascular dementia compared to controls (Table 2). Our study showed that the atherogenic index has a significant difference between control group and the group with ischemic stroke and vascular dementia (P <0.05). We also found a significant difference between the atherogenic index in the group with ischemic stroke and vascular dementia (P <0.05). (Table 3) Increasing concentrations of LDL and the atherogenic index values in the serum of patients with ischemic stroke influence the further development of atherogenesis and the development

of new stroke. According to our results the value of the ratio of cholesterol / HDL ratio (AHDL Risk factor) was significantly higher in the group with ischemic stroke compared to the control group (P <0.05). Results of our stady have show a significant difference between control group and the group with ischemic stroke and vascular dementia (P <0.05). It was not found significant difference between the group with ischemic stroke and vascular dementia (P <0.05).

Conclusions

From this study the following points we can concluded, that the level of total cholesterol and LDL cholesterol were significantly higher in patients groups (ischemic stroke and vascular dementia) in compared with controls. In our study we found lower HDL cholesterol levels in the ischemic stroke group and vascular dementia group in compared with controls. Longitudinal studies are needed in order to elucidate the possible role of HDL particles in the pathogenesis of thise disease. The possible limitation of our study are that we do not have informations about lipid levels before ischemic stroke or vascular dementia. The level of serum triglycerides was higher in ischemic stroke group than in control group. Therefore high concentrations of cholesterol, LDL cholesterol and c/ HDL (AHDL Risk factor) values in the serum of patients with ischemic stroke affects the possible further development of atherosclerosis and the development of new stroke.

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The analysis of psychological empowerment factors in Iranian public hospitals

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Abstract

Introduction: It is important to identify employee's empowerment factors in order to realize empowerment processes and enhance organization's capacity to make an empowered atmosphere. Undoubtedly, today strengthening the employee's capabilities in hospital can play an important role in improving the quality of the services and cut the costs.

Methods: The current study was done using a descriptive scalar method with 485 employees of the hospitals of the Medical University of Iran. We used stratified sampling method and chose an equal ratio of samples from hospitals. The data collection tool was a questionnaire consisting of 55 closed answer questions plus demographic characteristics. Data analysis was done using SPSS and factor analysis therein.

Results: Using exploratory factor analysis, the researchers identified 8 indices with regard to personnel's job responsibilities in psychological empowerment. They include the use of information, determination of appropriate indexes in performance and effectiveness feedbacks, teaching leadership skills, having clear goals, explaining the personnel's responsibilities and the role of others.

Discussion: It is necessary to hold employees' empowerment courses along with empowerment components training sessions for hospital managers and to use the identified characteristics in public hospitals. It is noteworthy that hospital managers should first study these factors in their own hospitals and then do the above mentioned procedures.

Key words: Psychological empowerment, Employees, Hospital.

Introduction

Appropriate management of the hospitals as the most important medical and health services center has always been the focus of attention. We have a slow and difficult trend in expanding the hospital facilities due to limitations in investment resources and their delayed feedback in health and treatment section and also factors such as investment for founding a hospital, expensive facilities and lack of skilled, expert and capable man power. So it is necessary to effectively use the existing resources with managerial elite techniques (1).

According to basic and widespread study of World Bank in developing countries from public hospitals, hospitals use between 50 to 80 percent of the public health resources. Based on the Iranian's budget law in 2011 for health ministry, there is a 74756792 million rial budget for the ministry including price and ownership credits. Medical service share for hospitals affiliated with the ministry is 39591799 million rials. The ratio of this credit to the total credit with respect to increments during the past year is between 52 to 80 percent in different medical universities of the country (2).

Total survey shows that, on average, about 10 % of hospitalization in medical institutes suffers from personnel and system flaws with 1% percent for death. Studies suggest that we can prevent these cases happening from 50 to 70 % with appropriate and in time planning and implementing the proper procedures. One such procedure is personnel's management and empowerment (3).

Today it is necessary for human resources managers to realize that empowerment is a vital tool for increasing the personnel's satisfaction and efficiency (4, 5). With empowerment trends in mind, everyone in every job status and organizational rank will have its own management and decision making capabilities (6). By empowerment, managers really multiply their efficiency and their organization will be more effective and perform better (7,8,9).

Scott and Jaffe hold that the reason for personnel's empowerment is that organizations are under attack from inside and outside of the organization. From the outside point of view, we need immediate response to worldwide competition, unbelievable swift changes and new demands for quality and services, and lack of resources. From the inside point of view, personnel feel that they are not treated honestly and thus will be disappointed and have more expectations from the organization. Meanwhile, personnel want more meaningful work, honesty and self-discovery. Changing the traditional organizations and emerging new organizations require more attention to empowerment. Traditional organizations needed men doing assigned jobs without any question (10), but modern job environments need men who choose the best method of performing the assigned job smartly and without any help and feel that they share the best ideas with their managers (11). Briefly, organizations need capability to effectively respond to the problems in risky and uncertain situations (12).

Today in most of the countries, health care industry needs the promotion of care quality with fewer resources. This need gives rise to many changes and challenges in health care organizations worldwide. Hospital managers and officials look for reengineering health care procedures to attain more effective results with lower prices. These changes include restructuring the health care system, redesigning the job procedures and developing the role and responsibilities of the personnel. Leaders, managers and officials of the organizations should identify the effective processes in personnel's attitudes and engender the proper atmosphere enabling the personnel to properly manage the changes. Hospitals are one of the important medical services organizations which play an important role in preserving the physical and mental health of the patients; hence, paying attention to empowerment of hospital personnel is more important than those in other organizations (13).

All the evidence shows that capable personnel are more effective, innovative and happier than others and produce more quality products. When we have capable personnel, we will have efficient organization too (6,8). Therefore, the aim of the present study is to identify empowerment factors in Iran's educational and medical hospitals.

Methods

The present research was done through a descriptive scalar method. In analytical model of the research, delegation, shared management, communication, information preparation, performance assessment, clear objectives, reward system, leadership, hospital culture, modeling, emotional excitement, grouping and vagueness in roles are considered as independent variables and psychological empowerment is considered as the dependent variable. With respect to research variables, the population was the personnel of medical and educational hospitals of medical sciences universities of health ministry. According to the type of services, health ministry has categorized medical sciences universities to 7 groups. From each group, one hospital, where organizational superiority model of board of trustees' hospitals passed in 2009 is implemented, was chosen. They included hospitals of Amin in Esfahan, Imam Khomeini in Kermanshah, Imam Hussein in Tehran, Al-Zahra in Tabriz, Faghihi in Shiraz, Razi in Ahwaz, and Hashemi Nejad in Mashhad. The Qualified population consisted of 3450 participants. According to Cochran's sampling formula, the required sample size was 485 persons. Due to 50 to 60 percent prediction for questionnaire return factor, 920 questionnaires were distributed from which 540 questionnaire were returned and 55 questionnaire were discarded because of incomplete response. finally, 485 questionnaire were analyzed. For questionnaire distribution, with reference to hospitals, we gathered data from personnel in a morning work shift. We used clustered and stratified sampling. Variance for a 50 pretest sample was 0.341 and we used just questionnaires as the data collection technique. Fifty five questions on a five-point Likert scale questionnaire were used. A primary sample including 30 pretest questionnaires to test the reliability of the questionnaire was tested, and it calculated the confidence coefficient via cronbach's alpha method. The obtained index of alpha was 93% (that was larger than 70%), indicating a desirable level of reliability. To control the content validity that assessed the sufficiency and content of the questions, 30 questionnaires were distributed among some experts and all ambiguities regarding the questions were eliminated, and then the designed models were reviewed.

The SPSS software version 15 and exploratory factor analysis to find out the variables underlying the phenomena and to summarize the data were used. Also, Kaiser-Meyer-Olkin (KMO) index was calculated as a measure of sampling adequacy to determine the appropriateness of factor analysis.

Results

The results of analyzing the main components of organizational dimension showed that 6 components of clear objectives, reward system, leadership, hospital culture, modeling and emotional excitement were reduced to 3 sub-components of clear objectives, leadership style and hospital culture and 2 items out of 25 were omitted. So we used exploratory factor analysis with maximum likelihood method and varimax rotation. The results show that Kaiser-Meyer-Olkin (KMO) sampling adequacy index equals to 1 and Bartlett's Test of Sphericity significance level was less than 0.001.

According to Table 1, the results showed that from organizational dimension component, three factors with special amount bigger than one emerged. The first three factors explained 44/93 %, 11/53 %, and 7/05 % of the variance of all the variables respectively; and altogether, they explained 63/54% of the variance of all the variables of organizational factors. In this component, items 23, 22, 21, 20, 13, 14, 15, 16, 17, 18, and 19 as the first factor were called hospital culture, items 4, 3, 5, 12, 11, 10, 9, 8, 7, and 6 as the second factor were called leadership style and finally items 3, 2, and 1 as the third factor were called clear objectives.

The results of analyzing the main components of structural dimension showed that 6 sub-components of delegation, shared management, communication, information preparation, performance assessment and support reduced to 3 sub-components of delegation, managerial support and information sharing. Two items out of 20 items were omitted. So we used exploratory factor analysis with maximum likeliho-

Table 1. Rotated matrix of organizational dimension component

Factor name	Item no.	Item	Factors		
			1	2	3
Clear objectives	1	My duties and responsibilities are clearly determined.	-		.852
	2	Expectations from me are carefully explained.	-		.846
	3	Organization objectives are clear to me.	-		.735
Leadership style	4	My supervisor always pursues our sector's superiority.	-	.757	-
	5	My supervisor always clearly defines our sector's special objectives.	-	.788	-
	6	My supervisor is known as a good model.	-	.867	-
	7	My supervisor emphasizes performance quality.	-	.797	-
	8	My supervisor pays attention to personnel's welfare.	-	.821	-
	9	My supervisor knows how to develop personnel's potentials.	-	.824	-
	10	My supervisor has good communication skills.	-	.832	-
	11	In our hospital, supervisors trust personnel.	-	.531	-
	12	In our hospital, supervisors support personnel appropriately.	-	.567	.501
Hospital culture	13	Humanitarianism is one of our hospital's important values.	-	-	.589
	14	Our hospital has a clear outlook.	-	-	.673
	15	Correct risk taking is admired in our hospital.	-	-	.753
	16	There are many self-guided work groups in our hospital.	-	-	.720
	17	There has been an attempt to explain the successful job performance.	-	-	.726
	18	Successful personnel who play pattern role will be admired.	-	-	.791
	19	Humanitarian models are made as elite models.	-	-	.804
	20	Works with significant effect in hospital will be highlighted.	-	-	.783
	21	There has been an attempt to make a friendly relationship among the personnel.	-	-	.751
	22	There is congruence between important personal values and organizational objectives.	-	-	.795
	23	Rewards are related to work progress.	-	-	.604

od method and varimax rotation. The results showed that Kaiser-Meyer-Olkin (KMO) index of sampling adequacy equaled to 0.923 and Bartlett's Test of Sphericity significance level was less than 0.001.

The results showed that from organizational dimension component, three factors with special amount larger than 1 emerged. The first three factors respectively accounted for 45/46 %, 9/75 %, and 8/47 % of the variance of all the variables; and altogether, they explained 63/69% of the variance of all the variables of organizational factors. In this component, items 41, 40, 39, 38, 37, and 36 as the first factor were called delegation, items 30, 29, 28, 27, 26, 25, and 24 as the second factor were called information sharing, and finally items 33, 32, 31, 35, 34 as the third factor were called managerial support.

Table 2. Rotated matrix of organizational dimension components

Factor name	Item no.	item	Factors		
			1	2	3
Delegation	24	I am allowed to do the delegated works via my own method	-	.701	-
	25	I am allowed to do what brings the best quality to my work.	-	.657	-
	26	To change things, I need not follow the redundant paperwork.	-	.721	-
	27	I have the necessary opportunity to express my ideas and interest in my job field.	-	.759	-
	28	My manager admires involving in decision making.	-	.747	-
	29	My manager tries to involve me in decisions affecting my job.	-	.688	-
	30	My manager uses my experiences in planning, determining goals, and choosing task performance methods.	-	.676	-
Information Sharing	31	I am aware of topics and discussions related to my job.	-		-
	32	I am capable of communicating necessary and sufficient information to others.	-	-	-
	33	Hospital personnel communicate their real feeling regarding different topics to others.	-	-	-
	34	I receive good feedback from my colleagues.	-	-	-
	35	I have all the necessary information to do my responsibilities	-	-	-
Managerial support	36	The manager assesses my performance based on determined objectives.	.770	-	.770
	37	The manager supervises personnel's performance based on predetermined criteria.	.809	-	.809
	38	The manager assesses me based on job behavior.	.828	-	.828
	39	I receive support when I do my job well.	.784	-	.784
	40	I receive required support and feedback repeatedly.	.778	-	.778
	41	I receive support regarding my work interests and benefits.	.757	-	.757

Table 3. Rotated matrix of job dimension components

Factor name	Item no.	Item	Factors	
			1	2
Grouping	42	Work groups are allowed to make decisions and actualize their suggestions.	.690	-
	43	Fair act in team work is ensured in this hospital.	.825	-
	44	People's rights are revered in teams.	.845	-
	45	Team members can stand each other.	.846	-
	46	Team members are liable for their acts upon society.	.768	-
	47	There exists cooperation in teams.	.775	-
Role ambiguity	48	My assigned role in the hospital is clear to me.	-	.824
	49	My role in the hospital is clear to others	-	.863
	50	Others' roles in the hospital are clear to me.	-	.841
	51	I feel satisfied playing my role in the organization.	-	.703

The results of analyzing the main components affecting psychological empowerment in the dimension of job showed that two components of group making and ambiguity in role were approved. Therefore, we used exploratory factor analysis with maximum likelihood method and varimax rotation. The results showed that Kaiser-Meyer-Olkin (KMO) index of sampling adequacy equaled 0.880 and Bartlett's Test of Sphericity significance level was less than 0.001. So based on both these factor, we concluded that factor analysis in sample group upon correlation matrix would be justified.

The results of factor analysis showed that from job factors component, the first factor (group making) and the second factor (role ambiguity) respectively accounted for 50.88% and 17.36% of the variance of all the variables and these two factors altogether explained 68/25% of the variance of all the variables of job dimension. In this component, items 42, 47, 46, 45, 44, and 43 as the first factor were called group making and items 51, 50, 49, and 48 as the second factor were called role ambiguity.

Discussion

The results showed that Kaiser-Meyer-Olkin (KMO) index of sampling adequacy was grater than 0.9 and Bartlett's Test significance level was less than 0.01. So based on both these factors, we concluded that factor analysis in sample group upon correlation matrix would be justified.

It is mentioned that if information is shared well in the organization, personnel will have better understanding of the concept and content of managerial decisions. Therefore, they will be more coordinated with organization's activities (14). In both these research studies, the use of information in performance feedback and efficiency is considered as the most important factor in learning, motivation and education. Furthermore, feedback is considered as a component of personal interaction. These research studies are compatible with the present study because all underscore the importance of using and sharing information which result in empowerment.

Thomas and Velthouse suggest the mechanism of clear statement of objectives and missions, education, support, delegation, and reward system and democratic leadership style for empowerment (15). Whetten, Cameron and Woods (1997) present nine managerial strategies for personnel empowerment according to Kanter's investigations (6, 16). Managers use these strategies to develop empowerment dimensions in personnel. These strategies are as follows: having clear objectives, developing personal domination experience, modeling, support, emotional excitement, information preparation, resources preparation, bond with results, and provision of confidence. Spreitzer in a study on organizational traits enabling personnel empowerment, state the clear and challenging outlook as the first factor leading to personnel empowerment (17). Components of information preparation, leadership style and clear objectives are in line with the suggested model of the present study and components of modeling, reward system and emotional excitement in factor analysis were omitted.

It was stated that when organizations do not reward personnel or rewards are not based on capabilities, the personnel feel they are weak. A reward system that is based on innovative or unusual performance strengthens the personnel's self-efficacy (18). In the current research, the purpose of the reward system is to motivate persons or groups in improvisation of their performance. In this research, the reward system in factor analysis was omitted.

Psychological empowerment with factors of organizational conditions (organization's objectives, reward system, resources preparation), factors of managerial strategies (giving information, delegation, team making and partnership atmosphere), is considered as a self-efficient resource in Abdollahi's empowerment model (9). Organization's clear objectives, giving information and delegation are congruent with the model of the current research.

Uniform empowerment model of Tae-Jon Chu holds that structural empowerment factors (delegation, information sharing, performance feedback, partnership in decision making), personal and total performance have effects on psychological empowerment (19). Components of information sharing and delegation are in line with current research.

Conclusion

Identified factors can be used in measuring the rate of personnel's empowerment of organizations in service sector. Compiled tools in this research can be used in medical and health system that is

part of the country's service organizations and thereby each organization will identify its weak and strength points and will provide itself a stable competitive benefit. In answer to the questions of the research including identification and determination of the characteristics of the empowerment of the personnel of the Iran's public hospitals, we briefly state that the researchers, using exploratory factor analytical method, could identify 8 out of 14 components which are influential in psychological empowerment. They include information sharing, using information, determination of suitable components in performance feedback, teaching leadership skills, having clear objectives, manager's support, duties' explanation and ambiguity removal from personnel's roles. Accordingly, holding educational courses for personnel's empowerment, teaching empowerment components to hospital managers and also using identified traits in public hospitals seem indispensable. It is noteworthy that hospital managers should first examine these factors in their own hospitals and then take the necessary action.

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Evaluation of depression problem solving education

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Abstract

Male patients with a diagnosis of depression in the experimental population of the study in order to evaluate the effectiveness of problem-solving training, Van Military Hospital psychiatric outpatients and patients receiving treatment for depression, and samples selected by random sampling in male patients, including 34 patients in experimental group and control group, 34 patients with a total of 68 established. Personel Information Form, Beck Depression Inventory, Problem Solving Inventory to evaluate the effectiveness of training, education, pre-and post-training was implemented after 1 month after the completion of training. Evaluation of the data frequency, distribution, chi-square test, independent Student's t-test, paired sample t-test is used. According to research, problem-solving training are not given training in the group of patients with depression, depression levels, differences were statistically significant. training in the group, problem-solving skills has been increased significantly.

Key words: Depression, education, problem solving.

Introduction

Depression, loss of energy, failures in school and at work, unable to meet responsibilities, lack of drive, insomnia, early morning waking, night sleep fragmentation, decrease in self-perception, decrease in sexual desire and appetite changes are (1, 2). The prevalence of community % 10-20 between. The incidence of depression in women 20%, men 10%. Depression is less common in men. Suicide is a serious risk for depression and suicide is four times more than women (5). Having been diagnosed with depression in male patients of this study, increases the importance of the research.

Due to the long duration of work loss and the treatment is expensive and makes a big burden on the country's economy (3, 4). The most important drugs for the treatment of depression. In addition, patients with educational programs have contributed to the improvement. One of these training programs, problem-solving training.

The problem is preventing it from reaching the purpose of an individual. The problem is to reach the target blocks. A difference between the desired state and the current situation is detected, begin the process of problem solving (7, 8).Problem-solving, exploring one's own abilities to meet the needs of development and makes it easy. People facing problems, knowledge and skills. Self-confidence increases. Problem-solving behaviors that are required to do in order to achieve the goal of knowledge, skills, attitudes and tools to choose to use it, in order to achieve the goals to establish a relationship between different events, include the person to reach the goal of preventing combat situations. Effectively tackle the problems facing the development of problem solving skills ensures that the person. Problem solving, intelligence, motivation, values, beliefs and affects such factors as the state of preparedness (7, 8, 9).

Due to the long duration of work loss and the treatment is expensive and makes a big burden on the country's economy (3, 4). The most important drugs for the treatment of depression. In addition, patients with educational programs have contributed to the improvement. One of these training programs, problem-solving training.

Include problem-solving steps (7, 9);

- Problem determination
- Problem analysis and evaluation
- The development of a solution for the problem
- The implementation of decision
- To evaluate the solution.

Problem-solving training has been proven efficacy in patients with depression (6, 10, 11).

Method

The sample of this pre test-post test study consisted of 68 cases among who were inpatient and outpatient treatment of depression with antidepressants in patients who were at least three weeks in Van Military Hospital. The sample consisted of 34 patients as an experimental group and 34 patients as a control group with a total of 68 patients (confidence interval 0.95, power analysis 0.90) The experimental group received eight sessions of "Program for Improving the Problem solving skills, which was developed by Liverpool University Psychiatry Department. Problem Solving Training in experimental group three days a week and six sessions of 60-minute individual interview method was applied.. Study two weeks completed. However, the control group did not receive any education. The data in both groups were collected before training, after training, after one month. Data collected with Personal Information Form, Beck Depression Inventory (BDI), and Problem Solving Scale (PSS). Problem Solving Scale developed by Heppner and Peterson (1982) investigating the information form Problem Solving Inventory, which measures an individual's perception of himself as a problem-solving skills, self-evaluation type scale. Applied to adolescents and adults. Consists of 35 items, scored 1-6 Likert-type scale. Factor analysis of the scale, "hasty approach", thinking approach " ,evaluative approach", self-confident, "" avoidant approach "," planned approach "6 factors were found to be known as (12, 13). The Beck Depression Inventory (BDI), created by Aaron and Beck, (1961) is a 21-question multiple-choice self-report inventory, one of the most widely used instruments for measuring the severity of depression. There are four choices at each of the 21 symptom category in the form. Obtained from the scale 10-17 points light, medium 18-29 points, 30-63 points indicates severe depression. The mean BDI cut-off score is considered to be 17.

The research ethics committee approval and with the permission applied.

The data were evaluated via computer. Evaluation of the data, the evaluation of demographic and disease characteristics of the frequency, distribution, chi-square test, evaluating the effectiveness of training, independent sample t-test, paired sample t-test was used.

RESEARCH PROCEDURE	
EXPERIMENTAL GROUP	**CONTROL GROUP**
Personal Information Form	Personal Information Form
Beck Depression Inventory	Beck Depression Inventory
Problem Solving Inventory	Problem Solving Inventory
↓	↓
AFTER TRAINING	**AFTER TRAINING**
Beck Depression Inventory	Beck Depression Inventory
Problem Solving Inventory	Problem Solving Inventory
↓	↓
1MONTH AFTER TRAINING	**1MONTH AFTER TRAINING**
Beck Depression Inventory	Beck Depression Inventory
Problem Solving Inventory	Problem Solving Inventory

Results

The mean age of the patients in the experimental group 23.03± 22.03, in the control group 23:03±2.13 were found. Educational level, marital status, financial status in terms of statistically significant differences between experimental and control groups to be determined. 24 in the experimental group who attempted suicide (70.6%) persons, the control group, 20 (58.8%) employees.

Between problem-solving scores of the experimental and control groups before and after training and a month later there isn't a statistically significant difference (Table 1). Problem-solving score of the experimental group before training is 124.29 ±21.95, after training is 94.79 ± 22.79 and one month after training is 78.00 ± 23.24 (Table 1). Problem-solving score of the control group before training is 120.38 ± 19.22, after training is 118.71 ± 19.33 and one month after training is 118.24 ± 18.94. After training, one month after the training, a statistically significant difference is found between the scores of the experimental group and the control group. ($t = -4.860$ $p = 0.001$, $t = -8.740$ $p = 0.001$) (Table 1).

Depression score of the experimental group before training is 30.32±9.12, after training is 29.94±8.76, and one month after training is 29.12±8.66 (Table 2). Depression score of the control group before training is 30.67±9.10, after training is 31.00±8.83, and one month after training is 30.94±8.85 (Table 2). Between depression scores of the experimental and control groups before and after training and a month later there isn't a statistically significant difference ($t = -0.160$ $p = 0.874$, $t = -0.496$ $p = 0.621$, $t = -0.859$ $p = 0.394$).

Table 1. Problem Solving Score

Problem Solving Score	Experimental Group (n=34) X±SD	Control Group (n=34) X±SD	t	p
Pre-Training	124.29±21.95	120.38±19.22	0.782	0.437
Post-Training	94.79±22.79	118.71±19.33	-4.860	0.001
After One month Training	78.00±23.24	118.24±18.94	-8.740	0.001

Independent Sample t testi

Table 2. Beck Depression Score

Beck Depression Score	Experimental Group (n=34) X±SD	Control Group (n=34) X±SD	t	p
Pre-Training	30,32±9.12	30,67±9,10	-0.160	0.874
Post-Training	29.94±8.76	31.00±8.83	-0.496	0.621
After One month Training	29.12±8.66	30.94±8.85	-0.859	0.394

Independent Sample t testi

Discussion

Depression is a disease that require attention due to the social and economic burdens. Antidepressants for being expensive, long-term hospitalization, personal motivation and joy of life is important to treat due to the decrease of suicide. World Health Organization in the coming years will increase indicates the existence of depression (4, 5, 14, 15).

In this study, patients with low problem-solving skills, suicide attempts were higher. Eskin, Akoğlu and Uygur in his study, problem-solving ability was found to be lower than suicide attempts in psychiatric patients (16). Problem-solving training in reducing the severity of disease symptoms in depressed patients and found to be effective. Townsend and et all (17), Teasdale and et all (18), Küçük and Işil's (19) Nezu and Ronan (20), depressed individuals revealed using less of a problem to solve, the problem-solving program for patients with depression scores applying the After you have found significantly reduced.

Goddard, Dritschel and Burton (21), involved in the development of depression in their study showed that the decrease in problem-solving abilities. In this study, patients were increased after the training problem-solving skills. Townsend and et all (17) problem-solving training, patients found to be effective in the development of problem solving skills. Problem-solving training, patients with depression, hopelessness levels were found to be having a positive effect in reducing their recovery. Küçük and Işil's (19) dialysis patients into their study of patients with problem-solving, problem-solving skills training was found to increase. Kelleci's (6), applied problem solving in female patients admitted to primary health care services in the education of women was found to increase problem-solving skills.

Conclusion

In the study, problem-solving training, the effect of patients' depression levels were not statistically significant. However, a statistically significant increase in problem-solving skills provided.

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Comparison of body weight gain and loss between male and female NMRI mice on a high fat-diet and treated by Orlistat

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Abstract

Background: In order to counteract the global epidemics of obesity, development of new medicines is warranted, and this research field is still dependent to animal models. Some of the obesity complications are more prevalent in one gender, thus, differences between obesity and weight loss pattern were investigated here in both genders of adult NMRI mouse-strain.

Material and Methods: Baseline of mice body weight was 24±4 g. Obesity was induced using a high fat-diet (for six weks), after what the effect of a switch to normal diet and use of orlistat was studied for six additional weeks. Body weight, and blood serum levels of triglyceride, cholesterol, LDL-(C) and HDL-(C) were measured.

Results: Percentage of average increased body weight were 7.94% (g) and 4.10%(g) in female and male mice respectively. Serum cholesterol levels and triglyceride levels showed significant increase in obese group ($p<0.001$) in both sexes. After orlistat treatment, percentage of average decreased body weight was 6.74% (g) and 2.40%(g) in female and male mice respectively. Cholesterol and serum triglycerides decreased significantly in treated groups of both sexes, but changes in serum LDL (C) were not significant in both sexes ($p>0.05$). Female serum HDL (C) of the treated group increased significantly compared to control, sham and obese after drug consumption but not (significantly) in the treated male mice.

Conclusion: Body weight increase (upon high fat diet consumption) and decrease (upon shift to normal diet and antiobesity medicine treatment) were found to be different in male and female adult NMRI mice. Female mice were more affected by both diet and treatment. These differences could be related to hormonal and metabolic rate differences but also to the strain that has been used. Further investigations are thus needed to clarify the exact underlying mechanism of this observation.

Key words: Weight gain, weight loss, NMRI mouse, antiobesity.

Introduction

Obesity is a widespread condition which rapidly evolving as a fundamental world health problem which is associated with many disorders with high mortality and morbidity [1].

Obesity results from an imbalance between energy intake and its expenditure [2] Obesity and excess body weight are estimated by the Body Mass Index (BMI). [2, 3, 4]. It is prevalent in developed populations of the world [3, 5], where abundance [3] and good taste of a variety of food [6] are numbered as contributing factors to this condition. Obesity is associated with an increased risk to prostate, colon, breast and endometrium cancers [3], as well as the incidence of diabetes mellitus, cardiovascular disease, hypertension, respiratory and gastrointestinal problems such as reflux and fatty liver and dyslipidemia [5].

In addition, excess weight and obesity have close relationship to osteoarthritis [2, 4, 5] primary and secondary infertility and menstrual irregularities are also related with this condition[4]. Maternal obesity may also affect the health of infants, causing congenital malformations. Studies show that infants born from mothers with a pre-pregnancy BMI of ≥30 were 2.0-4.3 folds as likely to die in the prenatal period as were infants born to mothers with a pre-pregnancy BMI of less than 20 [4]. It has been known that males and females differ in their consummatory behavior and weight maintenance, and in humans many obesity-related health risks are known to be gender dependent

[7, 8]. For instance women with type 2 diabetes are twice as likely to die from hypertension than men[7]. In animals, gender differences in feeding and body weight regulation appeared to be determined by perinatal brain androgenization and influences of circulating sex steroids in the hypothalamus followed that [7]. Additionally body fat distribution, metabolic rate and oxidative capacity are different between males and females [9]. The most critical factor in dietary obesity seems to be strain [9]. Physicians usually recommend behavioral methods and increase physical activities for reducing body weight and caloric intake, however these strategies are difficult to follow and the search for antiobesity medicine still continues [11]. Based on the above considerations, it seems that gender differences are required to be considered when new treatments for obesity are developed [7]. However the role of gender in resistance or susceptibility to obesity is not entirely clear [10], and necessitates further studies.

As the principal anti-obesity medicine currently in use, orlistat (tetrahydrolipstatin) [12], is a pancreatic lipase inhibitor [13 14]. This compound has been originally obtained from a soil bacteria and irreversibly binds to the active site of gastrointestinal lipases, inhibiting the systemic absorption of dietary fat to some extent [15, 16]. A small fraction of the drug absorbed after oral intake (bioavailability of orlistat is less than 1%) and the medicine is considered relatively safe [14].

The current study focused on the comparison of weight changes resulting from a high fat-diet as well as the effect of orlistat in the two genders of NMRI- mouse strain.

Materials and Methods

Animals

NMRI mice weighting 24±4 g were allowed to acclimatize to laboratory conditions for one week prior to the experiment. During this period they were fed standard laboratory chow and water *ad libitum*. All animals were maintained in the same room at a room temperature of 22 ± 2°C with relative humidity of 55-60% and a 12h light-dark cycle. After one week of acclimatization, mice were weighed and divided into four groups of control (C), Obese(O), sham and Experimental(E). n=5 in each group. C group was fed standard laboratory chow and water *ad libitum* during study. Three other groups were fed as described below for six weeks. Then C and O groups were anesthetized by ether and their blood samples were collected for biochemical analysis whereas sham group received the solvent used for the administration of orlistat, which was Phosphate Buffered Saline (P.B.S) and and E group received orlistat (9mg/Kg, daily, orally) for another six weeks. In this phase of study all mice received standard laboratory chow diet and water ad *libitum*. The body weight of all mice was measured weekly during the study period. The experimental protocol was approved by the Research and Ethics Committee of Science and Research Branch, Azad University, Tehran.

High fat diet composition

The three groups under obesity regime were fed a high fat diet to which they had free access. This high fat diet was prepared from the following ingredients: 15 g of mouse pellet standard chow, 10 g of roasted ground nut, 10 g of milk chocolate [17] and 5 g of sesame crackers. Ten-fold of these ingredients were mixed, to which 20 g roasted sesame was added, resulting into 5783kJ/g energy content . Water was added to this mixture which was made as dough, and then cut and dried. In addition, all groups which have obesity regime group were fed 110g creamy biscuits 1822KJ) .All groups received diet *ad libitum* during the study.

Obesity assessment

Body weight of all groups were measured weekly during the study.

Biochemical analysis

In the first phase of study (after six weeks) for the C and O groups, and in the second phase of study (at the end of the twelfth weeks) for sham and E groups, after 12 h of starvation, blood was collected by cardiac puncture under ether anesthesia. The blood samples were centrifuged at 2500 rpm for 5 min, at room temperature. Cholesterol, Triglyceride, HDL- cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were measured by autoanalyzer prinier with commercial kits (Cholesterol and Triglycerides kits were purchased from Pars Azmoon Co., Iran, and HDL-kit was purchased from ELItech Co., France).

Statistical Analysis

The results are presented as mean ± standard error for animals in each group. The data were analyzed by SPSS soft ware version 10 one way ANOWA tukey test. Differences were considered significant at ($p<0.05$).

Results

Body Weight: body weight of Control (C) group had differences compared with Obese(O), sham (sh) and Experimental (Exp) groups in the two sexes significantly($p<0.001$). In both sexes, body weight decreased in (Exp) group after orlistat consumption but was not significant ($p>0.05$) (Figure 1 a, b). The percentage of increased body weight of obese female (O) group was more than this parameter in the obese male group (Figure 1 c) (7.94%g compared to 4.10%g)

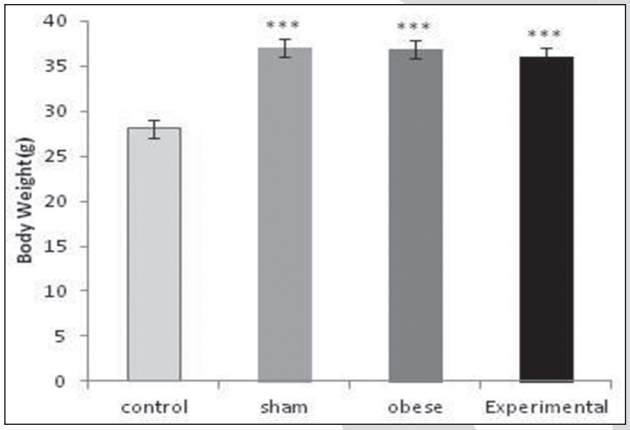

*Figure 1a. Histogram of the body weight of male different groups: Control, Obese, sham, Experimental mean±SEM) during 12 weeks; (**p<0.01, ***p<0.001).*

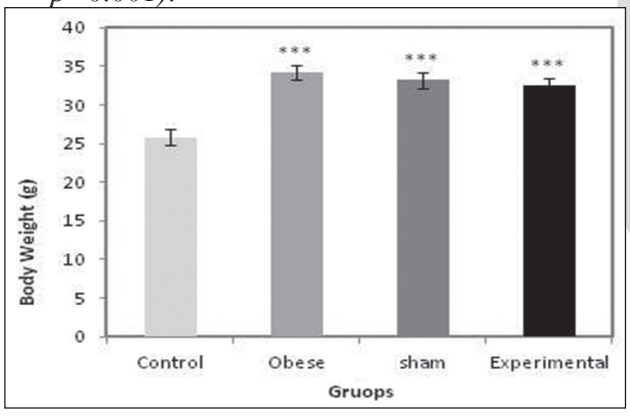

*Figure 1b. Histogram of the body weight of female different groups: Control , Obese, sham and Experimental. n=5, mean±SEM) during 12 weeks; (**p<0.01, ***p<0.001).*

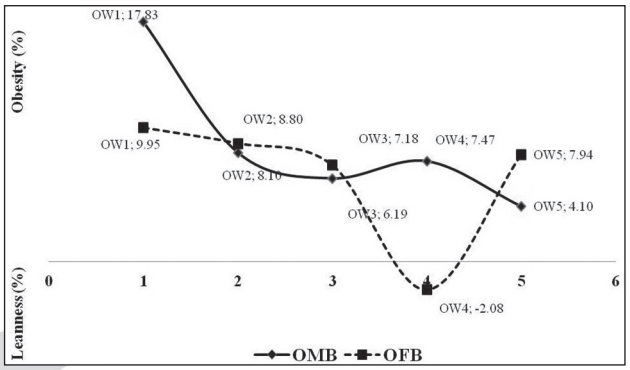

Figure 1c. The percentage of increased body weight between Obese male and female groups (n=5, mean±SEM) during 6 weeks.OMB: Obese Male Body weight. OFB: Obese Female Body weight.

The percentage of decrease of body weight in female (Exp) group was more than (Exp) male group (-6.74 g compared to -2.40 g) (Figure 1d).

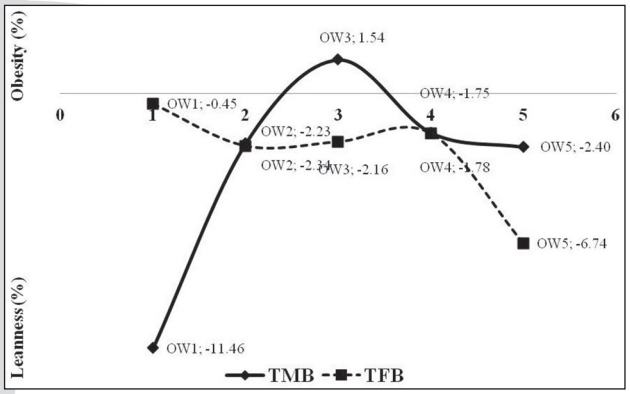

Figure 1d. The percentage of decreased body weight between Experimental male and female groups (n=5, mean±SEM) during 6 weeks.TMB: Treated Male Body weight.TFB: Treated Female Body weight.

Biochemical Analysis

Male serum cholesterol increased in (O) group compared to (C) group significantly ($p<0.001$), on the other hand this parameter decreased in (sh) group compared to (O) group significantly ($p<0.001$). In (Exp) group serum cholesterol decreased significantly compared to sh ($P<0.001$), O ($p<0.001$) and C ($p<0.05$) (Figure 2a).

Female serum cholesterol in (O) group had significant increase compared to (C) group ($p<0.001$); this factor was significantly lower in (sh) compared to (O) group ($p<0.001$). In (Exp) group it showed significant decreased level compared to (sh) and (O) groups ($p<0.01$) and ($p<0.001$) (figure 2b).

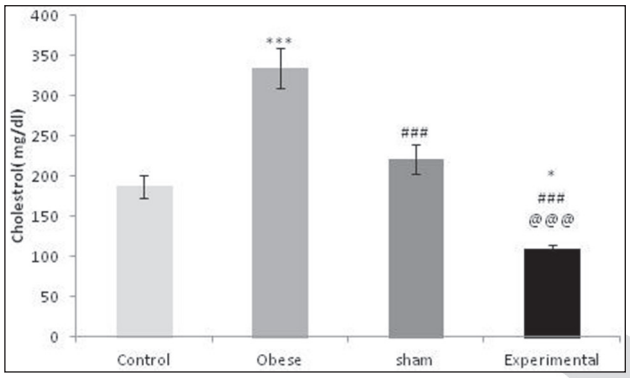

*Figure 2a. Histogram of the amount of cholesterol in male at different groups(Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group.(@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

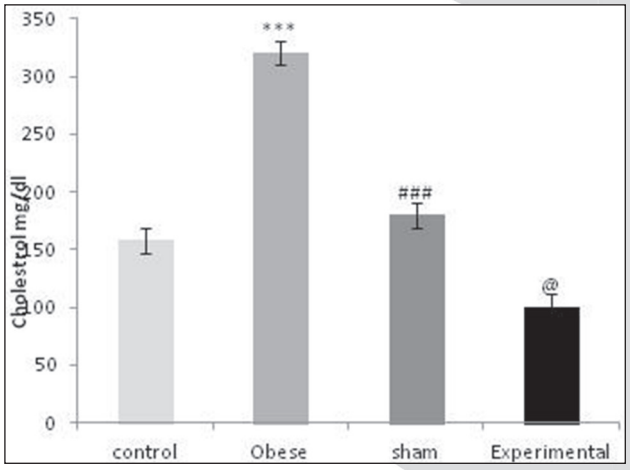

*Figure 2b. Histogram of the amount of cholesterol in female at different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

Female and male LDL(C) showed differences between groups but these differences were not significant statistically (p>0.05) (figure 3 a, b).

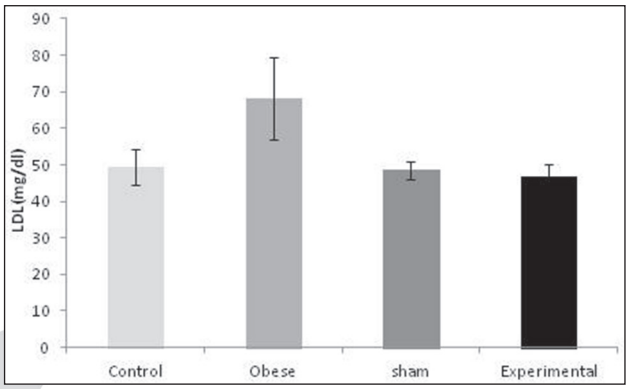

*Figure 3a. Histogram of the amount of LDL(C) in male at different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

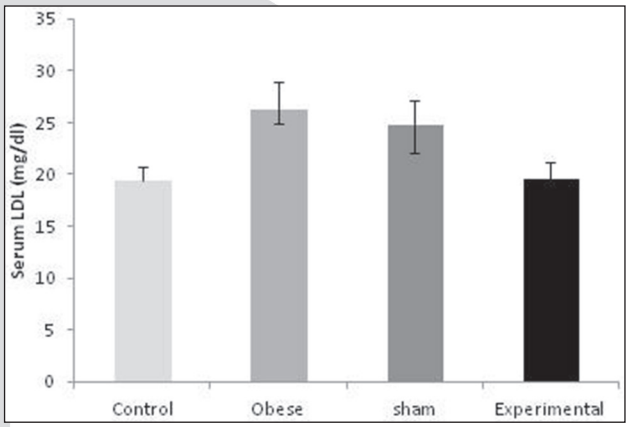

Figure 3b. Histogram of the amount of LDL(C) in female and different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

In male, HDL(C) had no significant differences between groups(p>0.05) but this parameter was lower in (O) group and higher in (sh) and (Exp) (figure 4a). In femal, HDL- (C) had significant difference in (Exp) group compared with (C) and (sh) groups (p<0.01), (p<0.001) respectively (fig 4b).

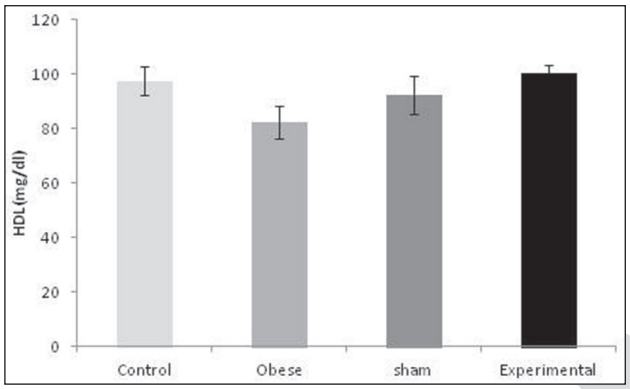

*Figure 4a. Histogram of the amount of HDL(C) in male at different groups(Control, Obese, sham and Experiment) (n=5, mean±SEM) during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001).#indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

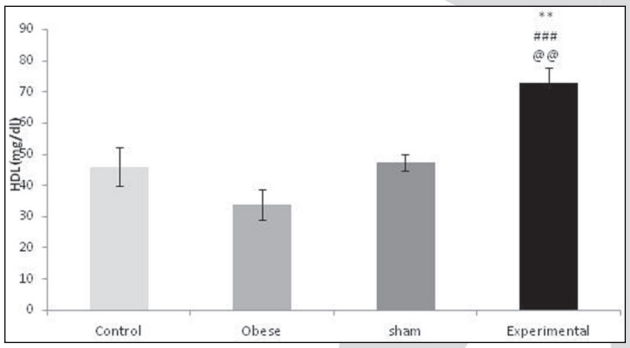

*Figure 4b. Histogram of the amount of HDL(C) in female at different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM) during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

Male serum triglyceride level in (Exp) group had significant differences compared with the three other groups which is described as below (Figure 5a):

(Exp) group compared to (C) group showed significant decreased levels (p<0.05).This group had significant decreased levels compare to (O) and (sh) groups (p<0.001).

Female triglyceride levels showed changes which are described as below (figure 5b):

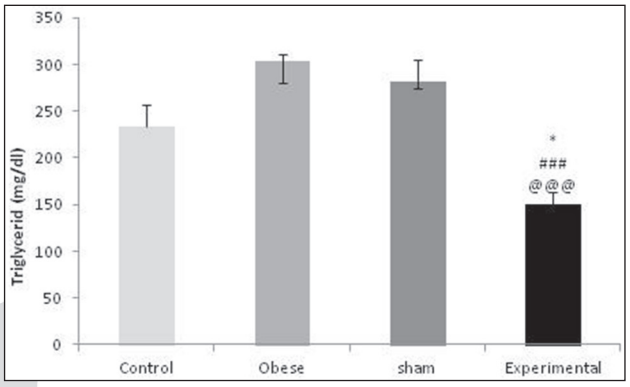

*Figure 5a. Histogram of the amount of triglyceride in male at different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

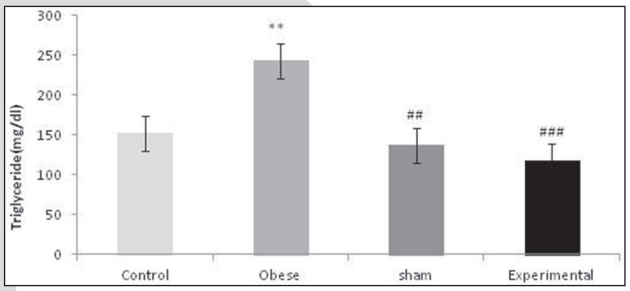

*Figure 5b. Histogram of the amount of triglyceride in female at different groups (Control, Obese, sham and Experiment) (n=5, mean±SEM)during 12 weeks; * indicates comparison to (C) group. (*P<0.05), (**p<0.01) and (*** p<0.001). #indicates comparison to (O) group. (#p<0.05), (##p<0.01) and (###p<0.001). @ indicates comparison to(sh) group. (@p<0.05), (@@ p<0.01) and (@@@ p<0.001).*

(O) group had significant increased level compared to (C) group (p<0.01). Both (sh) and (Exp) groups showed significant decrease compared to (O) group with (p<0.01) and (p<0.001) respectively.

All changes in blood serum lipid profiles showed reasonable relationship to diet changes and medicine that was consumed.

Discussion

Both men and women are susceptible to overweight or obesity but some differences exist between two sexes which are associated to pattern of fat deposition, mobilization, utilization, as well as metabolic rate, energy expenditure and oxidative capacity[8]. A critical factor which cause the appearance of different patterns of obesity between genders is the effect of sex hormones in both human and rodents. Additionally, strain, diet, age and nutritional state are important factors in animal models that relate to the obesity pattern in two sexes. [7]

As mentioned in the results section, body weight decreased in (sh) and (Exp) groups in two sexes that as a consequence of diet modification in (sh) and diet change alongside with orlistat consumption in (Exp) groups. However, t body weight decrease was not significant statistically. It should be mentioned that, as M.Grilo and M.Masheb(2007) have reported, the normal dose consumption of orlistat (120mg, three times daily) achieves a maximum of 30% weight reduction in human, and its efficacy is relatively limited [18].

Some parameters that are related to overweight status and/or obesity are linked to the gender; for instance blood levels of high density-lipoprotein and triglycerides are better predictors of coronary disease in women than in men [7]. Thus research on these subjects needs the use of the proper gender of the animal model; in the current study we focused on weight changes which are induced by a high fat-diet and orlistat treatment on both genders of NMRI mouse strain. This strain has some advantages including: fast adaptation to environment, easy and economical maintenance, and susceptibility to weight gain induced by a simple high fat-diet. Our results showed that female mice gained and lost more body weight than male sex.

Increase and decrease of parameters that are related to lipid profile at different groups in obese stage and experimental stage after specific diet consumption and medicine usage were according to our expectations. Biochemical serum blood factors related to obesity increased in (O) group compared to (C) group, and subsequently decreased in (sh) and (Exp) groups via diet modification or diet modification and orlistat consumption.

D.Taraschenko *et al*. (2011) studied sex differences in obese rats in which obesity was induced by high fat-diet (effects of 18-methoxycoronaridine) during 38 days. They showed that male Sprague-Dawley rats responded to high fat-diet and became more obese, and then responded to treatment better than female rats. In the beginning of the study, the average body weight of male rats was 275 g and they reached 400 g after 38 days while body weight of female rats changed from 275 g to 287g [7].

Schoroeder *et al*. (2010) studied the effect of exercise on obesity in male and female OLETF rats for 23 days. They indicated that the effect of exercise in OLETF was more successful compare with OLETF females, in females the effect was much more moderate[17].

A.Rushing *et al*.(1993) examined effects of Phenylpropanolamine (PPA) infusion on body weight and dietary in male and female Sprague - Dawley rats during 4 weeks. In both genders, PPA reduced body weight significantly compared with controls during two weeks of PPA administration ($p<0.001$). In contrast to males, females in both dosages of PPA groups continued to weigh less than controls significantly during two weeks after drug termination[18].

Tokuyama *et al*. (1982) examined the effects of wheel running on food intake and weight gain in the two sexes of Wistar King rats for 50 days, they concluded that food intake of two sexes in the exercising group increased after a transient decrease. The finding that food intake and wheel running activity attained plateaus within 20 days after the starting of exercise is suggesting that two genders acclimate to activity within 20 days although food intake in males and females increased after adaption to wheel running , the rate of that was higher in females. They concluded that there is a positive correlation between wheel running and increase in food intake in two sexes, there was no significant differences in the slopes of regression lines between two genders. Researchers suggested that sex differences in activity related partly to sex differences in rate of increase in food intake elicited by wheel running [19].

Hirsch et al. (1982) studied sex differences of voluntary activity on sucrose induced obesity in Sprague-Dawley rats. While high levels of activity

and obesity were found to coexist when normal female rats were fed a palatable diet, this form of obesity was eliminated by activity in male rats [22].

E.Bocarsly *et al.* (2010)investigated long term effects of high –fructose corn syrup(HFCS) on body weight and obesogenic parameters in two sexes of Sprague-Dawley rats for 6 or 7 months. Their results showed that male rats gained significantly more weight than females (males body weights reached to 260% percentage of baseline body weight while females gained 200% percentage baseline body weight) when both groups were maintained on 24-h access to HFCS during the study period (6-7 months) [23].

Gorman *et al*. (1977) investigated several factors such as age, sex and prior body weight under the effect of a high fat diet named super market diet in adult CFE rats, and found that male and female rats gained body weight equally[24]. Taraschenko *et al*.(2008) studied the effect of new antiobesity compound named 18-methoxycoronaridine in the rats which were fed by supermarket diet they showed that female became obese via this diet consumption. [25].

Priego *et al*. (2008) investigated differential expression of genes related to obesity in Wistar rats and found that female rats that consumed a high fat diet for six months became more obese than males, with more increase in the adiposity index [26].

As mentioned, several factors could be involved in the differences of body weight changes in male and female genders. A thorough understanding of the involved pathways is not straightforward and needs further studies, but some suggestions has been so far made. D.Taraschenko *et al*.(2011) stated that one possible explanation for the differential responses between male and female rats to diet induced obesity could be gender-specific changes in leptin or ghrelin occurring in high fat diet feeding. For instance male rats fed a high fat diet for five weeks were shown to become resistant to exogenous leptin and were not able to lost weight whereas female rat which were treated by the same method remained leptin–sensitive and lost weight after leptin usage. In another study serum levels of leptin and its gastric levels mRNA were shown to be higher in female rats compare to male rats consuming a high fat diet. [7].

Choi et al. (2012) discussed that male mice are known to be more likely to become obese compare to females and overectomized female mice mimic male mice in susceptibility to obesity [9]. Therefore ovary hormones could be important for body weight fluctuations in two genders. Generally, phenotypic differences could be associated with strain, diet, age, physiological signaling, and metabolic processes. Several studies indicated that these differences may reflect changes in diet consumption and energy availability consist of β-oxidation rate and fatty acid synthesis additionally oxidative capacity, mitochondrial activity, thermogenic capacity, abd glucose uptake could be critical in susceptibility to obesity in mice[9]. Hoyenga *et al*. (1982) referred to other differences between males and females such as: sex differences to obesity, starvation resistance, heat production, heat loss and circadian rhythm [10].

Conclusion

Overall, results variability is seen, depending on the diet and animal strain used, which makes it difficult to make a general conclusion as to the higher susceptibility of a particular gender toward both obesity and weight loss. However, this seems to be an important issue in obesity related studies, and needs further detailed studies, first on the animal models, and then on the cellular and molecular level in order to clarify the involved pathways.

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Evaluation of psychosocial problems experienced during treatment process by women successful in infertility treatment

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Abstract

Background: Infertility may cause serious physical, psychological, sociocultural, economic, marital and sexual problems. The objective of this study is to identify the psychological problems experienced in treatment process by women successful in infertility treatment.

Materials and Methods: This study is retrospective and descriptive. The research was conducted on women who had undergone and become successful in infertility treatment in Antalya. The women who had had at least one living child at the end of infertility treatment were included in the study. The data of 204 women who were accessed by means of snowball sampling method and accepted to participate in the study were assessed.

Results: It was found that for 77% of the women, having a child was the most important thing in their lives, 63.7% were uncomfortable about people asking questions concerning having a baby and 81.9% expressed that their families supported them in this process. It was also found that 62.3% of them got furious with the misbeliefs of people around them regarding in vitro fertilization, and that 57.4% of them perceived sexual intercourse as a duty.

Conclusion: Psychosocial problems encountered during treatment process by women who received infertility treatment were identified as stress, anxiety, depression, failure, hopelessness, disappointment, fury, anger, loss of status, misery, anguish, social isolation, guiltiness, stigma, insufficiency, reduction in self-respect, disruption of interpersonal affairs, ambivalence, loss, distraction, impairment of concentration, embarrassment, loss of privacy and secrecy of private life. Awareness on the psychosocial problems experienced in treatment process by women who succeeded in infertility treatment may facilitate the adaptation of infertile women to infertility and its treatment, and reduce their reactions to infertility.

Key words: Infertility, Infertile Women, Psychosocial Problems.

Introduction

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse on a regular basis. Infertility affects 10-15% of the couples who are at reproductive age. (1-4).

Infertility is a complex situation crisis which is often psychologically-threatening and emotionally stressful for both partners as well as economically expensive and causes physical pain due to the operations performed for diagnosis and treatment purposes (5-7).

Physical, psychological, social, emotional and financial impacts of infertility have been revealed by a number of international (7-9) and national (10-14) studies. They are stress, anxiety, depression, economic hardships, guiltiness, fear, loss of social status, helplessness, social stigma, and violence in some cases.

Infertile couples are under a great social pressure, and need to conceal the problem as it is exceptionally private for them. Infertile individuals are destitute of support when they do not share their pregnancy problems with their families and relatives. This may turn into a social loneliness and leave the infertile couple deprived of the sources of support in their troubled time. Couples define their infertility experience as the most stressful experience of their lives (12,15). Infertility may also be considered as a developmental crisis. Fertility is an important function of adult development. The failure of infertile couples to meet this need adversely affects their future plans, self-respect, marital relations and sexual lives. Loss of

bodily and sexual privacy in these couples is also possible (14-18).

Materials and Methods

The aim of the study

The objective of this study is to identify the psychosocial problems experienced during the treatment process by women who became successful in infertility treatment.

Participants

This study was conducted on women who lived in the city centre of Antalya, had received a successful infertility treatment and, as a result, had a child who was between 0-6 years old at the time of the study. The data of the study were collected between September and December 2010. Participants were accessed by phone after their phone numbers were obtained by permission of the In Vitro Fertilization Department of Akdeniz University. Among non-probability sampling methods, snowball sampling method was employed, and 204 women who were accessed and accepted to participate were included in the study. The sample size was calculated as 72, with a power of 80% and a significance level of 5%.

Means of Data Collection

The data of the study were collected by researchers employing face-to-face interview method by means of two different forms prepared in the light of literature knowledge (17,19). Interviews were conducted in approximately 20 minutes in the workplaces of those who were working and in the houses of the remaining participants.

Personal Information Form

Personal information form consists of 14 questions interrogating the socio-demographic characteristics and infertility story of the women.

Information Form Concerning the Infertility Treatment

This form contains 50 statements related to psychosocial aspects, marital relationship, sexuality and economic area (e.g., "I was uncomfortable with being in places with children"; "Having a baby was the most important thing in my life"; "I was angry with my spouse"; "I perceived sexual intercourse as a duty", etc.) The participants were asked to respond to the questions by answering "yes", "no" or "sometimes".

Data Analysis

In the evaluation of the data of this study, SPSS 15.0 statistical software was used, and percentage distributions, average and standard deviation methods were employed.

Ethical Considerations

In order to be able to conduct the research, the approval of Ethics Board of Health Sciences Institute in Atatürk University was obtained. Principles of "Informed Consent", "Privacy and Protection of Privacy" and "Respect to Autonomy" were observed by explaining the participants the objective of the study prior to the collection of research data, by stating that the information would be kept confidential and by accepting those who voluntarily wanted to participate in the study.

Results

When the information provided by the participants is examined, it is seen that 62.3% of the women are at the age group of 31-40, 19.1% are high school graduates and 47.1% are university graduates, 59.3% are employed, and 92.6% have social security. It was found that 62.3% of the women had an average financial situation and 66.7% had spent most of their lives in the province of Antalya. It was found that 84.8% of the women had a nuclear family, and 38.7% had a marriage of 6-10 years.

Distribution of the women by their infertility background is given in Table 1. It was found that 41.2% of the women could not have child for 6-10 years despite not using any birth control method, and 77.9% had a child after 1-4 attempts. It was found that 40.7% of the women could not have a baby due to a reason originating from themselves. It was also found that 75.5% of the women had not got pregnant before, 70.6% had used in vitro fertilization method as a treatment method, and that 72.5% had not used any birth control method before being diagnosed with infertility.

Distribution of the women by the psychosocial statements reported to have been experienced during the infertility treatment process is given in

Table 1. Distribution of the women by their infertility background.

Infertility Background of the Women (N=204)	N	%
Period of time in which no pregnancy was achieved without any birth control		
0-5 years	55	27.0
6-10 years	84	41.2
11-15 years	40	19.6
16-20 years	19	9.3
21 years and over	6	2.9
In which attempt pregnancy was achieved		
1-4 attempts	159	77.9
5 attempts and over	45	22.1
The reason for not being able to achieve pregnancy		
Female infertility	83	40.7
Male fertility	54	26.5
Both	19	9.3
Reason unknown	48	23.5
Any conception before		
Yes	50	24.5
No	154	75.5
Method of treatment		
In vitro fertilization	144	70.6
Intrauterine insemination	43	21.1
Ovulation induction	17	8.3
Use of birth control methods before being diagnosed with infertility		
Yes	56	27.5
No	148	72.5

Table 2-A. It was found that 57.8% of the women were affected by conversations on children, and 63.7% were annoyed by people around them asking questions about having a child. It was found that 81.9% of the women were supported by their own family, 65.2% by their intimates and 52% by the family of their spouses. 55.4% of the women were found to be not annoyed by being in the same environment with other individuals receiving treatment.

Continuation of the distribution of the women by the psychosocial statements reported to have been experienced during the infertility treatment process is given in Table 2-B. It was found that 62.3% of the women got furious with the misbeliefs of people around them regarding in vitro fertilization treatment, and that 67.6% had abandoned themselves to despair and 73% had been disappointed when they had learnt from the test results that they could not conceive. It was found that for 77% of the women, having a child was the most important thing in their lives.

It was found that 77.5% and 79.4% of the women frequently asked themselves whether they could get pregnant and whether they could become a mother, respectively, and that 65.7% felt very nervous at the beginning of the treatment and 56.9% got angry with themselves when they had menstruation.

85.8% of the women stated that they liked caressing and cuddling children. 57.8% of the participants stated that the health personnel (physician, nurse and midwife) had supported themselves in this process. It was found that 52.5% of the women did not get angry much when they saw a pregnant woman, and 59.8% did not think of splitting up with their husbands when no pregnancy was achieved.

Distribution of the women by their marital relationship characteristics during the infertility treatment process is given in Table 3. 70.1% of the women stated that they could calmly talk to their husbands about the treatment process, 71.6% that their husbands were always by them during the treatment and 67.2% that their relationship with their husbands had no change. 75.5% of the

Table 2-A. Distribution of the women by the psychosocial statements reported to have been experienced during the infertility treatment process

Psychosocial statements (N=204)	Yes		Sometimes		No	
	N	%	N	%	N	%
1. I was uncomfortable with being in places with children.	84	41.2	53	26.0	67	32.8
2. I was affected by conversations on children.	118	57.8	57	28	29	14.2
3. I was annoyed by people asking questions about having a child.	130	63.7	48	23.6	26	12.7
4. I spent less time with other people.	66	32.4	52	25.4	86	42.2
5. I did not say that I did not have a child among people.	77	37.7	54	26.5	73	35.8
6. I was annoyed by conversations on pregnancy.	83	40.7	61	29.9	60	29.4
7. My family gave me support.	167	81.9	24	11.8	13	6.3
8. My close acquaintances and relatives gave me support.	133	65.2	51	25.0	20	9.8
9. My husband's family gave me support.	106	52.0	41	20.1	57	27.9
10. I did not tell my close relatives that I was receiving treatment.	68	33.3	51	25.0	85	41.7
11. I was uncomfortable with being in the same place with others receiving treatment.	39	19.1	52	25.5	113	55.4
12. I did not want to share my worries with other people.	78	38.2	69	33.8	57	28
13. I spent my days at home.	89	43.6	32	15.7	83	40.7
14. I was annoyed to hear people's conversations on their children.	69	33.8	57	28	78	38.2

Table 2-B. Distribution of the women by the psychosocial statements reported to have been experienced during the infertility treatment process (Cont.)

Psychosocial statements (N=204)	Yes		Sometimes		No	
	N	%	N	%	N	%
15. I felt myself worthless.	77	37.7	49	24.1	78	38.2
16. I did not want to embrace a baby when I saw one.	69	33.8	39	19.1	96	47.1
17. I was annoyed to hear the question "Do you have a child?"	98	48.0	59	29	47	23.0
18. I was infuriated by the misbeliefs of people around me regarding in vitro fertilization.	127	62.3	38	18.6	39	19.1
19. I abandoned myself to despair when I learnt from the test results that I could not conceive.	138	67.6	38	18.6	28	13.8
20. I was disappointed when I learnt from the test results that I could not conceive.	149	73.0	35	17.2	20	9.8
21. Having a child was the most important thing in my life.	157	77.0	22	10.8	25	12.2
22. When the treatment had ended in failure, I did not want to start it again.	55	27.0	59	28.9	90	44.1
23. I got very furious when I saw a pregnant woman.	59	28.9	38	18.6	107	52.5
24. I could not understand what the doctor or nurse said when I was troubled.	60	29.4	83	40.7	61	29.9
25. I frequently asked myself "Will I ever be able to get pregnant?"	158	77.5	32	15.7	14	6.8
26. I frequently asked myself "Will I ever be able to become a mother?"	162	79.4	33	16.2	9	4.4
27. I felt nervous before beginning the treatment.	134	65.7	44	21.6	26	12.7
28. I did not feel myself healthy.	69	33.8	68	33.3	67	32.9
29. I got furious with myself when I had menstruation.	116	56.9	54	26.5	34	16.6
30. I considered my womanhood insufficient because I did not have a child.	60	29.4	58	28.4	86	42.2
31. I liked caressing and cuddling the children.	175	85.8	21	10.3	8	3.9
32. I thought that my life would continue anyway without having a child.	67	32.8	65	31.9	72	35.3
33. I thought of getting divorced from my husband when I could not get pregnant.	48	23.5	34	16.7	122	59.8
34. Health personnel (physician, nurse, midwife) supported me.	118	57.8	45	22.1	41	20.1

women desire that their husbands understand what they feel and 80.9% that their husbands behave in a considerate manner.

Distribution of women by the sexual problems they experienced during the infertility treatment process is given in Table 4. It was found that 52.9% of the women had been uncomfortable with their sexual intercourse scheduled by the health personnel, and 51% had had a change in their desire for sexual activity during the treatment process. 57.4% of the women were found to perceive sexual intercourse as a duty.

Discussion

The findings of the study were discussed along with the studies conducted directly or directly in infertility treatment process. It was found that 77.9% of the women had a child after 1-4 attempts (Table 1). The treatment may be promising for the couples who are unable to have a child by natural ways. This is because the treatment represents the hope. So, it may be motivating for those who commence the treatment for the first time.

Psychosocial Problems Experienced by Women During Infertility Treatment Process

Men's and women's responses to infertility treatment and reactions against the treatment process are different. Women seek for more social support, whereas men rather engage in their business. Whatever the cause of the infertility, women experience more intense emotional stress and feel more personal responsibility. For many women, conception anxiety leads to worries, hopelessness, depression and many other psychosocial problems. However, men are more comfortable with the hardships of infertility. This is because men do not undergo menstruation and do not need to deal with their biological clock.

Table 3. Distribution of the women by their marital relationship characteristics during the infertility treatment process

Marriage relationship characteristics (N=204)	Yes		Sometimes		No	
	N	%	N	%	N	%
1. I talked to my husband about the treatment process without quarrelling.	143	70.1	32	15.7	29	14.2
2. My husband always stood by me during the treatment process.	146	71.6	48	23.5	10	4.9
3. My relationship with my husband did not change.	137	67.2	29	14.2	38	18.6
4. My husband was not as eager as me for the treatment.	60	29.4	49	24.0	95	46.6
5. I expected from my husband that he would understand my feelings.	154	75.5	35	17.2	15	7.3
6. I desired that my husband would behave me in a considerate manner. **Please answer the two questions below if the reason for the failure to have a child was your husband.**	165	80.9	21	10.3	18	8.8
7. I felt that the problem was me	95	46.6	66	32.4	43	21.0
8. I was furious with my husband.	64	31.4	75	36.8	65	31.8

Table 4. Distribution of the women by the sexual problems they experienced during the infertility treatment process

Sexual Problems (N=204)	Yes		Sometimes		No	
	N	%	N	%	N	%
1. I was uncomfortable with the scheduling of the sexual intercourse by the health personnel.	108	52.9	41	20.1	55	27.0
2. Our sexual desire changed during the treatment process.	104	51.0	48	23.5	52	25.5
3. I avoided sexual intercourse deliberately.	73	35.8	41	20.1	90	44.1
4. I thought that the drugs used in the treatment affected my sexuality.	58	28.4	56	27.5	90	44.1
5. I perceived sexual intercourse as a duty.	117	57.4	31	15.1	56	27.5

This study reveals that 41.2% of the women are uncomfortable with being in places with children, 57.8% are affected by conversations on children, and 63.7% are annoyed by the questions of people around them regarding having a child (Table 2-A). It may be said that women do not want to talk about children and avoid being in places with children due to the feelings of embarrassment and guilt caused by the inability to have a child. Although the reactions of individuals against infertility vary, it is known that they have similar aspects as well. In her study, Devran (17) represented that 67.6% of women were annoyed by the questions of people around them regarding this matter. The pressure of getting pregnant and the frustration felt when pregnancy is not achieved are more intense for women than for men (20). Monga et al. (21) found that 83.3% of infertile couples were annoyed by the pressure of people around them regarding having a child. In the study conducted by Lau et al. (22), more than 60% of the women stated that either they or their spouses felt pressure due to their infertility.

In this study, 32.4% of the women stated that they spend less time with other people (Table 2-A). This result may make one think that women suffer from social isolation. The studies conducted have revealed similar results to findings of our research. All these findings make us think that the problem of infertility causes isolation from friends and family due to the great social and cultural expectations of couples wit respect to having a child (23).

This study revealed that 81.9% of the women were supported by their family, 65.2% by their intimates, and 52% by the family of their spouses in the treatment process (Table 2-A). In Turkish society, women having a child gain a status as well. It is thought that families may have activated a social support system due to the values attributed to children. As the case in our country, infertility is a problem that affects not only the couple, but also the people around them in societies with strong family ties. Support from the family and close relatives comes to the fore in the infertility treatment process. The studies conducted have revealed that 75.2% of women feel lonely most of the time (17), 10% are exposed to the reactions of their spouses, 26% of the family of their spouses, and 44% of the people around them due to their infertility, and that these women suffer from anxiety and loss of self-respect (24). The existence of support systems assists the women/couples in undergoing this process in an easier manner in psychosocial terms (24). Matsubayashi et al. (25) reported that stress and the lack of support from the spouse played a key role as sources of anxiety and depression in infertile women, and that infertile women felt more emotional pressure from relatives and their own family as compared to their husbands. Perception of stigma is associated to low social support for both genders, and it is reported that as the social support decreases, anxiety, depression and global infertility stress increases (26).

In this study, 77% of the women stated that having a child was the most important thing in their lives, 37.7% that they felt themselves worthless, 33.8% that they did not feel healthy, 29.4% that they found their womanhood insufficient on the grounds that they did not have a child. In our culture, most women believe that the biological basis of being a woman is giving birth to and raising a child. For men, having a child is considered as the proof of manhood. Women who are unable to have a child are exposed to social stigma (27). Infertile couples may feel themselves insufficient and worthless due to the importance attributed by society to children and continuation of the family name. Questions asked regarding this matter may annoy them and cause them to turn in on themselves. However, this trouble may not perceived by the environment. Women may feel anger for the society since the children are at the forefront of the family life. Thus, inability to have a child may lead to deep psychological impacts and cause the couples to consider themselves less sufficient and less precious than others. In this sense, it may be said that couples make having a child the focus of their lives (11). In her study, Devran (17) reported that 65.6% of women felt themselves incomplete because of pregnant women and people with children, and 58.4% felt like sick most of the time. Lau et al., (22) reported that 80% of women longed for having a child, 19.8% of men and 37.5% of women though that infertility was disparaging for women. In the studies conducted, women feel themselves empty, defected, incomplete and small. Thus, self-confidence and self-respect of women diminish (24,28-30).

In this study, 62.3% of the women reported that they got angry with the misbeliefs of people around them regarding in vitro fertilization (Table 2-B). Due to the failure to have a child despite receiving a treatment, women may feel anger for the misbeliefs of the society regarding in vitro fertilization, and thus for the society and themselves. Oguz (14) found that infertile couples were annoyed by the misinformation of people around them regarding the treatment. Devran (17) found that 76.6% of the women were annoyed by the misinformation of people around them regarding the treatment.

Our study revealed that 67.6% of the women abandoned themselves to despair and 73% were disappointed when they learnt from the test results that they could not conceive, and that 56.9% got angry with themselves when they had menstruation (Table 2-B). For many women, conception anxiety leads to worries, hopelessness, depression and many other psychosocial problems. Women may be said to be suffering from emotions of failure due to not having a child. It is considered that they feel hopelessness, disappointment, resentment and anger because of the emotions of failure. Studies indicate that infertility treatment taking long adversely affects both physical health (31) and psychosocial health (32,33) Ozcelik et al., (10) reported that the beginning of menstruation in women each month induced the sense of loss. Devran (17) reported that 76.6% of the women felt themselves bad and 52% felt angry on the beginning day of menstruation.

This study revealed that 59.8% of the women did not consider divorcing from their spouses when the pregnancy was not achieved. A reason for the unwillingness of women to get divorced from their husbands may be that they do not want to feel a sense of loss. Women feel a sense of loss when they are unable to have a child and become a mother. Due to the tendency to avoid a second loss (loss of the spouse), they may be unwilling to get divorced from their husbands when pregnancy is not achieved. Dilek (16) found that the ratio of those who do not want to get divorced from their spouses due to infertility was 97.5%. Although infertility is not a legal reason for divorce, it may be observed as a reason for divorce in some cultures where it affects family relations and harmony, particularly in large families. Furthermore, polygamous marriages which are widespread in some regions may be stemming from the infertility of the woman (34,35). According to the data provided by Turkish Statistical Institute (36), 7.2% of the women state that the man's inability to have a child may be a reason for divorce, whereas this ratio is 12.7% for men throughout Turkey. The reason for polygamous marriages which are at a level of 3% in Turkey is generally attributed to the infertility of women (37). Infertility is at the same time a crisis for the whole family. Responses of the family to infertility may be both supportive and offending. In many African countries such as Nigeria, women are often blamed for infertility, and men can be divorced from their wives or marry numerous women (6,36). In Korea, a man with no child can marry numerous times or get divorced from his wife. Men are not blamed by the society when they divorce from their wives, but women cannot get divorced from their husbands (38).

This study revealed that 57.8% of the women were supported by the health personel in this process, and 29.4% did not understand what the physician or nurse said in times of trouble (Table 2-B). As a result of distractibility and impairment of concentration stemming from such troubled times, women may have difficulty in understanding what the physician/nurse says. In the study conducted by Devran (17), 41% of the women reported that they were unable to understand what the physician said because of their distress. The informing of the couples sufficiently during the treatment and the accessibility of the health personnel anytime are factors that relieve the treatment stress (8).

Problems of women regarding marital relationship during infertility treatment process

Infertility may cause ruinous impacts on the partners and marital relationship in the short and long run.

In this study, 70.1% of the women stated that they could calmly talk to their husbands about the treatment process, 71.6% that their husbands were always by them during the treatment, and 67.2% that their relationship with their husbands had no change. 75.5% of the women desire that their husbands understand what they feel and 80.9% that their husbands behave in a considerate manner (Table 3). Women report that the factor of social support is important, and that primary support should come

from the spouse. These results make one think that couples are more supportive of each other and gain experience strengthening their relationship in this process. When the studies on marital adjustment in infertile couples are examined, one sees many discrepant results. Some studies suggest that the problem of infertility have positive effects on the marital relationship of the couple, make the partners closer to each other, and strengthen the marriage. According to such studies, the stress, grief and disappointment shared by the partners seem to enhance the harmony between them and contribute to the improvement of the marital relationship (39-41). Akyuz (5) found that 71% of women did not suffer from the problem of alienation from their husbands due to infertility. It was also found that in infertile couples, women reported lower marital adjustment and quality of life, and men had less stress, higher self-confidence and higher marital and sexual satisfaction as compared to women. Infertility allows the spouses to get closer to each other by means of the experiences of problem-solving (21,42). Boivin et al. (4) found that the distress of women related to the marital relationship was associated to the decrease in the success of treatment. Newton et al., (19) reported that in men and women who had a long infertility treatment, serious problems arose in terms of social, sexual and marital relationships. Ozkan and Baysal (43) found disruption in marital and sexual relationships due to infertility. In the study conducted by Gucsavas (44), it was found that the reactions of women against infertility were more intense, 73.3% of spouses alienated from each other due to the impacts of infertility in familial and social life and 81.7% of women felt insufficiency in their sense of self.

In this study, approximately half of the women (46.6%) reported that their spouse was as eager as they were for the treatment (Table 3). Willingness of the husbands for their wives' treatment during the process may enhance the compliance to treatment and assist the couples in coping with stress. It is important that both spouses agree to undergo the treatment. In the case that one of the spouses is more insistent, psychologically more intense emotions may arise, and in consequence, the couple may feel difficulty in adapting to the treatment throughout the process. As the interest of men in infertility treatment and their inclusion in the treatment increase, more favourable impacts are observed on the communication and marital relationship of spouses (39,45). St. Hill et al., (37) reported that men generally refrained from participating in diagnosis and treatment of infertility.

Sexual problems experienced by women in infertility treatment process

Infertility assessment is emotionally disturbing and a threat for the emotional and physical sex image of the spouses about themselves. Particularly, the requirement to have sexual intercourse in the fertile period of the cycle may result in the sexual intercourse losing its naturality and make it experienced like homework. The individual may feel herself sexually insufficient due to her infertility and become depressive when the joy from and interest in the marriage and sexual intercourse are lost. Scheduled sexual intercourse, purpose of impregnation in the intercourse and being directed to certain positions may be among the causes of the loss of joy and interest. It is reported that sexuality and sexual activity have significant meaning in the exhibition of feelings of intimacy and sincerity in the relationship of the spouses (41), and that one of the important factors in marital adjustment is sexual harmony (46).

This study revealed that 52.9% of the women had been uncomfortable with their sexual intercourse scheduled by the health personnel, and 51% had had a change in their desire for sexual activity during the treatment process. 57.4% of the women were found to perceive sexual intercourse as a duty (Table 4). The discomfort felt by the women for the scheduling of sexual intercourse by the health personnel may be stemming from embarrassment, loss of privacy and disclosure of private life. This suggests that the treatment process adversely affects the sexuality. There are studies in the literature, which reveal the adverse impacts of infertility on sexual life. Monga et al., (21) reported that men had more sexual dysfunction, whereas Newton (19) reported more sexual dysfunction in women. Lee et al., (42) report that sexual satisfaction of women is less in female infertility and infertility of both spouses. Lau et al., (22) report that more than half of infertile couples feel pressure during sexual intercourse. In a study conducted in our country, Guz et al., (24) reported that 58% of

the women had decreased sexual interest due to scheduled sexual intercourse. 37% of the women ascribe their dissatisfaction about their sexual life to the infertility treatment. Dilek (16) reported that infertility adversely affects the sexual life of 20% of the couples, and Oguz (14) reported that sexual life of 66% of women was adversely affected by infertility. Sexual dysfunction, loss of sexual drive and failure to have sexual arousal manifest themselves in the form of anorgasmia in women, which directly or indirectly makes it harder to get pregnant. Moreover, hormones used in the treatment adversely affect the sexuality.

Conclusions

This study reveals that women who succeeded in infertility treatment had many psychosocial problems in the treatment process. It is found that these women feel anger and fury for the misbeliefs of people around them regarding in vitro fertilization, abandon themselves to despair, are disappointed and feel senses of deprivation and loss when they learn from the test results that they are unable to conceive, and feel nervous, anxious and worried while beginning treatment. Furthermore, these women report that their family, intimates and family of their spouses support them in this process, which means they have social support systems. Finally, it is found that women are embarrassed and have a loss of privacy due to the scheduling of their sexual intercourse by the health personnel. Based on the results of the study, it is recommended that the society should be educated by means of the mass media so that they are more sensitive towards infertile couples and avoid offensive and stinging words and questions, and that infertile couples should be taught the ways of diminishing the adverse impacts of infertility and coping with them, and that particularly women should be supported in this respect. It is further recommended that the psychosocial problems experienced during treatment process by women who succeeded in infertility treatment should be assessed by qualitative research methods. Moreover, it is also recommended that the psychosocial problems experienced during treatment process by men who succeeded in infertility treatment should be assessed as well.

Limitations of the Study

The main restrictions of the study were that the registration system containing the treatment outcomes of couples who had undergone treatment in infertility treatment units was insufficient, and thus the treatment outcomes were not known by the health personnel. 33 women did not want to be involved in the study due to unwillingness to go down memory lane and keeping the treatment as a secret. These women even demanded their files to be removed from the in vitro fertilization units and destroyed if possible. Hence, the results of the research can only be generalized for the women within the scope of the study on the grounds that snowball sampling method was selected among the non-probability sampling methods. Retrospective collection of the data may result in the impacts of the past incidents being felt and expressed less or more intensely than they were.

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Authors' roles

NK**,** TP were responsible for the study conception and design, and drafting of the manuscript. NK performed the data collection. NK, TP performed the data analysis. NK, TP made critical revisions to the paper for important intellectual content. All authors have given final approval of the version to be published.

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Determinants of the choice of private hospitals by patients

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Abstract

Objective and Background: Today, patients show more sensitivity in selecting healthcare services compared to the past. Different factors, such as quality of service, costs, economic conditions, and social and cultural status, contribute to selecting hospitals. The present study attempts to examine reasons why patients prefer private hospitals for treatment.

Methodology: This cross-sectional descriptive study was conducted in 2009 on patients in Private Hospital of Tehran University of Medical Science. Random sampling was used (n=120). Data were collected using researcher-made questionnaire whose validity and reliability was then confirmed. Collected data were analyzed through t-test and ANOVA.

Findings: In selecting hospitals, patients gave the highest score to experienced and skilful doctors (mean = 3.55) and the lowest score to relatives working at the hospital (mean = 1.16). Mixture of quality factor (mean = 56.07) was the most important and mixture of economic factor (mean=32.59) was the least important factor.

Conclusion: Given the interaction between society and hospitals as well as constant need for healthcare services, for a proper objective management in hospitals and attracting customers (patients) consumers' opinions, needs, and expectations must be taken into account while improving quality and quantity of services and improving processes.

Key words: Patient, Private Hospital, Selection.

Introduction

Hospitals are integral parts of medical and social systems that provide the public with healthcare services. Variety of services is offered by hospitals. To maintain quality of service, hospitals should observe medical ethics and rights of patients [1]. Like everyone else, a patient has vital needs. Illness may prevent him from meeting his needs completely. Therefore, increased knowledge over patient's needs is helpful in identifying those needs and assisting patients in meeting them. Such needs provide a useful framework for delivery of healthcare services. Thus, treatment and healthcare staffs should possess required knowledge on needs, how to meet them, and situations where these needs can be satisfied [2].

Patients usually expect easily accessible nursing services and proper treatment in all stages. They prefer to deal with knowledgeable, reliable, and polite staff and have information on how and where these services are provided [3]. In the recent years, and due to competition over customers, reduced costs of healthcare services and increased income for hospitals, attentions have been drawn to evaluation of patients' opinions [4]. Therefore, hospitals cannot ignore patients' needs and offer services only based on their own interests. In today's competitive worlds, customers are of great importance for providers of healthcare services. In such conditions, of course, customer needs, behaviors, interests, and sensitivities become extremely important in designing and directing services [5].

Citing Okorafor, Yaghubi points out that today patients play more prominent roles in making decisions and selecting hospitals than they did in the past [6]. In his study, Sanayei notes that study of consumer's behavior is an important part of marketing since for proper marketing we need to properly understand consumer's needs and demands [7].

Taylor *et al.* showed that variables such as easy access, quality of care, hospital reputation, and waiting time are among the determinant factors

in selecting a hospital. They found that providing patients with proper information may help them make an informed decision [8].

Geber Michael *et al*. (2007) conducted a study on 1,657 patients in Eritrea. They found that important factors such as education, quality of healthcare services, wages, severity of disease, social status, and patient's place of residence statistically contribute to selecting healthcare providers [9].

Coulter *et al*. reported factors such as chances of successful surgery, waiting time, hospital reputation, follow-up capacities, doctors' reputation, doctors-hospital relationships, hygiene standards, and quality of service are likely to affect patient's decision in selecting hospitals [10].

Boshoff showed that quality of service, empathy from nursing staff, and insurance coverage from private section affect loyalty and satisfaction of customers as marketing targets [11]. In Iran, both private and public sectors offer healthcare services and given the fact that private sector covers 18.8 percent of healthcare organizations [12], a fundamental question to ask is that for what major reasons patients select private organizations. The present study attempts to address this question through examining determinant factors in selecting private healthcare providers by patients in private hospitals administrated by Tehran University of Medical Science.

Methodology

This descriptive cross-sectional study was conducted in 2009 on patients in four private hospitals supervised by Tehran University of Medical Science (Madayen, Eyvaz Zadeh, Shahryar, and Alvand). In different wards of these hospitals, 120 patients were selected by random sampling. In cases where, due to poor health conditions or small age, patients were not able to answer the questions, attendants were asked to answer the questions.

A researcher-made questionnaire was used for data collection. The first part of the questionnaire contained 9 questions on demographic profiles of patients. The second part (23 questions) was designed on Likert scale (1: a little to 5: very much) for four categories: external factors (6 questions: recommendation by doctor, access to doctor after surgery or treatment, skilful and experienced doctors, long waiting time and delay in other hospitals, hospital reputation and background, short home-hospital distance), quality factors (9 questions: doctor-patient relations and continuous presence of doctor at patient's bed, doctors' reputation, presence of patient's doctor in hospital, skillful and experienced nursing staff, good conduct of nursing staff and providing information, good conduct of hospital staff, orderly conditions in hospital, hygiene and cleanliness, advanced diagnosis and treatment equipment), personal factors (6 questions: presence of medicine and paramedic students in public hospitals, personal preferences, recommendations by relatives, previous experiences with the same hospital, Employment of relatives in the hospital, belief in cost-effectiveness of private hospitals), and economic factors (2 questions: personal income and treatment costs). In addition, two yes/no questions were designed to examine the effects of these factors on selecting a particular hospital.

We used previous studies and interviews with scholars to design questionnaires. We also gathered opinions of university professors to evaluate validity of questionnaires and remove potential problems. We used Cronbach's alpha for reliability evaluation and found r=0.78. Data obtained through questionnaires were analyzed using t-test and ANOVA.

Findings

The respondents were composed of 55% women and 45% men. Thirty percent of patients were above 60. Eighty percent were married and 37.5% were housewives. Among these patients, 41.7% had an income between IRR 300,000 and 600,000 and 34.2% did not have high school diploma. Thirty five percent were covered by social security insurance and 60% were referred by their personal doctor to hospitals. In addition, 76.7% had been previously hospitalized. Table I shows the distribution of demographic variables for patients.

The highest score was that of quality factors (mean = 56.07) while the lowest score belonged to economic factors (mean = 32.59) (Table II).

Among the personal factors, personal preference (2.86) had the highest mean and relatives working at the hospital (1.16) had the lowest mean value.

In the group of external factors, skillful and experienced doctors (3.55), access to doctors after treatment (3.22), recommendation by doctor

(3.06), and short home-hospital distance (1.71) had the highest and lowest scores, respectively.

Table 1. Demographic variables distribution of patients in selected hospitals

Demographic variables		Percent
Sex	man	55
	women	45
Age	Less than 15	1/7
	Between 16 to 30	15/8
	Between 31 to 45	25
	Between 46 to 60	27/5
	More than 60	30
Marital statues	single	20
	married	80
Education	illiterate	8/3
	below high school	34/2
	High school	30
	association	12/5
	Undergraduate and higher	15
Job	officer	10/8
	Self-employed	10/8
	student	5/8
	unemployed	2/5
	retired	26/7
	Housekeeper	37/5
	labor	3/3
	others	2/5
Monthly income	Less than 300 $	39/2
	Between 300 to 600 $	41/7
	Between 600 to 900 $	8/3
	More than 900 $	10/8
Type of Insurance	Health services	35
	Social security	29/2
	Armed forces	2/5
	Other Insurance	20/8
	Without Insurance	12/5
Referral way	Privet clinics	60
	Hospital clinics	5/8
	health system Referral	5/8
	Personal regard	16/7
	Friends and acquaintances	8/3
	others	3/3
History of hospitalization	Yes	76/7
	No	23/3

In the category quality of service, patient-doctor relationships, presence of the doctor in hospital, doctors' reputation, skillful and experienced nursing staff, hygiene and cleanliness with mean scores above 3 were among the determinant factors. The lowest score was that of orderly conditions (mean = 2.40).

In economic factors, personal income and treatment costs with the respective mean values 1.66 and 1.60 had the highest and the lowest scores, respectively.

In response to the question "If you became sick again, would you come to this hospital?", majority of respondents (88.3%) said yes. In addition, 78.3% of respondents confirmed that they would recommend the hospital to their friends and relatives after being discharged.

Table 2. Factors Mean and SD in the choice of selected hospitals

Reasons for preferring treatments from private hospitals	(Mean ± SD)
Economic factors	32/59 ± 22/69
External factors	50/16 ± 10/95
Personal factors	37/58 ± 15/17
Service quality factors	56/07± 17/58

T-test revealed significant relation between gender and quality of service; that is, women are more sensitive to quality of service compared to men.

ANOVA analysis suggested significant relationship between age groups, external factors, and quality of service (quality and external factors had more effects on older patients).

T-test also confirmed significant relationship between marital status and external factor (single patients cared more about external factors).

ANOVA indicated significant relation between education and external factors and between education, quality of service, and selecting a hospital. Individuals with higher levels of education paid more attention to external factors and quality.

ANOVA also showed that there is a significant relationship between quality of service and how a person is referred to hospital. Quality of service was more important to those referred from a doctor's office or those who came to hospital for personal preference.

T-test suggested significant relation between previous hospitalization and quality of service. For

Table 3. The relationship between demographic variables and factors in selected hospitals

Factors / Demographic variables	p-value			
	Economic factors	Personal factors	External factors	Service quality factors
Sex	0/09	0/61	0/241	0/013
Age	0/72	0/506	0/001	0/002
Marital statues	0/56	0/89	0/002	0/17
education	0/24	0/73	0/023	0/011
Job	0/384	0/123	0/238	0/295
Monthly income	0/274	0/443	0/451	0/538
Type of Insurance	0/91	0/43	0/82	0/72
Referral way	0/226	0/176	0/659	0/003
History of hospitalization	0/264	0/701	0/519	0/032

those who had been previously hospitalized, conduct of nursing staff and provision of information was the most important factor in selecting hospitals.

No relation was found between occupation, insurance, income, and these factors.

Discussion

Designing and planning services for attracting the public and customers should be based on the opinions of potential targets of those services [13].

In this study we examined four mixtures of determinant factors in selecting private hospitals. As our findings indicate, patients regard quality of service as the most important factor in selecting hospitals while economic factors do not play a significant role. This is in line with Taylor *et al.*[8] who found that easy access, quality of service, and hospital reputation were more important in patients' view compared to factors such as waiting time. The results also held for patients with higher levels of education.

Jackson argues that another determinant factor in selecting hospitals is satisfying experience of previous hospitalization [14]. In our study, majority of respondents (67.50%) had experienced previous hospitalization. This may show their satisfaction with previous hospitalization.

In "Determinant Factors in Selecting Healthcare Providers in Nigeria", Lloyd *et al.* (2007) found that two factors, namely distance and monetary value, encouraged people to seek healthcare services. However, money is of less importance in selecting healthcare service providers. Thorough more qualitative analyses, they found that monetary value of services is an important factor since many people with low income choose self-care. Moreover, their study revealed that older people are more likely to go to private hospitals [15]. Our findings are in line with the results obtained by Lloyd *et al.*

Coulter *et al.* [10] studied determinant factors in selecting hospitals in London. Quality of healthcare services and hygiene standards were of the highest priorities in their study. They found that for older individuals, factors such as hygiene and cleanliness and access to doctors are more important. We found similar results in our study. Furthermore, Coulter *et al.* found that the shorter the waiting time, the more important would become other factors, including facilities for parking, additional services offered by hospitals, reputation, and being a hospital of choice for celebrities. Although these factors were more or less present in our study, patients did not stress them.

Varmaghani [16] studied determinant factors in selecting private and public hospitals in Tehran. He pointed out that both private and public hospital patients based their choices on presence of experienced doctors in hospitals. Patients in our study stressed the importance of this factor.

"Reasons Why Patients Chose Treatment from Private Hospitals" [17], a study conducted in Amin Isfahan Institute, indicated that recommendation by doctors was the leading factor (70%). Fifty two percent of patients said that if their doctors worked at a public hospital, they would be prepared for hospitalization in those hospitals and 28% found no difference between public and private hospitals. Doctors' recommendation (mean = 3.06) in our study was a determinant factor in selecting hospital.

Limitations

Due to limited time, authors did not include other public hospitals as well as hospitals run by SSO, Armed Forces, NIOC, Relief Foundation, *etc*.

Conclusion

Unlike the past times, patients are not indifferent to services they receive while in hospitals. They acknowledge the value of money they pay and expect services of higher quality. Patients seek best available healthcare services. Given patients' increased awareness and sensitivity to their conditions and treatment processes, each hospital needs to make required changes in its service framework based on proper models.

Hospital managers must be aware of criteria for a good hospital and what patients expect from a hospital. They should identify what aspects are considered more important to patients and what issues results in their complaints. To win in the competitive environment, managers should take into account all these factors and make optimum use of available resources.

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Facial growth and rotation of the upper and lower jaw in patients with Cleft Lip and Palate (CLP)

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Abstract

Background/Aim: Clefts lip and palate are the most common congenital anomalies of the craniofacial complex, with a frequency of occurrence in 650-700 live births. People born with cleft lip and palate have characteristic changes in neurocranium and viscerocranium. Through the function of time and craniofacial growth and development they may lead to a specific feature of the skeleton of face and skull, and changes in facial aesthetics and way of orofacial functions, too. The aim of this study was to determine persistence of changes in the type of facial growth as a whole, as well as growth and rotation of the upper and lower jaw separately, which would be a consequence of the cleft lip and palate in this sample compared to control group.

Methods: The study included 80 patients of the Clinic of Orthodontics, School of Dentistry, University of Belgrade. Of that number, 40 patients had a diagnosis of cleft (20 unilateral - UCLP, 20 bilateral - BCLP), and the remaining 40 patients were without clefts (control group). On the lateral cephalometric radiographs were performed measuring and interpreting the following parameters: the angles NSAr, SArGo, ArGoMe, the sum of angles of Bjork's polygon, NGoAr, NGoMe, NSGn (Y), NS/SpP, NS/MP and linear parameters of S-Go, N-Me, S-Go:N-Me, S-SNP, SNP-Go, N-Sna, Sna-Me. Statistical analysis included the determination of average values, standard deviation, coefficient of variation and significance of differences using Student t-test.

Results: Cleft patients have characteristic increase in the sum of the angles of Bjork's polygon in terms of vertical facial growth, with the condition of the saddle and gonial angle. The anterior face height grows more intense than the posterior, thus changing their percentage ratio, which correlates with the growth of backward rotation. Lower jaw shows a trend backward down in patients with bilateral cleft, while the upper jaw grows forward and upward to the cranial base in the both: unilateral and bilateral clefts.

Conclusion: People with a cleft have characteristic of craniofacial morphology changes, in terms of vertical facial growth, increased gonial angle, increased anterior face height and, usually, divergent growth of the jaw bases.

Key words: Cleft lip and palate, cephalometric changes, facial growth, backward rotation, rotation of the upper/lower jaw

Introduction

Cleft lip, alveolar processes and palate are the most frequent congenital anomalies. They appear on the structures of the orofacial complex.

The question is, whether there are similarities and differences in craniofacial morphology of the patients with cleft lip and palate compared with patients without cleft who require orthodontic treatment.

The basic mechanism of cleft formation is the absence of healing or partial healing of facial processes. To structures, that constitute the primary and secondary palate could be adequately established, it is necessary to reach out to healing of facial processes by obliteration of the ectodermic furrow and ingrowths mesoderm of the each to the mesoderm of other structures. Causes of unhealing or incomplete healing are very different, such as: lack of mesenchymal tissue in the frontonasal processus, abnormal position of the embryo, the high position of the tongue with medial part distortion of frontonasal processus, the lack of amniotic fluid, late retroflexion of the embryo head, local mesenchymal tissue necrosis, excessively large or small tongue, excessively large head of the embryo, changes in the cervical vertebrae that occur in the Klippel-Feil syndrome, increased depth of the nasopharynx and changes in the cervical spine[1,2]. This causes significant morphological changes and functional disturbances, which are most

noticeable in the function of speech and irregular voice articulation[3].

The cleft etiology is multifactorial. A number of authors[4,5] believed that inheritance plays a dominant role in the development of the cleft. On the other hand, there are authors[6,7] who have completely rejected the significance of hereditary factors in the genesis of this anomaly. Besides heredity, development of the cleft contribute exogenous factors too: eating disorders[1,8], chemical teratogenic agents[9], pregnancy disease[1], effects of radiation[4,10,11] and psychological trauma[12,13].

Orthodontic problems in clefts are very complex. It is therefore essential that detailed and accurate study the characteristics of craniofacial morphology of those patients, with a view to proper planning of orthodontic treatment and to achieve better therapeutic results in the morphological, functional and aesthetic terms.

The aim of this study was to determine the type of facial growth characteristics in general, and separately growth and rotation of the upper and lower jaw, in patients with cleft lip and palate, and whether there are differences in the measured parameters compared with patients in the control group (without clefts).

Methods

This examination included 80 patients from the Clinic of Orthodontics, School of Dentistry, University of Belgrade. The first group comprised 40 patients with cleft lip and palate (20 bilateral - BCLP and 20 unilateral - UCLP). Second (control) group comprised 40 patients without a cleft. The average age in the first group of patients was 8 years and 7 months; in the second group was 8 years and 3 months.

All measurements and analysis (study casts, facial photos, orthopantomograms and lateral cephalograms) were done before the start of orthodontic treatment (Figures 1-4). All of patients were recorded by the standard lateral cephalometric image. The head was fixed in cephalostat. Recording was done from the distance of 1.5 meters.

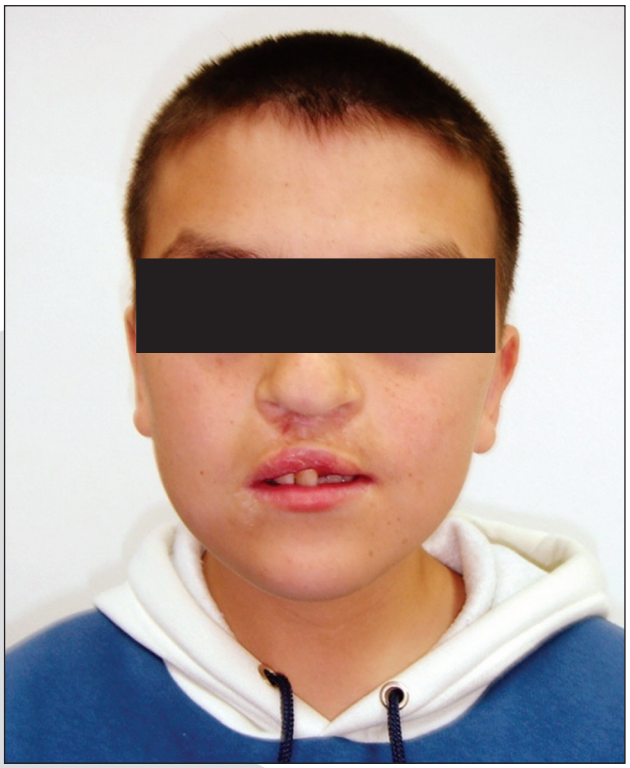

Figure 1. A boy, 9 years old, with CLP (frontal view)

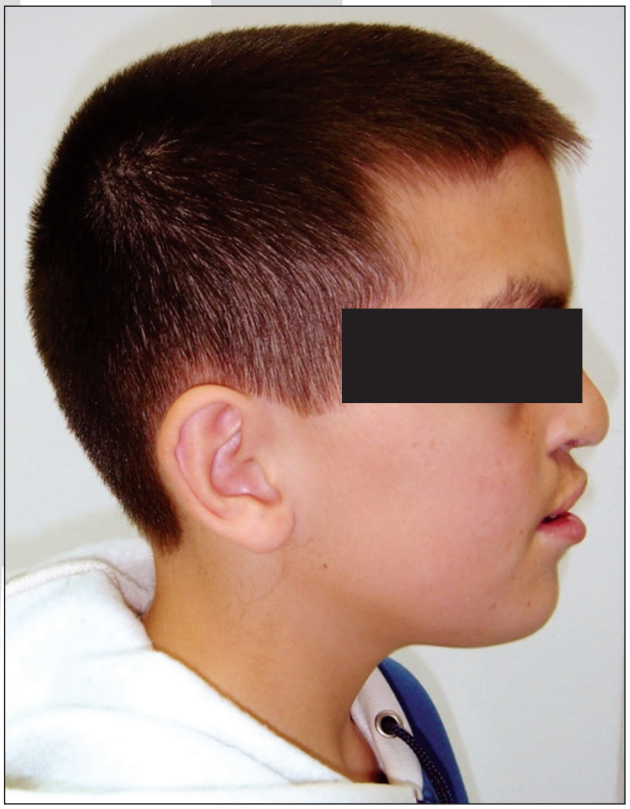

Figure 2. A boy, 9 years old, with CLP (lateral view)

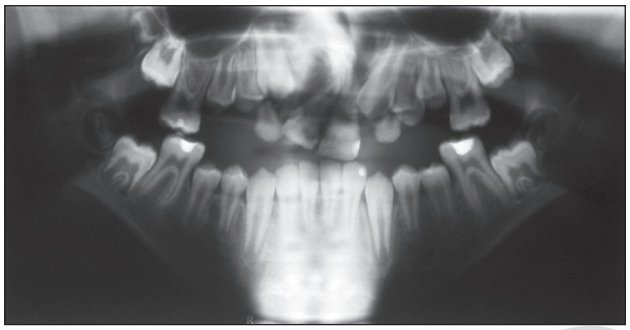

Figure 3. Orthopantomogram (unilateral CLP on the right side)

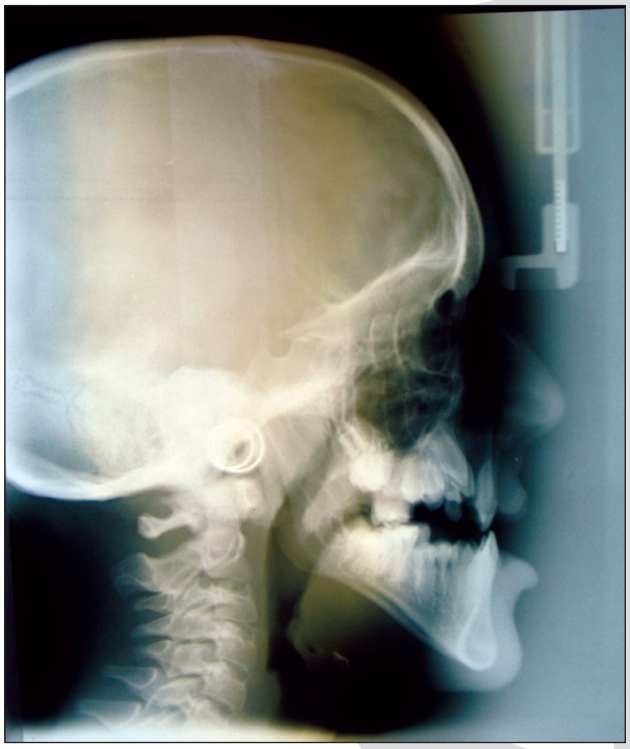

Figure 4. Lateral cephalogram of unilateral CLP

Measurements and analysis were performed on the following growth and rotation parameters of the face and jaws:

- angular parameters (Figure 5): the angle of the saddle (NSAr), articular angle (SArGo), gonial angle (ArGoMe), the sum of the angles of Bjork's polygon (NSAr + SArGo + ArGoMe), upper gonial angle (NGoAr), lower gonial angle (NGoMe), the angle of the lower jaw rotation (NSGn or Y axis),

- linear and angular parameters (Figure 6): posterior facial height (S-Go), anterior facial height (N-Me), percent ratio of S-Go:N-Me, posterior upper facial height (S-Snp), posterior lower facial height (Snp-Go), anterior upper facial height (N-Sna), anterior lower facial height (Sna-Me), the angle of the upper jaw rotation (NS/SpP), the angle of the lower jaw rotation (NS/MP).

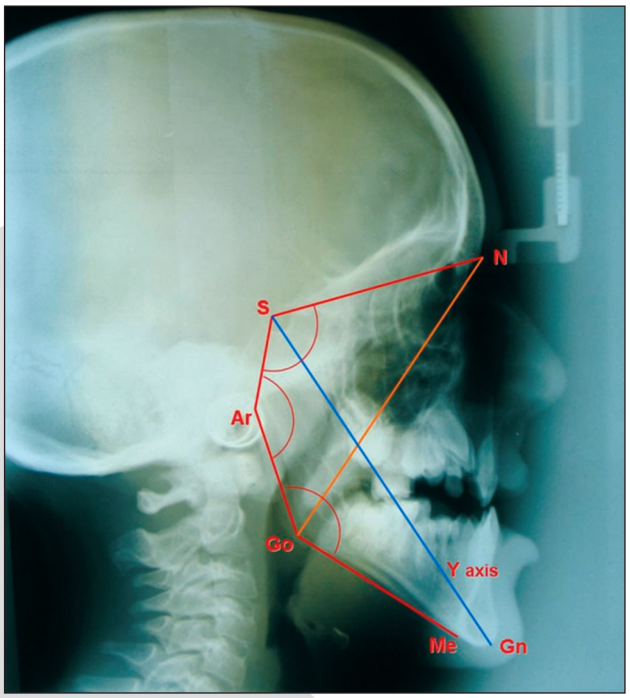

Figure 5. Lateral cephalogram with tracings (angular parameters)

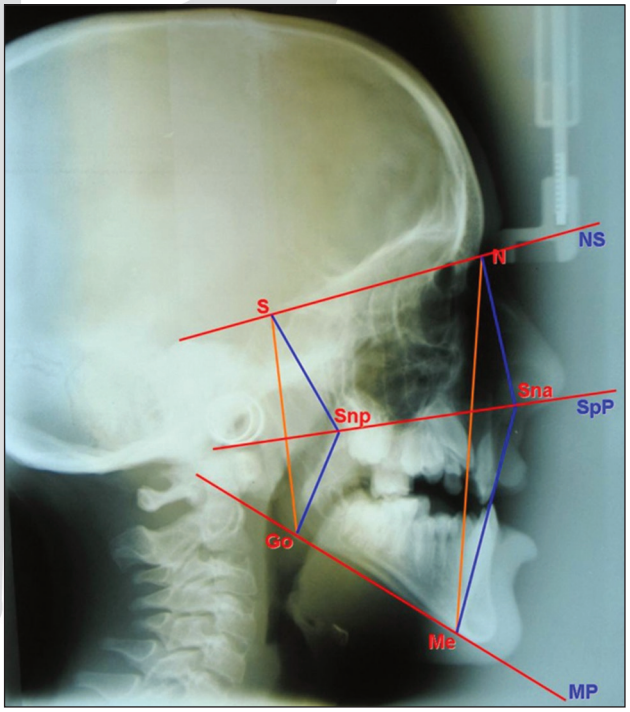

Figure 6. Lateral cephalogram with tracings (linear and angular parameters)

All of the parameters were calculated by the average value, standard deviation and coefficient of variation. Since the coefficient of variation was be-

low 30% (homogeneous sample), there was applied Student T test for statistically significant determination of differences between investigated groups.

Results

There are characteristic changes of analysed parameters in patients with cleft lip and palate.

Table 1. shows that saddle angle, NSAr, was increased in patients with BCLP. Also, articular angle, SArGo was increased in this group of patients. Increasing values of gonial angle, ArGo-Me, is tipical for whole sample with clefts. As a result of this changes exist enlarge value of sum of angles of Bojrk's polygon. It means that patients with CLP mostly have vertical growth, with backward rotation. Patients with BCLP have slightly increased upper gonial angle (NGoAr), while lower gonial angle was increased in whole sample.

There are no significant difference in values of posterior facial height, S-Go, between patients with and without CLP, as it indicates table 2. On the other side, whole sample with CLP has markedly increase od anterior facial height, N-Me. It causes changes in their relationship in terms of emphasizing the vertical growth. In sample with CLP 4 patients have forward rotation, 6 patients have neutral growth (without rotation), while 30 patients have backward rotation. Upper anterior facial height, N-Sna, as lower anterior facial height, Sna-Me, were increased, especially in patients with BCLP.

From table 3. we can conclude that patients with BCLP has backward rotation of the lower jaw with expressedY axis, while patients with UCLP have slight forward rotation of the lower jaw. Upper jaw has forward rotation in whole sample with CLP. Divergent growth of jaw basis has the worst prognosis in patients with vertical facial growth and backward rotation to the anterior cranial base.

Table 1. Values of angles of Bjork's polygon in patients with and without cleft lip and palate

Parameter	Control group (1)	Group with cleft			t-test		
		Bilateral (2)	Unilateral (3)	Total (4)	(1,2)	(1,3)	(1,4)
NSAr	121.23°	126.40°	122.75°	124.58°	p<0.001	p>0.05	p<0.01
SArGo	144.58°	137.75°	143.80°	140.78°	p<0.001	p>0.05	p<0.01
ArGoMe	128.20°	137.00°	133.40°	135.20°	p<0.001	p<0.001	p<0.001
∑	393.95°	401.15°	400.05°	400.60°	p<0.001	p<0.001	p<0.001
NGoAr	55.18°	57.60°	55.10°	56.35°	p<0.02	p>0.05	p>0.05
NGoMe	73.03°	79.40°	78.35°	78.88°	p<0.001	p<0.001	p<0.001

Table 2. Parameters of facial growth (Jarabak method) in patients with and without cleft lip and palate

Parameter	Control group (1)	Group with cleft			t-test		
		Bilateral (2)	Unilateral (3)	Total (4)	(1,2)	(1,3)	(1,4)
S-Go	71.50mm	69.40mm	70.65mm	70.03mm	p>0.05	p>0.05	p>0.05
N-Me	110.90mm	115.40mm	115.50mm	115.45mm	p<0.02	p<0.01	p<0.002
S-Go:N-Me	64.48%	60.07%	61.00%	60.55%	p<0.001	p<0.01	p<0.001
S-Snp	37.20mm	37.10mm	38.30mm	37.60mm	p>0.05	p<0.02	p<0.05
Snp-Go	34.30mm	32.30mm	32.35mm	32.30mm	p<0.02	p<0.01	p<0.01
N-Sna	50.70mm	52.75mm	53.30mm	53.03mm	p<0.03	p<0.001	p<0.001
Sna-Me	60.20mm	63.66mm	62.70mm	63.28mm	p<0.01	p>0.05	p<0.02

Table 3. Parameters of rotation of the upper and lower jaw in patients with and without cleft lip and palate

Parameter	Control group (1)	Group with cleft			t-test		
		Bilateral (2)	Unilateral (3)	Total (4)	(1,2)	(1,3)	(1,4)
NSGn	67.83°	70.50°	62.80°	69.65°	p<0.01	p>0.05	p<0.02
NS/SpP	13.30°	8.70°	10.20°	9.80°	p<0.001	p<0.01	p<0.01
NS/MP	32.60°	37.70°	35.20°	35.90°	p<0.001	p<0.001	p<0.001

Discussion

Characteric of the patients with cleft lip and palate is vertical growth type of the face, ie. backward and downward rotation. Those characteristics are more pronounced in bilateral than in unilateral clefts, whereas in the control group present a slight increase in facial forward rotation, which is considered a normal attribute of the population. All of the authors who have studied these researches[11,14-23] have got results that indicate the vertical type of growth (backward rotation) in patients with clefts.

In the Jaksic opinion[19,20] patients with bilateral cleft lip and palate retains the vertical growth tendency over time, whereas in patients with unilateral clefts can happen to change the overall type of the facial growth. Backward rotation as a growing trend continues through the time, as evidenced by papers of Nollet et al.[24] and Vettore and Campos[25] who worked longitudinal cephalometric study and monitoring of patients with complete unilateral cleft, in the age of 9, 12 and 18 years. Regardless of age and delayed surgical closure of the hard palate, there was a pronounced vertical facial growth, with an increase of anterior face height, especially of the anterior lower height (Sna-Me). In the cleft patients, increasing the angle NSGn and posterior rotation of the lower jaw is the most common finding described in the literature: Aduss[26], Rak and Muretic[27], Viteporn et al.[7], Da Silva et al.[28], Stamenkovic[21]. Some of authors like Bishara et al.[29], Capelozza et al.[30], Lisson et al.[31] and Holst et al.[32] (a sample of patients with bilateral cleft), point to a balanced growth of the lower jaw, without changing angulation of the Y axis in patients with clefts. At the same time Lisson et al.[31] and Corbo et al.[22] indicate a severe posterior rotation of the maxilla relative to the cranial base in a period of mixed dentition.

Relation between anterior and posterior facial height is changed, primarily due to increased distance N-Me. That's why patient have longer lower third of face, called syndrome ''long face''. Similar results suggest Narula and Ross[33], Krogman et al.[14,34], Vora and Joshi[35], Johnson[36], Mc Namara[37], Smahel[38], Ross[39], Horswell and Levant[40], Holst et al.[32], Semb[11], Capelozza et al.[30], Baek et al.[23], Ozturk and Cura[41], Jaksic[20], Stamenkovic[21], Vettore and Campos[25] and Horowitz et al.[42]. Spyropoulos and Linder-Aronson[43] and Cronin and Hunter[44] conclude that if we start in early age with multidisciplinary treatment of patients with cleft lip and palate and make insertion of bone graft analysed parameters have values closed to standard, with small deviation. On the other side, Ross[45] accentuate that values of anterior and posterior facial height are very similar, regardless of the age of surgical closure of cleft (6 or 18 months). Will[46] analysed craniofacial morphology of surgically untreated patients with cleft lip and palate. They didn't have significant deviations in vertical dimension compared with patients with cleft lip and palate who had prompt orthodontic and surgical treatment.

The opinion of Smahel[38] and Bishara et al.[47] upper anterior facial height (N-Sna) is decreased because of inhibition of growth of lower facial third in vertical direction. In contrast to this opinion, Jaksic[48] indicates that values of N-Sna distance is not changed in sample with clefts. Viteporn et al.[7] followed the changes of facial heights and concluded that anterior facial height is changed till 8 years of age, while increasing of posterior facial heights is the most intense between 12 and 16 years of age. On the sample of 11 patients with complete cleft Lisson et al.[31] determined significant decrease of posterior upper facial height (S-Snp) and increase of anterior lower facial height (Sna-Me) in repeated measurements in the age of 7 and 12 years.

Conclusion

Analysis of growth parameters indicates that patients with unilateral cleft lip and palate (UCLP) are characterized by vertical facial growth (backward rotation), increased gonial angle (especially lower - NGoMe), severe increased anterior facial height (N-Me), increased distances N-Sna and Sna-Me, moderate forward rotation of lower jaw and forward rotation of upper jaw.

Patients with bilateral cleft lip and palate (BCLP) are characterized by vertical facial growth (backward rotation), increased gonial angle (especially lower – NGoMe), increased saddle angle (NSAr), decreased articular angle (SArGo), severe increased anterior facial height (N-Me), increased distances N-Sna and Sna-Me, decreased posterior facial height (S-Go), backward rotation of lower jaw with expressed Y axis and forward rotation of upper jaw.

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Depression, sexual dysfunction and the affecting factors among the women treated for infertility

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Abstract

Objective: The present research was a descriptive study that was conducted in order to determine depression, sexual dysfunction and the affecting factors among the women treated for infertility.

Material and methods: The sample of the research was made up of 100 women selected using simple-random sampling who went to Private Çorum Infertility Policlinics in order to receive infertility treatment. The data were gathered with Data Collection Form for Infertile Women's Descriptive Characteristics designed by the researchers using the information in literature, Female Sexual Function Index and Beck Depression Inventory. The data were assessed with percentages, arithmetic means, standard deviation and ANOVA test in computer environment.

Results: It was found out that most of the participant women (72.0 %) underwent depression and nearly half of them (47.0 %) experienced sexual dysfunction. In the analysis, there was statistically significant correlation between women's sexual dysfunction and having health insurance, financial difficulties experienced during the infertility treatment, previous infertility treatment and depression experience whereas the correlation between women's sexual dysfunction and age, educational status, professional status, total monthly income, marriage duration and cause of infertility was statistically found insignificant.

Conclusion: It was detected that seven in ten women who received infertility treatment suffered from depression while nearly one in two women who had infertility treatment underwent sexual dysfunction.

Key words: Women's Infertility, Depression, Sexual Dysfunction, the Affecting Factors.

Introduction

Infertility means not being able to become pregnant despite a year of unprotected sexual relation. Infertility is both physically and emotionally a difficult process. Ambiguities about infertility that occur and financial and psychological difficulties experienced during the treatment-process may lead to biological crisis (1). Infertility, a sudden biological crisis, is a condition that is not expected, maybe not clarified, has a long diagnosis period, causes heavy stresses and challenges adjustment mechanisms (2).

Just as infertility itself may cause emotional and sexual problems, emotional and sexual problems may cause infertility, too (3). People may be depressed because they feel themselves sexually incompetent due to infertility and lose interest and satisfaction in marriage and sexual relation. Since couples think of preventive measures in their pre marriage or extramarital sexual relationships or they believe they are punished due to the illegitimate sexual relations; menstruation that occurs after premenstrual period during which couples expect pregnancy may lead to depressive feelings (4). Problems may occur in the relations of the couples that feel the pressure "Sexual relation must result in pregnancy." Additionally; timed-sexual intercourse, adopting certain positions during the sexual intercourse and hormonal drugs used in the treatment may cause sexual dysfunctions. The studies conducted report that infertile women experience more psychological problems such as depression and anxiety as well as more sexual dysfunction than men (5,6). As a result, women may experience sexual dysfunctions such as loss of sexual drive, lack of sexual arousal and anorgasmia (7-9).

When the literature is investigated, it is seen that there is a limited number of the studies that have aimed at the effects of infertility on emotional and sexual problems (10-16). It was the main

point of the present research. Our study was conducted in order to determine depression, sexual dysfunction and the affecting factors among the women who received infertility treatment.

Methods

Population and Sample of The Research

The population of the research was consisted of women who came to Private Çorum Infertility Policlinics for infertility treatment between March 2011 and June 2011. The sample of the research was made up of 100 eligible women selected using simple-random sampling between these periods.

Data Collection Tools

The data were gathered with Data Collection Form for Descriptive Characteristics of Women who Received Infertility Treatment designed by the researchers using the information in literature, Female Sexual Function Index and Beck Depression Inventory.

Data Collection Form for Descriptive Characteristics of Women who Received Infertility Treatment

The form contained a total of 11 questions that addressed some socio-demographic characteristics (such as age, educational status, professional status, health insurance, total monthly income, marriage duration) and infertility histories (such as having children, previous infertility treatment, cause of infertility, infertility diagnosis and treatment method).

Female Sexual Function Index

Female Sexual Function Index was developed by Rosen et al. (2000) in order to measure sexual function of the women who are clinically diagnosed with sexual arousal dysfunction (17). Cronbach Alpha coefficients were separately analyzed for six domains and the results ranged from 0.89 and 0.97. Turkish adaptation of the index was performed by Aygın and Eti (2005) and it was reported that the index was reliably suitable for use among Turkish women (18). The study of the index for Turkish version gave test-retest reliability coefficient as 0.75 and Croanbach Alpha coefficients for domains were found to be ranging from 0.89 to 0.98 in the internal consistency analysis (18).

Female Sexual Function Index assesses sexual function problems or sexual problems that occur during the last four weeks. There are six domains: desire (frequency and level of the sexual desire or sexual interest), arousal (frequency, level, confidence and satisfaction), lubrication (frequency, difficulty and frequency of maintaining lubrication), orgasm (frequency, difficulty and satisfaction), satisfaction (with amount of closeness with partner, with sexual relationship, with overall sex life) and pain (frequency during vaginal penetration, frequency following vaginal penetration, level during or following vaginal penetration (Figure 1). Below are the numbers of the relevant questions, score intervals, factor loads, minimum and maximum scores obtained after multiplying mean scores of the domains by factor loads. The highest raw score that can be obtained from the scale is 95 whereas the lowest raw score is 4. The highest score of the scale is 36 whereas the lowest score is 2; which are obtained by multiplying mean domain scores by factor loads. Cut-off point is recommended as 26 and those who have scores $\leq$ 26 are regarded as having sexual dysfunction. In our research, too, cut-off point was 26.

Beck Depression Inventory (BDI)

BDI, developed by Beck (1960), is one of the most used inventories in clinics and researches and can be administered for individuals aged between 13 and 80 (19). The scale is consisted of 21 statements and is used to objectively measure

Domains	Questions	Score intervals	Factor loads	Minimum Scores	Maximum scores
Desire	1,2	1-5	0.6	1.2	6
Arousal	3,4,5,6	0-5	0.3	0	6
Lubrication	7,8,9,10	0-5	0.3	0	6
Orgasm	11,12,13	0-5	0.4	0	6
Satisfaction	14,15,16	0-5 or 1-5	0.4	0.8	6
Pain	17,18,19	0-5	0.4	0	6

Figure 1. Female Sexual Function Index

the degree of depression and physical, emotional, mental and motivational symptoms seen during depression. The Turkish adaptation of the inventory was performed by Hisli (1988) and its validity and reliability coefficients are rather good (20). It was found out that test-retest reliability coefficient was 0.65 and split-half reliability coefficient was 0.78 for students and 0.61 for depressive patients.

Scores for the statements of the inventory range from 0 to 3. All of the scores are added and depression score is obtained. The highest score of the inventory is 63 (21 x 3). A higher total score means a higher level or severity of depression. Scores obtained from the inventory can be evaluated as follows:

Score Evaluation
0-9 Normal
10-15 Slight depression
16-23 Moderate depression
24-63 Severe depression

Procedure

The necessary permissions from the Private Infertility Clinic where pre implementation and implementation phases of the research were conducted were obtained. All patients were informed of the purpose the study with written information, were told of the research objective, were informed that all of the personal information will be undisclosed and oral consents of those volunteers were obtained. Administration of the data collection tools was performed at a time when the participant women felt comfortable and when they were not busy. Therefore, administration of the tools was mostly performed before medical examination at policlinic waiting rooms. Administration of the tools lasted averagely for 45 and 60 minutes for each women.

The data obtained were analyzed with SPSS 17.0 statistical package program. The data were assessed with percentages, arithmetic means and standard deviation and ANOVA test in computer environment.

Results

Findings about Some of Women's Demographic Characteristics

Most of the participant women were aged between 26 and 33 (36.0 %), most of them had primary school graduation (40.0 %), most of them were housewives (68.0 %) and most of them were married for 5-12 years (69.0 %). Nearly all of the women had a health insurance (91.0 %) and health insurances of most of them met their treatment expenses (76.0 %). Almost half of the women (48.0 %) had a monthly income of 1500-2001 TL and three fifth of the women did not have any economical difficulties about the treatment (60.0 %) (Table 1).

Table 1. Distributions about some of women's demographic characteristics

Characteristics	N	%
Age		
18-25	34	34.0
26-33	36	36.0
34-41	30	30.0
Educational Status		
Primary School	40	40.0
Secondary School	26	26.0
High School and above	34	34.0
Professional Status		
Having a profession	32	32.0
Not having a profession	68	68.0
Health Insurance		
Yes	91	0.91
No	9	0.09
Monthly Total Income (TL)		
500-1000	28	28.0
1001-1501	24	24.0
1501- 2001	48	48.0
Financial Difficulties about the Treatment		
Yes	40	40.0
No	60	60.0
Marriage Duration (years)		
5-12	69	69.0
13-20	20	20.0
21 and above	11	11.0
Total	**100**	**100.0**

Nearly two third of the participant women did not have any children (64.0 %) and nearly one of every two women (52.0 %) received previously infertility treatment. Most of the women with children (72.2 %) had children with a treatment. Infertility reason of one of each couple was female infertility (50.0 %). When the diagnosis about the infertility was analyzed, most couples suffered from low sperm count / low sperm motility (29.0 %), polycystic ovary syndrome (24.0 %) and idiopathic infertility (19.0 %). Most commonly used treatment was ovary stimulation (52.0 %) (Table 2).

Table 2. Distribution of some characteristics related to women's infertility histories

Characteristics	N	%
Alive Children	N=100	
Yes	36	36.0
No	64	64.0
Previous Infertility Treatment	N=100	
Yes	52	52.0
No	48	48.0
Having children with infertility treatment	n = 36*	
Yes	26	72.2
No	10	28.8
Cause of Infertility	N=100	
Female Infertility	50	50.0
Male Infertility	26	26.0
Both	24	24.0
Infertility Diagnosis	N=100	
Premature Ovarian Failure	15	15.0
Retroverted Uterus	3	0.03
Submucous Myoma	10	10.0
Polycystic Ovary	24	24.0
low sperm count / low sperm motility	29	29.0
Idiopathic	19	19.0
Treatments Received	N=100	
Over Stimulation	52	52.0
Insemination	26	26.0
In vitro Fertilization	22	22.0

** Answered by those who had children.*

Findings about depression status of the women and their husbands

According to the mean scores of the participant women obtained from Beck Depression Inventory (cut-off point: 10); it was detected that most of the women experienced depression (72.0 %). When the depression degrees of them were assessed, it was noted that nearly half of the women suffered from severe depression (48.6 %), nearly one fourth of them suffered from slight depression (26.4 %) and other one fourth from moderate depression (25.0 %) (Table 3.). Although not shown in the Table, only 10.0 % of the participant men experienced depression and these were slight depressions.

Table 3. Distributions of depression status and degrees of the women

Depression	N	%
	N=100	
Yes	72	72.0
No	28	28.0
Depression Levels	n=72*	
Slight Depression	19	26.4
Moderate Depression	18	25.0
Severe Depression	35	48.6

**Only those women who had depression.*

Findings about depression women's sexual function characteristics

When sexual dysfunction of all the female cases was examined according to FSFI cut-off points (cut-off point: 26), 47.0% of women had sexual dysfunction. The most affected domain was arousal (20.5 %) and orgasm (19.5 %) and the least affected domain desire (12.3 %). According to FSFI cut-off points, the most affected domain of the women with sexual dysfunction (n=47) was orgasm (19.1 %) and desire (17.9 %) while the least affected domain was satisfaction (Table 4).

Findings about the affecting findings of women's sexual dysfunction

Findings about sexual dysfunction according to some socio-demographic characteristics

It was found out that women with a profession (33.3 %) had fewer sexual dysfunctions than women without a profession (48.4 %) and similarly, women with a health insurance (33.3 %) had fewer sexual dysfunctions than women without a health insurance (48.4 %). Also, two third of the women (66.0 %) who had financial difficulties during the infertility treatment had sexual dysfunction while nearly half of the women (54.7 %) who did not have financial difficulties during the infertility treatment had sexual dysfunction. There were statistically significant correlation between sexual dysfunction and professional status, and financial difficulties during the infertility treatment ($p<0.05$) whereas the correlation between sexual dysfunction and age, educational status, monthly total income and marriage duration was statistically insignificant ($p>0.05$) (Table 5).

Table 4. Distributions of women's sexual function characteristics

FSFI	Mean ± SD	Min-Max	%
	N=100		
Desire	3.7920±.14318	1.20-6.00	12.3
Arousal	3.9660±.14842	.00-6.00	20.5
Lubrication	4.3380±.14190	.00-6.00	18.8
Satisfaction	4.2240±.14532	.00-6.00	17.5
Pain	4.5760±.13325	.80-6.00	16.4
Orgasm	4.0120±.14298	.00-6.00	19.5
Total Sexual Dysfunction	**24.9080±.75540**	**2.00-35.70**	**47.0**
FSFI of the women with sexual dysfunction	n=47*		
Desire	2.6936±.16713	1.20-5.40	17.9
Arousal	2.7128±.17069	.00-5.70	16.8
Lubrication	3.3191±.20103	.00-5.70	17.7
Satisfaction	3.1489±.19678	.00-5.60	19.1
Pain	3.6340±.18201	.80-6.00	16.7
Orgasm	3.2085±.18788	.00-6.00	18.8
Total Sexual Dysfunction	**18.7170±.95626**	**2.00-26.00**	**100.0**

** Only those women who had sexual dysfunction.*

Table 5. Distribution of Women's Sexual Dysfunction according to Some Characteristics

Characteristics	Sexual Dysfunction (%)		(%)	Chi Square	P
	Yes	No			
Age					
18-25	50.0	50.0	100.0		
26-33	45.0	55.0	100.0	12.985	0.678
34-41	51.0	49.0	100.0		
Educational Status					
Primary School	42.0	58.0	100.0		
Secondary School	38.9	61.1	100.0	8.654	0.879
High School and above	55.0	45.0	100.0		
Professional Status					
Yes	33.3	66.7	100.0	**0.742**	**0.389***
No	48.4	51.6	100.0		
Health Insurance					
Yes	33.3	66.7	100.0	**0.742**	**0.389***
No	48.4	51.6	100.0		
Monthly Total Income (TL)					
500-1000	30.0	70.0	100.0		
1001-1501	35.0	65.0	100.0	7.435	0.678
1501- 2001	28.2	71.8	100.0		
Financial Difficulties					
Yes	66.0	34.0	100.0	**1.311**	**0.252***
No	54.7	45.3	100.0		
Marriage Duration					
5-12	35.0	65.0	100.0		
13-20	35.0	65.0	100.0	5.232	0.564
21 and above	38.2	71.8	100.0		
Having children					
Yes	29.8	70.2	100.0	**1.486**	**0.223***
No	41.5	58.5	100.0		
Previous Infertility Treatment					
Yes	57.4	42.6	100.0	**1.054**	**0.305***
No	52.8	47.2	100.0		

**P < 0.05*

Findings about sexual dysfunction according to some characteristics of infertility history

It was detected that there was a positive correlation between sexual dysfunction and not having children and previous infertility treatment. In other words, there were more sexual dysfunctions among the women who did not have children and had previously infertility treatment. According to these findings; 41.5 % of the women who did not have children suffered from sexual dysfunction whereas this rate reduced to 29.8 % among the women who had children. Similarly, women who received infertility treatment previously (57.4 %) had more sexual dysfunction compared to those who did not receive any infertility treatment (47.2 %). In the analysis, the correlation between women's sexual dysfunction and having a child and previous infertility treatment was found to statistically be significant ($p< 0.05$) (Table 5) whereas the correlation between women's sexual dysfunction and the cause of infertility was statistically insignificant ($p>0.05$) (Table 5.).

Findings about sexual dysfunction according to depression status of women

In the study, it was seen that there was a positive correlation between depression and sexual dysfunction. According to the findings, 8 of 10 women who suffered from depression (79.2 %) had sexual dysfunction while nearly half of the women without depression had sexual dysfunction (53.2 %). The analysis performed revealed that the correlation between sexual dysfunction and depression experience was statistically significant ($p< 0.05$) (Table 6.).

Discussion

It is reported in the studies investigating emotional dimension of the infertility that women suffer psychological problems more than men and clinical depression and anxiety are seen commonly among the women who receive infertility treatment (21,22). Similar to the literature, our study pointed out that most of the women had depression and nearly half of them had severe depression. As for men, only 10 % had depression and nearly all of them were slight depressive symptoms. According to the literature, because women are emotionally under more pressure they experience more psychological problems compared to men in female infertility alone or both female and male infertility together (13, 23-25). Therefore; it was concluded that the fact that causes of infertility of most of the couples were female infertility and other couples' causes were both female and male infertility affected women's depression degrees and levels negatively. The study of Wischmann et al. (2001) reported that depression and anxiety scores of the women who had higher educational status and idiopathic infertility diagnosis were higher (22); which concurred with our results. On the other hand; the study of Matsubayashi et al. (2001) suggested that emotional stress of the infertile women was higher than normal pregnant women (21).

During the infertility treatment; such reasons as timed sexual intercourse, fertilization aim of the sexual intercourse, certain positions adopted during the intercourse and refraining from other positions may lead to sexual dysfunctions. There are different research results about the effect of the infertility on sexual life and marital adjustment. Some studies report that infertility affects quality of life, marital adjustment and sexual functions negatively (5) whereas others report that infertility leads to couple intimacy and reinforcement of the relations (6). The study of Nelson et al. (2008) reports that depression and sexual dysfunction are seen more commonly among the infertile women compared to men (3). Similar to our findings; the study of Jindal et al. (1990) indicated that 52.0 % of the infertile women suffered from sexual dysfunction (26) and the study of Öksüz and Malhan (2006) demonstrated that 48.3 % of the infertile women suffered from sexual dysfunction (27).

Table 6. Distribution of women's sexual dysfunction according to depression status

Depression	Sexual Dysfunction (%)		%	Chi Square	P
	Yes	No			
Yes	79.2	20.8	100.0	**1.596**	**0. 123**
No	53.2	46.8	100.0		

**P < 0.05*

When the findings were analyzed, the most affected sexual domains of the participant women were arousal and orgasm while the least affected domain was desire. Accordingly, it may be concluded that infertile women are generally aroused late and experience orgasm problems. Similarly, the most commonly seen problem was orgasm among the women with sexual dysfunction. Additionally, another domain affected most was sexual desire. Them most commonly seen problem among the infertile women was arousal problem in the study of Khademi et al. (2008) (28) ; which supported our research. The study of Jindal et al. (1990) indicated that women with sexual dysfunction experienced lack of sexual arousal, decreased frequency of sexual relation and anorgasmia (26). Other studies that investigated the same subject report that sexual dysfunction problems of the infertile women are loss of sexual desire, inability of sexual arousal and anorgasmia.

When findings about sexual dysfunction according to some socio-demographic characteristics of the participant women were analyzed, it was seen that educational status did not affect sexual dysfunction but those who had a profession had fewer sexual dysfunction problems. Similar to our findings, the studies of Güleç et al. (2011) and Oksuz and Malhan (2006) indicated that educational status did not affect sexual dysfunction (10, 27). Unlike our study, the study of Tashbulutova (2007) showed that sexual desire, satisfaction and orgasm increased as educational status increased (15). Also, similar to our study, Tashbulutova's (2007) study reported that women who had a profession and worked were sexually more eager and satisfied (15). The study of Lauman et al. (1999) demonstrated that sexual dysfunctions were seen less as educational status increased (29). According to the literature, one of the most important risk factor of female sexual dysfunction is age. The studies of Oksuz and Malhan (2006) and Önem et al. (2004) pointed out that female sexual dysfunction increased with age (27,30). It was detected in our study that age factor did not affect sexual dysfunction.

It is reported in the relevant studies that sexual dysfunction are seen less among the infertile couples as the income level increases (10,15). Unlike these studies; our study suggested that income level did not affect sexual dysfunctions but women who did not have any health insurance and had financial difficulties about infertility treatment suffered sexual dysfunction more. In other words, in our study, income level did not affect sexual dysfunction while a positive correlation between sexual dysfunction and lack of health insurance and financial difficulties during the treatment occurred. Considering this finding; it may be concluded that if infertile women do not undergo financial difficulties during the treatments they are not affected negatively in terms of sexual dysfunction even if they have low income.

When the literature is analyzed, it is seen that another factor of the female sexual dysfunction is marriage duration. The relevant studies report that sexual functioning may be deteriorated as the duration of marriage increase (10,31). However, it was detected in our study that duration of marriage did not affect female sexual dysfunction. But, we are of the opinion that the fact that marriage duration of most participant couples was between five and twelve years yielded the above mentioned result.

In the present study, there was a positive correlation between female sexual dysfunction and not having a child and previous infertility treatments. Particularly, sex out of fertile period may become meaningless and void for women because sex during the infertility treatment aims at reproduction and couples must follow menstrual cycle and ovulation (32). Sexuality corresponds to reproduction for infertile women; their perception of incompetence may cause sexual dysfunctions. The result of our study that there was a positive correlation between sexual dysfunction and not having children and previous infertility treatment supported this conclusion above. Similarly; the study of Leiblium et al. (1998) reported that women who were unable to have children after infertility treatment had poorer marital adjustment compared to those who had children or to those who adopted a child (33).

Another affecting factor on female sexual dysfunction that was examined in the study was the cause of infertility. When the findings were analyzed, it was seen that there was not a significant correlation between female sexual dysfunction and cause of the infertility. Accordingly; it may be concluded that the cause of infertility did not affect female sexual dysfunction. Some of the relevant studies suggested that there was not any difference

between women and men in idiopathic fertility in terms of sexual satisfaction but it was reported that sexual satisfaction of female infertility groups alone or both female and male infertility groups together was poor (34). On the other hand, some researches point out that cause of infertility do not affect female sexual dysfunction (8).

According to the literature, psychological causes and stress are among the important risk factors of sexual dysfunction. Tensions and psychological problems experienced during the infertility treatment may result in sexual dysfunctions. During this period, emotional and sexual problems may emerge as the cause or the result of one another. Similar to the literature; eight in ten women who had depression suffered from sexual dysfunction in our research. In the analysis, it was noted that there was a positive correlation between depression and female sexual dysfunction. That is to say that as the depression rates increased in the participant women so did rates of female sexual dysfunction. In this regard; similarly, the studies of Karlıdere et al. (2007) and Ramezanzadeh et al. (2006) indicated that sexual dysfunction may develop during the infertility treatment due to depression, anxiety and other emotional problems (8,13).

As a conclusion; it was detected that seven in ten women who received infertility treatment suffered from depression while nearly one in two women who had infertility treatment underwent sexual dysfunction. According to the findings; there was a positive correlation between sexual dysfunction and having health insurance, being subject to financial problems during the infertility treatment, having a child, previous infertility treatment and depression experience. The following recommendation may be presented in relation with these findings:

- Psychological Counseling and family therapy units should be established in infertility clinics.
- Planned training / counseling services should be provided by the nurses and midwives who work at infertility clinics to the infertile couples in order to terminate diagnosed emotional and sexual problems.
- The future researches should deal with anxiety, anxiety disorders and other emotional problems in relation with male sexual dysfunction, too.
- The future researches should include bigger sample size and control group in order to gain more reliable results.

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Comparison of the impact of the two educational methods (lecture and Group Discussion) on knowledge, attitude and behavior intention, and practice of the urban pregnant women; normal vaginal delivery or caesarean?

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Abstract

Background: Increasing rate of caesarian section is the one of the most important problems of maternal and neonatal health. Many women choose to attend childbirth classes to gather information and lessen their anxiety. Present study was implemented to compare the impact of two educational methods (lecture and Group Discussion) on knowledge, attitude and behavior intention, and practice of the pregnant women who seeking prenatal care from urban health facilities of Tehran Shahid Beheshti University of medical sciences in 2011.

Methods: This quasi-experimental study was done on the 200 urban, primiparous pregnant women with the least gestational age of 20-16 weeks, who had been eligible for study. After placing of the women in two groups (1. Group Disscussion and 2. Lecture), randomly, they educated and instructed about different aspects of safe delivery and after delivery cares. Data were gathered by pretest and post test questionnaires. Practice related data were gathered two months after delivery. All statistical analysis were performed by using SPSS version 18.

Results: Our findings did not show significant difference between two groups for knowledge and attitude mean scores, before intervention (respectively, P=0.91, P=2.43). Knowledge mean scores of group1 generally was more than group 2, significantly (p= P=0.03). Although attitude mean scores in both groups increased significantly, after intervention (p=0.001), but there was no significant difference between two study groups for attitude mean scores (p=0.5). However behavior intention of vaginal delivery in the group1 was increased more than group2, significantly (P=0.017). Despite of the presence of no significant difference between two groups for practice mean scores of after delivery cares (P=0.63), our findings show normal vaginal delivery in group1 was more than group 2, significantly (0.037).

Conclusion: We observed that educational methods effectively improved the knowledge, attitude and behavior intention of vaginal delivery in participant mothers. But group discussion improved the knowledge, behavior intention and practice of vaginal delivery in participant mothers, more than lecture. Our findings indicates on need to use of group discussion method in order to induce behavior intention and practice of normal vaginal delivery in pregnant mothers moreover educational program.

Key words: Group discussion, lecture, safe delivery, postpartum care, knowledge, attitude, behavior intention, and practice.

Introduction

In 2007, the Safe Motherhood Initiative celebrated its 20th anniversary [1, 2]. Many countries, including Iran, have been able to improve the health and well-being of mothers and newborns over the last 20 years[3]. However, countries with the highest burdens of morbidity and mortality have made the least progress. Several papers focus on important technical areas, particularly the management of post-partum complications, and saving pregnant women and newborns' lives by providing evidence and recommendations for policy changes and program implementation. But, the challenges to be met are not new technologies

or new knowledge about effective interventions, because we mostly know what needs to done to save the lives of mothers and newborns. The real challenges are how to deliver services and scale up interventions. Effective health interventions exist for mothers and babies, and several proven means of distribution can be used to put these in place. However, none will work if policies be absent where it matters most: at national and district levels [4].

Nowadays, increasing rate of caesarean section is one of the current and most important problems of maternal and neonatal health. Caesarean section rates are high and continue to rise in developed countries. However, the impact of guidelines and recommendations in curbing their growth has been limited: in 1985, representatives of a study group convened by the World Health Organization wrote, "there is no justification for any region to have caesarean section rates higher than 10–15%." [5]. In 2007, nearly one-third (32%) of all births were cesarean deliveries, the highest rate ever recorded in the United States and higher than rates in most other industrialized countries [6]. Nevertheless, little research exists on determinants of caesarean section utilization, at either the aggregate [7]or the individual level, [8, 9] and, until recently, the few randomized trials that have been published have found no effect for the intervention studied, on rates of caesarean delivery [10].

Cesarean delivery is associated with higher rates of surgical complications and maternal re- hospitalization, as well as with complications requiring neonatal intensive care unit admission [6]. There is concern that apparently inexorably rising rates of caesarean delivery have the potential to divert human and financial resources from other [11]. Over the last two decades, physicians have often permitted a "patient-choice" C-section, which allows women to avoid labor [12]. Many women choose to attend childbirth preparation classes to gather information and lessen their anxiety. Most health professionals would agree that prenatal classes are informative and highly recommended for expectant mothers to achieving positive health outcomes [13].

Despite the importance of pre-, intra-, and post-pregnancy counseling, no compiled program had ever existed in this regard in the health care system of Iran until 2007. It was then that the issue was discussed as a part of necessary care services for women when designing and implementing the nationwide integrated maternal health care project. As a part of nationwide Integrated Maternal Health Care Project, all women seeking maternity care from health facilities are to be educated and instructed about different aspects of the maternal and neonatal health [14].

The study on impact of the nationwide IMHCP on the cognition and behavior of the women who were given the maternity health care by health facilities show observed that the nationwide IMHCP effectively improved the knowledge and practice of the participant mothers. Opposite findings were observed for attitude. Findings indicates on need to use of educational methods such as role playing, visual tapes and specially group discussion in order to induce positive attitude and behavior intention moreover educational program [14].

Present study was implemented to compare the impact of two educational methods (lecture and Group Discussion) on knowledge, behavior intention and practice of the pregnant women who seeking prenatal care from urban health facilities of Tehran Shahid Beheshti University of medical sciences, about safe delivery and after delivery cares.

Methods and materials

Study population

The current study was a quasi-experimental, field trial designed to compare the impact of lecture and group discussion educational methods on the knowledge, attitude and behavior intention, and practice of pregnant women on the safe delivery and after delivery cares. The study sample consisted of 200 women who referred to urban health facilities of Tehran Shahid Beheshti University of medical sciences at the 16-20 weeks of gestation. Apparently primiparous women were eligible for the current study if they were Iranian, permanent resident of the city and able to read and write (literate).

Interventions

At the first, two midwives were trained for educating of pregnant women. The registered pregnant women allied in two intervention groups), randomly; 1.Discussion Group (6-12 persons) and 2. Lecture Group (10-15 persons). Each group pa-

ssed only one educational class during the second part of pregnancy. The educational content included data on:

A. Safe delivery and the benefits of normal vaginal delivery.
B. after delivery cares includes postpartum care such as postpartum visits and warning signs, supplement use, use of contraception, breast feeding and newborn care such as neonatal visits and warning signs, care of umbilical cord.

Data were gathered by pretest and post test questionnaires. Practice related data were gathered two months after delivery.

Measurements

Data were secured using a predetermined questionnaire consisted of 4 main components included demographic characteristics, knowledge, attitude and behavior intention, practice. Participants were classified based their levels of education into 4 groups of primary, less than diploma, diploma, and academic education, and also based their job into 2 groups of householder and worker. House ownership of participants' family was classified into rental, owner, with family.

Outcomes

The knowledge of participants was quantified by using scores assigned to correct answers. The overall knowledge variable was then calculated as the fraction achieved of the maximum point-total that participants were expected to achieve. The practice was quantified by using scores assigned to correct healthy behaviors. The practice variable was then calculated as the fraction achieved of the maximum point-total that participants were expected to achieve. For attitude participants were asked to complete phrases on a 5-point response scale in which 0 represents the absence of the theoretical construct and 4 represents the theorized maximum amount of the construct being measured. Scores of each dimensions of attitude were then multiplied by 5/100. Our method can be visualized by imagining an elastic rule with five equidistant numerals is stretched evenly to fit alongside a longer ruler with 100 numerals or one with 100 numerals compressed to fit alongside a ruler with five [15]. As such, we were able to quantify attitude on similar scale as knowledge and practice. Furthermore, results from other studies on an n-point scale could be comparable if multiplied by n/100.

Statistics

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Informed written consent was obtained from all participants and the Ethical Committee of Research approved this study. Data are presented as mean (SD) and frequencies (%) for continuous and categorically distributed variables, respectively. We set the statistical significance level at a two-tailed type I error of 0.05. All statistical analyses were performed by using SPSS version 18.

Results

Mean age of the participants was 25.76(4.8) years in group 1 and 25.44(6.3) years in group 2, (p=0.68). The majority of participants had diploma and were householder in both study groups, and there was no significant difference between two study groups by gestational age, maternal job, educational level and house ownership (respectively, p=0.19, p=0.91, p=0.64, p=0.90). Baseline characteristics of the participants are presented in table 1.

Table 1. The distribution of demographic characteristics across two study groups

Characteristics	Group 1 (discussion)	Group 2 (lecture)
	N (%)	N (%)
Education		
literate	12 (12)	19 (17.6)
Less than diploma	28 (28)	24 (22.2)
Diploma	45(45)	45 (41.7)
Academic	15 (15)	20 (18.5)
Job		
householder	94(94)	103(95.37)
Worker	6(6)	5(4.63)
House ownership		
rental	66(66)	74(68.50)
owner	20(20)	19(17.60)
with family	14(48.3)	15(13.90)

T test did not show significant difference between two groups for knowledge and attitude

mean scores, before intervention (respectively, P=0.91, P=2.43). Table 2 compared knowledge mean scores of two groups, before and after intervention. Not only knowledge mean scores increased in both groups significantly (p=0.001), after intervention, but also group 1 generally scored above than group 2, significantly (p= P=0.03).

Table 3 compared attitude mean scores of two groups, before and after intervention. Although attitude mean scores in both groups increased significantly, after intervention (p=0.001), but there was no significant difference between two study groups for attitude mean scores (p=0.5).

As shown in Table 4, despite of presence of no significant difference between two groups before intervention (p=0.07), Not only behavior intention of vaginal delivery increased in both groups significantly (p=0.001), after intervention, but also behavior intention of vaginal delivery in the group1 was increased more than group 2, significantly (P=0.017).

The majority of participants in both groups reported vaginal delivery healthier than c-section as the cause of their behavior intention for vaginal delivery, before intervention (group1:29.7%, group2:18.3%). Their cause of behavior intention for vaginal delivery was earlier wellness and lesser complications and morbidities (group 1:26.8%, group 2:21.8%). After intervention, not also the behavior intention of participants for c-section because of the fear and anxiety, and pain decreased in both groups (respectively, group one: 10.8% to 6.1% and 9.5 to 2.4%, group two: 4.2 to 1.3 and 9.9 to 2.6) but also, their behavior intention for vaginal delivery because of the emotional relation between mother and newborn increased in both groups (group1: 4.1% to 14.6%, group 2: 2.8% to16.8%).

Table 2. Differences in the knowledge of two study groups

Title	Pre intervention			post intervention		
	Group 1	Group 2	P value	Group 1	Group 2	P value
	mean±SD	mean±SD		mean±SD	mean±SD	
Safe delivery	2.71 ± 1.27	2.67 ± 1.42	0.895	4.49 ± 1.09	4.79 ± 0.51	0.21
Postpartum care	4.96 ± 2.27	4.97 ± 2.27	0.946	9.15 ± 1.06	8.82 ± 1.3	0.04
Contraception use	0.51 ± 0.5	0.5 ± 0.5	0.886	0.93 ± 0.24	0.88 ± 0.32	0.17
Breast feeding	1.53 ± 0.52	1.51 ± 0.52	0.874	1.91 ± 0.27	1.93 ± 0.25	0.7
Neonatal care	2.89 ± 1.29	2.88 ± 1.21	0.995	4.75 ± 0.71	4.67 ± 0.69	0.3
Total	13.31 ± 4.6	13.38 ± 4.5	0.918	22.94 ± 3.5	22.11 ± 1.95	0.03

Table 3. Differences in the attitude of two study groups

Title	Pre intervention			post intervention		
	Group1	Group2	P value	Group1	Group2	P value
	mean±SD	mean±SD		mean±SD	mean±SD	
Safe delivery	2.49 ± 1.2	2.77 ± 0.99	0.53	3.35 ± 0.95	3.54 ± 0.74	0.1
Postpartum care	5.74 ± 1.4	5.62 ± 1.6	0.72	6.57 ± 1.35	6.76 ± 1.14	0.26
Contraception use	2.84 ± 0.93	2.94 ± 1.04	0.41	3.47 ± 0.82	3.44 ± 0.79	0.8
Breast feeding	11.17 ± 2.97	11.63 ± 2.83	0.22	13.39 ± 2.33	13.58 ± 2.39	0.5
Neonatal care	5.64 ± 1.66	5.66 ± 1.56	0.81	7.18 ± 1.02	7.01 ± 1.16	0.2
Total	28.75 ± 5.82	27.88±5	2.43	34.32 ± 5	33.96 ± 4.96	0.5

Table 4. Differences in the behavior intention of two study groups

Title	Pre intervention				post intervention			
	Group1		Group2		Group1		Group2	
	N	(%)	N	(%)	N	(%)	N	(%)
Normal vaginal delivery	72	80	64	68.8	90	92.8	87	83.6
Cesarean section	16	17.8	19	20.4	6	6.2	6	5.8
Don't know	2	2.2	10	10.8	1	1	11	10.6
Total	90	100	93	100	97	100	104	100

Table 5. Differences in the practice of two study groups

Title	Group1	Group2	P value
	mean±SD	mean±SD	
Postpartum care	10.28 ± 3.02	10.47 ± 3.65	0.7
Contraception use	1.98 ± 0.15	2.03 ± 0.32	0.1
Breast feeding	4.2 ± 0.59	4.26 ± 0.69	0.5
Neonatal care	6.24 ± 2.7	2.46 ± 2.9	0.6
Total	27.58±5.62	27.53±6.3	0.63

Table 6. Differences in the mode of delivery of two study groups

Title	Group1		Group2	
	N	(%)	N	(%)
Normal vaginal delivery	42	47.7	27	32.1
Cesarean section	46	53.3	57	67.9
Total	88	100	84	100

Despite of presence of no significant difference between two groups for practice mean scores of after delivery cares (P=0.63), our findings show normal vaginal delivery in group one was more than group 2, significantly (0.037), (see tables 5, 6).

Discussion

In this study we compared the effectiveness of two educational methods (lecture and Group Discussion) on knowledge, attitude and behavior intention, and practice of the urban pregnant women about safe delivery and after delivery care. We observed that both of them effectively improved the knowledge, attitude and behavior intention of vaginal delivery in participants.

Few studies have examined the effectiveness of the nationwide Integrated Maternal Health care programs. It has been shown that such programs can effectively increase the knowledge of mothers. Whether increase in knowledge can be translated to improvement in behaviors, however, remained to be elucidated. In 2007, we observed that the Integrated Maternal Health Care program effectively improved knowledge and some behaviors of mothers. It has been shown that our finding of interest was that the IMHCP failed to improve the attitude of the participant mothers [14].

From a practical perspective, some policy makers and program managers believe that they are already conducting Safe Motherhood programs because, as they understand it, the activities comprise their usual activities-antenatal care, family planning, nutrition, etc. Other policy makers feel that although Safe Motherhood is a laudable goal, attaining it would require dauntingly vast efforts.

Most health professionals would agree that prenatal classes are informative and highly recommended for expectant mothers to achieving positive health outcomes. They are often found beneficial because of the socialization with other expectant mothers, rather than the knowledge and skills transferred [13]. This may produce positive results in terms of childbirth, but does not necessarily empower them to make informed health choices such as safe delivery [16].

Our findings show despite of presence of no significant difference between two groups for attitude and practice mean scores of after delivery cares (P=0.63), normal vaginal delivery in group 1 was more than group 2, significantly (0.037).

Our findings indicated on promotion of knowledge, attitude and behavior intention of normal vaginal delivery after educational intervention. But presence of no significant difference between attitude and practice mean scores of two groups can be due to the low number of participants of lecture group (10-15 persons) or participation of mothers only in one educational class during second part of pregnancy. However group discussion increased knowledge, behavior intention and practice of normal vaginal delivery of the urban pregnant women, more than lecture.

The conventional model for growth in caesarean section rates implies that caesarean delivery is a conventional economic good, in the sense that

the higher one's income the more one is inclined to "purchase" it, called such a model "demand-driven". A demand-driven model is consistent with the hypothesis that it is primarily women's choices that determine caesarean section rates. A supply-driven model would imply that, regardless of medical need, the greater capacity of the health system to deliver surgical obstetric care, the more will be delivered. Such a model suggests that "suppliers" of caesarean delivery (e.g. obstetricians) have substantial influence on delivery mode, and contribute importantly to rising caesarean section rate [5].

Our findings about causes of c-section show despite of upper ratio of c-section in group1, c-section because of the obstetrician order in grop1 was little more than group2 (92% versus 90.7%) and because of the maternal demand was lesser (8% versus 9.3%).

It is not easy to resolve major public health problem that do not require technological breakthroughs. In this case, the challenge is to put our knowledge to work. We need to challenge our policy-makers and program managers to refocus program content and to shift focus from development of new technologies towards development of viable organizational strategies that ensure a continuum of care and account for every birth and death [17].

Limitations and strengths

The strength of the current study lies in comparison the impact of two educational methods as a simple and applied strategy to promotion of mothers and newborns health through induce knowledge and ability of making informed decisions in pregnant mothers. The findings the study, however, should be interpreted in the context of its limitations. Small lecture group, passing only one educational class instead of the eight classes during second part of pregnancy according to nationwide maternal health care program, therefore, could be influence on the presence of no significant difference between two study groups for attitude and practice mean scores of after delivery cares. This limitations, however, is unlikely to affect differences that we found to be significant. Since such limitations tend to bias the estimates towards null.

Conclusion

We observed that educational methods effectively improved the knowledge, attitude and behavior intention of vaginal delivery in participant mothers of both groups. But group discussion improved the knowledge, behavior intention and practice of vaginal delivery in participant mothers, more than lecture. Our findings indicates on need to use of group discussion method in order to induce behavior intention and practice of normal vaginal delivery in pregnant mothers moreover educational program.

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Effect of Pentoxifylline on the healing of ischemic and non- ischemic left colon anastomosis

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Abstract

Background: Leakage of colorectal anastomosis is a major complication that causes high morbidity and mortality. Ischemia around the anastomotic region is one of the important factors for the healing of the anastomosis. Pentoxifylline (PTF) improves microcirculatory blood supply, decreases platelet aggregation and adhesion, and increases thrombolysis.

Aim: The aim of the study was to assess the effects PTF on the healing of the normal and ischemic left colon anastomosis.

Materials and Methods: A total of 40 adult male wistar rats were used. Rats were divided into four equal groups. Group 1 (Control): resection and anastomosis, Group 2 (PTF): resection, anastomosis, and administration 25 mg 2x1 PTF intraperitoneally (ip) during 7 postoperative days (POD), Group 3 (Ischemia Control): ischemia, resection, and anastomosis, Group 4 (Ischemia PTF) ischemia, resection, anastomosis, and administration 25 mg 2x1 PTF ip during 7 POD. The rats were sacrificed on 7th POD and their colonic bursting pressures were measured. The anastomotic area was excised for hydroxyproline level (HPL) assay and histopathologic examination.

Results: PTF increased statistically significant bursting pressures, neovascularitation, fibroblast and collagen dancity whereas decreased inflammation. HPL increased statistically significant in both normal and ischemic colonic anastomosis.

Conclusion: Pentoxifylline improves healing of colonic anastomosis under both normal and ischemic conditions.

Key words: pentoxifylline, ischemia, colon anastomosis, healing, hydroxyproline

Introduction

Leakage of colorectal anastomosis is a major complication that causes high morbidity and mortality. It remains a major threat for gastro-intestinal surgeons. In elective surgery, clinically detected leakage is reported to occur in up to 3-4% of colonic anastomosis [1-4] and in 11–12% of rectal anastomosis [5, 6]. Overall, leakage of colorectal anastomosis rate is 6,4% [7]. Many factors have been advocated as significantly impairing anastomotic healing. However, most of these factors compromise anastomotic perfusion, causing ischemia or hypo perfusion, or even worse ischemia/reperfusion [8-12]. Ischemia around the anastomotic region is one of the important factors for the healing of the anastomosis. It was proved that blood supply and the oxygenation of the tissues is very important. A poor blood supply may be responsible for anastomotic leakage because collagen synthesis is oxygen dependent. Another factor; reperfusion of ischemic intestines may lead to more severe damage with superoxide radicals than the injury produced by ischemia itself [13]. Reintroduction of oxygen to the ischemic tissues leads to the production of oxygen radicals [14]. High concentrations of the hydroxyl radical significantly inhibit contraction of the collagen matrix, and also causing damage to the collagen matrix [15].

Pentoxifylline (PTF) is a methyl xanthine derivative with proven hemorheologic properties. PTF administration has been shown to produce hemorheologic effects like improving erythrocyte flexibility and decreasing blood viscosity [16, 17]. PTF improves microcirculatory blood supply, decreases platelet aggregation and adhesion, increases thrombolysis thus it is used in treatment of peripheral antipathies of arteriosclerotic, diabetic, or inflammatory origin with intermittent claudication, leg ulcers and other disturbances [18, 19]. PTF decreases platelet

activating factor (PAF), [20] and increases prostaglandin I_2 synthesis during ischemia-reperfusion injury (IRI), [21]. Also, PTF is a potent free-radical scavenger, diminishing neutrophil degranulation and reducing the release of super oxides [22].

The aim of the present study was to assess the effects of PTF on the healing of the normal and ischemic left colon anastomosis.

Materials and methods

This study conformed to the guidelines for the care and use of laboratory animals established by the Ethics Committee of the University of Cumhuriyet. A total of 40 adult male wistar rats weighting between 250–350 g were used in this study. Rats were divided into four equal groups. Group 1 (Control): resection and anastomosis, Group 2 (PTF): resection, anastomosis, and administration 25 mg 2x1 PTF intraperitoneally (ip) during 7 postoperative days (POD), Group 3 (Ischemia Control): ischemia, resection, and anastomosis, Group 4 (Ischemia PTF) ischemia, resection, anastomosis, and administration 25 mg 2x1 PTF ip during 7 POD.

Rats were anesthetized by an intramuscular injection of ketamine (Ketalar, Parke-Davis, Eczacıbası, Istanbul, Turkey; 40 mg/kg body weight) and xylazin (Rhompun, Abdi İbrahim, Istanbul; 5 mg/kg body weight). All animals were allowed to breathe spontaneously during the experiments. A midline laparotomy was carried out, and the intestines were covered with sterile gauze pads soaked with saline at 37°C to minimize evaporation from the tissue. Body temperature was maintained between 36° and 38°C by the use of a heating lamp. In addition, 5 ml Ringer's Lactate solution was given subcutaneously to prevent dehydration in the animals during the experimental period.

Ischemic preparation

The model of ischemic colitis has been described elsewhere [23]. Briefly, the left colonic ischemia was induced by a division of the marginal artery and the ligation of the arteries at the beginning of the descending colon to the pelvic colon, assuring that the remaining blood supply to the left side of the colon was intramural. Profound morphological changes have been demonstrated in previous studies [24].

Operative procedure

A 1-cm left colon resection 2–4 cm above the peritoneal reflection was performed following the ischemic preparation of the left colon. Bowel continuity was restored with an end to end anastomosis of nine or ten interrupted sutures (6/0 monofilament polypropylene, Ethicon, UK). The abdominal muscle layer and the skin incision were closed separately with running sutures. All operations were performed by the same surgeon.

Analytic Procedures

Measurement of Anastomotic Bursting Pressure (ABP)

Laparotomy was done to the rats. The strength of each anastomosis was assessed by measuring its bursting pressure using a fluid pump (Becton Dickinson, Brezins, France), operating at 5 ml/min with a pressure transducer (Abbott, Monitoring Kit, Transpac II, Abbott

Ireland, Sligo, Ireland). The pressure was recorded in millimeters of mercury on a monitor (Petas KMA 260R, Ankara, Turkey), via a catheter having been passed per rectum to 2 cm below the level of the anastomosis, ligating the colon around the catheter below, and also at a point 2 cm proximal to the anastomosis. Bursting pressure and place were therefore measured in situ without disturbing any adhesions that had formed around the anastomosis. The pressure was observed, and leakage was visualized with a magnification lens or identified by a sudden loss of pressure.

Measurement of hydroxyproline Levels (HPL)

Tissue was rinsed with distilled water, dried with absorbent paper. The specimens were then dried in openmouthed beakers at 100˚C for 72 h. Dried specimens were hydrolyzed in 6 M HCl at 110˚C for 18 h. The specimens were then washed three times with distilled water and dissolved in 2 ml of buffer containing acetic acid 1.2%, sodium acetate 12%, citric acid 5% and sodium hydroxide 3.4%, pH 6. Chloramine-T (0.5 ml per 1 ml of specimen) was added to the specimens, which were then incubated at room temperature for 20 min. A mixture of perchloric acid 15.6% and dimethylaminobenzaldehyde, dissolved in 0.5 ml of propanol, was then added. Following incubation

at 60˚C for 15 min, the absorbance was read using a spectrophotometer at 550 nm. A standard curve was plotted for HPL content (µg/mg tissue) of the specimens [25, 26].

Histopathologic Evaluation

After resection of the anastomose region, this tissue opened longitunally, half of the tissue inserted in %10 formaldehide solution. Then fixation was done in 24 hours and paraphine blocks were prepared. Lastly cross-section of these tissues to be stained with haematoxyline and eosin. Anastomosis were graded histologically in a blind fashion, using a 0–4 Ehrlich and Hunt numerical scale as modified by Philips et al. [27]. Inflammatory cell infiltration (white blood cell count), collagen deposition and fibroblasts and neovascularization were graded from 0 to 4 as follows: 0=no evidence, 1=occasional evidence, 2=light scattering, 3=abundant evidence and 4=confluent cells or fibres.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS version 15.0, Chicago, IL, USA). All values were expressed as mean ± standard deviation (SD). Comparison of variables between the groups was performed with Kruscal-Wallis test and Mann-Whitney U test. Significance between histopathological scorings was determined with the chi-square test and Fisher's exact test. A value of $p<0,05$ was considered as statistically significant.

Results

During the experimental period, no mortality was observed. Wound complications and intra-abdominal abscesses were observed in two rats in group 3 (Ischemia Control) due to loss of anastomotic integrity. There was no statistically difference between groups in terms of wound complications, abdominal abscess and anastomotic dehiscence (p =0,104).

Anastomotic Bursting Pressure

In all subjects, the bursting was observed at the anastomotic line. Average ABP were; 136,80±30,39 mmHg in Group 1 (Control), 245,13±27,28 mmHg in Group 2 (PTF) , 74,27±36,95 mmHg in Group 3 (Ischemia Control), and 133,40±27,17 mmHg in Group 4 (Ischemia PTF). ABP were significantly higher in Group 2 than Group 1 (p=0,043). Similarly, ABP were significantly higher in Group 4 than Group 3 (p=0,009). Moreover, there was no differences between group 1 and 4 (p=0,739) with regards to ABP which may demonstrate the effect of PTF administration in healing of anastomosis. The average of ABP (Mean±SD) are exhibited in Table 1.

Table 1. Anastomotic Bursting Pressures (mmHg)

Groups	Mean±SD
Group 1 (Control)	136,80±30,39[a]
Group 2 (PTF)	245,13±27,78
Group 3 (Ischemia Control)	74,27±36,95[b]
Group 4 (Ischemia PTF)	133,40±27,17[c]

[a] *Difference between group 1 and 2 is significant, p=0,043*
[b] *Difference between group 3 and 4 is significant, p=0,035*
[c] *Difference between group 1 and 4 is not significant, p=0,739*

Tissue HPL

Averages of HPL were; 14,86±3,18 µg/mg in Group 1, 28,23±5,77 µg/mg in Group 2 , 11,66±3,85 µg/mg in Group 3, and 18,74±4,45 µg/mg in Group 4. HPL were significantly higher in Group 2 than Group 1 (p=0,019). Similarly, HPL were significantly higher in Group 4 than Group 3 (p=0,004). Moreover, There was no differences between group 1 and 4 (p=0,631) with regards to HPL which may demonstrate the effect of PTF administration in healing of anastomosis. The averages of HPL (Mean±SD) are exhibited in Table 2.

Table 2. Hydroxiprolline (µg/mg) levels in groups

Groups	HPL (mean±SD)
Group 1 (Control)	14,86±3,18[a]
Group 2 (PTF)	28,23±5,77
Group 3 (Ischemia Control)	11,66±3,85[b]
Group 4 (Ischemia PTF)	18,74±4,45[c]

[a] *Difference between group 1 and 2 is significant, p=0,019*
[b] *Difference between group 3 and 4 is significant, p=0,004*
[c] *Difference between group 1 and 4 is not significant, p=0,631*

Histopathologic evaluation

Inflammatory cell infiltration scores were significantly lower in Group 2 than Group 1 (p=0,001). Similarly, Inflammatory cell infiltration scores significantly lower in Group 4 than Group 3 (p=0,001). PTF administration in group 2 (p=0,001) and group 4 (p=0,001) increased fibroblast and collagen dancity compared to group 1

Table 3. Histopathologic findings in groups (X±SD)

Groups	Inflammatory* cell infiltration	Fibroblast + collagen deposition§	Neovascularation#
Group 1 (Control)	3,50±0,53[a]	3,0±0,18[a]	2,10±0,03[a]
Group 2 (PTF)	2,50±0,53	4,70±0,10	3,40±0,09
Group 3 (Ischemia Control)	4,30±0,44[b]	1,60±0,14[b]	1,72±0,14
Group 4 (Ischemia PTF)	2,80±0,35[c]	3,30±0,08[c]	2,80±0,61[c]

[a] *Difference between group 1 and 2 is significant, *p=0,001, §p=0,001, #p=0,001*
[b] *Difference between group 3 and 4 is significant, *p=0,029, §p=0,001*
[c] *Difference between group 1 and 4 is not significant, *p=0,393, §p=0,190, #p=0,481*

and 3. Neovascularation formations were significantly higher in Group 2 than Group 1 (p=0,001). A similar trend was observed between Group 4 and Group 3, but this trend was not statistically significant. Additionally, there were no differences between group 1 and 4 with regards to inflammatory cell infiltration, collagen deposition, fibroblasts, and neovascularization. Histopathological findings are shown in Table 3.

Discussion

Leakage of anastomosis remains a challenging problem in worldwide. Reversible or irreversible ischemia is one of the most important factors for the anastomotic healing (9). Insufficient blood supply to the large bowel is the crucial local factor that almost always effects the anastomotic healing, resulting in anastomotic dehiscence [28].

Effects of different dosages of PTF in different models and parts of gut anastomosis were the scope of many previous studies [11, 29-32]. Additionally, anastomotic healing were evaluated in different POD in those studies [11, 29-32]. Ender et al. [29] reported effects of PTF on the healing of experimental anastomosis of the left colon in rats. They divided rats in three groups as; group 1 (PTF 2,5 mg/kg ip), group 2 (PTF 20 mg/kg ip) and group 3 (control group).They observed significantly increased ABP in postoperative second and fifth days in group 1 compared to control group. Also, in the postoperative first and second days significantly increased ABP were detected in group 2 compared to control group. Additionally, in the first postoperative day they were found higher colonic blood flow in group 1 than control group. They also detected lower peritoneal reaction index in treatment groups than control group. They concluded while PTF treatment shortens the time needed for the healing, also may prevent failure of colonic anastomosis. Comert et al. [30] evaluated the effect of PTF (50mg/kg ip) on the healing of small bowel anastomosis in rats with experimental obstructive jaundice. Ileal anastomosis without resection was performed in their study. The qualities of anastomotic healing were measured on the fifth and tenth POD. They found that administration PTF to the jaundiced rats resulted in better anastomotic wound healing via increasing ABP and HPL. The results of those trials [29, 30] are compatible with our findings in normal anastomosis groups.

Tireli et al. [11] evaluated the effect of PTF (50mg/kg ip) on intestinal anastomotic healing after IRI. They found that administration PTF increased in ABP and HPL significantly. Aziz et al. [31] investigated that effect of PTF (50mg/kg ip) and vinpocetine on healing of ischemic colon anastomosis. They reveal that PTF treatment showed benefical effect in both ABP and HPL. However, statistically difference was detected only in HPL. Also, they assessed their results histopathologically, however only fibrosis grading was detected as statistically significant. Parra-Membrives et al. [32] evaluated PTF (50mg/kg ip) on the healing of ischemic colorectal anastomosis. They found a significantly favorable effect of PTF in treatment group on anastomotic healing confirmed by measurement of ABP and tension compared to control group. In addition, significant alteration was observed in histopathological analysis. In the present study, we specified that PTF administration may have beneficial effects in colonic anastomosis healing under both ischemic and non-ischemic conditions. We presented fairly by our findings that the treatment with PTF increased ABP, tissue HPL, neovascularation, fibroblast activity, collagen deposition and

decreased inflammatory cell infiltration may reflect this beneficial effects. The trials mentioned above [11, 31, 32] confirmed our findings. In conclusion, pentoxifylline improves both non-ischemic and ischemic colonic anastomosis healing.

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Predictors of perioperative cardiovascular deaths in non-cardiac surgery: Case study

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Abstract

Background: Perioperative complications are important cause of death in adult patients undergoing elective non-cardiac surgeries. We aimed in this prospective observational study to identify cardiovascular preoperative predictors for deaths after non-cardiac surgery.

Methods: We studied 215 patients older than 55 who underwent elective noncardiac procedures under general anaesthesia in a University hospital after prior cardiological examination. We used Lee-index to predict cardiovascular death and all-cause mortality. The primary endpoint was intrahospital all-cause and cardiovascular mortality while the total hospital stay was the secondary endpoint.

Results: In this study 7 (3.3%) patients died. Mortality was higher in patients who belonged to class II and III of NYHA classification [(5 vs. 2); p =0.021] and Lee Class III and IV (5/41 vs. 2/167; p = 0.006), were in poor functional state [5 (11.6%) vs. 2 (1,2%); p = 0.004], had positive anamnesis for coronary disease [4 (28.6 %) vs. 3 (1.5%); p <0.0005], stroke [2 (22.2%) vs. 5 (2.4%); p = 0.029)] and took nitrates [5(9.4%) vs. 2 (1.2%); p = 0,011] as part of regular therapy. Binary logistic regression shows that the outcome (living-dead) depends on: previous myocardial infarction (p = 0.001; Odds ratio = 594 (14 - 24740)) , previous stroke (p = 0.027; Odds ratio = 46 (1.5–1386.0)) and therapy with beta blockers (p=0.019; Odds ratio=0.007 (0.000–0.440)).

Conclusion: Positive medical history for ischemic heart disease, especially myocardial infarction, brain infarction, membership to NYHA classes II and III, and Lee classes III and IV, as well as presence of nitrates in therapy increase, whereas application of beta blockers reduces the risk of fatal outcome.

Key words: Risk evaluation, non-cardiac surgery, pharmacologic therapy, mortality.

Introduction

The number of surgeries is on the increase, especially in the developing countries[1]. Perioperative cardiovascular complications are still a significant cause of death during the perioperative period of elective non-cardiac surgeries[2]. The essence of electivity is the choice of the patient to be operated on, of the optimal surgical procedure and of the safest type of anaesthesia. Elective surgeries provide sufficient amount of time for perioperative evaluation. On account of the foregoing, perioperative cardiovascular complications are infrequent, averaging several percent[2]. Data from the relevant literature shows that the incidence of postoperative complications in the undeveloped and underdeveloped countries differs from that in the industrially developed countries[1, 2, 3]. The existing Recommendations on preoperative risk assessment and perioperative procedures for patients undergoing elective non-cardiac surgery had a strong influence on diagnostic and therapeutic procedures, primarily in terms of involvement of internists and cardiologists. Previously, the responsible doctors comprised only the surgeon and the anesthesiologist. Today, they are joined by a cardiologist. Frequent revisions of the Recommendations indicate that the attitudes towards 'optimal' perioperative

diagnostic and therapeutic procedures leading to reduction of perioperative cardiovascular complications are still evolving[2]. Each country has its own distinctions, especially developing countries and those in transition, so it is necessary that each should gain its own experience in dealing with this problem[4]. Sometimes, despite the best intentions, it is objectively not possible to implement the recommendations written, as a rule, by experts from developed countries.

The aim of our study was to examine the causes of perioperative mortality in elective non-cardiac surgery, determine the mortality caused by major adverse cardiovascular events (MACE), identify potential specificities related to our clime and analyze their influence on intrahospital death rate of operated patients.

Methods

Patients

The study was conducted as a prospective observational clinical study. The inclusion criteria for the study were: elective non-cardiac surgery under general anaesthesia, 55 plus years of age and at least one of the following cardiovascular risk factors: diabetes mellitus (DM), hypertension (AHT), hyperlipidemia (HLP), smoking habit and family history of coronary artery disease (CAD). Exclusion criteria were: emergent surgery and the disability to understand or to sign the Informed consent form. The study has been approved by the local Ethical board of the participating centre.

The design of the study

After the operative treatment was indicated, the patient was referred to an anesthesiologist who decided on patient's eligibility for the surgery, determined the type of anaesthesia and indicated consultative examinations, including cardiological. The cardiological examination consisted of anamnesis, physical examination and obligatory electrocardiogram (ECG) and radiogram of the heart and lungs.

Congestive heart failure (CHF) means at least two of the following medically documented signs of disease: swollen lower leg, radiographic lung congestion or cardiomegaly, and pleural effusion. As heart failure and other cardiac diseases manifested through a whole range of symptoms and signs of disease during the period of observation, we applied the New York Heart Association (NYHA) functional classification of cardiovascular patients to grade the severity of dispnea as a manifestation of cardiac disease[5].

The positive anamnesis for CAD meant positive coronarography, previous myocardiac infarction (MI) and any form of heart revascularization. To assess the intensity of anginal condition we used the classification of the Canadian Cardiovascular Society (CCS)[6].

By functionally poor capacity we meant Metabolic Equivalents (MET) < 4, determined by the questionnaire for self assessment of functional capacity - Duke Activity Status Index (DASI)[7]. Cerebrovascular morbidity meant stroke and transitory ischemic attacks.

The cardiologist indicated other consultative examinations and diagnostic procedures (echocardiography, ergometry, spirometry, coronarography) and modified the pharmacological therapy if necessary.

The prescribed therapy was maintained, with dosage titration, throughout the perioperative period. The exceptions were made in the case of patients with peroral anticoagulant therapy (OAC) which was cancelled five days prior to surgery and substituted by low-molecular-weight-heparin (LMWH), as well as in those who took antithrombocytic therapy involving acetyl salicylic acid and clopidogrel, where clopidogrel was cancelled five days prior to surgery. Preoperative prophylactic therapy for prevention of venial thromboembolism by LMWH was not prescribed only if patients had absolute contraindications for it, including scheduled neurosurgical operation. The period between this examination and the surgery did not exceed 28 days.

The opinion of the anesthesiologist was decisive when it came to operation, so that 24 -78 hours before the surgery, the anesthesiologist gave his definitive assent to the surgery under general endotracheal anaesthesia. The blood samples were taken from antecubital vein for hematological and biochemical control tests. On the day of the surgery, the cardiologist updated the study protocol and obtained data from the anesthesiologist and the surgeon regarding intraoperative events that might result in perioperative complications. The patients were observed on daily basis, and complications,

together with all the relevant events regarding treatment and diagnostics, were noted until the patient was either discharged from the hospital or dead.

Clinical endpoints of the study

We predefined all-cause mortality during hospitalisation and the combination of mortality and MACE: acute MI, cardiac arrest or ventricular fibrillation, cardio-pulmonary resuscitation (CPR) and acute decompensated CHF, or stroke as primary endpoints.

Total hospital stay was predefined as secondary endpoint. The cause of death was determined jointly by the surgeon, anesthesiologist and the cardiologist. The diagnosis of perioperative or postoperative MI was based on universal definition of MI[8].

Surgical procedures

In our study, elective non-cardiac surgeries were classified according to the currently valid recommendations into: low risk (< 1 %), medium risk (1-5 %) and high risk (> 5 %) operations for MACE[9]. They were all conducted under general endotracheal anaesthesia in accordance with clinical standards of University hospitals. A list of the different kinds of performed surgical procedures is presented in Table 1.

Revised cardiac index (RCRI) – Lee-index

Lee index was calculated prior to surgery on the basis of positive anamnesis for: MI, CVI, insulin dependent DM, as well as on the basis of signs of CHF, creatinemia higher than 177 μmol/L and operation risk[10]. The presence of each of these factors was scored by 1 point, so the Lee index was the total score ranging from 0 to 6. Thus obtained Lee index was connected to potential perioperative cardiological risk, and presented as membership to one of the four classes. According to Lee et al. high risk operations are: intraperitoneal, intrathoracal and suprainguinal vascular [10].

Statistical methods

To compare mean values of parameters between population, the t-test for independent samples and Mann-Whitney test were applied. Dependence of categorical parameters was examined by Chi-square and Fisher's test. Influence of parameters on fatal outcome was examined through univariant and multivariant binary logistic regression. For odds ratio, the trust interval of 95 % was given. For all statistical analyses the statistical software SPSS 17.0 (Statistical Package for the Social Sciences, Chicago, Illinois) for Windows was used.

Results

In the period from April 2007 to April 2008, 230 patients were recruited to participate in the study. Only 15 (6.5 %) patients were eliminated from the study: 8 (3.5 %) were operated on under spinal anaesthesia, 1 (0.4 %) died before the operation due to severe CHF, 5 (2.2 %) were referred to cardiac surgery (aortic valve or revascularization), while 1 (0.4 %) patient had a laparoscopic surgery. 215 patients proceeded to participate. In this study 7 (3.3 %) patients died.

Table 1. Surgical procedures and mortality

Surgical risk estimate	all Patients 215 (100,0)	survivors 208 (96,7)	decreased 7 (3,3)	Surgical risk observed
Low-risk <1%	54(25,1)	54(26,0)	0	0%
Breast	28(13,0)	28(13,5)	0	
Thyroidectomy	26(12,1)	26(12,5)	0	
Intermediate-risk 1-5%	154(71,6)	149(71,6)	5(3,2)	3,2%
Abdominal	112(52,1)	110(52,9)	2(1,8)	
Vascular	7 (3,2)	0(0,0)	0(0,0)	
Neurologic	13(6,0)	11(5,3)	2(23,1)	
Pulmonary	9(4,2)	9(4,3)	0(0,0)	
High-risk >5%	20(9,3)	5(2,4)	2(28,6)	10,0%
Vascular	20(9,3)	5(2,4)	2(28,6)	

Demographic characteristics of the patients are presented in Table 2**.** Both genders were equally present. The patients who survived were younger then patients who died (66.3 ± 6.67 vs. 63.42 ± 7.0 years), but there was no statistically significant difference in terms of age of those who survived and those who did not. Majority of the operated patients (47 %) was in their seventh decade, while 2 % of the operated patients were over 81 years of age. The percentage of fatal outcomes was the largest in the sixth decade (4.6 %) declining with older age (2.98 % in the seventh, 2.22 % in the eighth and 0 % in the ninth decade) as showed in Table 3.

Table 3. Ages and mortality

decade	All patients n(%) 215 (100,0)	decreased n(%) 7 (3,3)
6th decade	65 (30,0)	3 (1,4)
7th decade	101 (47,0)	3 (1,4)
8th decade	45 (21,0)	1 (0,4)
9th decade	4 (1,9)	0 (0,0)

Our patients most frequently suffered from AHT. Majority of patients (71 %) had two or more risk factors for CVD. Fatal outcome and conventional risk factors for CVD were independent. CAD, especially MI and membership to a group (living/dead)

Table 2. Baseline characteristics

	all Patients 215(100%)	survivors 208(96,7%)	decreased (3,3%)	p – value survivors vs. decreased
Gender (female) n (%)	109(50,7)	107 (51,4)	2 (28,6)	0,275
Age (years) AM ± SD	66,1±6,1	66,3±6,8	63,4±7,0	0,265
BMI (kg/m^2)	27,2±4,5	27,1±4,5	29,1±5,7	0,228
Obese BMI≥30 (kg/m^2)	50(23,3)	48(23,1)	2(28,6)	0,669
Hypertension n (%)	179(83,3)	175(84,1)	4(57,1)	0,094
Hyperlipideamia n (%)	76 (35,5)	75 (36,8)	1(14,3)	0,426
DM n (%) Insulindepedent n (%)	52(24,2) 6 (2,8)	50(24,0) 5 (2,4)	2 (28,6) 1 (14,3)	0,678 0,426
Active smoker n (%)	61 (28,4)	59 (28,4)	2 (28,6)	1,000
Familiar diposition n(%)	79(36,7)	78(37,5)	1(14,3)	0,427
CAD n (%)	14 (6,5)	10 (4,8)	4 (57,1)	**<0,0005**
Prior MI n (%)	12 (5,6)	8(3,8)	4(57,1)	**<0,0005**
Sings of HF	11(5,1)	11(5,3)	0(0.0)	1,000
CVI n (%)	9 (4,2)	7 (3,4)	2 (28,6)	**0,029**
AF n (%)	13(5,7)	11(5,4)	2(28,6)	0,060
Asthma n (%)	19(8,8)	18(8,7)	1(14,3)	0,482
Cancer n (%)	73(34,0)	69(32,7)	4(57,1)	0,231
NYHA I n (%) NYHA II-II n (%)	154(71,6) 61(27,8)	152(73,0) 56(27,0)	2(28,6) 5(71,4)	**0,021**
CCS II-III n (%)	54 (25,1)	50 (24,0)	4(57,4)	
DAS (MET<4) n(%)	43(20,0)	38(18,3)	5(62,5)	**0,004**
Systolic BP	147,5±22,9	140,0±23,0	136,4±13,7	0,187
Diastolic BP	87,9±13,0	88,0±13,0	82,9±9,5	0,298
Heart rate	72,0±13,1	72,0±13,1	75,9±11,6	0,440
Hb(g/L) Median (25th - 75th)	132,0 (122,0-141,0)	132,0 (122,0-141,0)	128,0 (119,0-149,0)	0,920
WBC (n·10^9/L) Median (25$^{th.}$-75$^{th.}$)	6,7 (5,5-8,1)	6,7 (5,5-8,1)	7,3 (6,7 -9,8)	0,199
Platelets (n ·10^9/L)	245,0 (207,0-300,5)	245,0 (207,0-294,5)	270,0 (193,0-356,0)	0,489
Kreatinin u (μmol/L)) Median(25th -75th)	79,6 (71,6 -97,2)	79,6 (70,8 –97,3)	70,8 (61,9-150,4)	0,898
ASA/Clopidogrel n (%)	68(31,6)	64(30,8)	4(57,1)	0,211
Beta blocker n (%)	90 (40,7)	88 (43,6)	2 (28,6)	0,702
ACEI/AT blocker	131(60,9)	127(61,1)	4(57,4)	1,000
Diuretics n (%)	43 (20,0)	41 (18,6)	2 (28,6)	0,629
Nitrates n (%)	53 (24,7)	48 (23,0)	5 (71,4)	**0,011**

were statistically dependent (χ^2 - test, p<0,0005). Only 5 % of patients had signs of CHF (NYHA IV) and there were no fatal outcomes in this group, while 28 % belonged to NYHA classes II or III preoperatively. Membership to NYHA classes II or III and fatal outcome were dependent (p = 0.021). The percentage of those who died in NYHA class I was 1.3 %, while in classes II and III it rose to 8.2 %. Poor functional state and mortality were dependent (p = 0.004). Perioperative fatal outcome and the presence of: malign disease, chronic obstructive pulmonary disease, peripheral vascular disease, renal failure and rheumatic diseases were statistically independent. Previous stroke and fatal outcome were statistically dependent (χ^2 - test, p = 0.029).

In accordance with current recommendations, there were 25.1 % low risk surgeries, 71.6 % medium risk surgeries and 9.3 % of high risk surgeries. The Lee index of 0 was present in 25.1 %, Lee index 1 in 53.5 %, Lee index 2 in 17.2 %, while Lee index 3 or more was found in 4.2 % patients. Mortality in low risk group was 0 %, in medium risk group 3.2 %, and in high risk group 10.0 % (Table 1). Total mortality per Lee's classes was: 1.8 % for class I, 0.9 % for class II, 10.8 % for class III and 11.1 % for class IV.

The Lee index (0,1 or 2,3) and fatal outcome were dependent (p = 0.006). In group with Lee index 0 or 1, 2 (1.2 %) patients died, in group with Lee index 2, 3 or higher 5 (10.9 %) patients died (Fisher`s test , p=0.006). Patients were hospitalized for a median period of 11 (IQR 7-17) days. Patients who survived had median of hospitalisation 12 (8 – 23), while deceased patients had 26 (30-47) days. Total hospital stay progressively increased with Lee-index: for Lee index 0 it was 8.0 (5.0 -9.5), for Lee index 1 it was 15.0 (8.0 – 27.0), for Lee Index 2 it 17.0 (7.5 – 26.5) and for Lee index 3 and higher 20.0 (17.5 – 37.0) days (p<0.0005; χ^2 - test) Figure 1.

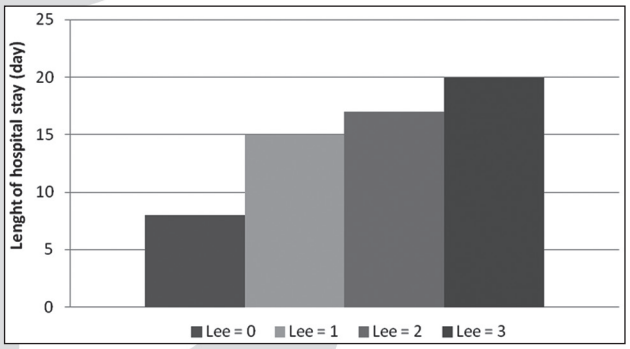

Figure 1. Length of hospital stay and Lee Index

Table 4. Lee Index, Lee risk class and mortality

Lee Index	Lee – class	Number	Death Number	Death observed %	Estimate surgical risk %
Lee Index 0	Lee–class I	54	1	1,8 %	0.4 %
Lee Index 1	Lee–class II	115	1	0,9 %	0.9 %
Lee Index 2	Lee–class III	37	4	10,8 %	6.6 %
Lee Index ≥ 3	Lee–class IV	9	1	11,1 %	11.0 %

Table 5. Lee-Index and mortality

Parameters	All patients number (%)		survivors number (%)		decreased number (%)		p*value survivors vs. decreased
Number (%)	215 (100,0)		208 (96,7)		7 (3,3%)		
Lee - Index 0 or Class I	54 (21,5)	169 (78,6)	53 (25,5)	167 (80,3)	1 (14,3)	2 (1,2) † (28,6)‡	<0,0005
Lee - Index 1 or Class II	115 (53,5)		114 (54,8)		1 (14,3)		
Lee - Index 2 or Class III	37 (17,2)	46 (21,4)	33 (15,9)	41 (3,4)	4 (57,1)	5 (10,9) (71,4)	
Lee - Index ≥ 3 or Class IV	9(4,2)		8(3,8)		1 (14,3)		

Table 6. Lee Index and length of hospital stay

Lee Index or class	LOS days, Median (25th -75th)	P - value
Lee Index 0 or class I	8,0 (5,0 – 9,5)	<0,0005
Lee Index 1 or class II	15,0 (8,0 – 27,0)	
Lee Index 2 or class III	17,0 (7,5 – 26,5)	
Lee index ≥3 or Class IV	20,0 (17,5 – 37,4)	

Length of hospital stay (days) (Median (25th -75th)

Antithrombocyte therapy, inhibitors of angiotensin converting enzyme (ACEI) or angiotensin 2 receptors blockers (AT2B), diuretics, statin therapy were not statistically different for groups of those who survived and those who did not, but the therapy with nitrates and fatal outcome were dependent (p = 0.011). Binary logistic regression shows that the outcome (living-dead) depends on: previous MI (p = 0.001; Odds ratio = 594 (14 - 24740)), previous stroke (p=0.027; Odds ratio = 46 (1.5–1386.0)) and therapy with beta blockers (p =0.019; Odds ratio = 0.007 (0.000–0.440)).

Analysis of fatal outcomes

In Lee class I, a 76-year-old female died after craniotomy and meningioma extirpation in the cerebellopontine angle. Her medical history was significant for long standing AHT and asthma. During the operation she had a cardiac arrest, which she survived after prolonged CPR. We suppose that cardiac arrest was the consequence of trigeminocardiac reflex which developed asystole. She died of CHF.

In Lee class II, a 67-year-old male died after total colectomy and ileorectal anastomosis L-T. He died of HF caused by sepsis. According to current classification, both patients belonged to medium risk operation group.

In Lee class III there were four fatal outcomes. The first patient, a 63-year-old male who underwent left hemicolectomy with rectal amputation (Miles' method) died of CHF. He had prior MI, AHT and DM. The second patient was a 65-year-old male, who underwent retrograde cholecystectomy and choledochectomy with T-draining of choledochus. He had AHT, atrial fibrillation and obesity in medical history, and died of stroke. According to current classification, both patients underwent medium risk surgeries. The third patient was a 59-year-old male who was obese, had prior MI and HLP, and died of HF. The fourth patient was a 56-year-old male who had prior MI, AHT and arterial fibrillation in medical history. He died of MI. Both patients underwent aorta-bifemoral supraingvinal bypass, a high risk procedure according to current classification. In Lee class IV a 59-year-old female died after craniotomy which was performed in order to reduce intercerebral tumor. She had stroke, MI and DM. The cause of death was a stent thrombosis and resultant MI with development of CHF.

Discussion

The aim of our study was to identify preoperative predictors associated with postoperative fatal outcome after elective non-cardiac surgery on patients older than 55 who had at least one of the conventional risk factor for cardiovascular morbidity. The surgeries being elective, all patients were cardiologically evaluated preoperatively. In spite of that, perioperative mortality in our study was 3.3 %, the rate similar to what other authors have reported[11, 12, 13, 14]. We have confirmed that major adverse cardiovascular events are, rare as they may be, still the most frequent cause of fatal outcome after non-cardiac surgery[11]. The death caused primarily by cardiovascular complications occurred in 5 (2.3%) patients, while in 2 (1.0%) patients cardiovascular morbidity was secondary cause of death. Two patients died of MI, one of stroke, and four of CHF. For stratification of poor perioperative outcome we used Lee index. We found a strong and stepwise association of adverse cardiac events to the revised cardiac index. That every operation is connected to risk for fatal outcome was confirmed by the fact that two patients belonging to low-risk Lee classes died. A female belonging to Lee class 0 undergoing a neurosurgical procedure died with signs of progressive HF after prolonged CPR following intraoperative cardiac arrest. We suppose that intraoperative cardiac arrest

was caused by excessive parasympatic irritation due to trigeminocardiac reflex, a rare complication that mostly occurs in cerebello-pontine angle meningeoma extirpation. Mortality in these procedures was almost the same as in high risk vascular procedures. Similar findings with larger cohort were reported by Boersma et al.[11]. It is interesting to note that Lee stratification model, being developed on patients who did not undergo neurosurgical operations, does not recognize neurosurgical procedures as high risk. In the current Guidelines, intracranial procedures receive the deserved, if somewhat underestimated, status of medium risk procedures when it comes to occurrence of fatal postoperative complications, which our study has confirmed[2, 9]. We have established that fatal outcome is dependent on CAD, and that patients who had a history of MI are primarily at risk of fatal outcome, which is in accordance with observations of other authors [10,11,15,16]. Since all the patients were cardiologically evaluated, such finding could suggest that preoperative cardiological evaluation of our patients was insufficient when it came to detecting patients at high risk of fatal outcome. This, however, is not true as only two patients died of postoperative MI occurring in specific circumstances. The patient undergoing neurosurgical procedure involving reduction of intracranial meningioma experienced a postoperative MI due to stent thrombosis 13 months after the implantation, when her acetyl salicyl acid therapy was discontinued. She belonged to the highest Lee class, and discontinuation of antiaggregation therapy was absolutely counter indicated on account of neurosurgical intervention operation with vital indication. The other patient who died undergoing a high risk vascular procedure (aortofemoral supraingvinal bifemoral bypass) belonged to Lee class III. He experienced gastrointestinal bleeding with moderate anemia that caused heart ischemia. In both cases, the impossibility to use adequate therapy for MI contributed to fatal outcome.

In this study not a single patient with the worst manifestation of CHF (at least 2 preoperative signs of CHF - NYHA IV) died, although Goldman showed as early as in 1977 that CHF is the most important risk factor for fatal outcome, which was later confirmed by other authors[10, 11, 15-17]. Only 5 % of our patients belonged to this group. These patients belonged to Lee class III, which meant that, apart from the risk from surgical intervention, they only had the risk caused by heart failure. To be honest, they were treated longer than other patients (median 31.50 (22.50 – 42.75) vs. 13.50 (22.50 – 42.75)). That patients with clinically stable HF did not have high perioperative mortality rates in association with elective major noncardiac surgery, but were more likely than patients without HF to have longer hospital stays, was shown by Xu-Cai Yo et al.[18]. It is obvious that awareness of the fact that it was a high-risk patient influenced the doctors to take more energetic measures to prevent fatal outcome, ranging from a detailed preoperative preparation aimed at achieving optimal stabile state and the choice of the procedure to enhanced medical control. The benefit of such an approach was obvious. However four of our patients died from HF. Two of them had shown no preoperative signs, including dyspnea. The other two patients, who manifested HF postoperatively, had previous MI in their medical history, belonged to NYHA class II or III, and had poor functional capacity estimated by DISI. Membership to NYHA class II and III and fatal outcome are connected in our study, as well as functional capacity (DASI), presented in our study as a dichotomous characteristic: good or bad. Patients who belonged to class II and III or poor general functional capacity often had previous history of MI, ATH, DM, COPD and obesity. It is well known that poor functional capacity is connected to numerous comorbidities which taken together may lead to fatal outcome, the fact confirmed in our study[19].

In our study, postoperative mortality was frequent in patients who had stroke in comparison to surviving patients without stroke. This is confirmed by findings of other authors, who suggested that there was association between CAD, especially MI and stroke, and fatal outcome[10]. The link is obvious. Both diseases are equivalent to atherosclerosis and are clinical manifestations of its severity, so it stands to reason that patients with severe manifestations of atherosclerosis ran the higher risk for fatal outcome.

Goldman et al. were the first to describe combination of advanced age and risk of postoperative mortality[16], which we failed to confirm. Our results are in accordance with observation of Kumar et al. that age is not related to adverse postoperative

events[20]. When age was regarded as dichotomous variable (cut-off on 75-years-of-age), the patients over 75 manifested lower frequency of adverse cardiac events, including fatal events, which was observed in our study as well. The reason for such a good survival rate in older population undergoing elective surgery probably lies in less risky procedures that they are exposed to, and the fact that elective surgical procedure is recommended to older patients in good general condition. The percentage of elderly operated patients in our sample cannot be compared to the percentage of those operated in the developed countries. This observation could suggest that there is no justification why population over 75 should be less frequently treated by surgical procedures.

The most frequent risk factor for CVD in our study was ATH, but not as independent risk factor for fatal outcome. This is in line with the conclusions of the meta-analysis of 30 observational studies examining influence of ATH on perioperative complication[21]. They found that there is a weak relationship between the values of blood pressure under 180 mmHg for systolic and 110 mmHg for diastolic blood pressure and perioperative complications. As all our patients were recruited for the study one month prior to the operation and as there was enough time to treat them and obtain stable values of blood pressure under 180/110 mmHg, it is our opinion that this is the reason why we have not observed negative influence of AHT on survival rate. One third of the total number of operated patients had HLP. We have failed to confirm the connection between HLP and fatal outcome, as well as the connection between smoking habit and fatal outcome, which is in accordance with findings of Wolters et al.[22]. Neither were combined conventional risk factors related to mortality of our patients. We have noticed a relationship between therapy with nitrates and fatal outcome. Nitrates were present in the therapy of all the patients with CAD and in great number of patients who belonged to NYHA classes II and III. In our cohort those patients were at the greatest risk of fatal outcome.

Our study has confirmed that therapy with beta blockers and fatal outcome are interdependent. To be precise, chronic use of beta blockers with dosage titration reduces mortality during the perioperative period for elective non-cardiac surgeries.

Considering the fact that cardiovascular complications are rare events, the most important limitation of our study is the small number of patients. The fatal outcomes which occurred in spite of preoperative measures of cardiologic evaluation, unequivocally indicate that surgical intervention is always accompanied by some risk for fatal outcome. We observed the highest mortality in the group of patients undergoing medium-risk surgeries, which were predominant in our study, so this group of patients should be the focus of attention if we aim to prevent and reduce postoperative fatal outcome.

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The relationship between serum hepcidin level and severity of chronic obstructive pulmonary disease

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Abstract

Aim: This study was designed to evaluate serum hepcidin levels in patients with chronic obstructive pulmonary disease (COPD).

Methods: Ninety male patients with COPD (43-66 years) were grouped as follows; Group I: Mild-moderate COPD (n:30) patients in stable period, Group II: Severe-very severe COPD (n:30) patients in stable period, and Group III: COPD (n:30) patients in exacerbation period. Healthy non-smoker males were included in Group IV (n:30) as control group. Also, in the patient groups with COPD, serum hepcidin level was compared with demographic data, blood iron parameters, pulmonary function tests and arterial blood gas results.

Results: Serum hepcidin level was highest in control group and serum hepcidin level was lowest in group I ($p<0.001$). In group II, serum hepcidin level was positively correlated with serum iron (Fe) level ($p=0.003$), arterial oxygen pressure (PaO2, $p=0.000$) and forced expiratory volume in one second/forced vital capacity (FEV1/FVC, %) ($p=0.006$). In group III, serum hepcidin level was negatively correlated with arterial carbon dioxide pressure (PaCO2, $p=0.027$), and positively correlated with FEV1/FVC (%, $p=0.008$), PaO2 ($p=0.000$), 02 saturation (Sa02, $p=0.047$) and ferritin ($p=0.049$). Serum interleukin-6 (IL-6) level was increased with the severity of COPD ($p<0.001$). However serum hepcidin level was not significantly correlated with serum IL-6 level.

Conclusion: The results of the present study suggest that serum hepcidin level is correlated with COPD severity.

Key words: COPD, hepcidin, IL-6, disease severity.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world and primarily characterised with the presence of airflow limitation resulting from airways inflammation and remodeling progressively. Chronic obstructive pulmonary disease exacerbations are an important outcome in COPD because patients with exacerbations have impaired health status, reduced physical activity levels, and accelerated declines of lung functions. Besides an increase in airway inflammation COPD exacerbations are associated with an increase in systemic inflammation (1). In future decades, burden of disease will increase and according to World Health Organization (WHO) data COPD is predicted to be third leading cause of death in 2030 (2). Airflow limitation secondary to inflammation and ventilation/perfusion mismatch due to alveoler wall damage lead to arterial hypoxemia and tissue hypoxia (3).

Iron dysregulation can be seen in many diseases and toxicological insults (4). Hepcidin (hepatic bactericidal protein), is secreted by the liver, which seems to be the "master regulator" of iron metabolism is a 25-amino acid polypeptide and was first discovered by Park et al. (5). Hepcidin coded by human hepcidin gene (HAMP;OMIM 606464) localized in chromosome 19q13.1 is an important inflammatory marker (6, 7). Hepcidin accepted as type 2 acute phase reactant has a regulatory role in inflammation, the immune system, and iron metabolism (8). Hepcidin secretion is suppressed by anemia and anoxia and hepcidin secretion increases with inflammation (9).

In the present study, serum hepcidin levels of COPD patients in mild- moderate and severe-very severe stages classified according to Global Initiative for COPD (GOLD) 2011 criterias and COPD

patients in exacerbation period were determined and compared with each other and with control group. Besides, relationships between serum hepcidin level with serum iron parameters, pulmonary function tests and arterial blood gas results were evaluated.

Methods

The study included patients with COPD diagnosis, according to the GOLD 2011, being at a stable period and exacerbation period of COPD. Ninety male patients without additional diseases aged between 40 to 75 years old, followed in Diskapi Yildirim Beyazit Training and Research Hospital Pulmonary Diseases Clinic, Ankara between June 2010 and December 2011 were recruited in our study. All participants provided informed consent. The study was planned in accordance with the suggestions of the Helsinki Document and Diskapi Yildirim Beyazit Training and Research Hospital Ethics Committee for Human Studies approved the protocol.

The patients were divided into three groups according to the GOLD criteria as follows Group I: Mild-moderate COPD patients in stable period (n: 30), Group II: Severe-very severe COPD patients in stable period (n: 30), and Group III: COPD patients in exacerbation period (n:30). 30 healthy volunteer subjects without smoking history over 40 years of age were in control group (Group IV, n: 30). Demographic data, laboratory findings, pulmonary function tests, posteroanterior chest X-rays and physical examinations of patient and control groups were evaluated. Chronic obstructive pulmonary disease patients over 40 years of age without additional diseases in stable and exacerbation period were enrolled to the patient groups of the study.

Patients with other chronic diseases, hematological disorders, malignancy, acute or chronic infections within last 3 months, blood transfusions within 6 months and anemia within 6 months were excluded from the study.

Treatment modalities were arranged according to GOLD criterias and disease severity was categorized into mild, moderate, severe and very severe.

Exacerbation of COPD was defined as a sustained (lasting 48 hours or more) worsening of dyspnea, cough and increase sputum production or purulent sputum leading to an increase in the use of maintenance medications and/or supplementation with additional medications for patients in exacerbation period according to GOLD. Group III patients were patients followed in our clinic [from Group I (n: 14) and Group II (n:16)] during exacerbation period.

Upper normal limit for C- reactive protein (CRP) level was 10 mg/l. Basal oxygen saturation was ≤%88, and haemoglobin (Hb) <12 g/dL was accepted for anemia(for males). White blood cell (WBC) number between 5.2-11.4 $10^3/\mu L$ was accepted as normal.

Body mass indexes (BMI**;** kg/m^2) were calculated for all groups. Pulmonary function tests were performed in all patients at the respiration laboratory of our clinic using a Jaeger spirometer according to Thoracic Society (ATS) guidelines. Each subject performed a maximal expiratory flow maneuver in the sitting position. Forced expiratory volume at one second (FEV_1) and forced vital capacity (FVC) were measured and then FEV_1/FVC was calculated.

In all patient groups and control group included in the study, blood samples were collected early in the morning after a minimum of 10 hours fasting. Serum Fe and total iron binding capacity (TIBC), ferritin, Hb, CRP, WBC values were assessed using standard laboratory methods. Also, a sample from the radial artery for arterial blood gas analysis was obtained. Serum samples were analyzed for IL-6 levels by an enzyme-linked immunosorbent assay (ELISA) (eBioscience,Vienna, Austria).

Serum hepcidin level was measured using a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding, according to manufacturer's indications (DRG Instruments, Marburg, Germany). Serum samples for hepcidin and IL-6 were centrifuged for 10 minutes at 3000 rpm and stored at –70 ºC.

The statistical method

Statistical analysis of the data and graphic presentation were accomplished using SPSS 15.0 (SPSS Inc; Chicago, III) package program. Results were presented as mean±standart deviation and median (min-max). Variables in four groups with normal distribution were compared by using one-way analysis of variance (ANOVA) and LSD test was used for post-hoc analysis. Welch test was performed for variances without homogenity and Dunnet T3 test was used to determine the different group. Variables without normal distribution

were compared with Kruskal-Wallis test. α=0.05 was used. P<0.05 was accepted as significant. Mann-Whitney U test and Bonferroni correction were performed to detect the different groups, and p<0.05/n, was used for significance level (n; number of comparision). Correlations between numerical parameters were analyzed with Spearman rho correlation coefficient. Correlations with P<0.05 were accepted as significant.

Results

Demographic data and laboratory results of the patient groups and control group were demonstrated in Table 1.

Age

The ages of the 90 male COPD patients included in the study were between 41 and 75. Control group and patient groups had similar age range.

Body mass index (kg/m^2)

BMI matched subjects in patient and control groups were included to the study.

Cigarette consumption per year (Cigarette pack-years)

Patients had at least 20 pack years smoking history. Subjects in control group were non-smokers.

Blood parameters related to iron (Fe, TIBC, Ferritin, Hb)

No subjects had anemia. Of the COPD patient groups, blood parameters related to iron were evaluated and median values were found to be within normal limits.

Arterial blood gas parameters (PaO2, PaCO2, SaO2)

Decrease in PaO2 (p<0.001) and SaO2 (p<0.001) and increase in PaCO2 (p<0.001) values were detected in COPD patients from group I through group III. There was no significant difference between group II and III in terms of mean $PaCO_2$ (p=0.945).

Table1. Demographic and laboratory data in COPD patient groups and control group

	COPD groups						Group IV Control (n:30)	
	Group I Mild-moderate (n:30)		Group II Severe-very severe (n:30)		Group III Exacerbation (n:30)			
Variables	**Mean±SD**	**Median (min-max)**	**Mean±SD**	**Median (min-max)**	**Mean±SD**	**Median (min-max)**	**Mean±SD**	**Median (min-max)**
Age (year)	58.8±7.19	58.5(45-66)	57.3±5.77	60.5(50-72)	56.4±5.6	55.5(46-67)	53.5±7.24	53 (41-65)
Cigarette (pack-years)	39.26±8.48	40 (20-56)	38.8±10.8	40(20-45)	40.63±9.24	40.5(26-60)	-	-
BMI (kg/m^2)	27.3±4.60	26.45(21-36.3)	25.52±3.55	24.5(20.8-35.2)	24.9±2.26	24.5(21.4-34.2)	25.1±2.31	24.75(22.7-31)
Hb(g/dL)	15.2±1.12	15.2(13-18.1)	15.3±1.09	15.2(13.9-18.1	15.3±1.2	15.1 (13.2-18.2)	15.2±1.17	15.3(13.6-17.3)
Fe(µg/dL)	89.1±28.5	84.5(59-202)	87.4±50.1	76.5(34-271)	82.7±36.3	75.5(34.4-171)	93.3±29.6	102(59-155)
TIBC(µg/mL)	302.5±58.6	298.5(153-491)	287.5±47.8	288.5(228-468)	293±28.7	270(201-368)	302.3±45.8	309(234-440)
Ferritin(ng/mL)	107.7±56.7	100(38-227)	101±62.5	85.5(42-357.3)	103.1±62.1	90(42-357)	111.2±54.8	95.5(42-208.7)
Hepcidin(ng/mL)	210.4±42.5	216.2(105.2-284.4)	224.3±34.5	224.1(101.1-288.3)	232.6±28.4	231.7(178.6-298)	242.4±38.4	248.5(164.6-301.1)
IL-6 (pg/mL)	1.6±0.5	1.4(1.01-3.1)	3.1±0.9	3.01(2.1-6.1)	9.1±4.1	9.2(3.2-16.25)	0.9±0.2	0.9(0.5-1.3)
Pa02(mmHg)	77.6±6.1	78.5(65-88)	59.6±6.7	58.9 (45-79.4)	52.4±5.3	53.9(41.3-65.1)	92.2±2.3	92(90-96)
PaC02(mmHg)	47.9±3.7	49(40.6-53.9)	56.8±6.02	55.5(46.1-71)	58.2±6.5	56.5(51.1-79)	35.4±1.6	35.4(33.1-38.1)
Sa02(%)	91±1.2	91(88.5-93.5)	85.4±4.1	86.2(73.2-89.6)	81.4±4.2	82.7(70.8-87.3)	95.2±1.4	95.4(92.9-98.9)
Fev1/FVC (%)	66.5±3.2	67(58.8-70)	46.7±4.5	47(33.2-56)	40.8±5.7	41.9(30.1-47.9)	95.7±3.4	96.2(90.7-100)

Std Devaition: *Standart Deviation*, **BMI:** *Body mass index*, **Hb:** *Hemoglobin*, **Fe:** *Iron*, **TIBC:** *Total iron binding capacity*, **IL-6:** *Inteureukin-6*, **Pa02:** *Arterial oxygen pressure*, **PaC02:** *Arterial carbon dioxide pressure*, **Sa02:** *Arterial oxygen saturation*, **FEV1/FVC**:: *Forced expiratory volume in one second/ Forced vital capacity*

Forced expiratory volume in one second/ Forced vital capacity (FEV1/FVC;%)

FEV1/FVC (%) was normal in control group. Patients in exacerbation period (group III) had lowest FEV1/FVC (%) (mean FEV1/FVC (%): 40.8±5.7%) (p<0.001).

Hepcidin

Median serum hepcidin level of control group was 248.75 ng/ml (164.64-301.16). Median serum hepcidin level was 216.12 ng/ml (105.12-284.42) in group I, 224.11 ng/ml (101.02-288.38) in group II, and 231.72 ng/ml (178.56-301.16) in group III (p<0.001). We found a significant difference in serum hepcidin level between control group and group II (p<0.001). While there was a significant difference between group I and II (p=0.010), no significant difference was found between group II and III (p=0.179). We also found a significant difference between group I and III in serum hepcidin level (p=0.04).

Figure 1 presented the boxplot graphs of the serum hepcidin leves in COPD patients and control group. Serum hepcidin levels were lower in the COPD patients than the control group, and decreased with severity of COPD.

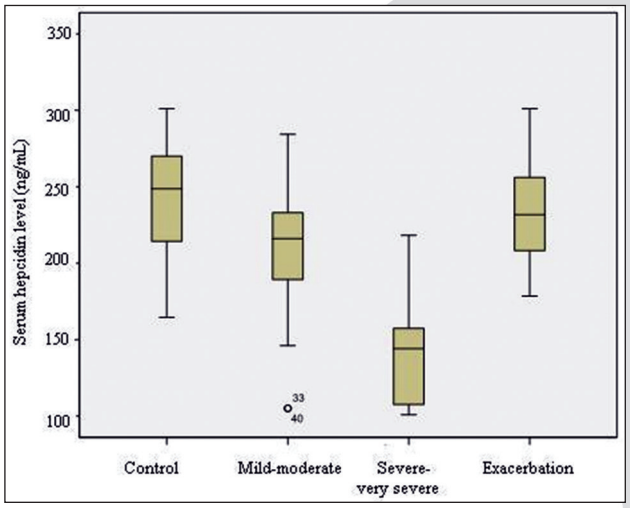

Figure 1. Serum hepcidin level in COPD patients and control group

Interleukin-6

Serum IL-6 level was significantly different among four groups (p<0.001). Mean IL-6 increased with disease severity. Positive correlations were found between IL-6 and age in group II (p=0.01) and group III (p=0.004).

Positive correlation was found between IL-6 and cigarette pack years as IL -6 levels were increased with increased cigarette pack years in exacerbation period (p=0.04). Negative correlation was detected between IL-6 and serum iron level in exacerbation period (p<0.001). We also determined a negative correlation between PaO_2 and IL-6 level in exacerbation period (p<0.001). Although serum IL-6 level increased with disease severity in COPD patient groups, we did not find significant correlation between serum IL-6 level and serum hepcidin level. IL-6 increase with disease severity was pointed out in figure 2.

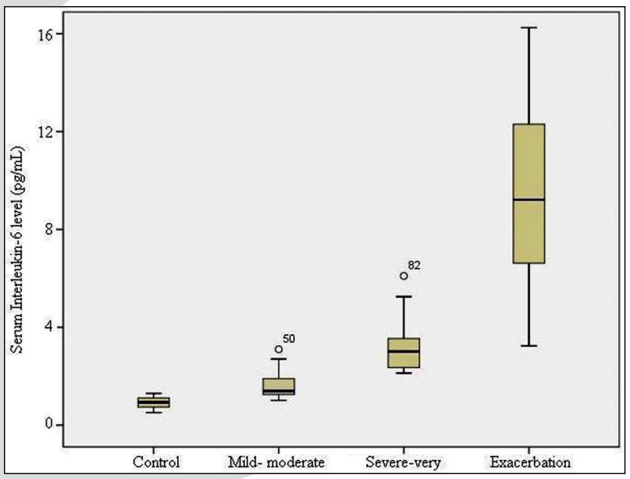

Figure 2. Serum hepcidin and Interleukin-6 level in patients with COPD and control group

The correlations between the serum hepcidin levels of the COPD patient groups with age, BMI, FEV1/FVC and laboratory parameters were shown in Table 2.

Serum hepcidin level in the mild-moderate COPD patient group:

There were no significant correlations between the serum hepcidin level and any other variables.

Significant correlations found between serum hepcidin level and other variables in severe-very severe COPD patient group (Group II):

Significant correlations between serum hepcidin level with serum Fe (r=0.556, p=0.003) and FEV1/FVC (r=0.530, p=0.006) were detected.

Table 2. Correlations between serum hepcidin values of COPD patient groups with other laboratory parameters

	Serum hepcidin levels (ng/mL)					
	Group I Mild-moderate COPD		Group II Severe-very severe COPD		Group III Exacerbation	
	r	p	r	p	r	p
Age(year)	0.017	0.931	0.268	0.152	0.268	0.152
BMI (kg/m^2)	-0.087	0.648	0.287	0.124	0.151	0.424
Cigarette (pack-years)	0.089	0.641	-0.052	0.784	0.096	0.615
Hb(g/dL)	0.080	0.674	0.133	0.482	0.155	0.413
Fe(µg/dL)	0.117	0.537	0.566	**0.003**	-0.246	0.189
TIBC(µg/mL)	0.121	0.523	-0.288	0.523	-0.170	0.370
Ferritin(ng/mL)	0.031	0.872	0.267	0.069	0.363	**0.049**
IL-6 (pg/mL)	-0.09	0.623	-0.102	0.591	0.225	0.232
Pa02(mmHg)	0.049	0.799	0.790	**0.000**	0.771	**0.000**
PaC02(mmHg)	-0.168	0.376	-0.000	0.998	-0.403	**0.027**
Sa02(%)	0.018	0.923	0.120	0.526	0.365	**0.047**
FEV1/FVC(%)	0.001	0.994	0.530	**0.006**	0.510	**0.008**

COPD:*Chronic Obstructive Pulmonary Disease,* **BMI:** *Body mass index*, **Hb:** *Hemoglobin*, **Fe:** *Iron*, **TIBC:** *Total iron binding capacity,* **IL-6:** *Inteureukin-6,* **Pa02:** *Arterial oxygen pressure,* **PaC02:** *Arterial carbon dioxide pressure,* **Sa02:** *Arterial oxygen saturation,* **FEV1/FVC**:**:** *Forced expiratory volume in one second/ Forced vital capacity (%).*

Significant correlations found between serum hepcidin level and other variables in the exacerbation period (Group III):

There was a negative correlation between hepcidin level and PaCO2 (r=0.402, p=0.027). There were positive correlations between hepcidin level with FEV1/FVC (%) (r=0.510, p=0.008), PaO2 (r=0.771, p=0.000), 02 saturation (r=365, p=0.047) and ferritin (r=0.363, p=0.049).

Discussion

COPD leads to progressive airway obstruction, ventilation/perfusion mismatch and hypoxemia. Increasing evidence indicates that COPD is a complex diseases involving more than airflow obstruction. It has been established that stable COPD is associated with low grade systemic inflammation (1). Especially in severe and very severe COPD stages, hypoxemia and inflammation increase disease severity. Hepcidin production decreases with anemia and anoxia although role of hepcidin in iron metabolism has not been well understood (10). However, in inflammatory processes hepcidin production and iron uptake of macrophages increase and plasma iron concentration decreases (11, 12). Thus, we were interested in how hepcidin production is affected by inflammation and/or hypoxia in COPD. We determined hepcidin levels in both stable and acute exacerbation period of COPD. Moreover, we investigated the relationships between serum hepcidin level with serum iron parameters, pulmonary function tests and arterial blood gas results. In previous studies different results have been found related to serum hepcidin levels in acute and chronic inflammation. For example, serum prohepcidin levels have been determined higher in patients with active rheumatoid arthritis than patients with inactive rheumatoid arthritis (13).

In another study, patients with RA had higher serum prohepcidin level than patients with systemic lupus erythematosus (SLE) and healthy volunteers although no correlations between serum prohepcidin level with RA disease activity scores, tumor necrosis factor-*alpha* (TNF-α) and/or IL-6 were determined (14).

In the present study, highest serum hepcidin level was in control group (242.43±38.46 ng(mL), and lowest serum hepcidin level was in patients with severe-very severe COPD (141.31±34.56 ng/mL).

In exacerbation group serum hepcidin level (232.16 ±28.45 ng/mL) was higher than in patients with, severe-very severe COPD. This result can demonstrate the positive correlation between serum hepcidin level and increased inflammation.

Moreover we think that the decrease in serum hepcidin level in severe-very severe COPD patient group may be related to hypoxemia rather than inflammation.

When disease severity increases especially in exacerbation period the partial oxygen pressure (Pa02, mmHg) decreases, the partial carbon dioxide pressure (PaC02, mmHg) increases and respiratory acidosis is seen together with prominent tissue hypoxia. Systemic hypoxia reduces hepcidin production in the liver. However, the molecular mechanisms in which hypoxia plays a role to repress hepcidin production have not been fully understood yet (15). We found positive correlation between serum hepcidin level with PaO2 ($p<0.001$), and negative correlation between serum hepcidin level and PaCO2 ($p=0.027$) in exacerbation period. These results show the increase of serum hepcidin production together with hypoxemia in COPD. Furthermore, in exacerbation period, increase in serum hepcidin level can indicate correlation between serum hepcidin level with inflammation.

The hepatic synthesis of hepcidin increases when serum iron concentration goes up. In contrast, hepcidin synthesis decreases when there is iron deficiency.

Hepcidin production also correlated with the serum ferritin level. According to our results, positive correlation between serum hepcidin level and serum iron concentration in severe-very severe COPD patients ($p=0.003$), and positive correlation between serum hepcidin level with serum ferritin level in exacerbation period ($p=0.04$) were detected.

In limited number of studies, correlation between serum hepcidin levels and BMI has been demonstrated (16, 17). In our patient and control groups BMI was similar and no correlation between BMI and serum hepcidin level was detected.

Disease severity increases from mild-moderate COPD to severe-very severe COPD and prognosis has been affected. Positive correlation between FEV1/FVC and serum hepcidin level was found both in severe-very severe COPD patients ($p=0.006$) and in exacerbation period ($p=0.008$).

This result can be attributed to correlation between disease severity and serum hepcidin level. mRNA of hepcidin is induced by inflammatory cytokines especially IL-6 which is a proinflammatory cytokine secreted from macrophages (18, 19). Previous studies has established that IL-6 level increases in systemic circulation and sputum especially in acute exacerbation period of COPD (20, 21). In our study, serum IL-6 level was lowest in control group (0.9±0.2 pg/mL) and highest in exacerbation group (9.1±4.1 pg/mL) ($p<0.001$). This result indicates the increase of IL-6 with inflammation in COPD patients. Moreover, in exacerbation period, increased IL-6 levels with increased cigarette pack years ($p=0.04$) demonstrates the propagative inflammatory effect of smoking. In concordance with increased IL-6 level in inflammation, increased hepcidin level induces ferroportin uptake into the cell and degradation of ferroportin in macrophages, hepatocytes, and duodenal erythrocytes. Thus, these cells keep iron and iron flow to plasma is inhibited. We determined negative correlation between serum iron level with serum IL-6 level in exacerbation period ($p<0.001$).

In a study by Kemna et al., in vivo human endotoxemia model was composed in 10 healthy subjects after lipopolysaccharide injection, and IL-6 increased within 3 hours after injection and urinary hepcidin level peaked within 6 hours followed by a significant decrease in serum iron (22). However, no significant increase in serum hepcidin level was found when compared with urinary hepcidin level.

In our study population, serum hepcidin level and serum IL-6 level were higher in patients during acute exacerbation period where intensive inflammation exists than in severe-very severe patient group.

On the other hand, no significant correlation was found between serum hepcidin level with serum IL-6 level in all patient groups in connection with the study by Kemna et al. Besides, serum hepcidin level and IL-6 level may be affected by delayed attention of patients in exacerbation period to hospital.

Limitation of the study

The number of patients enrolled to the study is low as finding COPD patients without comorbidities and abnormal results of hematological parameters is difficult.

Conclusion

In conclusion, serum hepcidin level increases in COPD patients. Decrease in serum hepcidin level may be related to hypoxemia in severe-very severe COPD patients and increase in serum hepcidin level may be related to inflammation in COPD patients during acute exacerbation. Serum hepcidin and IL-6 levels is correlated with COPD severity. Further studies with larger number of subjects are needed to explore these associations.

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Endoscopic excision of a maxillary sinus rudimentary giant nasolabial cyst in a child: Case report

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Abstract

Nasolabial cyst is a rare non-odontogenic cyst originating from the soft tissue, which is referred to as various names in the literature. Although these cysts originate from embryological tissue remnants, the clinical findings are generally observed in adult ages. It is rarely seen in childhood. Nasolabial cyst is typically known as a painless swelling in the canine fossa, upper lip, gingivobuccal sulcus, nasal ala and the vestibule. They usually do not cause bone erosion. The incidence of these cysts differs between genders and there is about four times female preponderance in the literature. Since nasolabial cysts cause cosmetic problems on the face in early periods, they should usually be managed when they are 2-3 cm in size. The treatment of the cyst is usually surgical excision through a sublabial approach. Cicatrizant agent injections into the cyst and endoscopic marsupialization have also been tried. In this case report, a 14-year-old boy with a giant nasolabial cyst, 6x5 cm in size, rudimentary in the right maxillary sinus has been presented and surgical excision of this lesion through endoscopy has been discussed.

In conclusion, nasolabial cysts may reach large sizes that may lead to bone deformation in facial development in pediatric patients. Such great cysts may be safely managed by endoscopy as this provides a good exposure.

Key words: Nasal congestion, Child, Endoscopy, Bone deformity, Nasolabial cyst.

Introduction

Nasolabial cysts are rare cysts of the jaw. These are non-odontogenic soft tissue-originated embryological rudimentary cysts of the maxillary area (1). Although these cysts originate from embryological tissues, the clinical findings are not usually observed until adulthood (2).

They typically cause congestion in the canine fossa, the upper lip, the gingivolabial sulcus, nasal ala and the vestibule (3). They are usually known as painless swellings unless they are infected. They lead to complaints of nasal deformation and rarely nasal congestion (3,4). Since nasolabial cysts cause cosmetic problems at smaller sizes, they are usually diagnosed and managed when they are 2-3 cm in size. Therefore, giant cysts that may cause deformation in the surrounding structures are rarely seen. This article discusses a 14-year-old boy with a rudimentary giant nasolabial cyst in the right maxillary sinus.

Case report

A 14-year-old boy presented with complaints deformation on the right cheek and the nose due to indurations, pressure feeling in the face and nasal obstruction. The patient stated that he had this swelling on his face for years, which had gradually increased in size, became painful by palpation in recent years, and right nasal obstruction also increased in the previous two years. On physical examination, the front wall of the maxillary bone was indurated anteriorly and this induration caused mild asymmetry, partially pushing the alar cartilage in the right nostril. On manual examination of the lesion, it was hard and painful when pressed. The roof of the mouth and the nasolabial sulcus were normal. On nasal endoscopy, the right nasal passage was completely obliterated due to lateral nasal wall swelling near the 1/3 anterior floor. This swelling was painful by palpation and could be partially lateralized by pressure. On paranasal computerized tomography (PNCT), a cystic mass 6x5 cm in size, filling the nasal cavity between the medial wall of the right maxillary sinus and the nasal cavity, rudi-

mentary in the sinus by displacing the medial wall of the maxillary sinus and medializing the lateral wall of the right nasal passage (Figure 1).

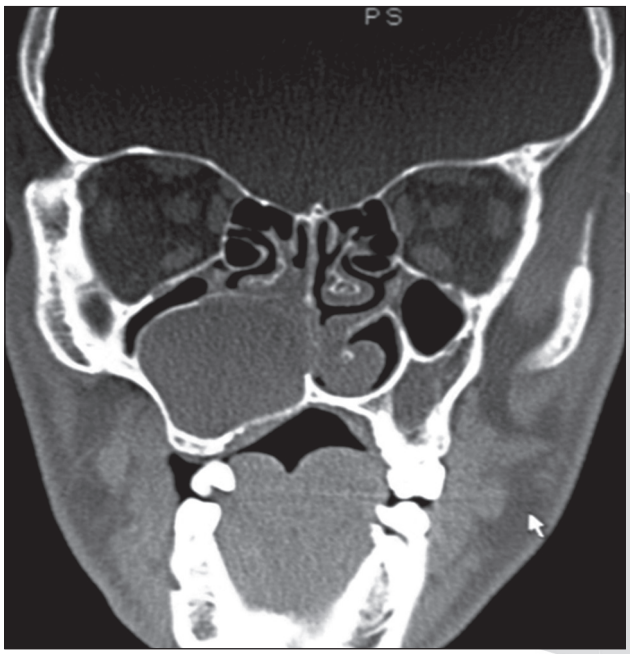

Figure 1. Nasolabial cyst on CT

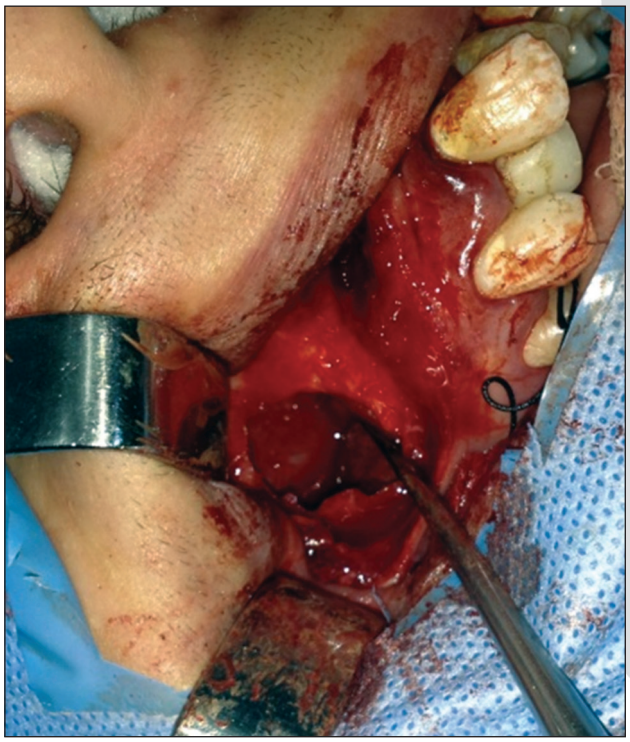

Figure 2. Defect formed by the cyst is seem in Caldwell window

With the prediagnosis of nasolabial cyst, a window 1x0.5 cm in size was opened using the Caldwell through the thin part of the anterior wall of the maxillary bone to access the cyst. The cyst content, which was of lucent yellow color, was partially emptied and under wide exposure using endoscopy, the sac of the cyst was dissected from the medial wall of the maxillary sinus and the nasal mucosa, and the sac was extracted from the Caldwell window as a whole (Figures 2, 3, 4). The potential space formed by the cyst was filled with irradiated acellular bone.

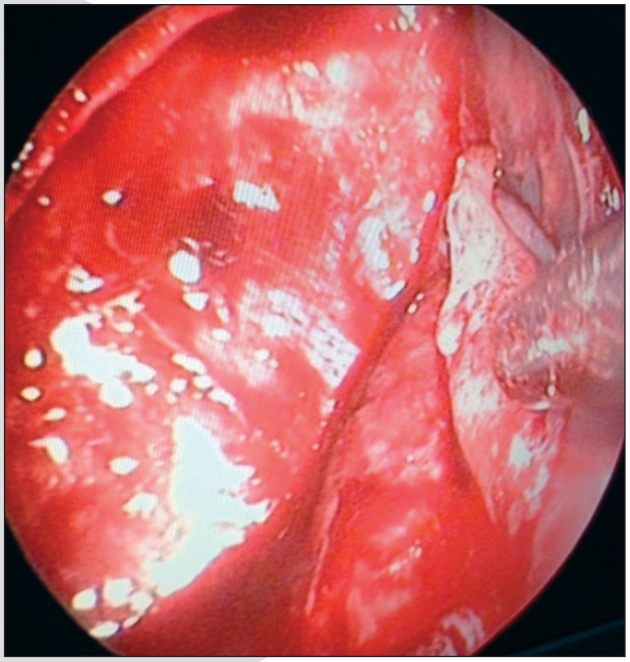

Figure 3. Endoscopic view of the nasolabial cyst defect

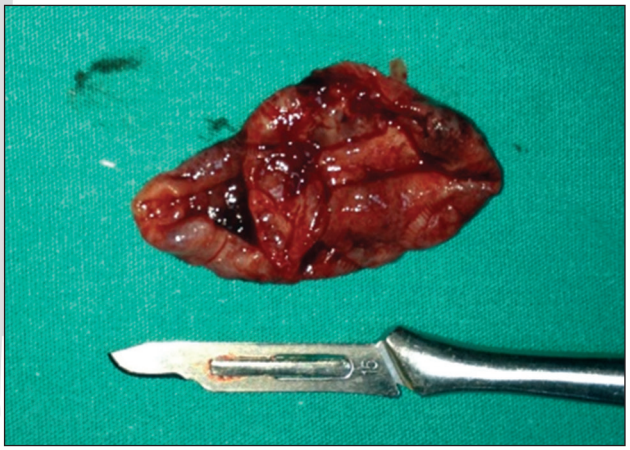

Figure 4. Cyst

Discussion

Nasolabial cyst is a benign extra-osseous lesion, which is slowly growing, and locally expanding under the alar cartilage and the medial nasolabial fold. It was first defined in 1882 by Emil Zuckerkandl,

an Austrian–Hungarian anatomist (5). Due to its location, it is referred to by different names in the literature, such as nasoalveolar cyst, mucoid cyst, subalar cyst, nasal vestibular cyst, and nasal wing cyst (6). Nasoalveolar cyst is a different form that causes erosion on the maxillary bone cortex (7).

Nasolabial cyst is among the rare cysts of the jaw and comprises 0.6% of all jaw cysts. Although these cysts are observed at all ages, it is quite rare at childhood. It is frequently seen between 30 and 50 years of age (2,3,8). The incidence of this cyst differs between genders and it is four times more frequent in women (9). The cyst is generally unilateral and tends to be more frequent on the left side, but it may be bilateral at a rate of 11.2% (10). Our case was 14 years old and the lesion was localized on the right maxillary region. On literature review, there was no child case with a nasolabial cyst. The right maxillary sinus localization was also rare.

Nasolabial cyst is typically seen as a painful swelling in the canine fossa, upper lip, gingivobuccal sulcus, alar cartilage and the vestibule (3). The increased size of the cyst may cause swelling anterior and inferior to the lower concha, and may result in nasal obstruction, rarely expanding to exert pressure on the contralateral septum. Furthermore, although these are soft tissue cysts and extraosseous, they may rarely result in erosion on the nasal floor or the premaxillary region by exerting pressure with its mass (7,11). Since these cysts are located near the distal region of the nasolacrimal canal, they may impair lacrimal drainage filling the lower meatus, and may simultaneously cause dacryocystitis or epiphora (12). Since nasolabial cysts usually result in cosmetic problems of the nose and the upper lip at early periods in adult age, the cyst is often managed when it is 2-3 cm in size (7,13,14). In our case, cosmetic deformation was relatively limited, although the cyst had developed to a giant size by displacing the maxillary sinus. There was mild induration on the upper lip and the alar wing. However, medial displacement of the cyst towards the septum had caused nasal obstruction.

There are two prominent views on the etio-pathogenesis of nasolabial cyst. One was reported by Klestadt which suggests the cause as impaired fusion of the medial nasal wall, the lateral nasal wall and the maxillary process, between intrauterine fourth and eighth weeks of fetal development (5). The second was defined by Wesley (1); he suggested that the nasolacrimal canal develops from the remnant of the distal part in embryologic life. In their immune-histochemical study in 2011, Toribio et al. (15) demonstrated that the cyst is a hamartoma, as a local remnant of the distal nasolacrimal canal. In the present case, the cyst was localized posterior to the anterior wall of the maxillary bone and hence, it had not expanded to the gingivobuccal sulcus. As the cyst did not extend beyond the bone aperture, did not cause erosion on the aperture and was localized behind the embryological fusion line, it is thought that the nasolabial cyst may develop from the hamartomatous remnant of the inferior part of the nasolacrimal canal.

PNBT imaging is an important assistant in the diagnosis of nasolabial cyst. On axial CT, nasolabial cysts are seen as extra-osseous soft tissue lesions in oval shape and well circumscribed on the lateral side of the apertura priformis and on the floor of the alar cartilage. It does not cause bone invasion, but the surrounding bones may be deformated due to external pressure exerted by the cyst in the development period (16). In our case, the giant nasolabial cyst, which was 6x5 cm in size, pressed onto the maxillary sinus and impaired its development and the maxillary sinus became rudimented by being displaced to the lateral side. It expanded towards the medial and pressed on the nasal septum and caused nasal obstruction. Moreover, it expanded anteriorly, which caused expansion and thinning on the anterior wall of the maxillary bone. Although Magnetic Resonance Imaging (MRI) helps in the diagnosis of nasolabial cyst, PNCT is the most effective imaging method for the diagnosis, particularly in the axial plain (16).

In the differential diagnosis of the cyst, one should consider the cysts localized on the midline, maxillary cysts, odontogenic cysts, periapical cysts, periapical abscess, periapical granuloma, epidermal inclusion cyst, nasal basis furuncles and nasal basis tumors (13). The general approach in cyst management is surgical excision through a sublabial approach (14). Furthermore, intracystic cicatrizant agent injection and endoscopic marsupialization may be performed (14,17). In case the sac is completely removed during the cyst excision, no recurrence is expected. Malignant transformation is rare; only one case has been reported

in the literature (18). In the present case, the mass and the cystic sac were completely excised through the sublabial approach using 0^0 endoscope. Since the cyst was large, the endoscopic approach expanded the exposure area in the dark area in the background and helped the cyst excision.

In conclusion, we have discussed the case of a boy with a rare giant nasolabial cyst expanding sufficiently to cause deformation in the maxillary sinus. In the surgical treatment of such great cysts, endoscopy providing a good exposure may safely be used.

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The effectiveness of maneuvers used to treat benign positional vertigo in the emergency department: A prospective study

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Abstract

Introduction: Management of benign positional vertigo (BPV) in the emergency department (ED) includes performing specific maneuvers at the bedside which relieve symptoms in a large proportion of cases. The aim of this study was to evaluate the effectiveness of therapeutic maneuvers in relieving BPV symptoms in the ED.

Materials and Methods: A convenience sample of patients who presented to our ED with vertiginous symptoms during an 80-day period were evaluated for the study. Patients were examined by a single physician using Dix-Hallpike and lateral canal testing to determine the character of each patient's nystagmus, if present. Those with chronic disease or already taking anti-vertigo medications were excluded. According to their test findings, patients were subjected to one of the following maneuvers: the modified Epley, the modified Semont, or the Brandt-Daroff maneuver. Before the maneuver, and 15 and 30 minutes after the maneuver, patients rated their vertigo symptoms on a 0-10 verbal rating scale. Using SPSS 12 for Windows®, before and after ratings were compared using chi-squared and Kruskal-Wallis testing.

Results: During the study period, 72 patients presented with vertiginous symptoms, and 40 were enrolled in the study. In the modified Epley (n=26) and modified Semont (n=11) maneuver groups, mean patient symptom scores decreased significantly between baseline and 15 minutes (p=0.0001 for both groups), and between baseline and 30 minutes (p=0.0001 and p=0.001, respectively). The number of patients (n=3) in the group subjected to the Brandt-Daroff maneuver was not sufficient for statistical testing.

Conclusions: All three performed therapeutic maneuvers are effective for the treatment of patients with BPV in the ED.

Key words: Benign positional vertigo, maneuvers, emergency department.

Introduction

Benign positional vertigo (BPV) is a common diagnosis among emergency department (ED) patients (1). Its main symptoms are the sudden onset of vertigo and nausea aggravated by head movement (2). Symptoms and signs are usually due to floating otoconia or debris particles in the posterior vestibular canals (2). The mainstay of treatment involves bedside maneuvers that aim to relocate the particles into the utricle (2); these maneuvers have been found to be useful in suppressing the recurrence of symptoms (3,4). The purpose of this study was to evaluate the effects of modified Epley, Semont, and Brandt-Daroff maneuvers on ED patients with BPV.

Methods

The emergency department has75,000 patient visits annually. Between March 15 and June 5, 2010, a convenience sample of patients with vertiginous symptoms presenting to our university hospital ED were approached for participation in this prospective study. A single physician performed the maneuvers after evaluating the patients directly or after receiving a call from other emergency physicians (EP). In the latter case, the physician arrived in the first half hour of patients' ED visits, and during that time, patients did not undergo any treatment or maneuvers. The EP hadexperience with BPV maneuvers, having performed them directly on a total

of 50 patients before the study. Inclusion in the study was based on historical and physical findings, and all included patients provided written informed consent. Patients who were using anti-vertigo medications, had chronic illnesses, or whose complaints had begun more than two hours prior to presentation were excluded from participation. Patients' symptoms were graded on a 0-10 verbal rating scale with 10 as the baseline.

The physician performed the Dix-Hallpike maneuver on all patients (1). In patients in whom nystagmus was not elicited by Dix-Hallpike testing, the 'lateral canal test' was performed with the patient lying on his/her back. With the neck at 30° of flexion, the examiner turned the patient's head to the right and waited one minute, and then to the left and waited one minute. If nystagmus occurred in the direction the head was turned, the patient was classified as having geotropic nystagmus, indicating the presence of lateral canalolithasis. If nystagmus occurred in the direction opposite of that in which the head was turned, the patient was classified as having ageotropic nystagmus, indicating the presence of lateral cupolithasis (5). According to the results of these tests (see Figure 1), one of the following therapeutic maneuvers was performed on the patient by the same physician: modified Epley, modified Semont, or Brandt-Daroff (3,4,5,6,7,8) (Diagrams 1-4, Figures 2-4). After the maneuver was performed, patients rested in sitting position. Fifteen and 30 minutes after the maneuver, patients again recorded their symptom scores. Patients with a new diagnosis of BPV routinely underwent brain computerized tomography (CT) imaging (1). This prospective study based clinical experience did not apply to the ethics board for approval. Results were analyzed using the chi-square test and the Kruskal Wallis test and SPSS 12 for Windows® analysis software.

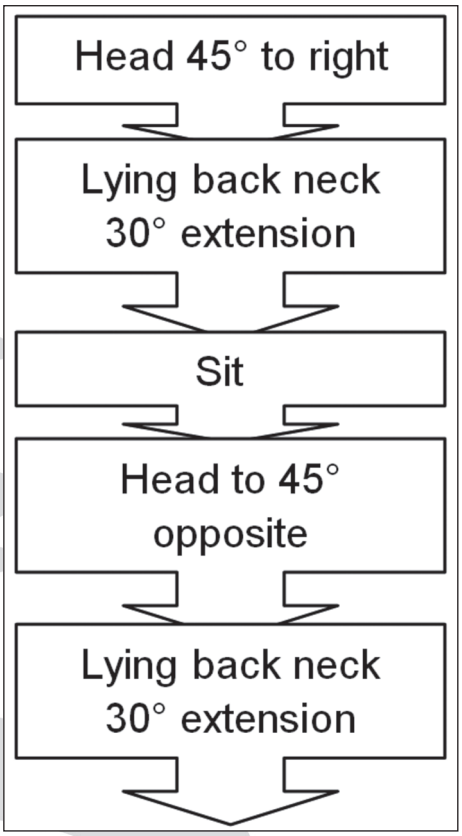

Diagram 1. Dix-Hallpike test (1)

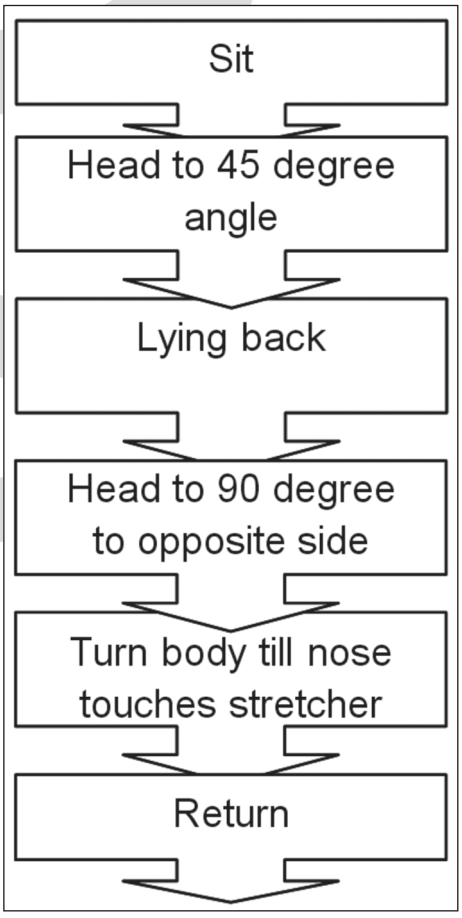

Diagram 2. Modified Epley maneuver (5,6). (3)

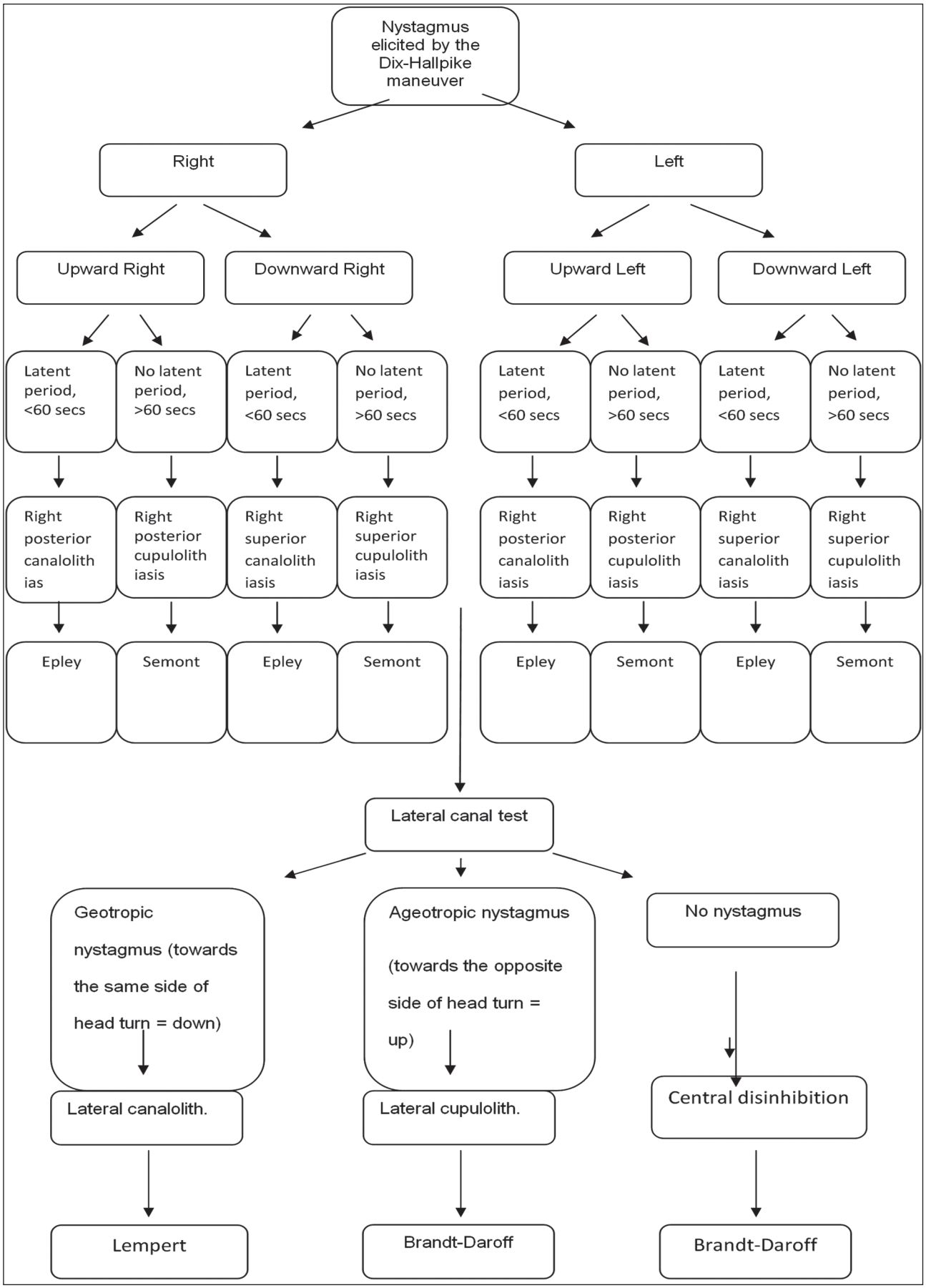

Figure 1. Testing of patients with vertiginous symptoms in the ED and maneuvers performed to resolve their symptoms (5)

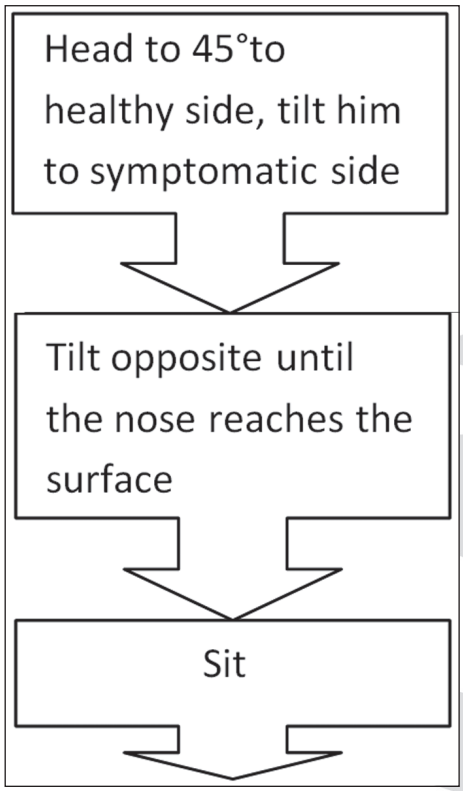

Diagram 3. Semont maneuver (3)

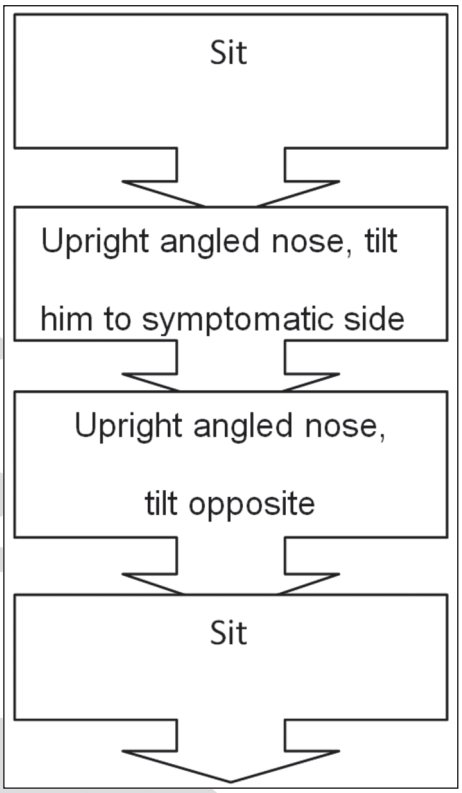

Diagram 4. Brandt-Daroff maneuver (5)

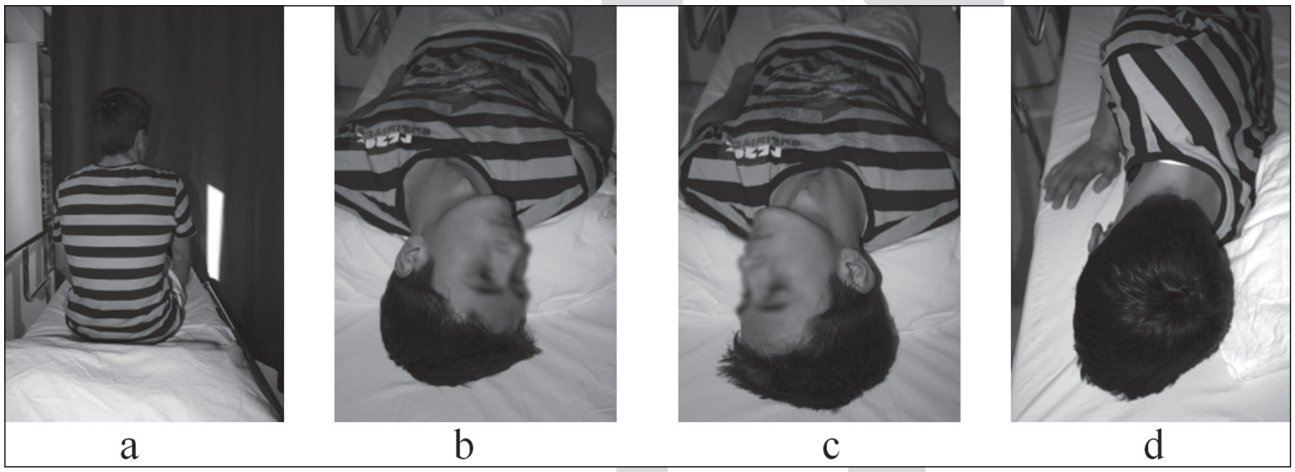

Figure 2. (a, b, c, d) The modified Epley maneuver used to treat symptoms of BPV due to right ear canalolithasis

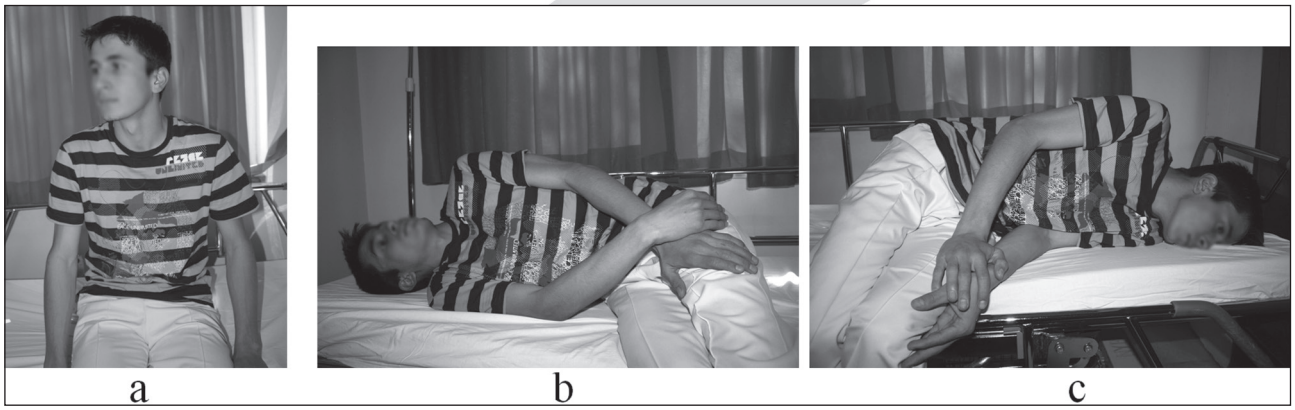

Figure 3. (a, b, c) The Semont maneuver used to treat symptoms of BPV due to right ear cupulolithasis

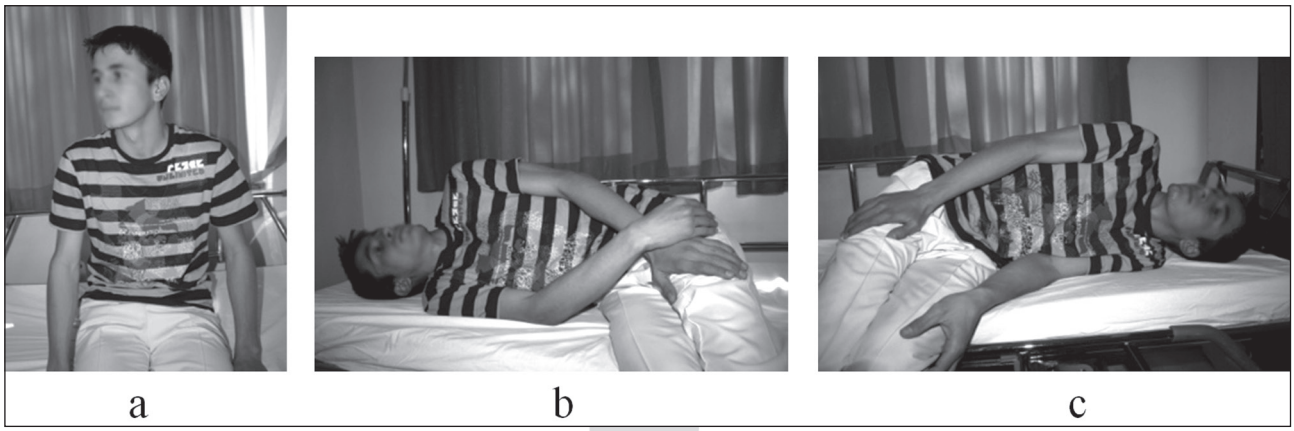

Figure 4. (a, b, c) The Brandt-Daroff maneuver used to treat symptoms of BPV due to right lateral cupulolithasis

Results

During the study period, 72 patients (mean age 46±14 years, range 18-69 years; 72% female) presented to our ED with symptoms of BPV. Of these, 40 were enrolled in the study. The other 32 patients were excluded due to anti-vertigo drug use, the presence of other chronic diseases, and/or arrival at the ED more than two hours after symptom onset. None complained of tinnitus, ear pain, or hearing loss. On physical examination, none had fever or abnormal central neurologic findings. Spontaneous nystagmus was seen in 67.5% (n=27) of patients (rightward in 12, leftward in 15 patients). Spontaneous nystagmus was absent in 13 patients (32.5%). Most patients (80%, n=32) had up-beating nystagmus; 12.5% (n=5) had down-beating; and 7.5% (n=3) had no nystagmus during the Dix-Hallpike test. The 17 patients (42.5%) with first-time BPV diagnose underwent brain CTs, and findings were normal in all cases.

Verbal ratings of vertigo symptoms (mean±SD) by patients who underwent the modified Epley test fell from 10 at baseline to 1.8±1.2 (95% CI=1-5; p=0.0001) at 15 minutes and 1.2±0.6 (95% CI=1-3; p=0.0001) at 30 minutes. Verbal ratings of vertigo symptoms (mean±SD) by patients who underwent the Semont test fell from 10 at baseline to 1.9±1.2 (95% CI=1-5; p=0.0001) at 15 minutes and 1.4±0.7 (95% CI=1-3; p=0.0001) at 30 minutesVerbal ratings of vertigo symptoms (mean±SD) by patients who underwent the Brandt-Daroff test fell from 10 at baseline to 1±0 (95% CI=1-1; p=0.083) at 15 minutes and 1±0 (95% CI=1-1; p=0.083) at 30 minutes. While the group treated with the Brandt-Daroff maneuver had the largest decrease in symptom scores at 30 minutes, the number of patients in this group was not sufficient for statistical testing. The decreases in symptoms of the various treatment groups were not significantly different at either 15 or 30 minutes (p=0.304, p=0.401). No complications occurred.

The symptom score reductions of males and females were not significantly different at either 15 or 30 minutes (p=0.360, p=0.107). The reduction in symptom scores decreased as age increased (18-29, 30-39, 40-49, 50-59, 60-69), but the number of patients in each group was insufficient for determining statistical significance (p=0.806, 0.603).

Discussion

BPV is caused by movement of particles in the labyrinthine semicircular canals (posterior, superior, and lateral) of the inner ear. BPV often recurs and may become a chronic health problem. The mainstay of treatment involves one or more maneuvers of the head that are designed to relocate the misplaced particles in the labyrinthine system. In addition to performing a head maneuver to resolve symptoms of BPV acutely, medications such as levosulpiride, meclizine, dimenhydrinate, metoclopramide, droperidol, diazepam, magnesium sulfate, piracetam, and gabapentine may be used in patients with severe exacerbations of BPV; however, in most cases of BPV, medications are not indicated (9).

Compared to the few studies in the literature reporting results of BPV maneuvers on emergency patients, our mean patient age was similar to those in the literature (46±14 years compared to 56 years,

49 years, and 63 years)(2,10,11). Most of our patients were female, as has been reported in other studies (10,12). As in other studies, the etiology of BPV in our patients was idiopathic in most cases (12). When the Dix-Hallpike test was applied on ED patients to confirm and characterize BPV, the rate of nystagmus was 92.5% in our study and 75% in a study of 22 patients by Chang et al. (1).

The Dix-Hallpike maneuver helps elicit nystagmus if not spontaneously present (1). We used the test to assign laterality. It was adequate in demonstrating the side on which nystagmus was worse for those who did not have obvious nystagmus at rest. Similarly to Çelebisoy et al., we classified the direction of nystagmus in our patients as posterior with up-beating, superior with down-beating, or horizontal (10). BPV is most frequently caused by canaloliths in the posterior canal (13). We found posterior canal BPV in 67.5% of our patients, while other studies reported the following proportions: 87.9%, 85.2%, and 96.5% (10,15,14). None of our patients had anterior canal nystagmus, and this form of BPV was also uncommon in other studies (1.3% in the study by Çelebisoy et al.) (10). While the right ear was most commonly affected in other reports (60.5%, 67.5%, 54.2%, 57.2%), the rate among our patients was only 47.5% (10,15,16,17).

Canalithiasis and cupulolithiasis are different mechanisms of BPV which can be identified by the type of nystagmus elicited by Dix-Hallpike testing. After the Dix-Hallpike maneuver was performed, nystagmus occurring after a latent period of 1-5 seconds, gradually slowing, and lasting less than 1 minute indicated canalithiasis caused by free particles in the posterior or superior canals. On the other hand, nystagmus occurring after the Dix-Hallpike maneuver without a latent period, without gradual slowing, and lasting more than 1 minute indicated cupulolithiasis (adherence of particles to the cupula of the canals) as the etiology of the BPV (5).

The essential treatment of BPV involves repositioning maneuvers; the Epley maneuver is the most suitable for posterior BPV (12,16). In their study of BPV maneuvers in the ED, Chang reported a decrease of 6 (on a scale of 10 points) in symptoms after the Epley maneuver versus a decrease of 1 after placebo treatment (1). In various studies, the Epley maneuver was 70-90% successful in resolving symptoms of BPV (6,7). The Semont maneuver, the first maneuver described for resolution of posterior BPV symptoms, had a success rate of 84% after the first application and 93% after the second (8). In studies of BPV patients by Radtke et al., the modified Epley maneuver was successful (over the course of a week) in 95%, the modified Semont maneuver in 58%, and the Brandt-Daroff maneuver in 23% (3,4). Casqueiro reported a 76.5% success rate ten days after applying the Epley maneuver to BPV patients (16). Both the Epley and Semont maneuvers have been reported to be highly effective in the literature (3,4,6,7). Massoud et al. found that 90-95% of patients with BPV improved after the Epley and Semont maneuvers, and Celebisoy et al. reported the success of the modified Epley maneuver to be 84%. However, Levrat observed resolution in only 68% of patients within one week after one application of the Semont maneuver (10,17,18). Korres reported that canalith repositioning procedures were successful in over 90% of their BPV patients (19).

In studies of BPV patients in non-ED settings that have followed patients for weeks or months, no significant differences in the efficacies of the maneuvers could be demonstrated (2,10,20). Cesarani mentioned that although BPV had a variety of etiologies, the Semont and Epley maneuvers and their modifications were effective in the relief of symptoms in the majority of cases (9). Cohen reported no difference in the improvement of symptoms in groups of patients undergoing the modified Semont and Epley maneuvers (21). In one study, however, 54% of patients had improved two weeks after the Semont maneuver, while only 25% had improved two weeks after the Brandt-Daroff maneuver (20).

Central neurologic pathology may also present with vertigo and nystagmus mimicking BPV (1). Therefore, we routinely performed a head CT to search for other diagnoses for patients with first time BPV-like symptoms. In these patients, however, no pathology was found.

The limitations of this study are that maneuvers were only applied once, and we did not follow our patients after discharge from the ED.

Conclusions

The acute symptoms of the vast majority of patients presenting to our emergency department and diagnosed with different kinds of BPV, as assessed by Dix-Hallpike and lateral canal testing, were successfully reduced by the modified Epley, modified Semont, and Brandt-Daroff maneuvers. In future studies, the effectiveness of various maneuvers should be compared after the placement of patients into categories based on nystagmus testing.

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Atypical meningioma in a patient with von Hippel–Lindau disease: A case report and literature review

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Abstract

Von Hippel-Lindau (VHL) disease is an autosomal dominant multicancer syndrome, which can involve multiple organs and exibit various clinical manifestations. The authors present the case of a 46-year-old man with an atypical meningioma in the left frontal region, a cerebellar hemangioblastoma, a choroidal melanoma in the right eye, pancreatic cysts, a gigantic renal carcinoma and multiple hemangiomas in the liver which was consequently confirmed to be a VHL. A genetic test for the tissue DNA from the meningioma revealed a point mutation, changing guanine to adenine, at nucleotide number 19 within exon 1. By reviewing the literature in the current, this appears to be the first recorded case with atypical meningioma involvement. However it is still unclear if meningioma is a member of the VHL family of tumors.

Key words: Gene test, meningioma, point mutation, von Hippel–Lindau disease

Introduction

Von Hippel-Lindau (VHL) disease is a rare disease caused by a mutation in the *VHL* gene with an incidence of 1/36 000 [1,2]. On the basis of the absence or presence of pheochromocytomas, VHL can be subdivided into type I (absence) and type II (presence). Type II can also be subdivided into type IIA and type II B based on the little or no tendency to develop renal cell carcinoma [3-5].The presence of pheochromocytomas without the other classical features of VHL constitutes type II C. It is difficult to diagnose VHL because the manifestation of VHL is various and approximately 20% of VHL disease patients do not have a family history [6].

VHL with meningioma is very rare. After systematic review of the current medical literature, only 3 cases of meningioma with VHL reported in detail were identified (Table 1) [7-9]. Here, we present the case of a 46-year-old man who was diagnosed to be a VHL with an atypical meningioma, a cerebellar hemangioblastoma, a choroidal melanoma in the right eye, a giant renal carcinoma, multiple pancreatic cysts and hepatic hemangiomas. To our

Table 1. Systematic review of the main reported VHL with meningioma in the current literature

Study	Age	Sex	Symptom	Location	Type of meningio	Side	Type of VHL	Family history	Resection
2001 Governale et al.	37	M	right frontal headaches	supratentorial frontal lobe	fibrous meningioma	right	I	NR*	Yes
2003 Kanno et al.	66	M	mild demention and memory disturbance	superolateral corner of the tentorium	meningothelial meningioma	left	IIA	Yes	Yes
2006 Santarpia et al.	26	F	no symptoms	supratentorial frontal	unknown	unknown	IIC	No	NR
Present case	46	M	recurrent dizziness	frontal region	Atypical meningioma	left	I	No	Yes

**unclear*

knowledge, this is the first VHL-patient with an atypical meningioma reported in the literature.

Case Report

A 46-year-old man with a history of resection of left cerebellar hemangioblastoma 10 years ago presented with recurrent dizziness and a progressive gait disturbance when admission to our hospital.

Enhanced magnetic resonance imaging (MRI) revealed a well-delineated mass, measuring 47×25×23mm, located in the right of cerebellar vermis and a enhanced lesion measuring 23×12 mm, located in the left of cerebral falx. The cerebellum focus was thought to be a hemangioblastoma, while the lesion of cerebral falx considered as a meningioma (Figure 1). Abdominal enhanced CT scan demonstrated multiple enhanced masses in the liver, numerous solid and cystic nodules in the pancreas and a renal mass, measuring 120×90 mm. The patient's laboratory data were approximately normal. Additionally, the maternal family history was negative for evidence of VHL disease. Details of the paternal history were not available.

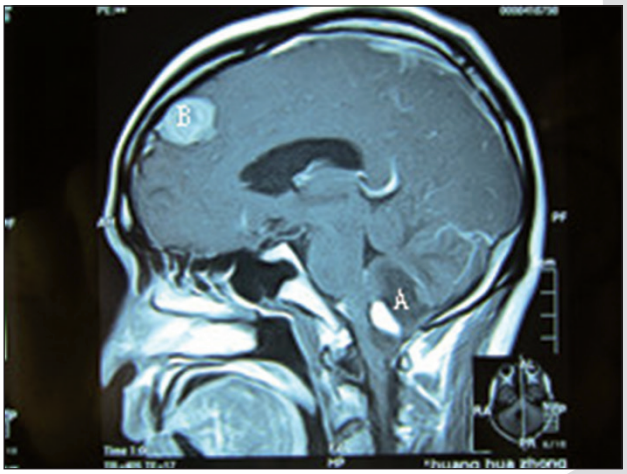

Figure 1. Enhanced MRI showing a well-defined with mixed long T1 and long T2 signals measuring 47×25×23 mm in the right of cerebellar vermis. After enhancement, an obviously enhanced nodule measuring 15×10mm can be identified(A). In the left of cerebral falx, a short T1, T2 signal measuring 23×12 mm enhanced lesion could be found(B).

The patient underwent a cerebellar tumor resection first. The tumor was found to be located on the fourth ventricle openings, firmly attached to medulla and underwent a complete resection. The histological examination of this mass was as a capillary hemangioblastoma. Half a month later, another resection of the tumor within left cerebral frontal region was performed. After opening of the dura mater, a soft, rubbery tumor, relatively rich in vessel tightly adherent to cerebral falx approximately 25×15×10 mm in size was identified. The tumor was clearly separated from the surrounding tissue and removed intactly. Histological and immunohistochemical of the cerebral tumor revealed highly cellular with scattered mitotic figures, and the Ki-67 staining index was 3-10%. The histological diagnosis was atypical meningioma (WHO, II) (Figure 2). After complete recovery, the patient underwent the third operation of radical nephrectomy for the renal tumor.

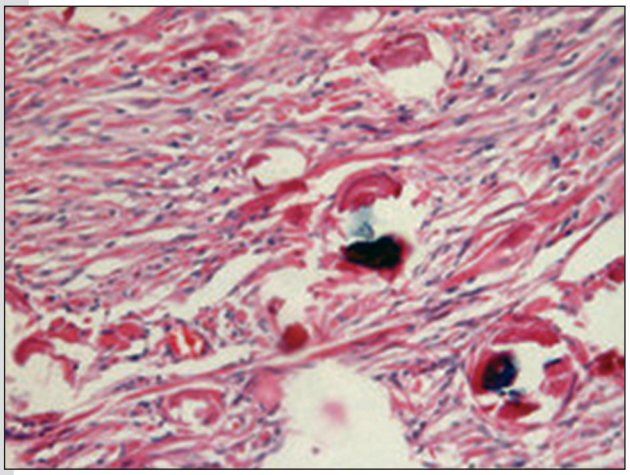

Figure 2. Photomicrographs of the left frontal region of the tumor showing a highly mitotic activity (HE stain,×100). Immunohistochemistry for Ki-67 antigen revealing that the Ki-67 staining index was 3-10% (E: ×100).

Informed consent for analysis of the VHL gene was obtained from the patient and his family. DNA extracted from the atypical meningioma and lymphocytes in the peripheral blood were performed. Each of the 3 exons of the VHL gene was individually amplified by polymerase chain reaction (PCR). The amplification products were purified and analyzed by direct sequencing using a DNA sequencer (Sequence of 3 730 Test Analyzer, USA, ABI company). As shown in Figure 3, The direct sequencing of the VHL gene revealed a point mutation, changing guanine to adenine, at nucleotide number 19 within exon 1.

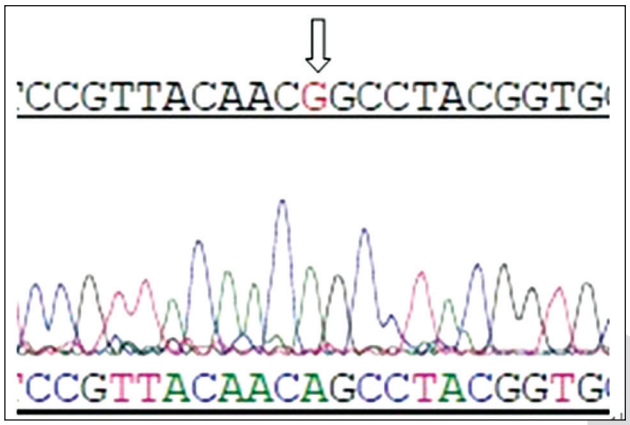

Figure 3. The same point mutation (arrow) from guanine to adenine is detected inboth DNAs from the leukocytes and the meningioma tissue.

Discussion

VHL disease is an autosomal-dominant familial cancer syndrome caused by a mutation in the VHL gene which usually functions as a tumor suppressor gene identified at chromosome 3p25–26 [10]. The VHL mRNA and protein is widely expressed in both fetal and adult tissues [11,12]. pVHL, the VHL gene product, negatively regulates hypoxiainducible mRNAs including vascular endothelial growth factor (VEGF) mRNA by forming a multiprotein complex [13]. The absence of pVHL itself has been shown to increase VEGF expression [14,15]. Therefore, vascular tumors, especially CNS hemangioblastomas are a cardinal feature of VHL disease [16].

Meningioma is the second most common tumor of the central nervous system. About 4.7% to 7.2% of meningiomas are atypical, which are a histologically defined subgroup of meningiomas that are associated with a significantly higher risk of local tumor recurrence than benign meningiomas, even after gross total resection [17]. Neurofibromatosis type 2 (NF 2) gene which located on the chromosome 22q are thougt to play an important role in the tumorigenesis of meningioma, and 70% of atypical meningiomas exbit NF2 gene mutations [18].

Numerous authors have previously reported that meningiomas associated with VHL disease, but only 3 reported in detail, with gene level detection [7-9]. Governale et al. reported a case that an incidental fibrous meningioma associated with VHL disease [7]. By useing SSCP to analyse the DNA extracted from the meningioma and hemangioma, they found that a loss of heterozygosity (LOH) at the NF2 gene locus was detected, whereas that of the VHL gene locus was not. So they considered that meningiomas may not be part of the spectrum of tumors combined with VHL disease. But Kanno et al. finding that The same point mutation from cytosine to guanine is detected in both DNAs from leukocyte and the meningioma tissue, but the peak of the wild type sequence at the point mutation in the tumor tissue showed significantly lower than the leukocyte [8]. So they suggested that meningioma should be considered as a member of the VHL family of tumors. Santarpia et al. found that missense mutation causing amino acid replacement in a pVHLregion might result in the formation of VHL-associated meningiomas [9]. As to our patient, somatic DNA extracted from the meningioma and lymphocytes both showed a point mutation on chromosome 3p. This result revealed a partial inactivation of the VHL gene in the meningioma. The limitation of our study is that we did not assay for LOH at both VHL and NF2 gene in our patient's tumor to clarify whether this patient's meningioma was associated with his VHL disease or just an incidental occurrence. From the present results we can conclude that there is some relationship between VHL and meningioma, but it is obscure that whether the meningioma is caused by this mutation or combined with other mutations.

Conclusion

We reported a VHL case with atypical meningioma in the left cerebral falx. It is difficult to judge if meningioma is a member of the VHL family of tumors and further basic research and clinical report are still needed.

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Dysphonia as a rare symptom occurring due to hypertrophic anterior cervical osteophyte: A case report

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Abstract

Introduction: Hypertrophic anterior cervical osteophyte (HACO) is an excessive formation of osteophytes along the anterior spine. The range of symptoms of HACO is quite wide depending on the organ that it made compression on and its prevalence increases with age.

Aim: In this article, we aimed to present a 68-year-old patient

with the complaint of dysphonia, which is very rare symptom, due to osteophytes of HACO.

Case Report: The patient was 68 years old and male. First laryngoscopy, then neck, larynx and virtual Computer Tomography (CT) examinations in 64 detected CT were performed for the patients admitted to our hospital with a complaint of dysphonia. I t has been detected that, in CT scan at the level of C3-C5, osteophytes at the anterior corpus vertebra narrowed pharynx and the left column of air of larynx and made indatation on left aryepiglottic fold and left pyriform sinus.

Conclusion: HACO should be considered as a rare cause of dysphonia when common causes were excluded. For the diagnosis, roentgenography, laryngoscopy, eusofagoscopy and eusofagoscopy examinations with barium may be performed and also for the complications of compression and 3-dimensional evaluation, CT and Magnetic Resonance Imaging (MRI) are sufficient.

Key words: Disphonia, severe osteophyte formation, cervical spine.

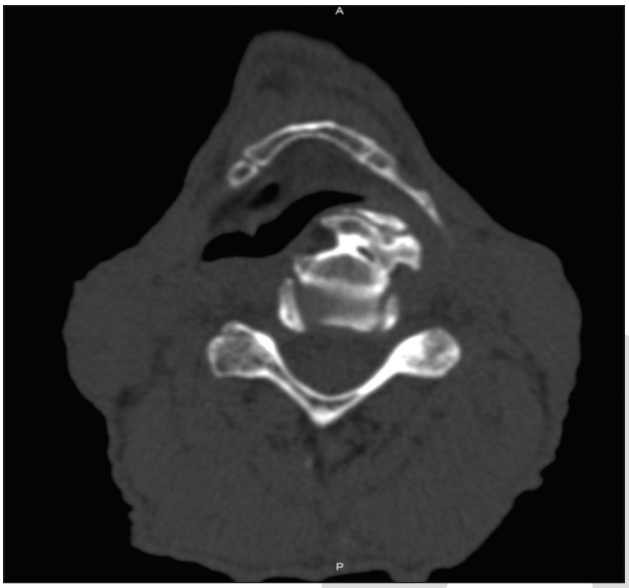
Introduction

Hypertrophic anterior cervical osteophytes (HACO) is seen quite often in the geriatric population but rarely symptomatic (1). Range of symptoms is quite variable. Symptoms are proportional to the size of hypertrophic spurs and it may vary from asymptomatic cases to severe cases of dysphagia, dysphonia and dyspnea (2). Clinical symptoms are often associated with the compression of the esophagus and larynx. Dysphagia devoloping due to obstruction of the esophagus in patients with symptomatic anterior cervical osteophyte is the most common symptom, only a few patients with dysphonia with laryngeal origin have been reported in the literature

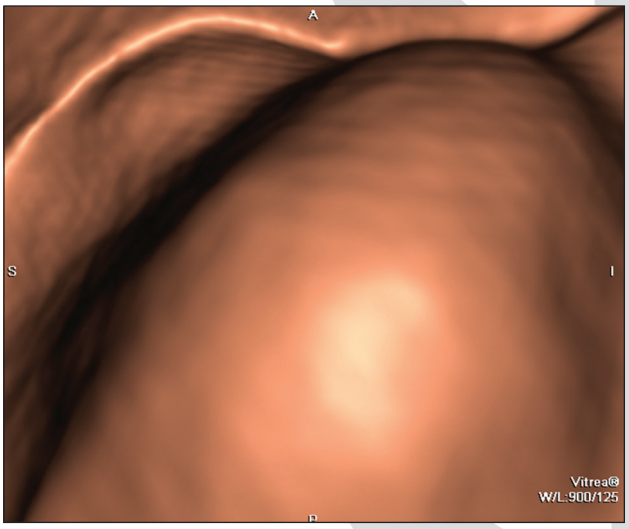
The purpose of this article is to attract the attention with the accompany of a case report and review of the literature that, HACO may be a rarely cause of dysphonia.

Case report

68-year-old male patient was admitted to ENT clinic of our hospital with the complaint of to dysphonia. In the laryngoscopy performed there, the presence of a mass compressing to larynx from outside and the patient was referred to our clinic for neck CT examination. 64 detected CT, laryngeal CT and virtual laryngoscopy were performed. In the CT, it has been detected that, the osteophytic formations at the anterior corpus vertebra narrowed pharynx air column at inferior oropharynx and larynx air column at the left half by starting from vallecula and made indatation on left aryepiglottic fold and left pyriform sinus (Figures 1a-b-c, 2).



a



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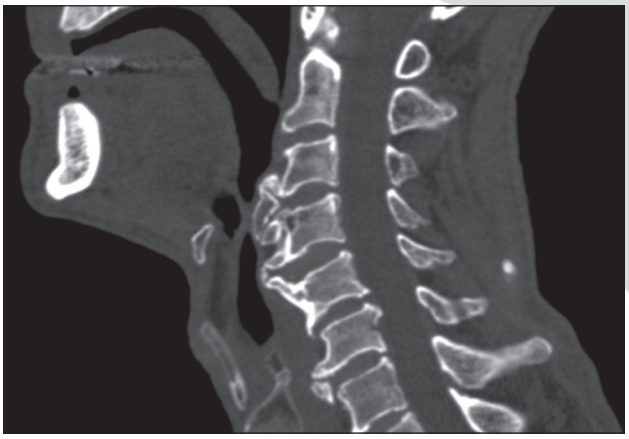

c

Figure 1a, b, c axial MRP (a), sagittal MPR (b) and virtual laryngoscopy (c) images; the osteophyte formation is seen which narrowed larinx air column by obliterating left vallecula

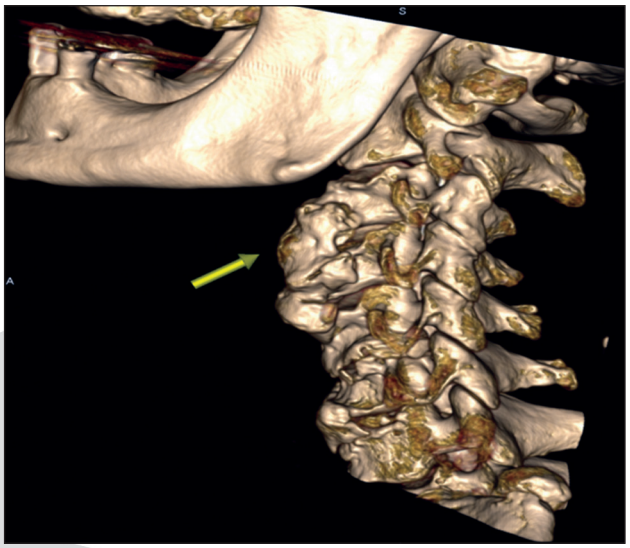

Figure 2. 3D CT image; the osteophytes formation at anterior corpus vertebra at the level of C3-C5 is seen

Discussion

The prevalence of HACO in the population is 20-30% and usually asymptomatic (3,4,5). HACO can be seen after diffuse idiopathic skelatal hypertrophy (DISH), ankylosing spondylitis, infectious spondylitis, postlaminectomi syndrome, cervical spondylosis, and trauma (3,6,7). However HACO is often seen as a part of the degenerative disc disease seen physiological or secondary to advanced age or (DISH), which is also known as Forestier disease (8).

Osteophytes often remains clinically silent, but sometimes can cause various symptoms secondary to compression to especially esophagus and larynx and nerve root, spinal cord and vertebral artery (5). Dysphagia occuring due to mechanical obstruction of pharingosafagial segment is the most common symptom of HACO when it is symptomatic (1). Dysphonia have rarely been reported in the literature (9,10,11,12,13,14,15,16,17,18). Cricoid ulceration takes places in the pathogenesis of laryngeal symptoms which is occured with laryngeal movements on a a large osteophyte.This may cause infectious, inflammatory edema and airway obstruction and bilateral cord paralysis (9). Outside of dysphonia due to compression of the upper air way, dyspnea, stridor, and cough may occur.

For the diagnosis roentgenography, laryngoscopy, esophagoscopy, CT, MRI, esophagoscopy examinations with barium can be used (19). However, CT is sufficient fort he diagnosis, which is able

to describe clearly the the relationship between esophageal and laryngeal-tracheal system, the size and morphology of the anterior osteophyte. MRI is useful for fully determine the level of the cricoid compression and demonstrating laryngeal necrosis and superinfection in patients with dyspnea. In asymptomatic patients, no treatment is necessary. The treatment of symptomatic anterior cervical hyperostosis is removal of osteophytes (7). In life-threatening airway obstruction, emergency tracheostomy is life-saving method. Osteophytes may occur again, therefore the long-term follow-up is recommended.

After excluding other common causes of dysphonia, the presence of HACO which is a rare cause of dysphonia, should be considered.

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Amyotrophic lateral sclerosis presenting in postpartum period after hormonal infertility treatment

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unclear etiology. It is more commonly seen in men compared to women. The lower prevalence and later onset of the disease in women is the reason of rare occurrence of this disease in obstetric population. A very small number of published studies have described association of ALS with pregnancy. Our study presents an unusual and rare case of rapid development of ALS in woman in postpartum period. Patent was previously treated for infertility. This case may indicate a significance of hormonal modifications in the ALS pathogenesis.

Key words: Amyotrophic lateral sclerosis, pregnancy, infertility treatment.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unclear etiology that is characterized by signs of damage to peripheral and central motor neurons. The disease has a progressive course and a lethal ending due to respiratory failure. ALS is a relatively rare disease. The average annual incidence of this disease is 0.4 to 1.8 patients per 100,000 members of general population, while the prevalence ranges between 4 and 6 patients per 100,000 persons, with a predominance of male patients. The ratio between male and female patients was 1.8: 1 (1). The disease usually occurs in the fifth and sixth decade of life. The onset of the disease in men occurs at a slightly earlier age than in women. Thus, for example, in the Irish population, the average age of men at the time of diagnosis was 59.7 years, while the average age of women is 62.7 years.(1). The lower prevalence and later onset of the disease in women are the result of a very small number of published cases of ALS among women of childbearing age, especially during pregnancy. The largest number of published cases of ALS among pregnant women comes from Guam, where the occurrence of the disease is about 100 times higher than in other parts of the world.

Except in cases of Guam, only a few cases of ALS during pregnancy were published in the world literature (2-5), with no description of cases of disease after treatment of infertility and artificial insemination. This case report described a woman in whom the disease appeared after a treatment of infertility, artificial insemination and after giving birth.

Case report

A 38-year-old female patient with progressive weakness of upper and lower extremities was presented. The first symptoms appeared gradually, soon after giving birth, six months before admission to our institution. The patient was treated for sterility for several years by a hormone therapy. After one ectopic pregnancy, she became pregnant by artificial insemination and gave birth to twins. A Caesarean section was performed for a delivery two weeks before the due date because of threatening eclampsia. Shortly after giving birth she began to complain of light weakness first of the left, then of the right hand, and of the general weakness and fatigue, which the patient interpreted as the result of tiredness due to the high involvement of taking care of the baby. Further along, the hand weakness became more pronounced, so it was more difficult for her to carry out normal daily activities. She noticed that the small hand muscles were becoming thinner. Gradually the leg weakness developed as well, causing her to fall repeatedly, without the loss of consciousness. In the period of 6 months she lost about 18kg in body weight despite the preserved appetite and normal food intake.

The clinical examination recorded hypotrophy of the small muscles of the hands and shoulder area with a lower strength of the same muscles (Figure 1 and 2).

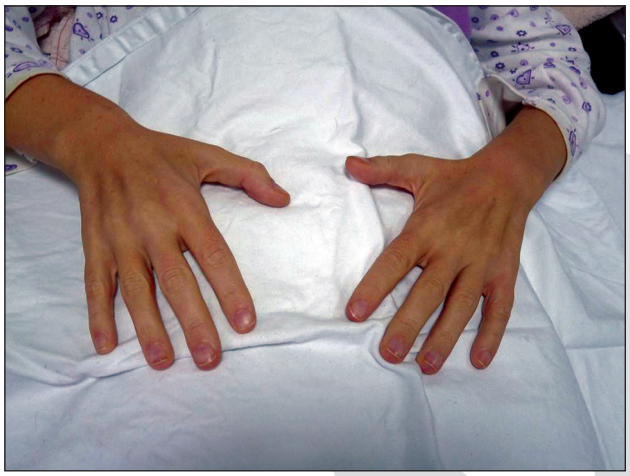

Figure 1.

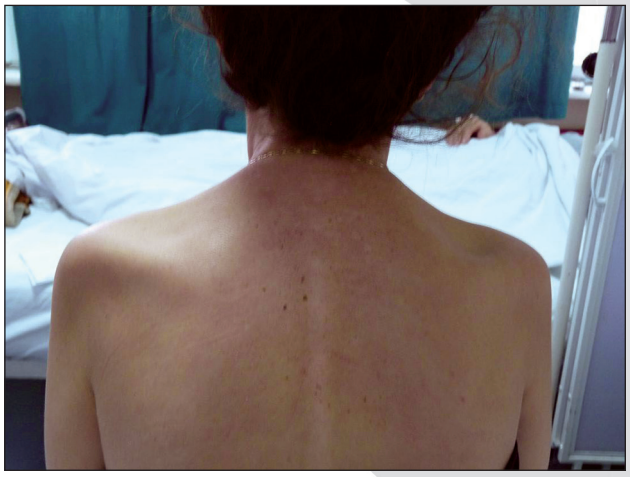

Figure 2.

The weakness of the proximal segments of the upper extremity was dominant, with a severe degree of manifestation and the evident difficulty with the abduction of the upper extremities (the movement of arms away from the body was possible to about 30 degrees) (Figure 3). The weakness of the neck flexor muscles and torso muscles was recorded, with a difficulty with getting up from lying down. She also demonstrated the light weakness of the lower extremities in general. Myotatic reflexes were generally increased, somewhat more ready at the right limbs. Mandibular reflex and the flexor reflex of the toes were enhanced. There was no change in sensibility.

Figure 3.

Detailed electromiographical (EMG) examination revealed the existence of denervation activity such as fibrillation in the examined muscles of the upper and lower extremities. In all examined muscles the potentials of motor units (motor unit recruitment) were significantly reduced, with high amplitudes, and of prolonged duration. A neurographic finding showed lower amplitude of compound muscle action potential (CMAP) of all tested nerves, with proper distal latency and motor conduction velocity (Figure 4). Sensitive neurograms of the examined nerves were regular.

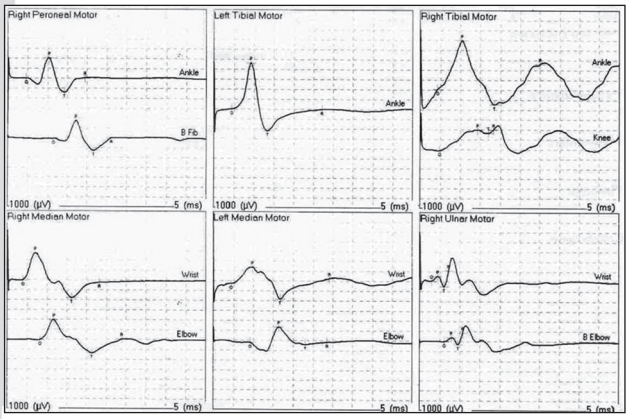

Figure 4.

Prostigmine test was positive. Test of repetitive nerve stimulation) was also positive with decrement of about 30% of the examined muscles of the upper extremities.

The results of serum biochemical tests, blood count, coagulation status and immunological analysis were regular. In the peripheral blood smear the mature formed elements of white blood cells of regular morphology were described. The result of a serum

protein electrophoresis was also normal. The levels of serum thyroid hormones, anti-thyroglobulin and anti-tireoperoxidase were normal. Anti-Yo and anti-Hu antibodies were negative. Anti-acetylcholine receptor antibodies and anti-MuSK antibodies were also negative. Results of cytochemical and immunological analysis of cerebrospinal fluid were within physiological values.

Lung roentgengraphy was regular. Brain and the cervical spine NMR findings were normal, as well as MSCT of the mediastinum.

Ultrasound examination of the thyroid, abdomen and pelvis showed no pathological changes. Gynecological examination was also normal.

Due to a positive prostigmine test and repetitive stimulation test the application of anticholinesterase and immunosuppressive therapy was started, after which there was a partial reduction of muscular weakness. With an attempt to exclude the anticholinesterase therapy, there was a worsening of muscle weakness, while the increase in doses led to the facial muscle fasciculations.

Therapeutic plasma exchange was also conducted on five occasions (amended 2000 ml, 2000 ml, 2000 ml, 1800 ml and 1800 ml) with adequate protein and electrolyte compensation and control of coagulation status. The patient coped well with the treatment without any complications, but also without a more significant impact on the clinical manifestation.

Discussion

The range of neurological conditions affecting women of reproductive age is extremely broad (6, 7). There are neurological disorders which occur more commonly during pregnancy and the puerperium than at other times. It is usually about simple neurological disorders, while serious neurological complications of pregnancy are rare. Several minor neurological disorders occur more often during pregnancy than at other times; Bell's palsy (8-11) and the carpal tunnel syndrome (12, 13) are common examples. Mononeuropathies, plexopathies, and radiculopathies may occur with increased incidence during pregnancy and the puerperium (Rosenbaum1994). Immunologically mediated disorders of peripheral nerve, neuromuscular junction, and muscle may be seen in obstetric population, too (9, 14). Pregnancy increases the risk of a cerebrovascular event (15-17) and neoplastic disease (18). Movement disorders occur infrequently during pregnancy (19). The restless legs syndrome is almost certainly the commonest movement disorder in pregnancy. On the other hands, few case report had described motor neuron disease (MND) especially ALS during pregnancy. ALS is the most common, progressive motor neurone disease but is extremely rare in the obstetric population. The scarcity of information about the disease in pregnancy is partly due to the fact that the mean age of onset of ALS is generally in the sixth decade that is beyond the reproductive years. Despite well-documented degenerative processes in ALS, there is no answer to the question of what causes or triggers this degeneration. So far, no single unifying hypothesis which could explain etiopathogenetic mechanisms responsible for the development of ALS has been defined. The significance of a number of biochemical, metabolic and other etiopathogenetic factors is assumed, including the endocrine disorders, especially thyroid function disorders, but also the significance of the estrogenic hormones. The fact that the disease is more common in men and that in women it occurs in older age than in men, suggests that the hormone estrogen may have neuroprotective effects. Study results indicate nueroprotective role of estrogen in the brain, but there is insufficient proof of the neuroprotective role of estrogen at the level of spinal motor neurons that are selectively affected in ALS. Some in vitro studies suggest a protective role of estrogen at the level of frontal horns of the spinal cord, in the sense of the protection of spinal motor neurons from the excitotoxic negative effects of glutamate and nitric oxide (20).

On the other hand, studies conducted in human populations, such as estrogen therapy in menopausal women with ALS, showed no differences in survival of female patients receiving and not receiving estrogen (21), so this hypothesis still remains controversial. A small number of cases of ALS among women of childbearing age and pregnant women, prevents further study of the influence of hormonal status on the occurrence and course of ALS. Chio et al (22) have reported four cases of pregnant women with ALS. In three pregnant women, the disease appeared during pregnancy, indicating a possible

trigger-effect of pregnancy on the occurrence of clinical manifestation of ALS. The authors hypothesized that hormonal modifications during pregnancy, with an increase in progesterone activity, may increase susceptibility to ALS. Our case report goes in favor of this hypothesis. We described a case of a woman in whom the disease occurred after the hormonal treatment of sterility, shortly after full pregnancy and childbirth. The first symptoms arose immediately after giving birth, in the form of the weakness of the upper extremities (UE).

The diagnosis of the disease was given based on clinical and electrophysiological tests, in accordance with El Escorial criteria for ALS (23). The presence of symptoms in the form of general weakness and fatigue, a positive prostigmine test, a neuromuscular transmission test, as well as the appearance of symptoms after giving birth, have led us to think about possible myasthenia gravis (MG), as well as associated diseases. This is supported by a certain reduction of muscle weakness after the administration of anticholinesterase therapy. On the other hand, the implementation of therapeutic plasma exchange did not provide a satisfactory therapeutic effect. Also, anti-nAChR antibodies and anti - MuSK antibodies were negative (<0,01). With these results in mind, the possibility of MG was excluded. However, these results do not categorically exclude the possibility of seronegative MG as well as comorbitities, which would be an extreme rarety in neurology. If we exclude MG, The impaired neuromuscular transmission in these circumstances could be explained by structural and functional abnormalities of neuromuscular coupling, which are described in patients with ALS (24). The results of previous histological findings indicate a small number or complete absence of nerve endings in patients with ALS. Acetylcholine receptor of the denervated muscle has characteristics of immature fetal receptors with reduced transport of sodium and lower safety factor for the initiation of muscle action potential (25). In this way, the postsynaptic defect of neuromuscular transmission is mimicked (26, 27). Repetitive stimulation test showed decrement in the proximal and distal muscles in patients with ALS in about 50% of cases.

Given the complexity of clinical manifestation, quick and progredient course of the disease, with progressive loss of body weight, the possibilities of paraneoplastic syndrome or systemic disease were considered, but were not confirmed by the used diagnostic procedures during this hospitalization and follow-up in inpatient - outpatient conditions.

Conclusion

This view illustrates an unusual and rare case of rapid development of amyotrophic lateral sclerosis in women in the postpartum period, and after years of hormonal treatment of infertility, which may indicate a etiopathogenetic significance of hormonal modifications in this disease. In this regard, further studies are necessary

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Alterations in serum vascular endothelial growth factor level in scald burn injury: An experimental study

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Abstract

Background: A severe burn is associated with release of inflammatory mediators which ultimately cause local and distant pathophysiological effects. Mediators including Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are increased in affected tissue, which are implicated in pathophysiological events observed in burn patients. Inflammatory cells surrounding the microvasculature can have a profound effect on promoting new vessel growth via vascular endothelial growth factor (VEGF) and VEGF receptors such as VEGF Receptor (Flt-1).

Aim: The purpose of the present study was to evaluate the alterations in serum levels of VEGF in severe scald burn injury in rats, and how sildenafil affects levels of VEGF.

Materials and Methods: Twenty-four rats were subjected to 30% total body surface area severe scald injury (except control group)and were randomly divided into three equal groups as follows: Control, sham, and 10 mg/kg sildenafil groups. Levels of malondialdehyde (MDA), vascular endothelial growth factor (VEGF), VEGF receptor (Flt-1) and activities of glutathione peroxidase (Gpx) and catalase (Cat), levels of total antioxidative capacity (TAC), and total oxidant status (TOS) were measured in serum.

Results: There were no differences between groups in terms of VEGF and Flt-1 levels. However, there was a significant difference between groups in terms of ratio of Flt-1/ VEGF levels. Sildenafil showed beneficial effects in oxidative balance.

Conclusions: Our investigations implicate, that the alteration in Flt-1/VEGF has the potency to play role in remote organ injury.

Key words: Burn, Vascular Endothelial Growth Factor, Inflammation, Angiogenesis.

Introduction

A severe burn is associated with release of inflammatory mediators which ultimately cause local and distant pathophysiological effects. Mediators including Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are increased in affected tissue, which are implicated in pathophysiological events observed in burn patients [1, 2].The response to the initial burn is often associated with secondary damage to vital organs such as, lung, liver, and kidneys that are distant from the injured site [3-6]. The pathophysiological mechanism of such tissue injury remains unclear. However, animal models and clinical trials of burn injury implicate that reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated by elevated pro-inflammatory mediators can act as causative agents in development of distant organ injury [1, 7-9].

Inflammation has been defined as a process induced by injury that normally leads to healing and is an essential component of physiological and / or pathological angiogenesis in most organs. Inflammatory cells surrounding the microvasculature can have a profound effect on promoting new vessel growth via vascular endothelial growth factor (VEGF) and VEGF receptors such as VEGF Receptor-1 (Flt-1) [10-12]. However, the roles of VEGF and VEGF decoy receptor Flt-1 in pathophysiological events, such as oxidative and nitrosative damage secondary to inflammation, are still in the area of active research [10, 12-16].

Sildenafil is a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase-5 (PDE-5). The cyclic nucleotide cGMP is a second messenger that plays major roles in various cellular processes, like inflammation [17]. Sildenafil has a relaxant effect on smooth muscle cells of the arterioles and via nitric

oxide (NO)-dependent mechanism and may induce blocking of VEGF activity by a neutralized antibody against VEGF receptors as well as augment angiogenesis [18]. However, most of these effects appear to be dosage dependent due to the levels of generated NO [13].

The purpose of the present study was to evaluate the alterations in serum levels of VEGF in severe scald burn injury in rats, and how sildenafil affects levels of VEGF.

Materials and Methods

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and approval has been received from the Institutional Animal Ethics Committee at Cumhuriyet University.

Animals

A total of 24 adult female Wistar Albino rats weighing between 200 and 250 g were included in the study. Animals were provided by the Experimental Animals Center, Cumhuriyet University, Sivas, Turkey. The animals were fed *ad libitum* with standard diet and water throughout the experiment. All animals were housed separately and kept under standard conditions of room temperature (22-24 °C) and a 12 h light/12 h dark cycle.

Burn Procedure

Animals were anesthetized with (i.p.) xylazine (5 mg/kg) and ketamine (30 mg/kg) during the scalding and burn procedure, and 1 mg/kg morphine was administered intra-muscular just before immersing each of them to the boiling water. The dorsal surfaces of the rats were shaved closely, and the rats were secured in a constructed template device. The surface area of the skin on the dorsal surface exposed through the template device was immersed in 98 °C water for 12 s. All test animals were quickly dried after each exposure to avoid additional injury. With the use of this technique, full-thickness dermal burns comprising 30% of the total body surface area (TBSA) were obtained [2, 19].

Chemicals

All the chemicals used in experiments were purchased from Sigma Chemical Co. (Munich, Germany). Sildenafil was obtained from Pfizer (Istanbul, Turkey).

Experimental Design

Animals were randomly divided into three equal groups as follows: Group I (control); no burn or no treatment was adminsitred; Group II (scald- sham) was administrated perorally (p.o.) 2 ml 0.09% NaCl, and group III, 10 mg/kg p.o. sildenafil, just after the scald burn injury. All animals (except group I) were administered 2 ml/100 g body weight of lactated Ringer's solution subcutaneously just after the burn injury for fluid resuscitation. Then, all animals were placed in their own cages and set free to reach food and water. The reason for the selection of 10 mg/kg dosage of oral sildenafil was that 10 mg/kg/day of sildenafil would result approximately in the same plasma concentration as 50 mg in humans [20]. All animals were sacrificed at the24th hour via an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). Blood samples from the all animals were collected in tubes for biochemical analysis.

Levels of malondialdehyde (MDA), VEGF, Flt-1, the activities of glutathione peroxidase (Gpx) and catalase (Cat), levels of total antioxidative capacity (TAC), and total oxidant status were measured in serum. Also, oxidative stress index (OSI) was calculated in serum.

Biochemical Investigation of Serum

Bloods were collected without using an anticoagulant, and then were allowed to clot for 30 minutes at 25°C. Later on, blood samples were centrifuged at 2,000 x g for 15 minutes at 4°C, and serum layers were pipetted off without disturbing the white buffy layers. Subsequently, serum was stored on ice and samples were frozen at -80°C.

Measurement of MDA, VEGF, FLT-1,GPX, and CAT

All analyses were made for each parameter according to the protocols of each kit manufacturer's requirements.

As an index of lipid peroxidation and free radical generation, MDA content in serum was mea-

sured by the MDA-586 method using a Bioxytech MDA-586 assay kit (Oxis Research, Oregon, USA), [21]. Protein concentration was determined with the Beckman Protein Assay on a Synchron® lx ٢٠ analyzer (Beckman Coulter, ٩٥٩٤٢ Villepointe–Roissy-CDG, France) using BSA as a standard. VEGF and Flt-1were measured in serum in order to identify how it's level changes in burn, and also to in order to identify effects of sildenafil in vascular permeability as well as angiogenesis and inflammation. The ratio of Flt-1/ VEGF was calculated in order to identify how it's level changes in burn. Concentrations of VEGF and Flt-1were measured using two ELISA kits (RayBiotech. Inc., Norcross, GA, USA and Cusabio Biotech Co., Wuhan, Hubei Province, China). Values were calculated and converted to picograms per milliliter for serum (pg/ml) [14, 15]. Gpx was measured as a marker of enzymatic defense against ROS. Gpx activity in serum was measured spectrophotometrically using Cayman's standard glutathione (GSH) assay kit [22]. Cat activity was measured as a marker of enzymatic defense against ROS. Cat activity in serum was measured spectrophotometrically with the Cayman's standard Cat assay kit [23].

Measurement of TOS, TAC, and OSI

TOS and TAC levels were measured using a spectrophotometric kit (Rel Assay Diagnostics, Gaziantep, Turkey) [24, 25]. Levels of TOS and TAC were assayed in an autoanalyzer (Beckman Coulter LX 20, Inc., Fullerton, CA, USA). Results of serum levels were expressed as millimolar Trolox (Rel Assay Diagnostics) equivalent per liter (L). The ratio of TOS to TAC was accepted as the OSI.

Statistical analysis

The Statistical Package for the Social Sciences for Windows 14 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All values were given as median and min-max. Anova test was used for multiple comparisons. Mann-Whitney U test with Tukey correction was used for dual comparisons between groups. Statistical significance was accepted as $p<0.05$.

Results

No mortality was detected during the study period.

Levels of VEGF, Flt-1, and Flt-1/ VEGF in groups

Levels of VEGF, Flt-1, and Flt-1/ VEGF in serum were detailed in Table 1 and Figure 1. There were no differences between groups in terms of VEGF and Flt-1 levels. However, there was a significant difference between groups in terms of Flt-1/ VEGF levels (p=0.014). The Flt-1/ VEGF levels were significantly higher in group II (p = 0.25) than in group I (p = 0.025) and in group III (p = 0.029).

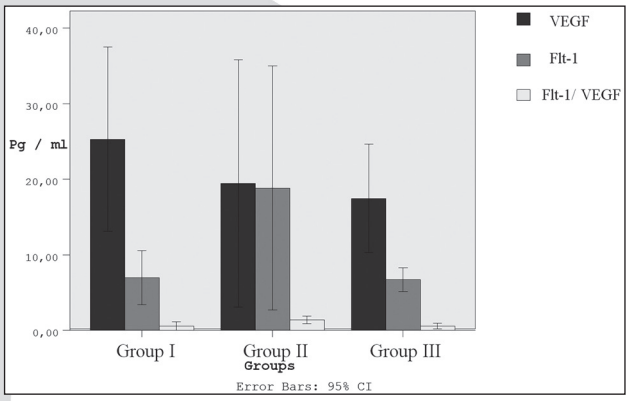

Figure 1. Levels of VEGF, Flt-1, and Flt-1/VEGF

Levels of MDA, Gpx, and Cat in serum

Levels of MDA, Gpx, and Cat in serum were detailed in table 2. The lowest MDA levels were detected in group I with a mean±SD value of 1.32±0.24 pmol/ml whereas the highest MDA levels were detected in group II 2.59±0.46 pmol/ml. There was a statistically significant difference

Table 1. Levels of VEGF, Flt-1, and Flt-1/VEGF in serum in rats

	Group I (n=8)	Group II (n=8)	Group III (n=8)	p Value
VEGF (pg/L)	26.59 (5.51-45.13)	9.78 (3.62-54.72)	19.77 (5.42-29.28)	0.560
Flt-1 (pg/L)	6.12 (2.68-14.86)	7.73 (5.92-58.19)	6.97 (3.97-9.21)	0.075
Flt-1/VEGF(pg/L)	0.16 (0.10-1.96)[b]	1.46 (0.16-2.20) [a, c]	0.37 (0.14-1.34)[b]	**0.014**

Results are median (min-max). n, number of rats.
[a] Significantly different when compared to Group I.
[b] Significantly different when compared to Group II.
[c] Significantly different when compared to Group III

between group I (p= 0.001) and group II, also a significant difference was found between group I (p=0.002) and group III whereas a similar significant difference was detected between group II (0.005) and group III. The highest levels of Gpx were detected in group I with a mean±SD value of 8.04±6.81 (nmol/min/ml) whereas the lowest levels were in group II 1.66±1.08 (nmol/min/ml). This decrease rate in group II was statistically different when compared to group I (p=0.028); also, Gpx levels were significantly higher in group III (p=0.038) than in group II; however, no difference was detected between group I and group III. Cat levels were significantly lower in group II (p=0.047) and in group I. There was no difference between other groups in dual comparisons. Although, the Cat levels detected higher in group III when compared to group II, it was far from a statistical significance.

Levels of TAC, TOS, and OSI in serum

There were no differences between groups in terms of TAC levels in both multiple comparisons, and dual comparisons. TOS and OSI levels were detected significantly higher in group II when compared group I (p=0.040, p=0.024). Also, OSI levels were detected significantly higher in group II when compared group III (p=0.041), whereas no difference was detecteted between group II and group III in terms of OSI levels.

Discussion

Burn injury induces the local and systemic release of different pro-inflammatory mediators. They play an important role in local tissue damage, systemic inflammatory response syndrome (SIRS), and multiorgan dysfunction syndrome (MODS). A central problem after burn injury remains the local and general tissue edema, especially during the first day, and later during the phase of wound healing and tissue repair [26]. Severe burn induces toxic mediators -such as ROS and RNS- that lead to lipid peroxidation, which may have a pivotal role in remote organ injury [2, 27, 28].

VEGF is one of the most potent mediators of vascular regulation in angiogenesis and vascular permeability [29], and may also have a role in inflammation [30, 31]; moreover, NO and VEGF may interact to promote angiogenesis [32, 33]. In animal models it has been shown that therapeutic application of VEGF had side effects and induced exacerbation or initiation of local edema and inflammatory reactions [34, 35] .

However, the results showed that a high concentration of NO donors downregulates VEGF expression in endothelial cells [33]. On the other hand, previous studies show endogenous NO enhances VEGF synthesis [18, 32, 33]. NO is known as an inducer of VEGF synthesis under normoxia. However, why NO shows conflicting effects on

Table 2. Levels of MDA, Gpx, and Cat in serum

	Group I, (n=8)	Group II, (n=8)	Group III, (n=8)	p Value
MDA (pmol/ml)	1.33 (0.99-1.63) [b, c]	2.59 (1.94-3.25) [a, c]	1.94 (1.74-2.47) [a, b]	**0.001**
GPx (nmol/min/ml)	7.13 (0.10-17.57) [b]	2.80 (0.10-8.15) [a,c]	2.94 (0.10-15.28) [b]	**0.017**
Cat (nmol/min/ml)	2.12 (0.47-3.91) [b]	0.87 (0.44-2.55) [a]	1.26 (0.43-3.91)	**0.033**

Results are median (min-max). n, number of rats.
[a] Significantly different when compared to Group I
[b] Significantly different when compared to Group II
[c] Significantly different when compared to Group III

Table 3. Levels of TAS, TOS, and OSI in serum

	Group I, (n=8)	Group II, (n=8)	Group III, (n=8)	p Value
TAC*	1.25 (1.02-1.51)	1.17 (0.94-1.46)	1.23 (1.05-1.96)	0.382
TOS*	18.70 (8.53-36.42)[b]	30.52 (14.45-36.42)[a]	19.60 (10.01-33.76)	0.039
OSI **	14.53 (5,64-29.13)[b]	25.57 (15.37-37.16)[a,c]	17.55 (4.58-23.44)[b]	0.016

** mmol Trolox quiv/L , ** Arbitrary Units*
Results are median (min-max). n, number of rats.
[a] Significantly different when compared to Group I.
[b] Significantly different when compared to Group II.
[c] Significantly different when compared to Group III

VEGF is still unclear. An optimal amount of NO may upregulate the VEGF in limited cell lines while an excessive amount of NO inhibits the VEGF expression through an unidentified pathway [13]. All this information reveals that VEGF governs the controlled and regulated phenomenon of angiogenesis, whereas it also may have a role in inflammation depended remote organ injury.

Flt-1 is known as a VEGF decoy receptor, serving to spatially control VEGF signaling and formation of angiogenic sprouts and in addition to its negative regulatory role in vascular development, Flt-1 is important in mounting an inflammatory response and inflammation-associated angiogenesis (denoted 'pathological angiogenesis') through recruitment of bone marrow- derived myelomonocytic cells followed by deposition of angiogenic growth factors [15].

Increase of Gpx and Cat enzyme activities following burn related injury protects tissues from the effects of free radicals and lipid peroxidation. TOS and TAC parameters instead of individual oxidant and antioxidant compounds such as MDA, Gpx, and Cat which act in combination with each other may reflect the total effect of oxidant and antioxidant balance in tissues and serum levels. The definition of oxidative stress index (OSI) shows either increased oxidant production or a decreased antioxidant capacity in cells characterized by the release of free radicals, resulting in cellular degeneration which reflects TOS vs TAC ratio [24, 25, 36].

In the present study, we specified that severe burn injury altered the ratio of Flt-1/VEGF, which lead us to assume that remote organ injury secondary to severe burn injury may be dependent upon this alteration. Also, the alteration in oxidative balance may have a supportive effect in this situation. Additionally; we specified that sildenafil may have protective effects against severe burn- induced remote organ injury, via decreasing oxidative and nitrosative stress, as confirmed by biochemical assays in serum. We presented fairly, by our findings that treatment with sildenafil decreased MDA and OSI.

Despite the clinical importance of tissue edema in the early and later stage of healing, less is known about the kinetics of ratio Flt-1/VEGF in burn injuries. Our investigations implicate, that the alteration in Flt-1/VEGF has the potency to play role in remote organ injury.

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Tripe palms and digital clubbing associated with metastatic lung adenocarcinoma - Case report and review of literature

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Abstract

Background: Tripe palms is a rare cutaneous paraneoplastic syndrome usually associated with internal malignancy. It refers to a characteristic velvety thickening of the palms, with the exaggeration of normal skin markings and often preceding a new or recurrent tumor.

Case report: We presented a 49-year old woman with adenocarcinoma of the lung in which tripe palms and digital clubbing were associated with the occurrence of metastases in the left suprarenal gland.

Conclusion: All patients with tripe palms, due to their strict paraneoplastic nature, should be evaluated for associated malignancy, particularly lung or gastric carcinoma.

Key words: Paraneoplastic dermatoses, lung carcinoma.

Introduction

Tripe palms or pachydermatoglyphy refers to an acquired palmar keratoderma with enhancement of normal dermatoglyphics. Typically, the texture is velvety or moss-like. It is a distinctive paraneoplastic cutaneous sign and rarely appears as an isolated finding. Reportedly, 77% of cases with tripe palms are associated with malignant acanthosis nigricans. More than 90% of reported cases with tripe palms alone are associated with an underlying malignancy, especially with carcinomas of the lung and stomach (1).

We present tripe palms, a rare cutaneous paraneoplastic sign and digital clubbing in a patient with lung adenocarcinoma.

Case report

A 49-year old woman with 8 month history of fatigue, coughing, loss of weight and pain in the left chest was seen for the evaluation of a expansive lesion in the left lobe that was detected on a chest radiography. The lesion extended from the left lobe base to the Nelson`s segment (Figure 1.). Computed tomography of the chest confirmed the mass in the left lobe and detected micronodular lesions in the upper part of the right lobe. There was no axillary, hilar or mediastinal lymphadenopathy. A metastatic 12 mm lesion in the left suprarenal gland was detected on computed tomography of the abdomen. Fine-needle aspiration of the tumor showed adenopapilar bronchogenic adenocarcinoma.

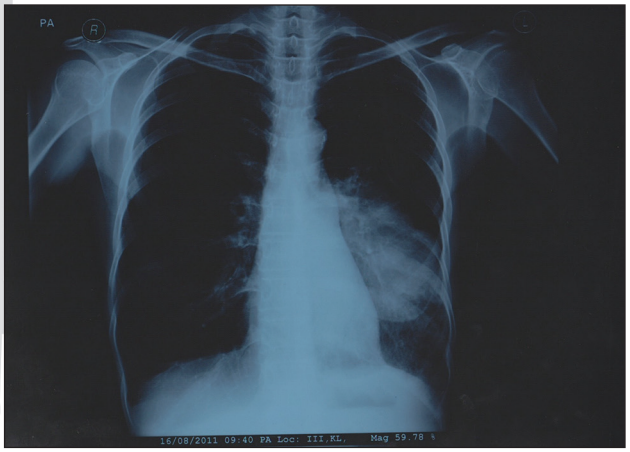

Figure 1. PA chest radiograph revealed tumor mass in left lung

The patient had smoked 35-50 cigarettes a day for the last 30 years. Approximately 4 months ago she noticed palmar lesions that were accompanied by the appearance of pain in her left lumbal and abdominal region. Family history was unremarkable.

Cutaneous examination revealed diffuse thickening of the palms with accentuation of the ridges and furrows (Figure 2). Digital clubbing was also present (Figure 3). Acanthosis nigricans, or other paraneoplastic cutaneous signs were absent.

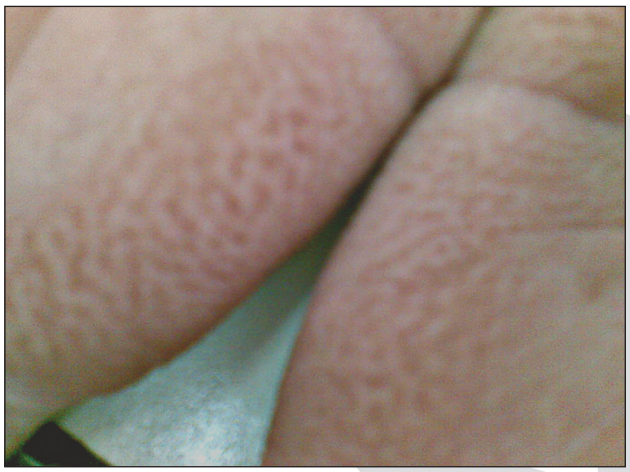

Figure 2. Tripe palms (accentuation of the ridges and furrows

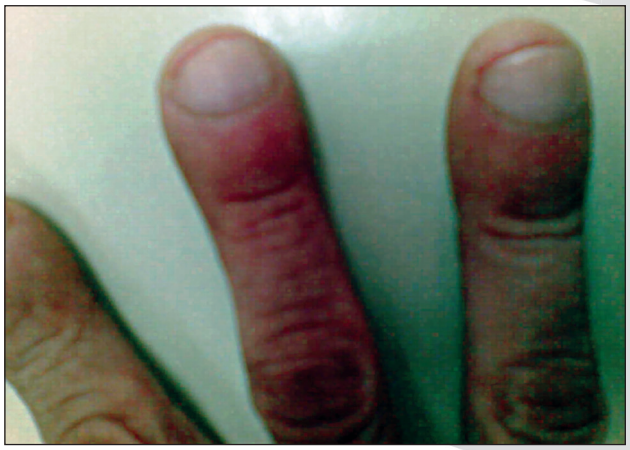

Figure 3. Clubbed appearance of distal phalanges

Discussion

Tripe palms is a rare paraneoplastic dermatosis. The term tripe palms was introduced in 1977 by Clarke and later made popular by Breathnach and Wells, who classified it as a form of palmar acanthosis (1). The distribution and morphology of the lesions in patients with tripe palms vary. Palmar changes can be diffuse or focal in distribution (2). The changes are more prominent over pressures areas, like thenar, hypothenar eminence and fingertrips, with accentuation of the ridges and furrows. Approximately a hundred patients with tripe palms have been described in the literature. The majority of published cases of tripe palms occured in patients with cancer; only five patients showed no evidence of an associated malignancy (1).

Tripe palms is often associated with other cutaneous paraneoplastic syndrome such as acanthosis nigricans, acrokeratosis paraneoplastica, bullous pemphigoid, florid cutaneous papillomatosis, hypertrichosis lanuginosa acquisita, hypertrophic pulmonary osteoarthropathy, paraneoplastic pemphigus (3). In cancer patients with tripe palms alone, the most common underlying neoplasm was pulmonary carcinoma (53% of cases) of squamous cell origin. Patients with both tripe palms and acanthosis nigricans frequently had gastric (35% of cases) or pulmonary (11% of cases) carcinomas (1). Rarely, a sign of Leser-Trelat may be associated with tripe palms and acanthosis nigricans. The association of these three cutaneous paraneoplastic signs have been recently presented in patients with ovarian and gastric cancers (4, 5). Khaled A et al, presented tripe palms associated with oligoarthritis as two rare paraneoplastic syndromes heralding a small cell lung cancer (6). Clubbing of digits, as observed in our patient, was noted in 14% of the patients with malignancy-associated tripe palms (7). Bladder (5%), breast (4%), cervical (4%), ovarian (4%) and renal (4%) carcinomas were also presented in association with tripe palms (1, 8, 9).

Tripe palms may be found prior to or at the time of the diagnosis of primary malignancy or, in some cases, later. Cohen et al. found that tripe palms occurs with carcinoma in 17%, before the diagnosis of carcinoma in 42%, and after the diagnosis in 23% cases (1). Some authors have noted that the appearance of tripe palms in a known cancer patient may be a sign of either recurrence or metastasis of the tumor (3). In our patient tripe palms occurred simultaneously with diagnosed metastasis in the left suprarenal gland.

Only 12 patients in the literature have shown an association with various benign conditions like bullous pemphigoid, benign hepatic neoplasia and systemic mastocytosis (10, 11, 12) .

The pathogenesis of cutaneous paraneoplastic syndromes is still under discussion. Since many of these syndrome, including tripe palms, acanthosis nigricans, Leser-Trelat syndrome are proliferative skin disorders, it is believed that products secreted by the tumor stimulate the keratinocytes to prolifer-

ate. High EGF levels were found in a patient with tripe palms (13). TGF-α also represents a key cytokine able to mediate epidermal cell proliferation. These stimulatory growth factors could reach a critical threshold and activate the EGF- receptor or insulin-like a growth factor receptor. The EGF-receptor is the common ligand for TGFα and EGF. Experimentally, TGFα has been synthesized by many tumor cells derived from solid tumours and all of these cells also produced the EGF-receptor mRNA (14).

The clinical appearance of idiopathic and malignant-associated tripe palms is similar. Histological examination of skin biopsies taken from tripe palms is not specific and invariably showed hyperkeratosis and acanthosis. Papillomatosis, dermal mucinosis and increased dermal mast cells, were also reported in some specimens (1, 8).

Conclusion

We want to point out, that all patients with tripe palms should be evaluated with a full diagnostic work-up for an underlying malignancy, particularly lung or gastric carcinoma.

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Laryngeal vascular malformation with atypical localization in an adult patient: Case report

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Abstract

Vascular lesions are rare anomalies of the larynx. Although all laryngeal vascular lesions in the literature have been evaluated under the title of hemangioma in general, lesions in adult and pediatric patients exhibit different characteristics. In this case report, a laryngeal vascular malformation with atypical localization in a 60-year-old female patient has been presented, underlining the discrepancies with similar lesions observed in childhood.

Key words: Larynx, Malformation, Hemangioma.

Introduction

Vascular lesions are the most common congenital anomalies observed in the head and neck area. However, vascular lesions localized in the larynx are regarded as rare anomalies, comprising only 1.5% of all congenital laryngeal pathologies (1). Laryngeal vascular lesions (LVL) are evaluated in two groups, namely lesions observed in adults and those observed in childhood (2). In childhood, these lesions are generally regarded as hemangioma. Similarly, most of the LVLs found in adults are also evaluated as hemangiomas in the literature; however, these lesions exhibit distinct variations from laryngeal hemangiomas in childhood in terms of incidence, gender, histology, localization, symptoms and treatment approaches. In this case report, a laryngeal vascular lesion with atypical localization found in a 60-year-old patient has been presented and variations from laryngeal hemangioma cases in childhood have been underlined.

Case

A sixty-year-old female patient presented to our outpatient clinic with long persisting symptoms of sensation of a foreign body in the throat, occasional hoarseness and sore throat of mild severity. Endoscopic examination of the larynx revealed a lobulated red-purple submucosal mass with irregular borders, filling the interarytenoid area, the right postcricoid area and the posterior region of the right sinus piriformis (Figure 1 and 2). Vocal cord movements were not affected by the mass. The remaining structures of the larynx had a normal appearance. Since the mass was considered to be a vascular lesion, no biopsy was performed. Oral, oropharyngeal, nasopharyngeal and nasal examination of the patient revealed no pathology. No additional mass was detected in the head and neck region. The patient was a non-smoker and did not consume alcohol. Medical history revealed no systemic disease, no continuous drug use and no history of trauma or surgical operation. The family history did not reveal any specific characteristics either. On laboratory examination, values related to the parameters of blood were within normal limits. On magnetic resonance imaging (MRI), the mass was seen to be hypodense in the posterior of the arytenoid on T1-weighted images, appeared as hyperdense on T2-weighted images, and exhibited an isodense contrast with muscle (Figure 3). The patient was diagnosed with laryngeal vascular pathology (cavernous malformation). Due to localization of the lesion and non-existence of any symptoms limiting the daily activities of the patient, no surgical intervention was performed. The patient was requested to comply with follow-up visits for regular endoscopic laryngeal examination as long as there was no change in the symptoms. The patient did not report any symptoms at the 6th month and 1st year visits; similarly, no change was detected in the shape and dimensions of the lesion on endoscopic examination of the larynx.

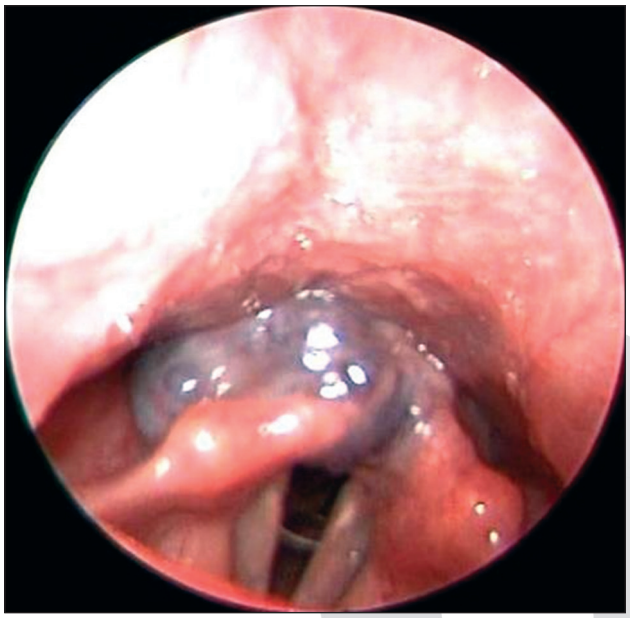

Figure 1. Vascular lesion in phonation, at the posterior of the left arytenoid and in the interarytenoid region

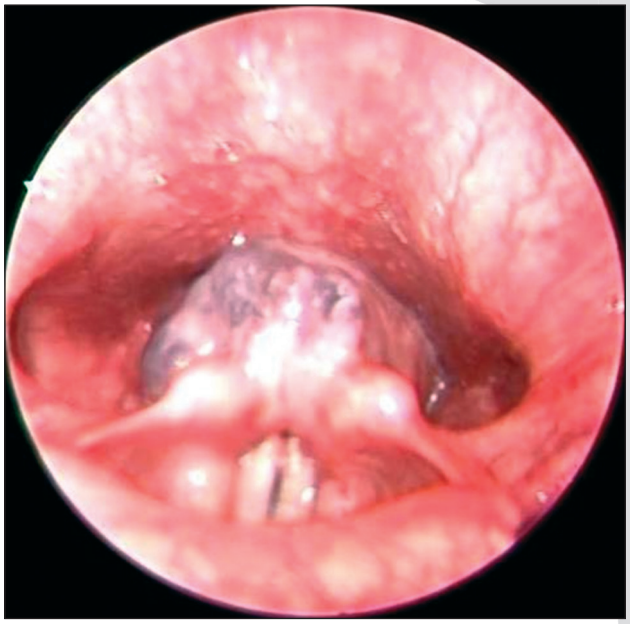

Figure 2. Appearance of the lesion during respiration

Discussion

Laryngeal vascular lesions were first described in 1864, but different characteristics of laryngeal vascular lesions in adulthood compared to pediatric cases were first reported by Sweetser in 1921 (3). The etiopathogenesis of vascular anomalies are still controversial. Similar controversies are observed in the classification of lesions and nomenclature of lesions in the literature. In 1982, Mulliken and Glowacki defined the most widely accepted classification of vascular lesions (4). The current classification of the "International Society for the Study of Vascular Anomalies" is based on this widely accepted classification (Table 1) (5). According to this classification, vascular lesions are evaluated in two groups as hemangiomas and malformations.

In the literature, most of the vascular lesions detected in childhood are infantile and in rare cases, congenital laryngeal hemangiomas. However, vascular laryngeal lesions found in adults are generally regarded as malformations and exhibit a histologically diverse structure. Histologically, hemangiomas are characterized by high endothelial cell cycle, while the clinical presentation comprises proliferative, plateau and involution phases (6). On the other hand, malformation has a normal endothelial cell cycle and is characterized by abnormal gross vascular anatomy with unvariable clinical phases (4). In the current case, sampling for histological evaluations was not performed in order to avoid bothersome complications.

LVL is frequently seen after the first 2 months and during the first year of life in 80-90% of the cases. The rate of LVL is decreased after the 30th month of life (2). Furthermore, in contrast to children, these lesions do not exhibit a proliferation phase in adulthood and they may be seen at every age of life (2,7). The current case was a 60-year-

Table 1. Classification recommended by "International Society for the Study of Vascular Anomalies" (5)

Tumors	**Malformations**			
- Infantile hemangioma - Congenital hemangioma - Pyogenic granuloma - Tufted angioma - Kaposiform hemangioendothelioma - Hemangiopericytoma	Simple		Mixed	
	Slow-flow Capillary Venous Lymphatic	Fast-flow Arterial	Slow-flow LVM CLVM	Fast-flow AVM CAVM

LVM: Lymphatic-venous malformation, CLVM: Capillary-venous malformation
AVM: Arterio-venous malformation, CAVM: Capillary arterio-venous malformation

old female patient and no change was observed in the course of the lesion during the follow-up. In pediatric cases, the incidence of hemangiomas are 3-4 fold more common in girls compared to boys (4), while LVLs are seen 2-fold more frequent in girls (2). On the other hand, a limited number of publications report a more common occurrence of laryngeal vascular lesions in adult male patients, most probably due to etiological factors (7,8). Due to the low number of adult patients in publications, it is difficult to prove the gender dominance. Besides, no difference was found in vascular malformations in terms of gender (4). The current case was a 60-year-old female patient.

LVL in children generally presents in the form of hemangiomas. In previous classifications, these hemangiomas were histologically regarded as capillary vascular lesions in most cases (2,9). In adult patients, LVLs generally present in the form of malformations with a cavernous vascular structure on histological examination (2,7). Cavernous vascular lesions are said to develop mainly from capillary vascular structures (2,7). In our case, a mildly protuberated light purple lesion covered with a thin layer of mucosa and pedicle supported the diagnosis of a cavernous vascular malformation of the venous structure. Since biopsy in a vascular structure may lead to serious adverse effects, no histopathological assessment was carried out.

A number of causes have been put forth for the etiology of hemangiomas in children. These may be summarized as hemangiomas developing from angioplasts comprising the placenta or emboli from placental cells, due to molecular or genetic developmental defects associated with angiopoetin and VEGF tyrosine kinase at the 6^{th}-10^{th} weeks of gestation, or due to somatic mutations in genes controlling the development of vessels (6). In several trials, environmental factors such as smoking, alcohol, laryngeal trauma and voice abuse have been blamed for the etiology of LVLs in adults; hence LVLs have been underlined to be more common in male patients (2,8,10). The current case was a female patient and did not possess any of the above-mentioned predisposing factors. We believe that vascular lesions detected in adulthood may be non-involuting capillary-type vascular malformations observed in childhood. These vascular lesions may transform into a cavernous form in time. The clinical characteristics of LVLs in adults also support this hypothesis.

Laryngeal hemangiomas in children are generally localized in subglottic regions and concurrent vascular skin lesions may be found in 50% of the cases (11). Skin lesions are typically localized in the beard area (trigeminal nerve area) (6). Symptoms usually follow a severe course with dyspnea, biphasic stridor and dysphagia. In particular, in cases with increased venous congestion as in crying spells, the symptoms may exacerbate to life threatening levels (6,12,13). LVLs in adults are generally localized in the supraglottic regions (2). In rare cases, they may be found in the interarytenoid region, postcricoid region and the vocal cords (7,10). In most cases, skin lesions or additional anomalies do not accompany the lesions. Symptoms generally follow a milder course with hoarseness, sensation of foreign body in the throat, cough, dysphagia and in rare cases, hemoptysis and dyspnea (7,8,10). Localization of the lesion in the current case was a rare localization for LVL, with the lesion being located in the posterior of the interarytenoid, postcricoid and right sinus piriformis. Since the lesion did not constrict the glottic area and did not affect cord movements, the symptoms followed a mild course. These findings were in compliance with the literature in general.

The diagnosis of LVL is generally based on anamnesis and endoscopic examination. Since the symptoms are more prominent in pediatric patients, the diagnosis is more readily confirmed compared to adult patients. Misdiagnosis and mistreatment are inevitable in some pediatric cases due to stridor and cough, and in a few of the adult cases with mild subjective symptoms. Imaging techniques such as Doppler ultrasonography, computerized tomography with dynamic contrast and magnetic resonance may be beneficial in the diagnosis of these patients, as well as in estimating the spread of the lesion and in determining the relation with surrounding vascular structures (14). In the current case, the lesion was diagnosed by indirect endoscopic examination of the larynx, and spreading of the lesion was detected by computerized tomography with contrast.

There is no consensus on the treatment of LVLs. Treatment is planned according to the age of the patient, localization of the lesion, histological type and dimension of the lesion and severity of symptoms.

In pediatric cases, lesions generally develop after the 2nd month of life, except in cases of congenital forms of hemangiomas, and show proliferation until the 12th-24th months. After this period, lesions enter an involution phase in general, exhibiting a major amount of involution until the 30th month (1,2,4,6,13). Non-involuting pediatric laryngeal hemangiomas, which pose a risk in terms of airway obstruction and lead to complications like bleeding and thromboembolism, may be treated by various approaches such as medical treatment (corticosteroid, interferon, propranolol), surgical intervention (laser, tracheostomy, cryotherapy), radiotherapy and embolization (6,12,13). Currently, the most preferred methods are propranolol, vaporization with CO_2 laser, surgical excision and tracheostomy (12). In symptomatic adult patients, lesion-oriented treatment may be utilized, while non-symptomatic patients may be followed up with endoscopic examination (2). In the management, small lesions may be treated by vaporization with CO_2 laser, while larger lesions may be treated by vaporization with CO_2 laser, embolization, surgical excision and tracheostomy (2,7,8). In the current case, the lesion was not symptomatic and localization carried a risk of serious complications in terms of excision; therefore, the patient was requested to comply with regular follow-up with endoscopic examination and no change was observed in the lesion during a follow-up period of 9 months.

In conclusion, LVLs in adult patients are rare lesions of the larynx, exhibiting different clinical characteristics compared to laryngeal hemangiomas observed in pediatric cases. Therefore, LVLs in adults should be evaluated under the heading of vascular malformations, unlike pediatric cases. In the management, follow-up with endoscopic examination may be recommended in non-symptomatic patients.

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A case of lymphoid tissue amyloidosis of neck and literature review

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Abstract

Objective: To review clinical features and pathogenesis of primary systemic amyloidosis with the analysis of a recent case of primary lymphoid tissue amyloidosis of neck in our hospital.

Methods: Analyzing patient cases and reviewing literature.

Results: The pathogenesis of this disease is still not clear. The primary characteristic of this disease includes infiltration of multiple organs, such as heart, kidney, gastrointestinal tract, skin. The diagnosis on this disease is based on histopathological and immunohistochemical examination and there's no good therapeutics on this disease yet. We have not seen any similar case reported in China and other countries.

Conclusion: Early diagnosis and early treatment to this disease may improve the survival opportunity, and it is shown from this case that the amyloidosis may also exist in the lymphoid tissues.

Key words: Systemic, lymphoid tissue, amyloidosis.

Introduction

Amyloidosis is a disorder in which variant protein is formed by amyloid fibrils accumulation in the extracellular tissue or organ [1]. It is a kind of the protein conformational disease, and a specific protein conformation change in the pathogenesis process plays an important role. The process of abnormal protein conformation and the physiological protein folding process can happen simultaneously, causing the soluble protein in normal circumstances changed into insoluble pathogenicity protein, which is formed into a beam of deposition in tissues with β lamellar structure in the form of fibrin. Insoluble pathogenicity protein affects the normal function of cells and tissues, and gradually replaces the normal structure; eventually leads to tissue damage and organ failure. Clinical manifestations are often complex and severe systemic symptoms are observed of the pathological process. Due to its rarity and delitescence, this disease is often misdiagnosed, and primary systemic amyloidosis of lymphoid tissue is even rarer. In the rest of this paper, we will discuss this disease with a systemic lymphoid tissue amyloidosis case in our hospital.

Case Report

Patient

Female, 68 years old, with generalized lymphadenopathy for 1 year, heavily stuffed nose, sniveling, breathing with mouth during sleep, intermittent epistaxis for six months. She was hospitalized on November 4 2008. The patient developed generalized lymphadenopathy one year ago, especially in neck. There was no local pain and fever. With the biopsy to neck lymph node, the disease was diagnosed as "lymph node amyloidosis" in the People's Liberation Army 301 Hospital. Half a year later, the symptoms increased with stuffed nose, snivel, mouth breathing in sleep, intermittent epistaxis, and nasopharyngeal lymphoid tissue hyperplasia. Examination: Lymph nodes can be touched in bilateral neck, armpits, and groin intumescent. The biggest is around 5 × 4 cm. Bilateral tonsil enlargement is in degree II with smooth surface. Laryngoscopy report: pharynx nasalis filled with lymphoid tissue down to the level of the soft palate (Figure 1). Nasopharyngeal tumor biopsy: mucosal chronic inflammation with organizational amyloidosis. Clinical diagnosis: nasopharyngeal and oropharyngeal tumor, generalized lymphadenopathy of unknown origin.

After general physical checkup and conventional chemical examination, no surgical contraindication was found. Nasopharyngeal CT: pharynx nasalis filled with soft tissue, bilateral neck lymph nodes intumesced. In order to resolve the symptoms of stuffed nose, mouth breathing in sleep, we performed surgery to remove the nasopharyngeal

tumor and tonsil with sinoscope on November 6th 2008. In the surgery we adopted tracheal intubation anesthesia, ventilator-assisted breathing, and carefully examined the nasopharyngeal tumors with sinoscope. The tumor was with smooth surface, filled the nasopharynx and intruded into bilateral posterior naris. We hold some tumor for pathology which was fragile like flour under polypus forceps (Figure 2), then removed most nasopharyngeal tumor by the cutter, and also cut the tonsils which were like flour with the tonsillectome; stopped bleeding and filled nasal cavity with formyl triiodide carbasus. The patient turned to be better after surgery. Two days later we removed the formyl triiodide carbasus. The patient felt good and was released form the hospital in another six days. Postoperative pathology: <nasopharynx, tonsil>: nasopharyngeal mucosa and tonsil filled with substantial interstitial amyloid deposition, special stains: Congo red (+), Methyl Violet (+), which matches the preoperative diagnosis (Figure 3).

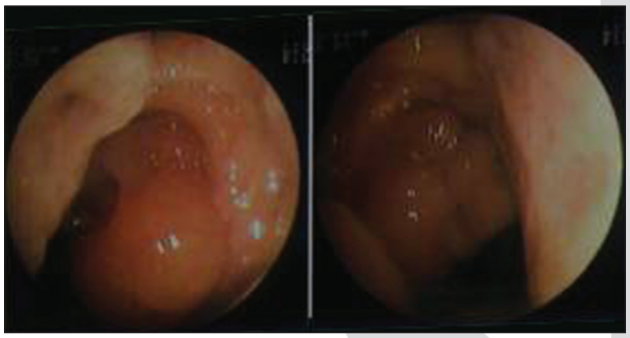

Figure 1. Laryngoscopy showed that pharynx nasalis filled with lymphoid tissue down to the level of the soft palate

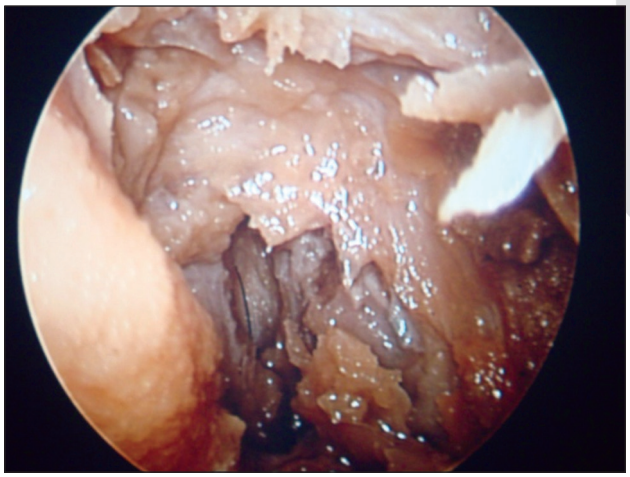

Figure 2. The tumor was fragile like flour under polypus forceps

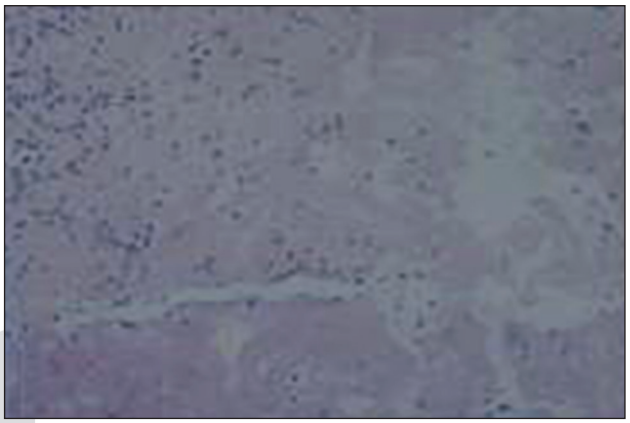

Figure 3. Postoperative pathology: nasopharyngeal mucosa and tonsil filled with substantial interstitial amyloid deposition, special stains: Congo red (+), Methyl Violet (+), which matches the preoperative diagnosis

Discussion

Amyloidosis is a heterogeneous group of diseases characterized by extracellular deposition of proteins that polymerize into fibrils [2]. These disease states are often associated with plasma cell dyscrasias and chronic inflammatory conditions, respectively, and affect the heart, liver, and kidneys most frequently [3]. Amyloidosis has a variety of clinical classification criteria. It can be roughly grouped into four types: 1) immune cells associated amyloidosis; 2) reactive systemic amyloidosis; 3) family hereditary amyloidosis; 4) limitations amyloidosis. From the molecular pathogenesis, the amyloid is primarily composed of a variety of amyloid peptides and additional amyloid components, and its pathogenesis is mainly due to the abnormal folding process of specific protein and the formation of wrong spatial structures. The loss of its biological function leads to disease. Although this precise mechanism about the occurrence of protein misfolding and amyloid formation are not clear so far, it is considered to be related to the changes in protein stability, genetic factors, age factors, environmental factors, hydrolysis of proteins. Amyloidosis, therefore, is not a single disease. It is caused by a number of different pathogenic mechanisms, and the biochemical components of these amyloid proteins are vicissitudinous with the type of clinical pathology. At present, a growing number of scholars have agreed to classify the disease based on the amyloid polypeptide components.

In which organ the amyloid is deposited may be determined by interaction between the heterogeneity of precursor substances and the tissue-specific components. Genetic factors can also impact the amyloid deposition in specific organs, while the role of different amyloid proteins in the same organ can also lead to significantly different prognosis. For human, more than 20 different, unrelated proteins are known to form amyloid fibrils [4]. No matter how the classification is made, whenever a type of amyloid deposition is constantly involved in a local or global systemic organs and tissues, the corresponding symptoms will appear. When the amyloid deposition happens in kidneys, fatigue and weight-loss are often the earliest symptoms, and later there will be substantial proteinuria and progressive renal failure. When the deposition involves the heart, the symptoms usually include restrictive heart disease, progressive congestive heart failure, and frequent unexplained myocardial infarction, which may lead to clinical misdiagnosis, especially when the symptom is typical angina due to cardiac amyloid variability; When deposition involves autonomic nerves and peripheral nerves, the symptoms include symptomatic orthostatic hypotension, gastrointestinal abnormalities power, carpal tunnel syndrome, limb numbness, gloves/sock-like changes; When the deposition involves digest organs, hepatomegaly is a common symptom. With amyloid deposition gradually increased in vital organs, organ failure and death will emerge. With poor prognosis of this disease, there is still no effective treatments. Therapeutic targets currently are focused on the following aspects: reducing the amyloid precursor protein synthesis or decreasing its concentration, inhibiting amyloid formation and extracellular deposition, and promoting the re-absorption of the amyloid. The diagnosis of amyloidosis is based on Congo red staining on tissue biopsy, which leads to apple-green birefringence on polarized microscopy [5]. Under an optical microscope, the primary pathological characteristic of this disease is that there is homogeneous red staining of amyloid deposition between tissues and around blood vessels. With Congo red staining (+) under electron microscopy, the amyloid is lump-shaped with fine non-branching fiber structures forming a beam or a network. From the above-mentioned characteristics, the disease can be diagnosed.

The patient has no long-term history of chronic inflammation, and there is no heart, lung, and abdominal abnormalities. The lymph nodes are enlarged without any apparent systemic lymphadenopathy incentives. Cervical lymph node biopsy and nasopharyngeal, oropharyngeal lymphoid tissue pathology showed there is amyloidosis and hyaline degeneration around the blood vessels, so we considered the disease as lymphoid tissues of amyloidosis of neck. Previous reports and studies about this disease were primarily limited to real organ and skin amyloidosis. The patients can be cured with surgeries and systemic lymphoid tissues amyloidosis of neck is not mentioned. E. KAISERLING and S. KRÖBER found in their studies that patients with systemic amyloidosis have amyloidosis in the large intestine, small intestine, lung, heart, and kidney lymphatic system. Especially in the intestinal tract, lymphatic amyloidosis was seen in the submucosa and lower serosa; and there was a case which had also been confirmed with electron microscope [6]. The case presented in this paper which has substantial systemic lymphoid tissue amyloid, however, is different from the above mentioned cases, making this case particularly valuable. Amyloidosis is a disease caused by the deposition of amyloid polypeptide and additional amyloid components in tissue, and the degeneration of amyloid peptides is the key reason for the disease. This change appeared in the lymphoid tissue may have two reasons, 1) Using the most common type of amyloidosis - immunoglobulin light chain-related amyloidosis (AL)- as an example, the accumulation position of denatured proteins not only depends on the properties of the precursor protein, but also impacted by gene. Therefore, this was a new distribution which were caused by new precursor protein or new genes of known precursor protein; 2) pathology of the disease showed that the degeneration mainly occurred between the tissues and around the blood vessels, and the space between the tissues is filled with amyloid. Since the lymphatic system plays an important role in the interstitial protein re-absorption process, and also participates in the amyloid re-absorption, the systemic lymphoid tissues amyloidosis has become a decompensation result. The scope of the case mentioned in this paper is much broader than those reported in previous studies, and further studies on this case may leads to new evidence for the pathogenesis of this disease.

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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

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Table 1. Page layout description

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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)

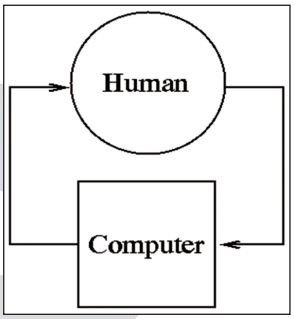

Figure 1. Text here

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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