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The effects of overt hypothyroidism on adipose tissue and serum betatrophin levels

Serum aşikar hipotiroidizmin adipöz dokuda etkileri ve serum betatrophin düzeyleri

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Abstract

Aim: Betatrophin, also known as angiopoietin-like peptide-8, is an adipokine in glycoprotein structure synthesized from adipose tissue and liver. Betatrophin plays a role in fat and energy metabolism by inhibiting lipoprotein lipase, the key enzyme in the hydrolysis of plasma lipoproteins. Although thyroid hormones have an active role in energy metabolism, their relationship with cholesterol and lipid metabolism is not clear. In our study, we aimed to investigate the relationship of serum betatrophin levels with energy and lipid metabolism in patients with overt hypothyroidism.

Methods: This is a case-control study. The mean age of 44 patients (20 males, 24 females) with hypothyroidism was 44.7 (13.8). A total of 40 healthy volunteers, including 19 males and 21 females, were included in the study as a control group. The mean age of healthy volunteers was 44.6 (14.4) years. Fasting blood glucose, AST, ALT, urea, creatinine, TSH, free T3, free T4, HDL-cholesterol, LDL-cholesterol, triglyceride (TG), total cholesterol, anti-TPO, anti-TG, insulin, HOMA-IR levels and serum betatrophin levels were measured by ELISA and compared in both groups.

Results: Serum betatrophin levels were significantly higher in patients diagnosed with hypothyroidism compared to the control group (P=0.001). Serum betatrophin levels were positively correlated with TSH, TG and total cholesterol, and negatively correlated with HDL, free T3 and free T4 levels. There was no significant difference in the comparison of patients regarding anti-TPO levels in the hypothyroidism group (P=0.78).

Conclusion: In our study, we found that serum betatrophin levels were high in hypothyroid patients. This study may be useful in the development of treatments targeting betatrophin in clinical practice.

Keywords: Hypothyroidism, Betatrophin, Lipoprotein lipase

Öz

Amaç: Angiopoietin like peptit-8 olarak da bilinen betatrofin, adipöz doku ve karaciğerden sentezlenen glikoprotein yapısında bir adipokindir. Betatrofin, plazma lipoproteinlerinin hidrolizinde anahtar enzim olan lipoprotein lipaz'ı inhibe ederek yağ ve enerji metabolizmasında rol oynar. Tiroid hormonları enerji metabolizmasında aktif rol almasına rağmen kolesterol ve lipid metabolizmasıyla ilişkisi net değildir. Yaptığımız çalışmada hipotiroidi tanılı hastalarda serum betatrofin düzeylerinin enerji ve lipid metabolizması ile ilişkisini incelemeyi amaçladık.

Yöntemler: Bu bir vaka kontrol çalışmasıdır. Çalışmaya katılan hipotiroidi tanılı 44 hastanın (20 erkek, 24 kadın) yaş ortalaması 44,7 (13,8) idi. 19 Erkek ve 21 kadın olmak üzere toplam 40 sağlıklı gönüllü de kontrol grubu olarak çalışmaya katıldı. Sağlıklı gönüllülerin yaş ortalaması: 44,6 (14,4) yıl idi. Her iki grupta da açlık kan şekeri, AST, ALT, üre, kreatinin, TSH, serbest T3, serbest T4, HDL-kolesterol, LDL-kolesterol, trigliserit (TG), total kolesterol, anti-TPO, anti-TG, insülin, HOMA-IR düzeyleri ve ELISA yöntemiyle serum betatrofin düzeyleri ölçüldü.

Bulgular: Kontrol grubuna kıyasla hipotiroidi tanısı konan hastalarda serum betatrofin düzeyleri anlamlı derecede yüksekti. (P=0,001). Serum betatrofin düzeyleri ile TSH, TG ve total kolesterol arasında pozitif yönde; HDL, serbest T3 ve serbest T4 ile negatif yönde korelasyon vardı. Hipotiroidili hasta grubunda anti-TPO yüksekliğine göre yapılan karşılaştırmada anlamlı farklılık yoktu (P=0,78).

Sonuç: Çalışmamızda hipotiroidi hastalarında serum betatrofin düzeylerinin yüksek olduğunu bulduk. Bu çalışma, klinik uygulamada betatrofeni hedef alan tedavilerin geliştirilmesinde faydalı olabilir.

Anahtar kelimeler: Hipotiroidizm, Betatrofin, Lipoprotein lipaz

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Introduction

Overt hypothyroidism affects between 4 and 10% of the world population [1]. Thyroid hormones regulate energy metabolism and affect the tissue response of other hormones in the organism [2,3]. Changes in the levels of thyroid hormones may affect muscle and visceral fat tissue mass, and may be involved in the pathogenesis of insulin resistance, type 2 diabetes mellitus and cardiovascular events [4]. TSH affects the function of the TSH receptor (TSHR) protein and stimulates adipokine production in abdominal adipose tissue and preadipocytes in humans [5]. Despite the important effects of thyroid hormones on intermediate metabolism and energy homeostasis, its relationship with cytokines secreted from adipose tissue is still unclear [6]. Betatrophin, also known as ANGPLT-8, is a 188 amino acid and 22 kDa glycoprotein produced in the liver and adipose tissue [7,8]. Betatrophin inhibits LPL, one of the key enzymes for lipid metabolism [9]. LPL is the enzyme that catalyzes the breakdown of triglycerides to free fatty acids [10]. LPL hyperactivity was associated with decreased plasma TG levels and decreased cardiovascular risks, whereas LPL function loss was associated with hypertriglyceridemia and increased cardiovascular risk [10,11]. Serum betatrophin levels and triglyceride levels were positively correlated in previous studies [8,12].

Betatrophin, unlike the other members of the ANGPTL family, does not carry the fibrinogen-like region, coiled spiral area and disulfide bond [13]. Therefore, betatrophin is considered as an atypical member in the ANGPTL family and a newly emerged player in lipid metabolism [9,14].

There are recent studies investigating the effects of betatrophin on beta-cell expansion and islet function [15]. In this study, we tried to explain the relationship between hypothyroidism and fat metabolism by serum betatrophin levels. This study may help demonstrate the effects of hypothyroidism on adipose tissue.

Materials and methods

Design and patients

44 patients between the ages 20 and 69, who were diagnosed with overt hypothyroidism in Internal Medicine Department between September 2017 and February 2018 and 40 healthy volunteers were included in this study.

In our study, TSH> 4.94 mIU / L, FT4 <9.01 ng / dL and / or freeT3 <2.5 pg / mL were defined as overt hypothyroidism. Patients with diabetes mellitus, acute and chronic renal failure, hypertension, coronary heart disease, congestive heart failure, peripheral arterial disease, cerebrovascular accident, malignancy, acute and chronic liver diseases, pituitary gland and hypothalamus diseases, major operation history in the last three months, rheumatic diseases, severe psychiatric conditions, malabsorption syndrome, pregnancy, alcohol intake and smoking were excluded. Blood was obtained from all participants for routine laboratory tests including fasting glucose, urea, creatinine, AST, ALT, TSH, free T3, free T4, TPO-Ab, TG-Ab, HDL-cholesterol, LDL-cholesterol, triglyceride, and total cholesterol levels. Routine laboratory tests were performed using Cobas Elecsys 601 (Roche Diagnostics, Switzerland). An additional tube of blood was obtained from the volunteers for the measurement of serum betatrophin levels. Blood tubes rested at room temperature for 30 minutes, then centrifuged at 4000 rpm for 10 min. The resulting sera were stored at -80 ° C.

BMI was obtained by dividing the patient's weight in kg by the square of height in meters. Insulin resistance was calculated by HOMA-IR index: Fasting serum insulin (uIU / mL) * Fasting serum glucose (mg / dL) / 405. Patients with HOMA-IR index> 2.5 were accepted as insulin resistance.

Measurements of betatrophin

Human ANGPTL-8 (betatrophin) levels were measured by using an enzyme-linked immunosorbent assay. Performance characteristics of betatrophin assay were as folows: The analytical (linear) measurement range was 11.4-1200 ng / mL and the minimum detection limit was 10.2 ng / mL. The reported intraassay and interassay CV’s were 8.5% and 7.7%, respectively.

Ethical committee approval

Our study was approved by the Committee of Ethics Committee of Okmeydanı Training and Research Hospital of Health Sciences University and it was complied with the principles of Helsinki Declaration (date: 19.12.2017; no: 784).

Statistical analysis

IBM SPSS version 25.0 (SPSS Inc, Chicago Illinois) statistical program was used. The distribution of variables was measured by Kolmogorov-Smirnov test. The data were expressed as mean (standard deviations, SD) for normally distributed variables and % frequencies for non-normally distributed and categorical variables. In the comparison of parametric data between two independent groups, independent sample t-test was performed. Mann-Whitney U test was used for the analysis of non-normally distributed data. The correlation of betatrophin, a non-normally distributed variable, to other parameters was analyzed by non-parametric Spearman test.

Multivariate regression analysis and stepwise option were used to evaluate all independent variables with serum betatrophin as the dependent variable. As a result of the regression analysis, independent variables other than TSH and triglyceride were excluded, because they were not related to serum betatrophin levels. All calculated *P*-values were bidirectional and *P*-values <0.05 were considered statistically significant.

Results

The demographic and laboratory data of study groups are summarized in table 1.

When compared with healthy controls, TSH, anti TPO, anti TG, serum triglyceride and serum betatrophin levels significantly increased in patients with hypothyroidism, while free T4 and free T3 decreased. Age, sex, BMI, Fast glucose, HOMA-IR, AST, ALT, urea, creatinine, HDL cholesterol, LDL-cholesterol and total cholesterol levels were not different.

The results of the correlation analysis between serum betatrophin levels and other parameters are summarized in table 2 and figure 1. There was a positive correlation between betatrophin levels of all participants and TSH, TG and total cholesterol levels. A negative correlation was found between

betatrophin levels of all participants and free T3, free T4 and HDL-cholesterol levels.

Multivariate regression analysis results are presented in table 3. Comparison of Anti-TPO> 9 and Anti-TPO <9 patients using the Mann-Whitney-U test is presented in table 4.

Table 1: The demographic and laboratory features for the two groups of hypothyroidism and euthyroidism (control group)

	Control group mean (SD) (n:40)	Hypothyroid patients mean (SD) (n:44)	*P*-value
Age (year)	44.65 (14.4)	44.7 (13.83)	0.986[a]
Sex			
Male (%)	19 (47.50%)	20 (45.45%)	0.788[b]
Female (%)	21 (52.50%)	24 (54.55%)	
BMI (kg/m^2)	25.80 (3,27)	26.75 (3.95)	0.236[a]
Fasting glucose (mg/dL)	92.52 (8.68)	91.93 (16.18)	0.795[c]
Fasting insülin (mg/dL)	5.33 (1.29)	8.25 (2.42)	0.056[c]
HOMA-IR	2.77 (1.96)	3.05 (1.84)	0.078[c]
Urea (mg/dL)	28.25 (8.97)	29.23 (10.12)	0.642[a]
Creatinine (mg/dL)	0.74 (0.18)	0.67 (0.14)	0.250[a]
AST (U/L)	19.45 (7.16)	20.15 (6.73)	0.492[c]
ALT (U/L)	19.55 (7.94)	18.02 (7.28)	0.422[c]
HDL-cholesterol(mg/dL)	50.15 (7.88)	44.25 (9.10)	0.308[a]
LDL-cholesterol(mg/dL)	117.78 (36.41)	124.02 (35.35)	0.428[a]
Triglyceride(mg/dL)	117.07 (50.15)	148.36 (55.93)	0.007[c]
Total cholesterol(mg/dL)	185.13 (36.88)	198.75 (35.62)	0.089[a]
TSH (µIU/mL)	1,91 (0.82)	9.30 (3.36)	0.001[c]
Free T4 (ng/dL)	11.99 (4.34)	3.53 (2.12)	0.001[c]
Free T3 (pg/mL)	2.85 (0.86)	1.46 (0.74)	0.001[c]
TPOAb (IU/mL)	6.35 (3.19)	12.90 (3.54)	0.001[c]
TGAb (IU/mL)	3.24 (1.56)	12.72 (2.60)	0.001[c]
Betatrophin (ng/mL)	324.58 (92.48)	417.40 (127.84)	0.001[c]

a: t-test; m, b: chi-square test, c: Mann–Whitney U test; ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body-mass index, HOMA-IR: homeostatic model of assessment-insulin resistance, LDL-cholesterol: low-density lipoprotein-cholesterol, HDL-cholesterol: high-density lipoprotein-cholesterol, TSH: thyroid stimulating hormone, Anti TPO: thyroid peroxidase ab, Anti TG: thyroglobulin ab

Table 2: Correlations between serum ANGPTL-8 (betatrophin) levels and covariates

Covariates	Correlation coefficient	*P*-value
BMI (kg/m^2)	0.03	0.808
HOMA-IR	0.03	0.750
HDL (mg/dL)	-0.23	0.034
LDL (mg/dL)	0.21	0.058
TG (mg/dL)	0.40	0.001
T.CHOL (mg/dL)	0.29	0.008
TSH (µIU/mL)	0.32	0.003
FT4 (ng/dL)	-0.30	0.006
TPOAb (IU/mL)	0.09	0.433
TGAb(IU/mL)	0.25	0.220

BMI: body-mass index, HOMA-IR: homeostatic model of assessment-insulin resistance, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol, T.CHOL: total cholesterol, TSH: thyroid stimulating hormone, TPOAb: thyroid peroxidase ab, TGAb: thyroglobulin ab

Table 3: Multivariate regression analysis

Covariates	Coefficient B (SE)	%95 CI	*P*-value
Constant	220.8 (30.5)	158.9 - 281.6	0.001
TSH	7.7 (2.5)	2.768 – 12.6	0.003
TG	1.1 (0.2)	0.679 – 1.5	0.001

Model R^2: 0.40 and p<0.001. Regression equation for serum betatrophin on the basis of this model was 220,782 -10,290 * TSH +1,070 * TG

Table 4: Comparison of TPOAb >9 (n=21) and TPOAb ≤9 (n=23) patients with Mann-Whitney-U test

	P-value[1]
BMI	0.026
HOMA-IR	0.176
TSH	0.235
Free-T4	0.842
Triglyceride	0.823
HDL-C	0.999
LDL-C	0.823
Total-cholesterol	0.565
Betatrophine	0.778

BMI: body-mass index, HOMA-IR: homeostatic model of assessment-insulin resistance, TSH: thyroid stimulating hormone, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, [1] Mann-Whitney U test

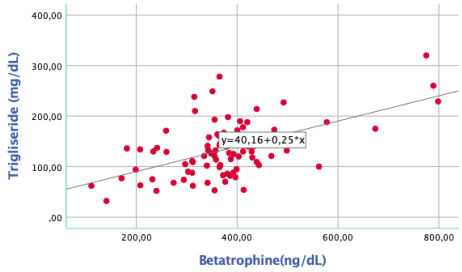

Figure 1: Relationship between serum betatrophin and triglyceride levels

Discussion

Thyroid hormones govern energy metabolism by activating glucose, fat and protein oxidation in tissues. In hypothyroidism, decreased T3 and T4 levels and increased TSH levels are characteristic. With the decrease in thyroid hormones, instead of being oxidized for energy, lipids are stored in the body. Consequently, body weight increases and lipid profile changes [16,17].

As is known, visceral adipose tissue is the main adipose tissue producing various adipokines. Numerous evidences have shown that cytokines secreted from adipose tissue lead to endothelial dysfunction and cause atherosclerosis with dyslipidemic effects [18-21]. In a study, visceral adipose tissue volume was increased in patients with hypothyroidism [22]. Despite the intense interaction of thyroid hormones and regulatory hormones secreted from adipose tissue, this relationship has not been fully elucidated. In our study, we investigated the level of ANGPTL family cytokines secreted from adipose tissue in patients diagnosed with overt hypothyroidism and found it was closely related to lipid metabolism, in accordance with the previous studies.

ANGPTL-8, also known as betatrophin, is a regulator and stabilizer in lipid metabolism by its LPL inhibition, which is also a feature of other cytokines in the ANGPTL family [23,24]. As is known, LPL provides clearance of TG-rich plasma lipoproteins [25]. LPL is located in the capillary endothelium and catalyzes the hydrolysis of endogenous VLDL-TG and exogenous chylomicron-TG to glycerol and FFAs [26].

A few studies in the literature demonstrate the relationship between hypothyroidism and serum betatrophin levels. In one study, the relationship between betatrophin levels and subclinical and overt hypothyroidism was investigated and serum betatrophin levels were found to be increased in hypothyroidism [27]. The same study suggested that betatrophin was a metabolic regulator that may affect lipid and glucose metabolism. However, it was stated that serum betatrophin elevation may be associated with thyroid autoimmunity. In our study, we also found that serum betatrophin levels were higher in patients diagnosed with overt hypothyroidism compared to the control group. Regardless of autoimmunity, we believe that betatrophin plays a regulator role in fat metabolism by its inhibitory effect of LPL, due to the fact that serum betatrophin levels were similar in patients with hypothyroidism having high or normal anti-TPO values.

In our study, although there were similar features regarding BMI, fasting plasma glucose and HOMA-IR levels in patients with hypothyroidism and control group, triglyceride levels were significantly different between both groups.

The relationship between betatrophin levels and TG levels could be detected even in patients with subclinical hypothyroidism, suggesting that hypertriglyceridemia in hypothyroidism may be mediated by increased betatrophin release [27]. In our study, there was a strong positive correlation between TG and betatrophin levels. The elevated betatrophin level inhibits LPL and prevents the breakdown of triglycerides into free fatty acids and glycerol. In hypothyroidism, lipid oxidation for energy production decreases as a result of the

decrease of fatty acid. On the other hand, elevated TG in the serum may initiate triggering mechanisms for obesity, insulin resistance and cardiovascular events, and especially for hepatosteatosis.

In a study in which we investigated the relationship between ANGPTL-4 and hepatosteatosis, we found that LPL inhibition further induces hepatosteatosis by causing TG accumulation in the tissues [14]. Betatrophin is known to exhibit the main characteristics of the ANGPTL family, with some structural differences. Stress, insulin resistance, inflammation and thermogenesis may affect betatrophin expression and signaling pathways [28]. The contradictory results in the previous studies suggest that betatrophin may be a multidimensional adipokine that can function differently under changing conditions.

Limitation

The small number of patients was the most important limitation for our study. Further studies are needed with more patient groups.

Conclusion

In our study, we found that serum betatrophin levels were high in hypothyroid patients. Consequently, considering the relationship between betatrophin and TG levels, it may give promising results as a therapeutic target.

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Research article
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Pattern of muscle involvement according to needle electromyography findings in clinically unaffected extremities of polio survivors with lower extremity weaknesses

Alt ekstremite güçsüzlüğü olan polio hastalarının klinik olarak etkilenmeyen ekstremitelerinde iğne elektromiyografi bulgularına göre kas tutulum paterni

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Abstract

Aim: Late neuromuscular deterioration may be seen in patients with a history of paralytic poliomyelitis. One of these problems is the development of a new weakness in clinically unaffected muscles. We aimed to determine needle electromyography (EMG) findings in these clinically unaffected limb muscles and to contribute to the physiotherapy strategies of poliomyelitis.

Methods: Patients with sequelae of poliomyelitis were included in this retrospective cohort study. Needle EMG findings of the patients were reviewed. If there were neurogenic needle EMG findings in the limb or muscle with no weakness, this muscle or limb was considered to be a subclinically affected muscle or limb.

Results: Eighteen patients were included in the study. Needle EMG findings of 190 muscles were analyzed. In the lower extremities, 18 (72%) of 25 clinically unaffected muscles had neurogenic needle EMG findings, and 14 (35%) of 40 upper extremity muscles had subclinical involvement. In the lower extremity muscles, this subclinical involvement was significantly higher than in the upper extremity muscles (P=0.004). In clinically unaffected upper and lower extremity muscles, the most prominent neurogenic needle EMG findings were in the deltoideus and vastus lateralis muscles, respectively (P=0.022 and P=0.028, respectively).

Conclusion: Subclinical involvement was more prominent in the lower extremity than in the upper extremity in polio survivors with weakness of lower extremity. The most prominent subclinical muscle involvement in the lower and upper extremities was the vastus lateralis and deltoideus muscles, respectively. We think that physical therapy strategies considering these findings will be beneficial for polio survivors.

Keywords: Needle electromyography, Poliomyelitis, Subclinical involvement

Öz

Amaç: Paralitik poliomiyelit öyküsü olan hastalarda geç dönem nöromüsküler kötüleşme görülebilir. Bu sorunlardan birisi, klinik olarak etkilenmemiş kaslarda yeni bir güçsüzlüğün gelişimidir. Bu klinik olarak etkilenmemiş ekstremite kaslarındaki iğne elektromyografi (EMG) bulgularını saptamayı ve poliomiyelitin fizik tedavi stratejilerine katkıda bulunmayı amaçladık.

Yöntemler: Poliomiyelit sekeli olan hastalar bu retrospektif kohort çalışmasına çalışmaya dahil edildi. Hastaların iğne EMG bulguları gözden geçirildi. Güçsüzlüğü olmayan ekstremite veya kasta nörojenik iğne EMG bulguları varsa, bu kas veya ekstremite subklinik olarak etkilenmiş olarak kabul edildi.

Bulgular: Onsekiz hasta çalışmaya dahil edildi. Yüzdoksan kasın iğne EMG bulguları analiz edildi. Alt ekstremitelerde, klinik olarak etkilenmeyen 25 kasın 18'inde (%72) nörojenik iğne EMG bulguları vardı, 40 üst ekstremite kasının 14'ünde (%35) subklinik tutulum mevcuttu. Alt ekstremite kaslarındaki bu subklinik tutulum, üst ekstremite kaslarına göre anlamlı olarak daha fazla orandaydı (P=0,004). Klinik olarak etkilenmemiş üst ve alt ekstremite kasları içinde, en belirgin nörojenik iğne EMG bulguları sırasıyla deltoid ve vastus lateralis kaslarında idi (sırasıyla; P=0,022 ve P=0,028).

Sonuç: Alt ekstremite güçsüzlüğü olan polio hastalarında subklinik tutulum alt ekstremitede üst ekstremiteden daha belirgindi. Alt ve üst ekstremitelerde en belirgin subklinik kas tutulumu sırasıyla vastus lateralis ve deltoid kaslarındaydı. Bu bulguları dikkate alan fizik tedavi stratejilerinin polio hastaları için faydalı olacağını düşünüyoruz.

Anahtar kelimeler: Elektromiyografi, Poliomiyelit, Subklinik tutulum

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Introduction

Poliomyelitis was a disease that caused deaths and disabilities in children and young adults in between the 1940s and 1950s. It is caused by the RNA enterovirus affecting brain and anterior horn cells of spinal cord and brainstem. Limb weakness due to death of motor neurons is observed in 1-2% of patients infected by this agent [1,2]. Due to the development of vaccines, it is rarely seen in developing countries today [1]. However, late neuromuscular deterioration and musculoskeletal problems in patients with a history of poliomyelitis continue to be a problem. Although some patients with a history of non-paralytic poliomyelitis do not have weakness, it is known that motor neurons are damaged in these patients [1,3]. In addition, it has been shown that the motor units of the limb muscles with no weakness are affected in patients with a history of paralytic poliomyelitis [4,5] because poliovirus affects most of the motor neurons during the acute phase of the disease [1,6,7]. Approximately 50% of these affected motor neurons are destroyed [7]. After the acute phase, the stable disease may worsen as a result of damage to motor neurons. The causes of this neuromuscular deterioration are controversial [1,8]. Furthermore, studies have shown that the death of motor neurons in polio survivors is faster than normal aging. [5,9]. Many factors such as chronic poliovirus infection or excessive exercise can accelerate the death of motor neurons. [1,8]. At this point, it is important to protect the motor neurons and axons of clinically unaffected muscles. According to clinical and histopathological findings, studies on muscle involvement pattern are available in the literature [10,11]. In this study, we wanted to discuss needle electromyography (EMG) findings in clinically unaffected extremities and muscles in polio survivors with lower extremity weaknesses. Thus, we wanted to contribute to the literature and to the physiotherapy strategies of poliomyelitis.

Materials and methods

Subjects

Patients older than 18 years with sequelae of poliomyelitis referred to the EMG Laboratory of Adana City Training and Research Hospital (ACTRH) between July 2018 and July 2019 were included in this retrospective cohort study. EMG laboratory records were reviewed retrospectively. Patients were included in the study if they had a definite history of poliomyelitis and weakness in at least one limb. The ages of the patients, ages of patients at the time of poliomyelitis, neurological examination findings, nerve conduction study and needle EMG findings were included in the analysis. Patients were excluded from the study if they had one of the following: a disease that can cause polyneuropathy such as diabetes mellitus, a surgical history of cervical or lumbosacral region, a major surgical history of extremities and findings suggestive of polyneuropathy in nerve conduction studies. In addition, patients who met the diagnostic criteria of post-polio syndrome were excluded from the study [12]. In neurological examination, the limb with weakness was considered a clinically affected limb, and otherwise, it was considered a clinically unaffected limb. Ethics committee approval was obtained from the ethics committee of ACTRH (number 37/514).

Electrodiagnostic tests

Nerve conduction studies and needle EMG were performed with Cadwell Sierra Summit EMG unit (Cadwell laboratories, Kennewick, Washington, USA). In the nerve conduction studies, surface electrodes were used for recording and stimulation. Nerve conduction studies and needle EMG were performed if the extremities were above 32°C; otherwise, the extremities were heated with a hair dryer. Band-pass filters for sensory and motor nerve conduction studies and needle EMG were set at 20 Hz to 2 kHz, 20Hz to 10kHz and 10Hz to 10kHz, respectively. Nerves were stimulated supramaximally. Sensitivity was 2 mV/division and 10 μV/division in motor and sensory nerve conduction studies, respectively. Sweep speed w6as 5 ms/division and 1 ms/division in motor and sensory nerve conduction studies, respectively. Compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) amplitudes were measured from peak to peak. Sensory nerve conduction velocity was calculated using peak latency. Median and ulnar sensory nerve conduction studies were performed orthodromically by stimulating the 1st, 2nd, 3rd and 5th fingers and the palm. Sural sensory nerve conduction study was performed antidromically. Median, ulnar, posterior tibial and peroneal nerve minimum F-wave latencies were determined by evaluating at least 10 responses. We used the reference values for nerve conduction studies recommended by American Association of Neuromuscular and Electrodiagnostic Medicine [13]. According to these suggested values, lower limits of normal median, ulnar, posterior tibial and peroneal CMAP amplitudes were considered to be 4.1, 7.9, 4.4 and 2.6 mV, respectively. Needle EMG was performed visually using a concentric EMG needle electrode (length=50mm, diameter=0.46mm, Bionen medical devices, Florence, Italy). Sensitivity was 50μV/division for the analyses of spontaneous activity. It was 200-1000μV/division for motor unit potential (MUP) and interference pattern evaluation. Sweep speed was 10 ms/division for the analyses of spontaneous activity and MUPs, and it was 100 ms/division for the analyses of interference patterns. Positive sharp waves (PSW) and fibrillations were carefully evaluated. According to tolerability of the patients, at least 10-20 MUPs were recorded during mild muscle contraction. Duration, amplitude, phase number and interference pattern of MUPs were recorded. MUP was considered neurogenic if: MUP peak to peak amplitude was ≥4mV and/or MUP duration ≥15ms or MUP could not be obtained. In our EMG laboratory, as a protocol for poliomyelitis, needle EMG is applied to the muscles of the extremity or extremities with weakness, the muscles of the contralateral extremity with weakness and the muscles of another upper or lower extremity. According to tolerability of the patient, needle EMG was performed to the tibialis anterior, medial gastrocnemius, peroneus longus, vastus lateralis, iliopsoas, first dorsal interosseous, biceps brachii, deltoideus and trapezius muscles. If needle EMG findings in muscle or limb with no weakness showed neurogenic features, it was accepted that muscle or extremity was affected subclinically.

Statistical analysis

The Shapiro-Wilk test was used to determine the distribution of the data. Mean (standard deviation) of numeric data were calculated for descriptive statistics. Pearson's and

Fisher's Chi-squared tests were used to analyze categorical variables. Kruskal-Wallis and Mann-Whitney U tests were used in the analysis of quantitative data. A p value less than 0.05 was considered significant. Statistical Package for the Social Sciences (SPSS IBM Corp; Armonk, NY, USA) 22.0 was used to perform the statistical analysis.

Results

Twenty-nine patients with a history of poliomyelitis were referred to our EMG laboratory. One patient was not included in the study due to diabetes mellitus. In addition, two patients had a suspicious history of poliomyelitis, and seven patients met the criteria of post-polio syndrome, so these patients were also excluded from the study. Eighteen patients were included in the study. The mean age of the patients was 46.5 (8.3) years. Six patients were female. None of them met the post-polio diagnostic criteria. The patients had no pain or paresthesia or newly occurring muscle weakness. Table 1 shows the clinical and demographic characteristics of the patients. Asymmetric clinical features were clearly present in 16 patients. Neurological examination did not reveal any evidence of bulbar involvement. Median and ulnar motor nerve conduction studies were normal in all patients. In 11 patients, the peroneal or posterior tibial nerve CMAP amplitude was reduced, or CMAP could not be obtained. Two patients had mild carpal tunnel syndrome. Median sensory nerve conduction studies of 16 patients were normal. Ulnar and sural sensory nerve conduction studies of all patients were normal.

Muscles of 56 extremities and the trapezius muscle of 16 patients were examined by needle EMG. Three extremities (two lower and one upper extremities) per patient in 16 patients and 4 extremities per patient in 2 patients were included in the study. One of the upper extremities of 16 patients was not included in the study because needle EMG was not performed on these extremities. Needle EMG was performed in 190 muscles. The mean number of muscles examined by EMG per patient was 10.6 (1.8) (min 8, max 16). In 138 (73%) of the 190 muscles, needle EMG findings showed neurogenic features. The needle EMG findings of the all examined muscles are shown in Table 2. Thirty-four (42%) of 81 clinically unaffected muscles had neurogenic needle EMG findings. Two of the 16 trapezius muscles were subclinically affected. Needle EMG findings in clinically unaffected muscles are shown in table 3. Among clinically unaffected lower extremity muscles, vastus lateralis (86%) had the highest rate of subclinical, whereas deltoideus (75%) had the highest rate of subclinical involvement among the clinically unaffected upper extremity muscles. The MUP duration of the vastus lateralis muscle was significantly longer than that of the medial gastrocnemius and tibialis anterior muscles (P=0.028, Table 3). The MUP duration and amplitude of the deltoideus muscle were significantly higher than the MUP duration and amplitude of the first dorsal interosseous and biceps brachii muscles (P=0.046, P=0.022, Table 3). In 18 (72%) of 25 clinically unaffected muscles examined in the lower extremities, needle EMG findings showed neurogenic features, and 35% of the upper extremity muscles were subclinically affected. This subclinical involvement was significantly higher in the lower extremity muscles than in the upper extremity muscles. (P=0.004, Table 4). Fifteen of the 188 muscles had PSW or fibrillation or fasciculation potentials. Positive sharp wave or fibrillation potentials were observed in the tibialis anterior, medial gastrocnemius, peroneus longus, vastus lateralis or iliopsoas muscles of 5 patients. Fasciculation was seen in the tibialis anterior muscles of 2 patients.

Clinically, 28 of 36 lower extremities had weakness, and at least one muscle of these lower extremities had neurogenic needle EMG findings. When needle EMG findings were taken into consideration, abnormal needle EMG findings in at least one muscle of extremity were present in 35 of 36 lower extremities. Two patients had weakness in one upper extremity. Ten upper extremities were affected subclinically. Two patients with clinically affected upper extremities had neurogenic needle EMG findings in other upper extremity muscles with no weakness. There were no weaknesses in 28 extremities (20 upper extremities, 8 lower extremities). In 15 (54%) of 28 clinically unaffected extremities (8 upper extremities, 7 lower extremities), neurogenic needle EMG findings were present in at least one muscle of the extremity. Subclinical involvement in the lower extremities was significantly higher than in the upper extremities (P=0.029, Table 4). The affected body regions according to the clinical findings of the patients and the needle EMG findings of the muscles are shown in figure 1.

Table 1: Clinical features of patients

Parameter	Value
Age mean (SD) (min-max) (year)	46.5 (8.3) (31-59)
Age at acute poliomyelitis mean (SD) (min-max) (year)	2 (1.3) (0.4-5)
The interval between age at the acute poliomyelitis and age mean (SD) (min-max) (year)	46 (8.5) (29-58)
Sex Female / Male	6 / 12
Clinically affected limbs of patients	
Number of patients with weakness in one lower extremity	7 patients (6 with weaknesses in the left lower extremity, 1 with weakness in right lower extremity)
Number of patients with weakness in two lower extremities	9 patients (4 with weaknesses in left>right lower extremities, 3 with weakness in right>left lower extremities)
Number of patients with weakness in upper and lower extremities	2 patients (1 with weaknesses in the left upper extremity + right>left lower extremities, 1 with weakness in the left upper extremity + 1 right lower extremity)
Weakness of bulbar muscles	None
Number of patients with asymmetric clinical features	16 patients

SD: Standard deviation

Table 2: Needle EMG findings of the all examined muscles

Muscle	Right	Left	Total (%)
Tibialis anterior	15 / 18	15 / 16	30 / 34 (88)
Medial gastrocnemius	16 / 17	13 / 15	29 / 32 (91)
Peroneus longus	2 / 2	2 / 2	4 / 4 (100)
Vastus lateralis	16 / 17	11 / 12	27 / 29 (93)
Iliopsoas	13 / 14	15 / 17	28 / 31 (90)
First dorsal interosseous	7 / 16	3 / 4	10 / 20 (50)
Biceps brachii	3 / 16	1 / 3	4 / 19 (21)
Deltoideus	3 / 3	1 / 2	4 / 5 (80)
Trapezius	1 / 8	1 / 8	2 / 16 (13)
Upper extremities muscles			18 / 44 (40.9)
Lower extremities muscles			118 / 130 (91)
Upper extremities, lower extremities and bulbar muscles			138 / 190 (73)

Number of muscles with neurogenic needle EMG findings; total number of muscles examined with needle EMG, EMG: Electromyography

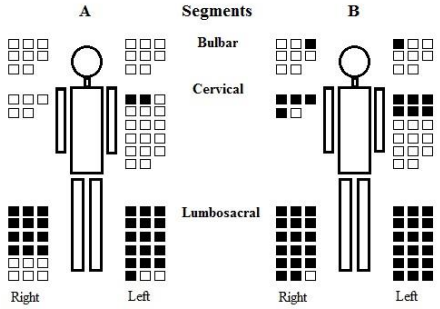

Figure 1: Affected body regions according to clinical features and needle EMG findings. Each box shows an extremity or a trapezius muscle. 1A was made using the clinical features of the patients. In case of clinical weaknesses in the examined extremity or bulbar segment, the box was stained black, and in regions with clinically unaffected muscles, the box was stained white. 1B was made using needle EMG findings of the muscles examined. If neurogenic needle EMG findings were present in at least one muscle of the extremity

or bulbar segment, the box was stained black; otherwise, the box was stained white.

Table 3: Needle EMG findings of clinically unaffected muscles

	Number of muscles of unaffected extremities with neurogenic needle EMG findings / total number of unaffected muscles examined with needle EMG (%)	MUP amplitude of unaffected muscles (mV) Mean (SD)	MUP duration (ms) of unaffected muscles Mean (SD)
Lower extremity muscles			
Tibialis anterior	5 / 7 (71%)	5.1 (2.9)	16.6 (4.4)
Medial Gastrocnemius	4 / 7 (57%)	4.4 (3.5)	14.3 (6.0)
Vastus lateralis	6 / 7 (86%)	8.4 (5.1)	23.0 (3.8)
Iliopsoas	3 / 4 (75%)	5.5 (2.3)	18.0 (4.3)
P-value		0.299	0.028*
Upper extremity muscles			
First dorsal interosseous	8 / 18 (44%)	3.5 (2.9)	13.3 (5.2)
Biceps brachii	3 / 18 (17%)	2.5 (2.6)	14.9 (4.1)
Deltoideus	3 / 4 (75%)	7.9 (4.4)	23.5 (8.1)
P-value		0,046**	0.022**
Bulbar muscles			
Trapezius	2 / 16 (13%)	1.9 (2.2)	12.1 (2.9)
Lower extremity muscles	18 / 25 (72%)	5.9 (3.9)	17.9 (5.6)
Upper extremity muscles	14 / 40 (35%)	3.5 (3.3)	15.1 (5.8)
Total	34 / 81 (42%)		

EMG: Electromyography, MUP: Motor unit action potential, *: The MUP duration of the vastus lateralis muscle was significantly longer than that of the medial gastrocnemius and tibialis anterior muscles, **: Both the MUP amplitude and duration of the deltoideus muscle were significantly higher than the MUP amplitude and duration of the other two upper extremity muscles, Kruskal-Wallis test was used, and a *P*-value less than 0.05 was considered significant.

Table 4: Comparison of subclinical involvement of upper and lower extremities

	Lower extremity	Upper extremity	*P*-value
Number of clinically unaffected muscles with neurogenic needle EMG findings / total number of unaffected muscles examined with needle EMG (%)	18/25 (72%)	14/40 (35%)	0.004
Number of clinically unaffected extremities with neurogenic needle EMG findings in at least one muscle / total number of clinically unaffected extremities (%)	7/8 (88%)	8/20 (40%)	0.029
Mean MUP amplitude of clinically unaffected muscles (SD) (mV)	5.9 (3.9) (n=25)	3.4 (3.2) (n=40)	0.007
Mean MUP duration of clinically unaffected muscles (SD) (ms)	17.8 (5.7) (n=25)	15.1 (5.8) (n=40)	0.026

EMG: Electromyography, MUP: Motor unit action potential, n: number of muscles. Pearson's and Fisher's Chi-squared test were used. A *P*-value less than 0.05 was considered significant.

Discussion

Although poliomyelitis is rarely seen in developing countries due to the use of vaccines, the problems of polio survivors continue to be a health problem. In some patients, the disease is stable for many years after acute poliomyelitis, and later neuromuscular and musculoskeletal deteriorations may develop [1]. One of these conditions is post-polio syndrome. The causes of these disorders are controversial. Chronic poliovirus infection, excess weight gain, overuse weakness, changes in muscle fiber metabolism and immune-mediated mechanisms are some of the reasons responsible for these neuromuscular deteriorations [1,8]. Regardless of the cause, the death of motor neurons is faster than normal aging [5,9]. As motor neuron death increases, muscle weakness increases [5,6]. It is known that the motor units of unaffected limb muscles are affected [4,5]. Excessive effort may exacerbate the death of motor neurons [1,8,14]. For these reasons, protection of motor neurons and axons of the unaffected extremity muscles is important. Our study showed that 42% of clinically unaffected limb muscles and 54% of clinically unaffected limbs had neurogenic needle EMG findings, and this finding was consistent with the literature [5]. In our study, considering the clinically unaffected limb muscles and limbs, neurogenic needle EMG findings were found to be more prevalent in the lower extremity muscles than in the upper extremity muscles. It is known that lower extremity muscles are more clinically affected than the upper extremity muscles in poliomyelitis [15], and we thought that it was important to demonstrate a similar situation in clinically unaffected extremities in our study. We think that physical therapy strategies considering these findings will be beneficial for patients with sequelae of poliomyelitis. Patients with a history of poliomyelitis should stay away from activities that will over-exercise their muscles, especially the lower extremity muscles. However, this finding should not mean over-exercising the upper extremity muscles because poliovirus affects most of the motor neurons [1,6,7]. Note that two patients with upper limb weaknesses had subclinical involvement on the other upper extremities in our study. It may also be important to treat each patient separately. Treatment can be planned considering needle EMG findings because muscles with no weakness may be severely neurogenic in needle EMG examination. In our study, PSW, fibrillation or fasciculation potentials were observed in 5 of (28%) 18 patients and in 15 (8%) of 190 muscles. These findings were consistent with the literature [5]. Presence of active denervation findings in EMG can be seen in patients with poliomyelitis, and it is known that detection of active denervation is not used to differentiate patients with post-polio syndrome from those without [5,15,16].

There have been studies on the pattern of muscle involvement in the upper and lower extremities in poliomyelitis [10,11]. However, these studies were performed according to clinical and histopathological findings rather than needle EMG findings. We found that the most affected subclinical muscle in the upper extremity was the deltoid muscle. The most commonly paralyzed muscle has been shown to be the deltoideus muscle [10]. It has been reported in the literature that the muscles innervated by the upper lumbar spinal segments were more affected than the muscles innervated by the sacral segments [11]. In our study, we detected more neurogenic effects in the vastus lateralis muscle innervated by the upper lumbar spinal segments than in the medial gastrocnemius and tibialis anterior muscles innervated by the lower lumbar spinal and sacral segments. These findings may suggest that there are similar patterns of muscle involvement in clinically affected and unaffected extremities.

Limitations

There were some limitations in our study. Firstly, the number of patients and the number of unaffected limbs were low. Secondly, needle EMG was performed to three different muscles in the upper extremity, and more accurate results would be obtained by evaluating the muscles such as triceps or abductor pollicis brevis with needle EMG. Finally, the retrospective nature of the study can be considered as a limitation.

Conclusion

This study showed that clinically unaffected limb muscles could have neurogenic needle EMG findings and that this was more pronounced in the lower extremity muscles in polio survivors with lower extremity weaknesses. Subclinical involvement was most prominent in the deltoideus muscle among the examined upper extremity muscles and vastus lateralis among the examined lower extremity muscles. These findings should be taken into consideration, and physical therapy strategies should be planned to protect motor neurons and axons in polio survivors.

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Investigation of fungal flora in hammams, Turkish baths: A field study

Türk hamamlarında fungal floranın araştırılması: Bir saha çalışması

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Abstract

Aim: Hammams, also known as Turkish Hammams or Turkish Baths, have preserved their traditional importance and popularity in various countries, namely, Turkey, Morocco, Yemen, and Algeria. In this study, we aimed to evaluate the fungal flora in hammams and its effects on public health.

Methods: This cross-sectional study was performed by collecting two hundred forty samples from different areas of the baths and the tools used. Collected samples were inoculated on Sabouraund dextrose agar and Potato dextrose agar for mycological evaluation. Agar plates were incubated at 25 °C and 37 °C for four weeks and fungal growth was observed every day. For identification of isolated fungi, micro and macro morphology was evaluated; germ tube test, biochemical tests and VITEK®2 Compact (Biomerieux, France) equipment were used.

Results: We determined that molds are the most common fungi in Turkish hammams. *Aspergillus spp.* (n=20), *Scedosporium apiospermum/boydii* (n=5), *Alternaria ulocladium* (n=1), *Rhizomucor spp.* (n=1) and *Penicillium spp.* (n=1) were isolated in collected samples. Isolated yeasts were *Trichosporon spp.* (n=6), *Candida albicans* (n=1) and *Candida tropicalis* (n=1). *Trichophyton tonsurans*, the dermatophyte, was isolated in two samples. Fungus was most commonly isolated from slippers, and not at all isolated from towels or peshtemals.

Conclusion: In our study, the most isolated molds were fungi, found in the nature, and the isolation rate was exceptionally low. Dermatophytes are the most common culprit of fungal transmission in public places such as baths. Compared to previous studies conducted in swimming pools, wrestling cushions, mosque carpets and slippers, our study showed that Turkish baths with high humidity and temperatures are not rich in fungal flora and that the risk of fungal contamination is low.

Keywords: Hammam, Turkish bath, Fungi, Dermatophytes

Öz

Amaç: Türk hamamı veya Türk banyosu olarak da bilinen hamamlar, günümüzde Türkiye, Fas, Yemen ve Cezayir gibi bazı ülkelerde geleneksel önemini ve popülerliğini korumaktadır. Bu çalışmada hamamlardaki fungal floranın ve halk sağlığına etkilerinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Bu kesitsel çalışma, hamamın farklı alanlarından ve kullanılan aletlerden iki yüz kırk örnek toplanarak gerçekleştirildi. Toplanan örnekler mikolojik değerlendirme için Sabouraund dekstroz agar ve Patates dekstroz agara inoküle edildi. Agar plakları dört hafta boyunca 25°C ve 37°C'de inkübe edildi ve her gün fungal büyümeleri gözlendi. İzole edilen fungilerin identifikasyonu için; mikro ve makro morfoloji, germ tüp testi, biyokimyasal testler ve VITEK®2 Compact (Biomerieux, France) cihazı kullanıldı.

Bulgular: Çalışmamızın sonunda, Türk hamamlarında küflerin en yaygın funguslar olduğu belirlendi. Toplanan örneklerden izole edilen küfler, *Aspergillus spp.* (n=20), *Scedosporium apiospermum/boydii* (n=5), *Alternaria ulocladium* (n=1), *Rhizomucor spp.* (n=1) ve *Penicillium spp.* (n=1) idi. İzole edilen mayalar *Trichosporon spp.* (n=6), *Candida albicans* (n=1) ve *Candida tropicalis* (n=1) idi. Dermatofit olarak iki örnekte *Trichophyton tonsurans* izole edildi. En sık fungal izolasyon terliklerde görülürken, havlu ve peştemallerde fungal izolasyon görülmedi.

Sonuç: Çalışmamızda en fazla izole edilen küfler doğada ve çevrede oldukça yaygın bulunan fungilerdir ve çalışmamızda izolasyon oranı oldukça düşük bulunmuştur. Hamam gibi ortak kullanılan yerlerde fungal bulaş için en önemli grup dermatofitlerdir. Yüzme havuzları, güreş minderleri, cami halı ve terliklerinde önceki çalışmalar ile karşılaştırıldığında, bizim çalışmamız, yüksek nem ve sıcaklığa sahip Türk hamamlarının fungal flora açısından zengin olmadığını ve fungal kontaminasyon riskinin düşük olduğunu göstermiştir.

Anahtar kelimeler: Hamam, Türk Banyosu, Fungi, Dermatofitler

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Introduction

For thousands of years, people have retreated to hammams to sweat, clean, get scrubbed and socialize with other people. In daily life, hammams are not only traditional cleaning areas, but also centers for health, social and cultural activities. Studies report more than four hundred species of pathogenic fungi commonly isolated from humid locations such as pools, saunas, and public baths, some of which are responsible for serious infections [1]. It is well known that fungal infections are directly transmitted through infected persons, animals, and soil, and indirectly through infected skin and hair follicles [2]. In addition, it is thought that humid environments such as swimming pools and public baths are the main sites for fungal contamination and spreading [3]. Fungi are responsible for allergic conditions and respiratory, dermatological, and central nervous system diseases. Considering their frequent use, hammams are particularly important and effective in terms of public health. However, studies on fungal flora and fungal contamination on Turkish hammams are limited. This study on hammams in cities with medium-sized populations (40,000-260,000 persons) may provide general information about the fungal flora of Turkish hammams.

Materials and methods

This study was performed in accordance with the principles of Declaration of Helsinki. No human or animal material was used in the study and permission was obtained from all bath-hammam establishments. Nine traditional hammams from five different cities in Turkey were included in this study: Tokat, Kırıkkale, Çorum, Yozgat, and Havza. A total of two hundreds forty samples were collected from different areas of the hammams and the tools used: The marble floor, central massage platform, sauna, dressing room, *kurna* edge, body shaving area, slippers, bowl, bath loofahs, towels and *peshtemals*. *Kurna* is a round, mostly marble basin, located under a tap in Turkish hammam, for storing water. *Peshtemal* is a traditional pareo-like cloth, made from special cotton or silk, used to cover and dry the lower part of body. Central massage platform or the navel stone, which is the hottest area of the hammam with underfloor heating, is made from marble, and used for lying down on and having a massage. Body shaving area is the place where body hairs are shaven before entering the hammam. Bath loofah, which is produced from plant or animals, is a coarse bath scrub used for exfoliating the skin. The traditional Turkish hammams usually work in split-schedules specified for male and female customers. Some hammams have separate rooms for men and women, while others serve men and women at different times and days. In our study, four hammams were used by men and women at different times, and five hammams were used by men only. The hammams are usually open from 08.00 to 22.00 on weekdays, and from 08.00 to 24.00 on weekends and vacation days. The hottest places of the hammams were the central massage platforms and saunas. Temperature ranged between 20-60 ºC in different areas. After closure, all surfaces were cleaned daily with 5% sodium hypochlorite. For this reason, the samples were collected in the afternoon and before the evening cleaning.

Sample collection and identification method in hammams

Dry sterile cotton swabs were used for wet surfaces. Cotton swabs, moistened with sterile saline (0.09% NaCl), were used for dry surfaces. All samples were collected by sweeping an area of 10 cm in diameter with circular motions. Collected samples were inoculated on unsupplemented and chloramphenicol-and-cycloheximide-supplemented Sabouraund dextrose agar (Oxoid Limited, Basingstoke, Hampshire, UK) for mycological evaluation. Plates were incubated at 25 °C and 37 °C for four weeks and fungal growth was observed every day. The isolated fungi and suspicious dermatophyte samples were subcultured on plates of Potato Dextrose Agar (Oxoid Limited, Basingstoke, Hampshire, UK) for fungal growth. Growing dermatophytes in the plates were observed and sufficiently grown dermatophytes were examined under the microscope using lactophenol cotton blue. Yeasts were identified by using several conventional methods including evaluation of macro-morphology, germ tube test and biochemical tests if necessary. VITEK®2 Compact (Biomerieux, France) equipment was used for advanced mycological identifications.

Results

In our study, thirty nine fungi were isolated from two hundred and forty samples of nine Turkish hammams. The provinces where the Turkish Baths where fungal specimens were collected are shown in Figure 1. Microscopic views of fungal specimens collected from Turkish baths are shown in Figure 2. Among the isolated fungi species, molds were the most common. The isolated molds were as follows: *Aspergillus spp*. (n=20), *Scedosporium apiospermum/boydii* (n=5), *Alternaria ulocladium* (n=2), *Rhizomucor spp*. (n=1) and *Penicillium spp.* (n=1). As yeasts, *Trichosporon spp*. were detected in six samples, *Candida albicans* in one sample and *Candida tropicalis* in one sample. As a dermatophyte, *Trichophyton tonsurans* was isolated in two samples. Among personal tools, fungi were most commonly isolated from slippers, and not at all detected in towels and *peshtemals* used in hammams.

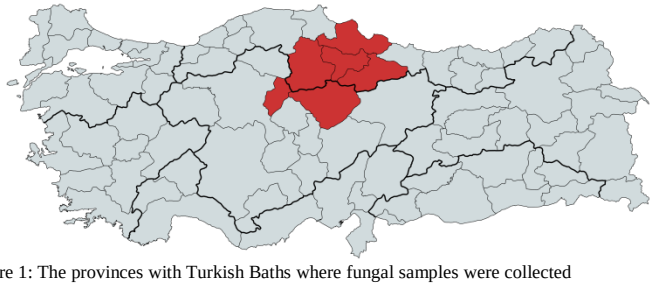

Figure 1: The provinces with Turkish Baths where fungal samples were collected

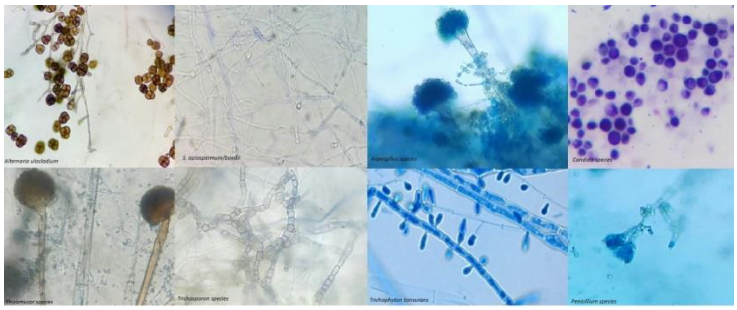

Figure 2: Microscopic views of fungal specimens collected from Turkish baths

Table 1: Fungi distribution in different parts of the hammams and using tools

Fungi	n(%)	Dressing Room Carpet	Floor	Slippers	Bowl	"Kurna"	Body shaving area	Sauna	Central massage platform	Bath loofah	Towel	"Peshtemal"
Mold												
Aspergillus fumigatus	14(35.9)	2	3	5	1	0	2	0	1	0	0	0
Aspergillus terreus	2(5.1)	0	1	0	0	0	0	0	1	0	0	0
Aspergillus niger	2(5.1)	1	0	0	0	0	1	0	0	0	0	0
Aspergillus flavus	1(2.6)	0	0	0	0	0	0	0	1	0	0	0
Aspergillus species	1(2.6)	0	0	0	0	0	1	0	0	0	0	0
S. apiospermum/boydii[a]	5(12.8)	0	1	0	0	2	1	1	0	0	0	0
Alternaria ulocladium	2(5.1)	0	0	2	0	0	0	0	0	0	0	0
Rhizomucor species	1(2.6)	1	0	0	0	0	0	0	0	0	0	0
Penicillium species	1(2.6)	0	0	1	0	0	0	0	0	0	0	0
Yeasts												
Trichosporon species	6(15.4)	1	2	1	1	0	0	0	1	0	0	0
Candida albicans	1(2.6)	1	0	0	0	0	0	0	0	0	0	0
Candida tropicalis	1(2.6)	0	0	0	0	0	0	0	0	1	0	0
Dermatophytes												
Trichophyton tonsurans	2(5.1)	0	0	1	0	0	0	0	1	0	0	0
Total	39(100.0)	6	7	10	2	2	5	1	5	1	0	0

S. apiospermum/boydii: Scedosporium apiospermum/boydii

Scedosporium apiospermum/boydii was isolated from one sample collected from the sauna, one of the hottest area of hammams. *Aspergillus spp.* (n=3), *Trichosporon spp.* (n=1) and *Trichophyton tonsurans* (n=1) were isolated from another hot area, the central massage plattform. *Trichophyton tonsurans*, a dermatophyte, was isolated once from a slipper sample and once from a central massage platform sample. Among common tools, fungal colonization was most frequently seen in slippers, no colonization was detected on towels and peshtemals. *Candida albicans* was isolated from the dressing room carpet in one sample. *Candida tropicalis* was isolated from a bath loofah in one sample. *Aspergillus fumigatus*, and *Trichosporon spp*. were each isolated once from the bowls used in hammams. The distributions of the fungi isolated from different parts of the bath and the tools used are shown in Table 1.

Discussion

Turkish hammams are Finnish sauna-variants, only humid. The average temperatures in Finnish saunas and Turkish hammams are 80°C and 60°C, respectively [4]. In previous studies, the presence of dermatophytes was shown on the wrestling mats, floors of swimming pools and carpets [5]. Positive results were obtained for fungal contamination in all studies conducted in hammams. Physical environmental factors, including humidity and temperature, not only initiate fungal growth, but also stimulate fungal colonization [6]. Dermatophytes can survive for several years on the epithelium sheds and be transmitted indirectly to healthy people through the remnants of the epithelium sheds in the environment [7]. Hannuksela et al. [8] investigated the benefits and risks of Finnish saunas on human health and reported that fungal infections increased the risk. Although there are many scientific reports on fungal infections, investigations about fungal diversity and contamination in hammams in Turkey still do not suffice. Up until now, only one study regarding fungal flora conducted in two hammams has been published by Goksugur et al. [4] in 2006 in Turkey. In that study, Goksugur et al. [4] isolated 16 fungi species from 209 samples collected from traditional Turkish hammams. The isolated fungi species in this study were *Aspergillus spp.* (n=4), *Penicillium spp.* (n=4), *Trichophyton rubrum* (n=3), *Trichophyton mentragrophytes* (n=1), *Epidermophyton floccosum* (n=1), *C. albicans* (n=2), and *C. tropicalis* (n=1). The results of this study were similar to ours.

Being the most isolated mold, *Aspergillus* is known to cause many diseases in humans, from allergic reactions to respiratory tract colonization, aspergilloma and tissue invasions, mostly through pre-existing colonization [9]. In our study, *S. apiospermum/boydii* was isolated in five samples. *Scedosporium* is a rare pathogen which most frequently causes sinopulmonary and central nervous system diseases in both immunocompromised and immunocompetent humans [10]. These fungi are airborne saprobes that survive and grow in decomposing organic matter, which is why they reside in sewage areas, places with polluted water and urban soil, affecting nearby populations and causing serious infections [11]. Molds are quite common in the environment we live in. Reports show that *Fusarium* induces onychomycosis and lower-extremity skin lesions in neutropenic patients, and *Rhizopus* induces sinus infections in patients with diabetic ketoacidosis. *Alternaria* is an important culprit of paranasal sinusitis in both healthy humans and immunocompromised patients [9]. In our study, common tools were evaluated for fungal reproduction. Although there was no fungal growth or activation on towels and peshtemals, slippers were most frequently found to house fungal colonies. Washing towels and peshtemals in the washing machine at high temperatures after each use and providing disposable articles for each customer may be effective for preventing fungal contamination. Insufficient disinfection and washing of the slippers only once at the end of the day may explain the rich fungal colonization. The central massage platforms and saunas are the hottest parts of the Turkish Hammams with an average temperature around 60°C. In our study, *S. apiospermum/boydii* was isolated in only one sample collected from saunas. The high temperature limit for growth of any thermophilic eukaryotic organism is about 62-65 °C, so dermatophytes and other infective microorganisms are thought to be easily killed by the high temperatures in the Hammams [12]. In our study, five fungal colonies were grown from samples collected from the central massage platform, which were *Aspergillus spp. (n=3), T. tonsurans (n=1) and Trichosporon spp. (n=1)*. Central massage platforms and saunas had similar ambient temperatures, so what was the reason for the difference in fungal colonization? This question can be answered by comparing the use of saunas and central massage platforms. In traditional hammam culture, customers use slippers and sit in the saunas with peshtemals. The saunas in hammams are used for sweating purposes only. There is little skin contact with the floor and sitting areas, which is quite different from the central massage platforms. The customers lie on the marble central massage platforms with their

whole bodies after removing their slippers while they receive the exfoliation and massage services. At this time, the feet and almost all skin surfaces are in contact with the marble floor. We think that the epithelium, poured on the marble floor during massage and exfoliation, may increase the risk of fungal contamination. As mentioned in some studies, it has been reported that central massage platforms are quite rich for keratin derived from skin and other skin waste products, which are nutritional source of fungi [13,14]. Yenisehirli et al. [5] detected dermatophytes in 113 out of 144 samples in a study on mosque carpets. Interestingly, in our study, no dermatophyte could be isolated on the hammam dressing room carpets. However, six fungal colonies were isolated from samples collected from the dressing room carpets in hammams: *Aspergillus spp*. (n=3), *Rhizomucor spp.* (n=1), *Trichosporon spp.* (n=1), and *C. albicans* (n=1). More frequent disinfection, cleaning and washing of hammam dressing room carpets than mosque carpets may explain this significant difference. However, it is advisable to use an easier-to-clean flooring material in the dressing rooms instead of the carpets. Benammar et al. [1] isolated eight yeast species, *C. albicans*, *C. glabrata*, *C. tropicalis, C. lipolytica, Geotrichum spp., Trichosporon spp., Rhodotorula spp*. and *Cryptococcus spp.* in a traditional hammam study in Algeria. In our study, *C. albicans*, *C. tropicalis* and *Trichosporon spp.* were isolated. Being opportunistic fungi, *Candida* species can cause clinical infections in all organs, which include superficial mucosal infections, cutaneous candidiasis and extensive hematogenous spread involving organs such as the liver, spleen, heart and brain. Mucosal infections (known as oral candidiasis) linked with *Candida* species may be limited to the oropharynx or may spread to the esophagus and the whole gastrointestinal tract [9]. *Trichosporon* species are normal flora members of the gastrointestinal and genitourinary tract in humans but may temporarily colonize the skin and respiratory system [15]. In such studies, the reasons for reduced isolation of yeast groups may be related to the sample collecting method, or some fungi such as the *A. flavus*, which may exert antifungal effects against *C. albicans* and *C. Tropicalis* [16]. On the other hand, filamentous fungal hyphae can cause this result by rapidly growing and limiting the growth of yeasts in the environment [17]. The isolation rates of dermatophytes in hammam studies were reported as 0.98% by Benammar et al. and 2.39% by Goksugur et al [1,4]. In our study, the rate of dermatophyte isolation was 2.3%, which was similar to other studies. In our study, *T. tonsurans* were isolated from two samples only. Tinea corporis and Tinea capitis are the two most common clinical forms of *T. tonsurans* infection [18].

The limitations of this study are the use of conventional methods and the absence of molecular methods in fungal isolation.

Conclusion

In our study, the most isolated molds were fungi, as found in the nature. The isolation rate was very low. Dermatophytes are the most important culprit for fungal transmission in public places, such as baths. Compared to previous studies in swimming pools, wrestling cushions, mosque carpets and slippers, the outcomes of our study showed that Turkish Hammams had a very low risk for fungal contamination. Especially in hammams, regular disinfection and cleaning of the common areas and the tools, use of personal or disposable slippers, and informing customers about fungal infection transmission routes and prevention could reduce risk of fungal contamination.

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Antibiotic susceptibility pattern of *Enterococcus* isolates in a five year period at a tertiary care hospital

Üçüncü basamak bir hastanede beş yıllık bir sürede izole edilen *Enterococcus* izolatlarında antibiyotik duyarlılık profili

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Abstract

Aim: Enterococci are present as a part of the normal gut flora which can cause many community- and hospital-acquired infections. It is essential to determine the antibiotic resistance profile in treatment of Enterococcus spp. In this study we aimed to determine the sub-species of Enterococcus spp and their antibiotic resistance profiles isolated from a tertiary hospital in a five-year period.

Methods: The antibiotic resistance profiles of 2995 Enterococcus spp isolated from various clinical specimens of patients between January 2014 and December 2018 were reviewed in this retrospective cohort study.

Results: Ampicillin resistance was very low (5.6%) in *E. faecalis*, but it was very high in *E. faecium (91.8%)*. High level gentamycin, high level streptomycin and levofloxacin resistances were very high in all Enterococcus species and especially in *E. faecium*. Linezolid, tigecycline or daptomycin resistance was not determined in any Enterococcus isolates. Nitrofurantoin resistance (61.9%) and parenteral penicillin resistance (82.4%) were also very high in *E. faecium* isolates. Teicoplanin resistance was very low in *E. faecalis (1.5%)* isolates but approximately half (44.9%) of the *E. faecium* isolates were resistant to Teicoplanin. Vancomycin resistance was determined in 1.5% of *E. faecalis* isolates and in 45.5% of *E. faecium isolates.*

Conclusion: In conclusion, we determined high resistance rates to many antibiotics in both *E. faecalis* and *E. faecium* isolates. Tigecyclin, linezolid and daptomycin resistance was not determined in any Enterococcus isolates. Vancomycin resistance was determined in 1.5% of *E. faecalis* isolates and in 45.5% of *E. faecium* isolates. This high rate of vancomycin resistance should be taken into account and studies should be conducted to eliminate this resistance.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, Enterecoccus antibiotic resistance, Enterococcal infection

Öz

Amaç: Enterokoklar, normal bağırsak florasının bir üyesi olup, birçok toplum- ve hastane- kökenli enfeksiyona neden olabilmektedirler. Enterococcus spp. tedavisinde antibiyotik direnç profilinin belirlenmesi son derece önemlidir. Bu çalışmanın amacı, beş yıllık bir süre içerisinde üçüncü basamak bir hastaneden izole edilen Enterococcus spp. alt tiplerini ve antibiyotik direnç profillerini belirlemektir.

Yöntemler: Ocak 2014-Aralık 2018 tarihleri arasında çeşitli klinik örneklerden izole edilen 2995 Enterococcus spp. nin antibiyotik direnç profilleri bu retrospektif kohort çalışmada incelendi.

Bulgular: *E. faecalis* 'te ampisilin direnci çok düşük (%5,6) iken *E. faecium* 'da (%91,8) çok yüksekti. Yüksek düzey gentamisin, yüksek düzey streptomisin ve levofloksasin dirençleri tüm Enterococcus türlerinde ve özellikle *E. faecium* 'da çok yüksekdi. Enterococcus izolatlarında linezolid, tigesiklin veya daptomisin direnci saptanmadı. *E. faecium* izolatlarında nitrofurantoin (%61,9) ve parenteral penisilin direnci (%82,4) de yüksekti. *E. faecalis*'te (%1,5) teicoplanin direnci çok düşüktü, ancak *E. faecium* izolatlarının yaklaşık yarısı (%44,9) teicoplanine dirençliydi. Vankomisin direnci, *E. faecalis* izolatlarının %1,5'inde, ancak *E. faecium* izolatlarının %45.5'inde belirlenmiştir.

Sonuç: Sonuç olarak, hem *E. faecalis* hem de *E. faecium* izolatlarında birçok antibiyotiğe yüksek direnç oranları belirledik. Enteroccus izolatlarında tigesiklin, linezolid ve daptomisin direnci yoktu. Vankomisin direnci, *E. faecalis* izolatlarının %1,5'inde, ancak *E. faecium* izolatlarının %45,5'inde belirlenmiştir. Bu yüksek vankomisin direnci oranı göz önünde bulundurularak bu direnci ortadan kaldırmak için çalışmalar yapılmalıdır.

Anahtar kelimeler: *Enterococcus faecalis*, *Enterococcus faecium*, Enterecoccus antibiyotik direnci, Enterokok enfeksiyonu

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Introduction

Enterococci are present as a part of the normal gut flora and can cause many community- and hospital-acquired infections, including bloodstream infections, endocarditis, meningitis, and urinary tract infections [1]. Enterococcal infections are important since they may contribute to patient mortality, increased length of hospital stay and higher healthcare costs. *Enterococcus faecalis* and *Enterococcus faecium* are the most common enterococcal species. Especially *E. faecium* has emerged as an important multidrug-resistant nosocomial pathogen [2,3]. Unfortunately, Enterococci are intrinsically resistant to many antimicrobials and easily acquire the high-level drug resistance via horizontal gene transfer. Resistant Enterococci species, especially vancomycin resistant enterococci may cause difficulties in treatment [4,5]. In that aspect, it is essential for every hospital to determine their antibiotic profile in treatment of Enterococcus spp.

In this study we aimed to determine the sub-species of Enterococcus spp and their antibiotic resistance profiles isolated from a tertiary hospital in a five-year period.

Materials and methods

This study was performed in Health Sciences University Okmeydanı Education and Research Hospital, Medical Microbiology Department. The antibiotic resistance profiles of 2995 Enterococcus spp isolated from various clinical specimens of patients between January 2014 and December 2018 were retrospectively reviewed. Among patients with reproduction in more than one sample, only one strain was included in the study. Repeated samples were excluded from the study and different samples of the same patient were not included in determining susceptibility rates. Demographic features of the infected patients were also investigated.

Blood cultures were assayed on a fully automated blood culture device, BACTEC 9240 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA). The passage of the detected vials in the automated blood culture device to the Macconkey, chocolate and 5% sheep blood agar was performed. Cultures of urine, tissue-abscess, tracheal aspirate, catheter tip, sterile fluids were evaluated according to the material and using standard microbiological techniques in accordance with the procedure [6].

Colonies thought to be effective, especially for inpatients, were identified at the species level by the Phoenix ™ - 100 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA) automated system and antibiotic susceptibilities were studied. Isolated colonies from an outpatient group, Gram positive cocci with positive colony morphology on blood agar, having negative catalase test, forming blackness on bile esculin medium, growing on medium containing 6.5% NaCl and positive for pyrrolidonyl arylamidase (PYR-Oxoid) test were defined as Enterococcus spc. Antibiotic susceptibilities of isolated enterococci were determined by Kirby-Bauer disc diffusion method. Antibiotic susceptibilities were evaluated in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [7] in January 2014-December 2015, and of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6] in January 2016- December 2018. The study was approved by Okmeydanı Training and Research Hospital ethics committee (Date: 27.8.2019; Number:1415)

Statistical analysis

Statistical analyses were performed with SPSS 19.0 (IBM Company, Chicago, IL) software. The conformity of the parameters to the normal distribution was evaluated by Kolmogorow-Smirnov test. Descriptive statistics (number, percentage, mean and median) were performed. Comparison of descriptive data between groups was performed with cross tables and chi square test. One-way ANOVA test was used to compare the antibiotic resistance rates of different enterococcus spp. Results with *P*-value <0.05 were considered statistically significant.

Results

A total of 2995 Enterococcus spp were investigated. The mean age of the study participants was 52.45 (28.67) (median age: 62, range: 1-100) years. Among the patients, 1427 (47.6%) were male and 1568 (52.4%) were female. Enterococcus spp were isolated from 1250 (41.7%) patients who were admitted from the outpatient clinics while remaining 1745 (58.3%) were isolated from the hospitalized patients. The subgroups of isolated Enterococcus spp are summarized in Table 1. *E. faecalis* and *E. faecium* were significantly more common in hospitalized patients ($P<0.001$) while Enterococcus spp were significantly more common in out-patient admissions.

Enterococcus spp were isolated from different tissues and body fluids. The most commonly infected body fluid was urine (Table 2). Distribution of main Enterococcus subtypes in main tissues and body fluids infected are summarized in Table 3. In urine the most commonly isolated subtype was *E. faecalis* and in rectal swabs the main subtype was *E. faecium.* The most commonly isolated subtypes in some different wards are summarized in Table 4. Most common isolates were obtained from the intensive care unit and the most common subtype isolated in intensive care unit was *E. faecium.*

The resistance rates of Enterococcus spp to different antimicrobiotics are summarized in Table 5. Ampicillin resistance was very low in E. *faecalis*, but it was very high in *E. faecium*. Ciprofloxacin, High level Gentamycin, High level Streptomycin and Levofloxacine resistances were very high in all Enterococcus species, and especially in *E. faecium*. All *Enterococcus* isolates were susceptible to Linezolid, Tigecycline and Daptomycin. Nitrofurantoin resistance and parenteral penicillin resistance was also very high in *E. faecium* isolates. Teicoplanin resistance was very low in *E. faecalis* isolates but approximately half of the *E. faecium* isolates were resistant to Teicoplanin. There were significant differences between Enterococcus species regarding antibiotic resistance rates (Table 5). Vancomycin resistance was determined in 1.5% of *E. faecalis* isolates but in 45.5% of *E. faecium* isolate. Among Vancomycin resistant isolates, 175 (4 *E. faecalis* and 171 *E. faecium* isolates) were obtained from rectal swabs. Since rectal isolates are regarded as colonization, we should ignore those isolates. In that aspect, Vancomycin resistance was determined in 1.1% of *E. faecalis* isolates but in 30.4% of *E. faecium* isolates.

We also investigated the distribution of Enterococcus species in time and we determined that there was a significant

increase in *E. faecalis* isolates compared with *E. faecium* isolates, in time. In general, female patients were slightly higher when distribution of genders of patients infected with Enterococcus species is evaluated (Figure 2).

Vancomycin resistance rates of different Enterococcus spp in time is summarized in Figure 3. Regarding these findings, Vancomycin resistance rates in *E. fecalis* and *E.spc* were very low in time without significant alterations. However we determined very high Vancomycin resistance rates in *E. faecium* which showed a significant decrease in last 2 years.

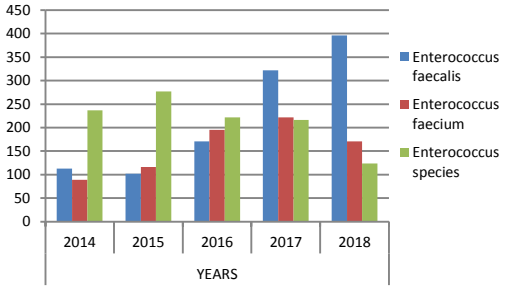

Figure 1: Distribution of Enterococcus species in time

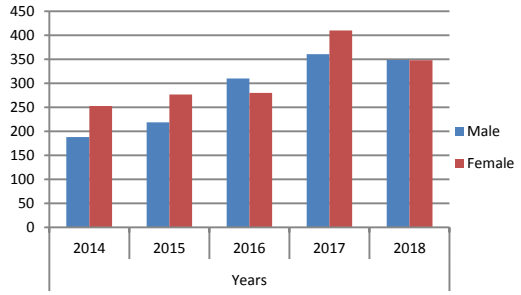

Figure 2: Distribution of gender of patients infected with Enterococcus species in time

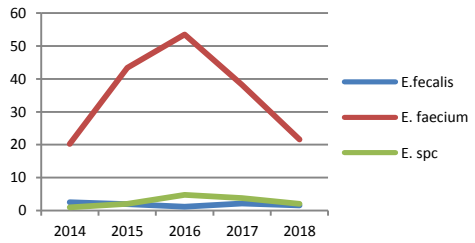

Figure 3: Vancomycin resistance rates of different Enterococcus spp in time

Table 1: The subgroups of isolated Enterococcus spp

Subgroups	In-patient	Out-patient	Number of isolates (%)
Enterococcus faecalis	648	456	1104 (36.8)
Enterococcus spp	337	739	1076 (35.9)
Enterococcus faecium	750	43	793 (26.5)
Enterococcus casseliflavus/gallinarum	4	10	14 (0.46)
Enterococcus raffinosus	5	1	6 (0.21)
Enterococcus durans	1	1	2 (0.07)

Table 2: Different tissues and body fluids from which Enterococcus spp were isolated

	Number of isolates (%)
Urine	2331 (77.8)
Blood	366 (12.2)
Rectal swab	182 (6.1)
Wound swab	58 (1.9)
Catheter	13 (0.4)
Abscess	11 (0.37)
Tissue	11 (0.37)
Peritoneal fluid	8 (0.26)
Cerebrospinal fluid	8 (0.26)
Throat swab	4 (0.13)
Sputum	1 (0.03)
Tracheal aspirate	1 (0.03)
Urethral effluent	1 (0.03)

Table 3: Distribution of main Enterococcus subtypes in main tissues and body fluids infected

	E. faecalis n=1104	*Enterococcus spc* n=1076	*E. faecium* n=793	Total
Urine	845	1030	438	2313
Blood	192	21	150	363
Rectal swab	5	0	177	182
Wound swab	32	16	9	57

Table 4: Distribution of main Enterococcus subtypes in some wards

	E .faecalis n=1104	*Enterococcus spc* n=1076	*E. faecium* n=793	Total
Intensive care unit	241	125	385	751
Internal medicine	215	117	142	474
Pediatrics	16	17	47	80
Hematology	15	13	44	72
Urology	30	12	17	59

Table 5: Antibiotic resistance rates

	E. faecalis n=1104 (%)	*Enterococcus spc* n=1076 (%)	*E. faecium* n=793 (%)	*P*-value
Ampicillin	62/1093 (5.6)	275/1072 (25.6)	729/794 (91.8)	0.01
Ciprofloxacin	365 /769 (47.5)	531/1067 (49.7)	284/371 (76.5)	0.01
Fosfomycin	0 /48	259 /992 (26.1)	6/45 (13.3)	0.01
High level gentamycin	493/1103 (44.7)	359/1055 (34.0)	481/790 (60.8)	0.09
Levofloxacin	449/953 (47.1)	490/ 1065 (46.0)	437 /563 (77.6)	0.02
Linezolid	0/1103	0/1077	0 /785	
Daptomycin	0/212	0/4	0 /220	
Nitrofurantoin	46 /950 (4.8)	100/1019 (9.8)	161 /260 (61.9)	0.01
Penicillin (parenteral)	67 /258 (25.9)	158 /1008 (15.7)	215 /261 (82.4)	0.01
Quinupristin/Dalfopristin	175/213 (82.1)	12 /639 (1.8)	1 /4 (25.0)	0.01
High level streptomycin	446 /885 (50.4)	13 /59 (22.0)	433/584 (74.1)	0.01
Teicoplanin	17 / 1098 (1.5)	56/1073 (5.2)	355 /790 (44.9)	0.01
Tigecycline	0 /881	0/58	0/580	
Vancomycin	17/1092 (1.5)	48/1073 (4.4)	360/791 (45.5)	0.01

Discussion

In this study we analyzed the antimicrobial resistance in 2995 Enterococcus isolates and we determined that approximately half of the E. *faecalis* isolates were resistant to ciprofloxacin, gentamycin, levofloxacin and streptomycin while approximately 80% of *E. faecalis* isolates were resistant to Quinupristin/Dalfopristin. On the other hand, among *E. faecium* isolates, more than 90% were resistant to ampicillin, approximately 75% were resistant to ciprofloxacin, levofloxacin, and streptomycin, and 60% were resistant to gentamycin and nitrofurantoin and approximately half of the isolates were resistant to teicoplanin. Tigecyclin, linezolid and daptomycin resistance was not determined in any Enteroccus isolates. Vancomycin resistance was determined in 1.1% of *E. faecalis* isolates but in 30.4% of *E. faecium* isolates. Moreover, we also determined that, there was a significant increase in *E. faecalis* isolates compared with *E. faecium* isolates, in time.

Enterococci are associated with both community-acquired and nosocomial infections and their antibiotic resistance potential and multidrug resistant isolates poses an important therapeutic challenge [8]. Sattari et al. [9] reported that more than 92% of *E. faecium* isolates were resistant to ampicillin (92.5%), ciprofloxacin (96%), erythromycin (100%) and clindamycin (96%) while a high frequency of resistance to clindamycin (100%), erythromycin (98.5%) and ciprofloxacin (80.5%) was reported in *E. faecalis* isolates, with a less frequent resistance to ampicillin (7%) in a children's hospital. In a study from Korea, Liu et al. [10] reported the ampicillin and penicillin resistance in *E. faecalis* blood strains was as 0.6% and 26.3%, respectively. On the other hand, they reported that resistance to vancomycin (34.0%) and teicoplanin (18.8%) was more frequent in *E. faecium* strains. Mamtora et al. [11] reported that *Enterococcus* spp were highly susceptible to linezolid (96%), vancomycin (92%), and teicoplanin (93.3%) while being resistant to erythromycin and ciprofloxacin. Zallipour et al. [12] reported the highest antibiotic resistance rates against tetracycline (93.5%), erythromycin (87%), and ciprofloxacin (80%) in *E. faecalis* isolates. They did not determine any resistance to fosfomycin or linezolid. Our results were similar with the results of previous studies. In another study performed in our country, more than 80% of the enterococci were reported

to be resistant to tetracycline and erythromycin; but Vancomycin resistance was not defined in any of the 235 Enterococcus isolates obtained in an animal study [13]. In a literature review, *E. faecalis* was reported to have a high resistance rate against erythromycin (67% resistance), gentamicin (65%), trimethoprim/sulfamethoxazole (54%), ciprofloxacin (51%) and oxacillin (49%), whereas nitrofurantoin (4% resistance) and teicoplanin (9%) were the most active agents against this species. On the other hand, *E. faecium* isolates were reported to be mostly resistant against erythromycin (78%), norfloxacin (84%), imipenem (82%) and trimethoprim/sulfamethoxazole (81%), whereas linezolid with no resistance and nitrofurantoin (16%) were the most effective antibiotics [14]. Huang et al. [15] also reported greater resistance rates of *E. faecium* than *E. faecalis* as in our study. They also reported the resistance rates of *E. faecium* to ampicillin and quinolones were more than 80%. Besides, the authors reported that, linezolid resistance in *E. faecalis* increased from 1.6% in 2008 to 2.97% in 2016, and linezolid resistance was higher in *E. faecalis* than in *E. faecium.* However, in our study, we did not determine any linezolid resistance in *Enterococcus spp*.

Vancomycin is regarded as the main treatment option in resistant enterococci infections. However we determined the Vancomycin resistance as 1.1% in *E. faecalis* isolates, but as high as 30.4% in *E. faecium isolates.* An increasing prevalence of vancomycin resistant enterococci (VRE) has been reported in previous literature [16,17]. Zallipour et al. [12] reported that 22.8% of 232 *E. faecalis* isolates were vancomycin resistant (MIC $\geq$ 256 μg/ml). Linezolid is the main treatment option in patients with VRE. Although linezolid resistance was also reported previously, the rates are still very low [18,19]. We also did not determine any isolates resistant to linezolid. Our results were compatible with the previous literature regarding the resistance rates, except very high Vancomycin resistance rates in *E. faecium isolates*.

Another interesting finding of this study was a significant increase in *E. faecalis* isolates compared with *E. faecium* isolates, in time. Due to the lower resistance potential of *E. faecalis* isolates than *E. faecium* isolates, this increased prevalence may be favorable and should be investigated in further studies. Interestingly, we also determined a decrease in Vancomycin resistance rates in *E. faecium* isolates within the last 2 years. In previous literature, the majority of vancomycin-resistant *Enterococcus* isolates were also defined as *E. faecium* and the resistance rates were reaching more than 80% [20]. High Vancomycin resistance rates were associated with long hospital stays and extended use of antibiotics [21]. This decrease in Vancomycin resistance rates in recent years may be associated with an increased awareness of this condition by clinicians and precautions taken to decrease this resistance which should also be investigated in further studies.

The main power of this study was the high number of isolates included in the study. However, these results were obtained from a single center and we did not perform any resistance analyses at molecular or genetic level, which are the main limitations.

Conclusion

We determined high resistance rates to many antibiotics in both *E. faecalis* and *E. faecium* isolates. Tigecyclin, linezolid and daptomycin resistance was not determined in any Enteroccus isolates. Vancomycin resistance was determined *in 1.1%* of *E. faecalis* isolates and in *30.4%* of *E. faecium* isolates. This high rate of vancomycin resistance should be taken into account and studies should be conducted to eliminate this resistance. However, we also determined a decrease in Vancomycin resistance in last two years in *E. faecium*, which should also be confirmed with prospective clinical studies.

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The efficacy of very low-density sodium hypochloride washes in preventing healthcare-associated infections in pediatric intensive care units

Çocuk yoğun bakım ünitelerinde sağlık hizmeti ilişkili enfeksiyonları önlemede düşük konsantrasyonlu sodyum hipokloritli banyo uygulamalarının etkinliği

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Abstract

Aim: Healthcare-associated infections (HAIs) have increased in pediatric intensive care units (ICUs) within the last decade. Maintaining hand hygiene, performing invasive interventions in accordance with aseptic techniques, contact precautions and chlorhexidine gluconate showers are the usual prevention methods against HAIs. However, despite all prevention methods, HAI incidence has globally increased in pediatric ICUs. The purpose of this study is to investigate the preventive effects of 0.005% sodium hypochlorite (NaOCl) showers against HAIs in pediatric ICUs.

Methods: This case control study was conducted in a 17-bed pediatric intensive care unit. Patients were washed with water and soap during the first six months and water and 0.005% sodium hypochlorite during the following six months, after which the incidence of HAIs was compared. The diagnosis of HAIs was made according to Centers for Disease Control and Prevention National Healthcare Safety Network guidelines.

Results: Two hundred thirty patients (118 patients in control group, 112 patients in NaOCl group) who met the inclusion criteria were included in the study. 26 patients among the control group and 20 patients among the NaOCl group were diagnosed with HAIs. In the NaOCl group, we detected 100% and 66% reductions in *P. aeruginosa* and *S. aureus* infections, respectively. There was no statistically significant difference between the groups in terms of overall HAI incidences (P=0.510). Most frequently encountered HAIs in both groups were ventilator-associated pneumonia and bloodstream infections. The rates of multidrug resistant gram-negative bacterial isolation were 77.8% (14/18) in the control group and 66.7% (5/15) in the sodium hypochlorite group. The rates of extensive drug resistant gram-negative bacterial isolation were 38.9% (7/18) in the control group and 26.7% (4/15) in the NaOCl group. There was no statistically significant difference between the two groups (P=0.458). We did not encounter any local or systemic side effects in any of our patients.

Conclusion: We found that weekly 0.005% NaOCl showers reduced *P. aeruginosa* and *S. aureus* infections, although it did not change length of hospital stay, incidence of total HAIs and the sensitivity of gram-negative bacteria to antibiotics.

Keywords: Antibiotic resistance, Chlorhexidine gluconate, Gram negative bacteria, Healthcare-associated infections, Sodium hypochlorite

Öz

Amaç: Sağlık hizmeti ile ilişkili enfeksiyonlar (SHİE) son on yılda çocuk yoğun bakım ünitelerinde (YBÜ) artış göstermiştir. El hijyeni sağlamak, girişimsel uygulamalarda asepsi şartlarına uymak, temas önlemleri ve klorheksidin glukonat banyoları, SHİE'leri önlemenin en temel yollarıdır. Ancak, tüm önlemlere rağmen, SHİE insidansının global olarak pediatrik yoğun bakımlarda artış gösterdiği görülmektedir. Bu çalışmanın amacı çocuk YBÜ'de %0,005 sodyum hipokloritli (NaOCl) banyo uygulamalarının dirençli bakteriler ile ortaya çıkan SHİE önleyip önlemeyeceğini değerlendirmektir.

Yöntemler: Bu vaka kontrol çalışması, 17 yataklı çocuk BYÜ'de prospektif olarak yapıldı. Çalışmanın ilk altı ayında hastalar sadece su ve sabun ile yıkanırken ikinci altı aylık dönemde NaOCl ile yıkandılar. SHİE tanıları hastalık kontrol ve önleme merkezinin rehberine göre konuldu.

Bulgular: Çalışma kriterlerini karşılayan 230 hasta (118 kontrol grubu, 112 NaOCl grubu) çalışmaya dahil edildi. Kontrol grubundan 26 hasta ve NaOCl grubundan 20 hastada sağlık hizmeti ile ilişkili enfeksiyon saptandı. NaOCl grubunda kontrol grubuna göre *P. aeruginosa* ve *S. aureus* enfeksiyonlarında sırasıysa %100 ve %66 oranında azalma tespit edildi. SHİE sayısı açısından gruplar arasında istatiksel olarak anlamlı fark saptanmadı. Her iki grupta da en sık görülen SHİE ventilator ile ilişkili pnömoni ve kan dolaşım yolu enfeksiyonlarıydı. Çoklu ilaç dirençli gram negatif bakteri oranı kontrol grubunda %77,8 (17/18) iken, NaOCl grubunda %66,7 (5/15) olarak saptandı. Yaygın ilaç dirençli bakteri oranı kontrol grubunda %38,9 (7/18), NaOCl grubunda %26,7 (4/15) idi. Gruplar arasında anlamlı fark yoktu (P=0,458). NaOCl uygulanan hastalarda herhangi bir lokal veya sistemik yan etki gözlenmedi.

Sonuç: Haftalık %0,005'lik NaOCl banyo uygulamalarının *P. aeruginosa* ve *S. aureus* enfeksiyonlarında azalma sağladığı, ancak hastane kalma süresi, total SHİE sayısı ve gram negatif bakterilerin antibiyotik duyarlılıklarında ise bir değişikliğe sebep olmadığı gösterilmiştir.

Anahtar kelimeler: Antibiyotik direnci, Klorheksidin glukonat, Gram negatif bakteriler, Sağlık hizmeti ilişkili enfeksiyonlar, Sodyum hipoklorit

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Introduction

Healthcare-associated infections (HAIs) affect approximately 30% of patients in intensive care units (ICUs). It increases mortality and morbidity rates, length of hospital stay, and medical expenses [1]. Therefore, prevention and reduction of HAIs in ICUs is among the most imperative issues.

HAIs include surgical-site infections, bloodstream infections (BSI), central-line-associated bloodstream infections, urinary tract infections (UTI), and ventilator-associated pneumonias (VAP). Patients' skin may be colonized with methicillin-resistant *S. aureus* (MRSA) and carbapenem–resistant *Enterobacteriaceae* during hospitalization [2]. Nowadays, prevention of these infections has become the first step in the fight against HAIs. The usual prevention methods include hand hygiene, contact precautions, and aseptic techniques in performing invasive interventions. Chlorhexidine gluconate (CHG) shower has emerged as a new strategy to prevent skin colonization [3].

Sodium hypochlorite (NaOCl) application prevents *S. aureus* colonization and infections, including MRSA, in patients with atopic dermatitis (AD) [4,5]. At concentrations of 0.025-0.5%, NaOCl is used for treatment of wounds, burns, and decubitus ulcers [6]. Several studies have shown that NaOCl is safe at the concentration of 0.005% [6-10].

Intensive bacterial colonization is an important risk factor for HAIs [11]. Increasing antibiotic resistance and difficulties in treating infections have encouraged novel studies aimed at reducing colonization. In this study, we aimed to evaluate the efficacy of NaOCl wash at a bactericidal, non-toxic concentration in reducing the incidence of HAIs in pediatric ICU patients. We also evaluated patients for any metabolic or allergic side effects of NaOCl.

Materials and methods

Patients (between the ages of 1 month to 18 years old) without any dermal lesions, open wounds or any known allergic reactions to NaOCl, and who were hospitalized in pediatric ICUs for more than 72 hours were included in the study. A washing solution of 0.005% NaOCl was prepared by mixing 100 ml 5% NaOCl with 100 liters of water. Patients' whole bodies, except the eyes and mucosal membranes, were washed with the NaOCl solution using a washcloth for 30 minutes, after which they were washed with pure water. This washing procedure was performed once a week to all patients. During the research period, routine cleaning procedures and infection control measures, such as contact precautions for cases who were colonized or infected by multidrug-resistant organisms (MDRO), were continued. We did not actively survey MDRO colonization. Routine oral hygiene with 0.12% CHG was continued in both groups.

We evaluated the incidence of HAIs as a primary outcome and positive culture samples as a secondary outcome. HAI diagnoses were based on The Centers for Disease Control and Prevention guidelines [12]. We took samples from patients suspected of having infections to demonstrate the etiological agent.

The study was initiated in March 2015. During the first six months, patients were washed with soap and water. After a month-long gap, NaOCl-wash procedure was initiated in October 2015 and lasted until April 2016. Prospective active surveillance continued. The patients' demographic data, primary diseases, reasons for hospitalization in the pediatric ICU, hospitalization and discharge (or death) dates, presence of central or urinary catheters, duration of mechanical ventilation, isolated microorganisms, and their sensitivity to antimicrobials were recorded.

Statistical analysis

We used Statistical Package for Social Sciences Version 21.0 (SPSS, Chicago, IL, USA) for statistical analysis. *P*-value <0.05 was deemed statistically significant. Shapiro-Wilk test was used for normality analysis of numerical data. Mann-Whitney U test was used to compare numerical data that are not normally distributed, and Chi-square was used to compare categorical data.

Results

During the first and second six-month long periods, 420 and 405 patients, respectively, were hospitalized in the pediatric ICU. 118 patients in the first group and 112 patients in the NaOCl group met the inclusion criteria. The mean duration of hospitalization was 27.4 days in the control group and 32.8 days in the NaOCl group. 14 (11.9%) patients from the control group and 14 (12.5%) patients from the NaOCl group died during the study. The demographic data of the patients and statistical analysis results are presented in Table 1.

Among the control and NaOCl groups, 26 and 20 patients were diagnosed with HAIs, respectively. There was no difference between the two groups in terms of total HAI incidences (*P*=0.510) (Table 2). The most commonly isolated microorganisms were *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* (Table 3). Antibiotic resistance of Gram-negative bacteria (GNB) did not differ among groups (Table 4). The rates of multidrug resistant Gram-negative bacteria (MDRGNB) responsible for HAIs were 76.5% (13/17) in the control group and 66.7% (10/15) in the NaOCl group. There were no significant differences between the groups in terms of MDR rate (*P*=0.472). The rates of extensively drug-resistant bacteria isolates (XDRGNB) were 41.2% (10/17) in the control group and 26.7% (4/11) in the NaOCl group, which did not differ among the two groups (*P*=0.538) (Table 5).

Toxic, allergic, or metabolic reactions against NaOCl were not detected in any of our patients during the course of this study.

Table 1: Demographic data

		Control*	NaOCl*	Total*	*P*-value
Age (month)		43 (2-204)	41 (2-190)	43 (2-204)	0.440
Duration of hospitalization (days)		21 (4-217)	23 (4-179)	33 (4-217)	0.112
Duration of mechanical ventilation (days)		3 (0-217)	4 (0-156)	4 (0-217)	0.144
Duration of central catheters (days)		0 (0-34)	0 (0-19)	0 (0-34)	0.053
Duration of urinary catheters(days)		0 (0-20)	0 (0-34)	0 (0-34)	0.532
		Control n (%)	NaOCl n (%)	Total n (%)	*P-value*
Gender	Male	64 (54.2)	59 (52.7)	123 (53.5)	0.895
	Female	54 (45.8)	53 (47.3)	107 (46.5)	
Dead		14 (11.9)	14 (12.5)	28 (12.2)	1.000

* median (min-max)

Table 2: Total number of HAIs and their percentiles

	Control n (%)	NaOCl n (%)	Total n (%)	*P*-value
VAP	9 (7.6)	6 (5.4)	15 (6.5)	0.597
BSI	9 (7.6)	7 (6.3)	16 (7)	0.798
CABSI	1 (0.8)	2 (1.8)	3 (1.3)	0.614
Pneumonia	3 (2.5)	3 (2.7)	6 (2.6)	1.000
USI	2 (1.7)	1 (0.9)	3 (1.3)	1.000
CNSI	1 (0.8)	0 (0)	1 (0.4)	1.000
SSTI	1 (0.8)	1 (0.9)	2 (0.9)	1.000
Total	26 (22)	20 (17.9)	46 (20)	0.510

BSI: Bloodstream infection, CABSI: Central-line associated bloodstream infection, CNSI: Central nervous system infection, HAIs: Healthcare associated infections, SSTI: Skin and soft tissue infection, USI: Urinary system infection, VAP: Ventilator associated pneumonia

Table 3: Distribution of HAIs according to pathological agent (n,%)

HAIs	VAP		BSI		CABSI		Pneumonia		USI		CNSI		SSTI	
Pathological Agents	CG	SG	CG	SG	CG	SG	CG	SG	CG	SG	CG	SG	CG	SG
A.baumannii	1(0.8)	2(1.8)	0	1	1(0.8)	0	0	2(1.8)	0	0	1(0.8)	0	0	1(0.9)
C. albicans	0	0	1(0.8)	0	0	1(0.9)	0	0	0	0	0	0	0	0
C. parapsilosis	0	0	0	1(0.9)	0	0	0	0	0	0	0	0	0	0
C. freundii	1(0.8)	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter Spp.	1(0.8)	1(0.9)	0	2(1.8)	0	0	0	0	0	0	0	0	0	0
E. faecium	0	0	1(0.8)	0	0	0	0	0	0	0	0	0	0	0
E. coli	1(0.8)	0	0	0	0	0	0	0	1(0.8)	0	0	0	0	0
K. oxytoca	0	0	1(0.8)	0	0	1(0.9)	1(0.8)	1(0.9)	0	1(0.9)	0	0	0	0
K. pneumoniae	2(1.7)	2(1.8)	1(0.8)	1(0.9)	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	2(1.7)	0	2(1.7)	0	0	0	1(0.8)	0	1(0.8)	0	0	0	0	0
S. marcescens	1(0.8)	0	1(0.8)	0	0	0	0	0	0	0	0	0	0	0
S. aureus	0	1(0.9)	1(0.8)	0	0	0	1(0.8)	0	0	0	0	0	1(0.8)	0
S. epidermidis	0	0	1(0.8)	1(0.9)	0	0	0	0	0	0	0	0	0	0
S. malthophilia	0	0	1(0.8)	1(0.9)	0	0	0	0	0	0	0	0	0	0
Total	9(7.6)	6(5.4)	9(7.6)	7(6.3)	1(0.8)	2(1.8)	3(2.5)	3(2.7)	2(1.7)	1(0.9)	1(0.8)	0	1(0.8)	1(0.9)

BSI: Bloodstream infection, CG: Control Group, CABSI: Central-line associated bloodstream infection, CNSI: Central nervous system infection, HAIs: Healthcare associated infection, SG: Study Group SSTI: Skin and soft tissue infection, USI: Urinary system infection, VAP: Ventilator associated pneumonia

Table 4: Antibiotic resistance rate of gram negative bacteria

Antibiotics	Control n (%)	NaOCl n (%)	*P*-value
Cefepime and ceftazidim	15/17 (88.2)	12/15 (80)	0.645
Piperacillin-tazobactam	15/17 (88.2)	11/15 (73.3)	0.383
Aminoglycoside	9/17 (52.9)	8/15 (53.3)	1.000
Carbapenem	11/17 (64.7)	7/15 (46.7)	0.503
Fluoroquinolone	12/17 (70.6)	9/15 (60)	0.978
Colistin	0/17 (0)	0/15 (0)	-

*Number of resistant bacteria/ number of total bacteria

Table 5: MDR and XDR rates of GNB

	MDR n (%)			XDR n (%)		
	CG	SG	*P*-value	C	SG	*P*-value
Escherichia coli	2 (100)	0	(-)	1/2 (20)	0	(-)
Klebsiella Spp.	2/5 (40)	3/6 (50)	0.740	1/5 (20)	1/6(16.7)	0.887
Acinetobacter Spp.	3/3 (100)	6/6 (100)	0.635	1/3 (33.3)	3/6 (50)	(-)
Pseudomonas Spp.	5/6 (83.3)	0	(-)	4/6 (66.7)	0	(-)
Enterobacter Spp.	1/1 (100)	3/3 (100)	0.248	1 (100)	1/3 (33.3)	(-)

CG: Control Group, GNBI: Gram negative bacteria, MDR: Multidrug resistant, SG: Study Group, XDR: Extensively drug resistant

Discussion

Around 30% of ICU patients are affected by HAIs. Along with mortality and morbidity rates, HAIs also increase duration of hospitalization and healthcare costs [1]. Prolonged hospital stay increases skin colonization, which in turn leads to an increase in HAIs, blood culture contamination, and hand contamination in healthcare personnel [13,14]. Skin colonization with resistant bacteria such as MRSA, vancomycin-resistant *enterococci* and *A. baumanii* cause severe HAIs [15-17].

Incompliance with hand hygiene and barrier precautions as well as disagreements about cost-effectiveness decreases the efficacy of infection control [18,19]. Infection control precautions are generally focused on patients, infected fomites, and contact with environmental surfaces. HAIs can develop despite contact precautions, compliance with hand hygiene as well as aseptic conditions during the performance of invasive interventions [18,19].

One of the most significant factors in decreasing the rate of HAIs is to decrease skin colonization, for which the scientists are always looking for new methods. One of the most frequently used methods to reduce HAIs is CHG shower, which is currently recommended by several guidelines [20]. It has been indicated that CHG application decreased *A. baumanii*, vancomycin-resistant *enterococci*, MRSA colonization and BSI rate [13,21,22]. Although the efficacy of this method has been proven, its application is not practical in daily routine because of the inadequacy of healthcare personnel. Besides, application of CHG is costly; and unnecessary application of CHG could cause an increase in bacterial resistance [23,24].

The objective of our study was to investigate whether 0.005% NaOCl solution could be an effective, low cost, and easily applicable agent in prevention of HAIs. We gave weekly 0.005% NAOCl washes. Several studies have reported that 0.005% NaOCl showers were effective and safe in reducing *S. aureus* (including MRSA) infections and colonization in patients with atopic diseases (AD) [6-10]. NaOCl has been safely used in environmental cleaning and disinfection. It is known to be bactericidal in concentrations that are used to prevent *S. aureus* infection and colonization in AD [25,26]. At concentrations of 0.025-0.5%, NaOCl is used for antiseptic purposes for the treatment of burns, wounds and deep ulcers [4,27-31].

When mixed with water, NaOCl is converted to hypochloric acid (HOCl), which has strong antibacterial and antifungal effects. HOCl produces superoxide radicals that cause oxidative damage and cell death. HOCl is quite effective against Gram-negative and positive bacteria, spores, fungi, and viruses [25,26].

Decolonization of patients is known to prevent HAIs [32,33]. Although we did not evaluate colonization of patients in this study, we evaluated HAIs, which is an indirect indicator of colonization. We found an insignificant reduction in HAIs in the NaOCl group compared to the control group. We also found a decrease in VAP and BSI which was not statistically significant. Despite the lack of significant difference between the groups, reduction in HAIs with NAOCl wash remains an important finding.

Although the rates change according to geographical regions, GNB are responsible for 70% of VAPs and UTIs and 30% of BSIs [34]. Moreover, the GNB are responsible for up to 97.8% of all HAIs in developing countries [35]. In this study we found that GNB were responsible for all VAP and UTI infections and 84.3% of HAIs. In addition, GNB were responsible of 83.1% of HAIs in the control group and 85.7% of HAIs in the NaOCl group. There were three HAIs caused by *S. aureus* and one caused by *E.faceum* in the control group, whereas there was only one HAI caused by *S.aureus* in the NaOCl group. Although sample size was small, this study shows that very low density NaOCl is effective in reducing Gram-positive infections (especially *S. aureus*) up to 66%.

MDRGNB and XDRGNB have become major problems in the ICUs. In some developing countries, MDR and XDR rates are as high as 96% and 43.3%, respectively [35]. Therefore, besides the usual precautions to prevent HAIs, daily wash with CHG or very low-density NaOCl (which was used in this study) gained importance. Although statistically insignificant, it is promising to find reductions in HAIs rates, GNB resistance rate, MDR rate, XDR rate, and 66% reduction in *S.aureus* infection rates with NaOCl.

The CHG and NaOCl washes cannot get ahead of contact precautions. These strategies are important in terms of

preventing infections that develop despite all infection control precautions. They should be considered as complementary applications.

Limitations

We gave NaOCl washes just once a week owing to the limited number of healthcare staff. The NaOCl wash period was short (6 months). We were not able to use higher concentrations of NaOCl because of safety precautions (in the literature, higher concentrations were used locally only). We could not prevent lung colonizations with this method, which posed a risk for development of VAPs (responsible for most of HAIs).

Conclusions

We could not show that NaOCl wash was effective in reducing HAIs and epidemiologically important GNBS infections, except for *P.aeruginosa*. However, we demonstrated a significant decrease in Gram-positive bacterial infections, especially those caused by *S.aureus*. Although we could not detect a significant difference between two groups, the diminution in HAI rates is promising. We proved that NaOCl wash (at concentrations used in our study) does not have any toxic, metabolic, or allergic side effects on patients. Further multicenter studies with longer durations are required to determine the efficacy of 0.005% NaOCl in prevention of HAIs.

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Prognostic performance of optic nerve sheath diameters in CT images and serum procalcitonin levels in traumatic brain injury patients in the intensive care unit: A retrospective cohort study

Yoğun bakım ünitesinde travmatik beyin hasarlı hastalarda BT görüntülerinden elde edilen optik sinir kılıf çapı ve serum prokalsitonin değerlerinin prognostik performansları: Retrospektif kohort çalışma

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Abstract

Aim: Traumatic brain injury (TBI) is one of the common emergencies with a high mortality rate. It is difficult to determine the mortality and prognosis of TBI in the intensive care unit (ICU). The aim of this study is to assess the prognostic relationship of optic nerve sheath diameters (ONSD) as seen on computerized tomography (CT) images as well as serum procalcitonin (PCT) levels to mortality and Glasgow Outcome Scale (GOS) scores of patients with traumatic brain injury in the ICU.

Methods: Data from 78 traumatic brain injury patients who were admitted to an ICU, underwent brain CT and had serum PCT levels measured, were investigated retrospectively. Patients' data were gathered from ICU medical records. The ONSD was measured at 3 mm behind the globe.

Results: The mean age of the patients was 57.11 (17.07) years. 57.7% of the patients were males, and 42.3% were females. The cut-off ONSD and serum PCT values were evaluated to determine mortality and prognosis (Cut-off values: right ONSD: 5.44, left ONSD: 5.37, PCT: 3.95 for mortality; right ONSD: 5.26, left ONSD: 5.28, PCT: 2.29 for GOS, respectively).

Conclusion: ONSD measurements and serum PCT levels are associated with mortality and prognosis in traumatic brain injury patients.

Keywords: Optic nerve sheath diameter, Procalcitonin, Traumatic brain injury

Öz

Amaç: Travmatik beyin hasarı (TBH) yüksek mortalite oranı ile sonuçlanan sık karşılaşılan acillerden biridir. Yoğun bakım ünitesinde (YBÜ) TBH için mortalite ve prognozu belirlemek zordur. Bu çalışmanın amacı, YBÜ'deki travmatik beyin hasarı olguların mortalite ve Glasgow outcome skalası ile bilgisayarlı tomografi (BT) görüntülerinden elde edilen optik sinir kılıf çapı ve serum prokalsitonin seviyeleri arasındaki ilişkiyi göstermek ve optik sinir kılıf çapıyla prokalsitonin seviyesinin prognostik değerini ölçmektir.

Yöntemler: Beyin BT görüntülemesi yapılmış ve serum prokalsitonin düzeyi çalışılmış 78 travmatik beyin hasarı hastasının verileri retrospektif olarak incelendi. Hasta verileri yoğun bakım tıbbi kayıtlarından elde edildi. Optik sinir kılıf çapı göz güresinin 3 mm arkasından ölçüldü.

Bulgular: Hastaların yaş ortalaması 57,11 (17,07) iken %57,7'si erkek, %42,3'ü kadındı. Optik sinir kılıf çapı ve serum prokalsitonin cut-off değerleri, mortalite ve prognozu belirlemek için hesaplandı. Mortalite için cut-off değerleri sırasıyla: Sağ ONSD: 5,44, sol ONSD: 5,37, PCT: 3,95, GOS için sağ ONSD: 5,26, sol ONSD: 5,28, PCT: 2,29'du.

Sonuç: Optik sinir kılıf çapı ölçümü ve prokalsitonin seviyeleri travmatik beyin hasarlı hastalarda mortalite ve prognoz ile ilişkilidir.

Anahtar kelimeler: Optik sinir kılıf çapı, Prokalsitonin, Travmatik beyin hasarı

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Introduction

Traumatic brain injury (TBI) is one of the common emergencies with a high mortality rate [1]. Many factors, such as gender, age, the severity of the injury, comorbidities, anticoagulant use, initial Glasgow Coma Scale (GCS), the affected region of the brain, and the prevention of secondary brain injury determine the prognosis of TBI [2]. Intracranial pressure (ICP) monitoring is essential to decrease morbidity and to prevent patients from secondary brain injury in moderate and severe TBI in order to improve functional outcome and mortality [3].

The optic nerve is the most accessible part of the brain meninges. Previous studies have shown that examining the optic nerve sheath diameter (ONSD) calculated from the computed tomography (CT) images is one of the easiest ways to detect elevated ICP [4]. Serum procalcitonin (PCT) level is another indicator of the severity of TBI [5]. In theory, the combination of these two parameters should prove a better way to evaluate patient prognosis in TBI.

In this study, we aimed to assess the prognostic relationship of ONSD (calculated from CT images) as well as serum PCT levels to mortality and Glasgow Outcome Scale (GOS) in TBI patients.

Materials and methods

After obtaining approval from the ethical committee for clinical research of Mugla Sitki Koçman University on 08.08.2019 (approval number: 9/4), adults with TBI who were admitted to the Anesthesiology Intensive Care Unit of Mugla Sitki Koçman University Research and Training Hospital between January 2017 and June 2019 were enrolled in our study. We excluded patients who had facial trauma affecting the eyeballs, a pre-existing orbital disease affecting the orbital nerve, globe pathology, incomplete medical records, unavailable CT brain scans, and whose PCT levels were not obtained within the first 12 hours of admission.

Age, sex, initial GCS, Acute Physiology and Chronic Health Evaluation score, a mortality predictor, Revised Trauma Score, and GOS values were obtained from the medical records. Serum PCT and ONSD were recorded if they were tested within the first 12 hours. The ONSD was measured from brain CT images, at 3 mm posterior to the optic disc exit site in both eyes.

Statistical analysis

Data were analyzed using the IBM SPSS Statistics package version 23.0 (IBM Corp., Armonk, NY). Descriptive analysis was performed to calculate percentages and proportions. A t-test was used to compare the means of the two groups. A chi-square test was used to compare qualitative data for the level of significance. The values for PCT and ONSD were evaluated with a receiver operator characteristic (ROC) curve to determine the cut-off value. Regression analysis was used to examine the relationship between PCT, ONSD and mortality.

Results

A total of 109 patients with TBI were examined from the ICU registry. Among them, 31 patients were excluded based on the reasons listed above. A total of 78 patients with the following injuries were included in the study: subdural hematoma (4 patients), epidural hematoma (6 patients), subarachnoid hemorrhage (21 patients), brain edema (18 patients), intracerebral hematoma (6 patients), concussion (10 patients), and combined hemorrhage (13 patients). The mean age of the patients was 57.11 (17.07) years. Among them, 57.7% were males, and 42.3% were females (Table 1).

A statistically significant relationship was observed between ONSD (left and right) and mortality (t: -7.157, P<0.001; t: -6,853, P<0.001; respectively). A ROC curve was plotted to assess the prognostic value of the left and right ONSD measurement. The area under the curve (AUC) of the left and right ONSD were 0.883 and 0.879, with a 95% CI of 0.800 to 0.966 and 0.799 to 0.959, respectively. Results for the left ONSD at a cut-off point of 5.44 mm had a sensitivity of 92.77%, specificity of 86.21%, positive predictive value of 77.8%, and a negative predictive value of 78.4%. Results for the right ONSD at a cut-off point of 5.37 mm had a sensitivity of 96.56%, specificity of 84.43%, positive predictive value of 84.43%, and a negative predictive value of 81.5%.

According to the evaluation results of serum PCT with respect to mortality, the AUC was 0.841 with a 95% CI of 0.753 to 0.928. A cut-off value of 3.95 had a sensitivity of 92.19%, specificity of 83.25%, a positive predictive value of 58%, and a negative predictive value of 92%. A statistically significant negative correlation (-31.70) was found between PCT and mortality (t: -5.995, P<0.001).

The GOS was used to assemble two groups: poor recovery and good recovery. Those who scored two to five on the GOS were included in the poor recovery group while the good recovery group consisted only of those that scored one on the GOS. We compared ONSD and serum PCT with the poor and good recovery groups. The analyses of ROC and AUC are presented in Table 2. There was a statistically significant difference between the left-right ONSD and serum PCT, according to the GOS (P=0.002).

Table 1: Patient demographic characteristics and results

		Death (n=27)	TBI (n=51)	Total (n=78)	*P*-value
Gender %, (n)	Men	16.7% (13)	41% (32)	57.7% (45)	0.237
	Women	17.9% (14)	24.4% (19)	42.3% (33)	
Age		50.70 (17.7)	45.21 (16.59)	47.11 (17.07)	0.179
APACHE-II		30.22 (8.51)	20.49 (8.19)	23.85 (9.44)	<0.001
GCS		3.74 (1.65)	10.29 (3.48)	8.02 (4.31)	<0.001
RTS		4.59 (3.30)	6.62 (3.83)	5.92 (3.76)	0.022
PCT (ng/mL)		40.86 (29.41)	9.16 (17.33)	20.13 (26.78)	<0.001
ONSD (mm)	Left	5.57 (0.123)	5.06 (0.355)	5.24 (0.371)	<0.001
	Right	5.56 (0.147)	5.08 (0.314)	5.24 (0.382)	<0.001

Results are presented as number, mean (standard deviation) or percentage according to the normality of distribution. APACHE-II: Acute Physiology and Chronic Health Evaluation II, GCS: Glasgow Coma Score, ONSD: Optic nerve sheath diameter, PCT: Procalcitonin, RTS: Revised trauma score, TBI: Traumatic brain injury

Table 2: The results of the diagnostic scan and ROC curves for ONDS and PCT compared to mortality and GOS

		Diagnostic scan					ROC curve		*P*-value
		Cut-off	Sensitivity	Specifity	Positive Predictive Value	Negative Predictive Value	AUC	95% Confidence Interval	
Mortality	Left ONSD (mm)	5.44	92.77	86.21	77.8	78.4	0.883	0.800-0.966	<0.001
	Right ONSD (mm)	5.37	96.56	84.43	81.5	78.4	0.879	0.799-0.959	<0.001
	PCT (ng/mL)	3.95	92.19	83.25	85.2	86.9	0.841	0.753-0.928	<0.001
GOS	Left ONSD (mm)	5.26	72.69	81.75	98.9	97.6	0.711	0.578-0.842	0.002
	Right ONSD (mm)	5.28	70.28	81.67	98.9	97.6	0.710	0.579-0.842	0.002
	PCT (ng/mL)	2.29	74.56	70.61	88.9	91.2	0.715	0.600-0.830	0.002

AUC: Area under the curve, GOS: Glasgow Outcome Score, ONSD: Optic nerve sheath diameter, PCT: Procalcitonin

Discussion

Our study demonstrated that ONSD measurements and serum PCT values are useful parameters in predicting the mortality and prognosis of patients with TBI. Neurological examinations are important for determining TBI, but not enough to predict the prognosis. The present prognostic scores are complex, require numerous data and are not easily applied [6]. Thus, there is a need for a prognostic indicator that is non-invasive, simple and includes a small amount of data.

Many previous studies have demonstrated that ONSD measurement can be used for detecting increased ICP and that monitoring ICP is important for proper management of TBI [4]. Moreover, it has been shown that an enlarged ONSD on brain CT is an independent factor for mortality and poor prognosis [6]. A similar study by Lee et al. demonstrated that the cut-off value for ONSD on CT was 4.13 mm, and the AUC was 0.986 (95% CI: 0.939 to 0.989) [4]. The results of our study are similar with previous studies concerning ONSD values for the prediction of mortality and prognosis, but our cut-off value for ONSD on CT was higher. This difference could be explained by the fact that the brain CT scan was obtained within 12 hours after the trauma in our study.

Diverse techniques have been utilized for ONSD measurement. It was more frequently measured with ultrasound but this requires an experienced practitioner [2]. Magnetic resonance imaging (MRI) is another technique proposed for ONSD evaluation, however, limited accuracy and thicker brain slices may change the measured ONDS value. Most reported ONSD values are measured on CT and MRI [6]. In our study, we measured ONSD with CT scans.

PCT is an inflammatory marker present in the pathogenesis of severe complications such as sepsis or organ failure. Recent studies have demonstrated the role of PCT in neurotrauma that is caused by TBI or resuscitation [5,7]. When inflammatory process is triggered, messenger ribonucleic acid synthesized by calcitonin I gene is upregulated, giving rise to increasing PCT levels within a two to three-hour period. PCT reaches plateau levels within 6 to 12 hours and has a half-life of 20 to 24 hours. Via the same pathogenesis, TBI leads to increased PCT levels in blood circulation. However, PCT levels in patients with TBI are lower than those after extracranial injuries [5]. In our study, high PCT levels were detected in patients with multiple trauma.

Limitations

There are several limitations to this study. First, this is a single-center study with a retrospective design. Therefore, the results may have limited applicability. We could not standardize the data because of the small number of patients; and we could not group the data according to hours passed after trauma.

Conclusion

Traumatic brain injury is still one of the most mortal diseases amongst patients in intensive care units. The best and safest method used to determine mortality and prognosis in TBI is currently under investigation. In our study, there is a well-documented relationship between ONSD measurements, serum PCT levels, prognosis and mortality. In patients with TBI, ONSD measurements via CT and serum PCT can be safely used to determine prognosis and mortality.

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Effects of preoperative fine needle aspiration biopsy on surgical strategy in patients with papillary thyroid carcinomas

Papiller tiroid karsinomlu hastalarda ince iğne aspirasyon biyopsinin cerrahi strateji üzerine etkileri

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Abstract

Aim: Papillary thyroid carcinomas (PTCs) usually have good prognosis. In the presence of lymph node metastasis, capsular invasion and extra-thyroidal extension, some PTCs may display aggressive behavior. Early diagnosis of these cases is extremely important. Ultrasound-guided fine needle aspiration biopsy (FNAB) is an important diagnostic procedure and may identify the PTC likely to behave aggressively. Our study aimed to examine the effects of FNAB on surgical strategy in patients with PTC.

Methods: The data of 269 patients who underwent total thyroidectomy were evaluated in our retrospective cohort study. FNAB had been performed in 188 (70%) patients. Patients with non-incidental diagnoses were compared with patients who had incidental diagnoses in terms of parameters likely to be related to aggressive PTC.

Results: Patients ≤45 years old were more common in the non-incidental group (P=0.041). Incidental diagnoses were common in females (P=0.014), those with nodules larger than 2 cm in diameter and in patients with ≥2 nodules (P=0.001). Postoperative remnant thyroid tissue was observed more commonly in incidental cases (P=0.008). Lymph node metastasis (P=0.044), capsular invasion (P=0.009), and extra-thyroidal extension (P=0.022) were more common in the non-incidental group.

Conclusion: It is difficult estimate the behavior of PTCs preoperatively. Only a small number of PTCs can be preoperatively diagnosed with ultrasound-guided FNAB. The extent of surgery generally is defined by FNAB results. Detailed examination of all suspicious nodules is of great importance for earlier detection of aggressive PTCs and avoiding surgical over-treatment.

Keywords: Papillary thyroid carcinoma, FNAB, Incidental, Non-incidental

Öz

Amaç: Papiller tiroid karsinomları (PTK) genellikle iyi prognozludur. Lenf nodu metastazı, kapsül invazyonu ve ekstratiroidal yayılım varlığında, bazı PTK'lar agresif seyir gösterebilir. Bu vakaların erken teşhisi son derece önemlidir. Ultrason eşliğinde ince iğne aspirasyon biyopsi (İİAB) önemli bir tanı prosedürüdür ve agresif davranış gösteren muhtemel PTK'ları erken tanıyabilir. Çalışmamız, PTK'lı hastalarda İİAB'nin cerrahi strateji üzerindeki etkilerini incelemeyi amaçlamıştır.

Yöntemler: Retrospektif kohort çalışmamızda total tiroidektomi uygulanan 269 hastanın verileri gözden geçirildi. Preoperatif İİAB 188 (%70) hastaya uygulanmıştı. Non insidental tanı konan hastalar, insidental tanı konan hastalar ile PTK'nın agresif davranışına bağlı olabilecek parametreler açısından istatistiksel olarak karşılaştırıldı.

Bulgular: Non-insidental grupta yaşı ≤45 olan hastalar daha sıktı (P=0,041). İnsidental vakalar kadınlarda (P=0,014), çapı 2 cm'den büyük nodüllerde ve nodül sayısı ≥2 olan hastalarda daha sıktı (P=0,001). Postoperatif rezidü tiroid dokusu insidental grupta daha sık gözlendi (P=0,008). Lenf nodu metastazı (P=0,044), kapsül invazyonu (P=0,009) ve ekstratiroidal yayılım (P=0,022) non-insidental grupta daha sıktı.

Sonuç: PTK'ların biyolojik davranışlarını preoperatif olarak tahmin etmek zordur. Ameliyat öncesi ultrason eşliğinde İİAB kullanılarak PTK'ların az bir kısmı teşhis edilebilir. Genellikle ameliyatın kapsamı İİAB sonuçlarıyla belirlenmektedir. Tüm şüpheli nodüllerin detaylı muayenesi, agresif davranış gösteren PTK'ların daha erken tespiti ve aşırı cerrahi tedavinin önlenmesi açısından çok önemlidir.

Anahtar kelimeler: Papiller tiroid karsinomu, İİAB, İnsidental, Non-insidental

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Introduction

Carcinoma of the thyroid gland is the most common endocrine malignancy. In the recent years, the incidence of papillary thyroid carcinomas (PTCs) has increased globally due to widespread utilization of high-resolution ultrasound-guided fine needle aspiration biopsy (FNAB) [1-8]. The clinical significance of this increase is controversial in terms of surgical strategy. One of the most common findings of thyroid disease is the presence of thyroid nodules. The prevalence of thyroid nodules ranges from 19% to 67% [9-11]. Some ultrasonographic findings, such as microcalcifications, hypoechogenicity, absence of a halo, increased intranodular vascularity, nodular shape or irregular margins indicate increased malignancy risk [12-14].

The prognosis of PTC is usually excellent and often does not require aggressive treatment. However, the presence of lymph node metastasis on the first admission of PTC patients indicates poor prognosis. Early diagnosis and formulation of the appropriate surgical strategy are very important in these patients. Ultrasound-guided FNAB is an important diagnostic method for early diagnosis of patients with PTC [14-19]. Our study aimed to examine the effects of results of FNAB on the surgical treatment of patients with PTC.

Materials and methods

Ethics committee approval was obtained from Diskapi Yildirim Beyazit Training and Research Hospital, University of Health Sciences (No: 19.02.2018-46/11) and Declaration of Helsinki was complied. Our retrospective cohort study included 269 patients who underwent thyroidectomy between January 2014 and December 2018 and was diagnosed with PTC according to the final pathology report. All data including age, gender, nodule size, nodule findings (number, size, and presence of calcification), number of biopsies, FNAB results, extent of thyroidectomy, tumor diameter, subtype of tumor, multifocality, presence of residual thyroid tissue after thyroidectomy, lymph node metastasis, capsular invasion, and extra-thyroidal extension were recorded. Demographic features of the cases were detailed in Table 1. Ultrasound-guided FNAB was performed preoperatively in 188 (169 females and 19 males) patients. The mean age of these patients was 47.94 (11.60) (min=19, max=80) years. FNAB results were reported as malign in 32 (17%) patients and suspicious for malignancy in 37 (20%), who were included in the non-incidental group. FNAB results of 119 patients, who constituted the incidental group, were preoperatively reported as benign in 31 (16%), non-diagnostic in 30 (16%) and atypia of undetermined significance (AUS) in 58 (31%) patients. Incidental and non-incidental patients' data were compared. The presence, localization, and size of the postoperative residual tissue were evaluated with thyroid scintigraphy.

Technetium-99m (Tc-99m) pertechnetate thyroid scintigraphy was performed in all patients before the initiation of levothyroxine therapy. Anterior and oblique images were acquired 15 minutes after intravenous injection of 5 mCi ± 1 (185 MBq ± 37) Tc-99m pertechnetate. Prior to imaging, patients drank a glass of water to eliminate esophageal activity. Images were obtained by a gamma camera (Siemens ecam-signature; Siemens, Hoffman Estates, Illinois, USA) in a 256x256 matrix, using a pinhole collimator with 1.78 zoom, over 100kcounts. The photopeak energy was 140 keV, with a 20% window width. Any increased activity distinguished from background activity was interpreted as a presence of residual thyroid tissue.

Statistical analysis

Statistical Package for Social Sciences 18.0 (SPSS inc., Chicago, USA) program was used for statistical analysis. Descriptive statistics were presented as frequency and mean (standard deviation). Variables were compared with Chi-Square, Mann-Whitney U, and Kruskal-Wallis nonparametric tests. McNemar's test was used for the analysis of the dependent groups. *P*-value less than 0.05 was considered statistically significant.

Table 1: Demographic features of the cases

	n (%)
Female/Male	169/19
Age of mean (SD), years	47.94 (11.60)
≤45 age	75 (40)
Solitary nodule	47 (25)
Nodule diameter ≤2cm	105 (56)
Preoperative FNAB results	
Malignancy/suspicious malignancy	69 (37)
AUS/non-diagnostic/benign	119 (63)
Type of Surgery	
Total thyroidectomy	99 (52.6)
Total thyroidectomy + CLND	89 (47.4)
Histopathological diagnosis	
PTMC	104 (55)
PTC	84 (45)
Mean tumor diameter (SD), mm	12.24 (10.96)
Residual thyroid tissue	135 (72)
Multifocality	75 (40)
Lymph node metastasis	13 (14.6)
Capsular invasion	22 (11.7)
Extrathyroidal extension	8 (4)
Recurrence	5 (2.6)

FNAB: fine needle aspiration biopsy, AUS: atypia of undetermined significance, CLND: central lymph node dissection, PTMC: papillary thyroid microcarcinoma, PTC: papillary thyroid carcinoma

Results

Among patients who underwent FNAB (n=188), 104 (55%) were diagnosed with papillary thyroid microcarcinoma (PTMC) and 84 (45%) were diagnosed with PTC. Malignant or suspicious for malignant cells were detected in 24% of patients with PTMC, and 52% of patients with PTC (*P*=0.001). Among the incidental diagnosis group, 35% (n=42) were ≤45 years old. 48% (n=33) of patients younger than 45 years were in the non-incidental group (*P*=0.041). There was a significant difference between the incidental and non-incidental groups in terms of the extent of thyroidectomy. Extended thyroidectomy with central lymph node dissection (CLND) was carried out more commonly in non-incidental group (*P*<0.001). Malignancy or suspicious for malignancy were more commonly reported in FNAB results of male patients (64%) compared to females (34%) (*P*=0.014). According to FNAB results, malignancy and suspicious for malignancy were more commonly reported in solitary nodules (*P*=0.001). Presence of a report of malignancy and suspicious for malignancy were significantly more common in nodules ≤2 cm (46%) compared to nodules larger than 2 cm (23%) (*P*=0.001). Capsular invasion was observed in 13% (n=9) of the incidental group and 19% (n=13) of the non-incidental group (*P*=0.009). Extra-thyroidal extension was seen in a limited number of cases from each group, but was significantly higher in the non-incidental group (*P*=0.022). Similarly, recurrences were observed in a few patients among both groups. Malignancy was not reported in 56% of calcified nodules in preoperative FNAB

examination (P=0.042). Number of biopsies did not differ among the two groups. There was a significant difference between the incidental and non-incidental group patients in terms of thyroidectomy extension. Extended thyroidectomy (with CLND) was more commonly carried out in non-incidental group patients. The comparison of the parameters of the two groups is presented in Table 2.

Table 2: Demographic characteristics of incidental and non-incidental cases

	Incidental n (%)	Non-incidental n (%)	P-value
Age			
≤45	42 (35)	33 (48)	0.041
>45	77 (65)	36 (52)	
Gender			
Female	112 (66)	57 (34)	0.014
Male	7 (37)	12 (63)	
Nodule diameter			
≤2 cm	56 (54)	49 (46)	0.001
>2 cm	63 (77)	20 (23)	
Nodule number			
Single	22 (47)	25 (53)	0.001
>2	97 (69)	44 (31)	
Calcification			
Yes	47 (56)	37 (44)	0.042
No	72 (69)	32 (61)	
Biopsy number			
less than 2	100 (84)	59 (85.5)	0.514
2 and more	19 (16)	10 (15)	
Type of Surgery			
TT	75 (63)	24 (35)	0.001
TT+CLND	44 (37)	45 (65)	
Histopathological diagnosis			
PTMC	79 (76)	25 (24)	0.001
PTC	40 (48)	44 (52)	
Residual thyroid tissue	96 (81)	39 (56)	0.008
Multifocality	51 (43)	24 (35)	0.175
Lymph node metastases	5 (11)	8 (18)	0.044
Capsular invasion	9 (13)	13 (19)	0.009
Extrathyroidal extension	4 (3.4)	4 (5.8)	0.022
Recurrence	4 (3.4)	1 (1.4)	0.034

TT: total thyroidectomy, CLND: central lymph node dissection, PTMC: papillary thyroid microcarcinoma, PTC: papillary thyroid carcinoma

Discussion

Despite the increasing incidence of PTC, there is no consensus about the optimal treatment. It is widely accepted that PTC has excellent prognosis [6,15,20]. Some non-incidental tumors behave more aggressively and tend to recur [21]. Many authors suggest that early detection and aggressive treatment are necessary in these cases [22]. The incidence of incidental PTMCs have increased in the recent years [23]. Patients incidentally diagnosed with PTC are reported to comprise about 45-75% of all PTC patients [21-24]. It has been known that factors such as slow growth rate, absence of specific symptoms, clinical characteristics and potential co-occurrence with benign thyroid disease make diagnosis difficult.

FNAB is considered the most accurate procedure to identify malignant nodules. However, nodule characteristics are not reliable enough to diagnose malignancy. PTC was more frequently diagnosed incidentally in nodules larger than 2 cm, which can be explained by increased heterogeneity in larger nodules. FNAB is not always applicable in terms of cost effectiveness and results are usually obscure. Positive prediction of malignant thyroid nodules is restricted due to limited diagnostic methods, such as FNAB [24]. Currently, only 10-25% of patients with PTC can be diagnosed preoperatively with FNAB. In our study, the incidence of incidental diagnosis was 63%.

Presence of lymph node metastasis, capsular invasion and extra-thyroidal extension on admission indicates aggressive PTC. Surgical treatment of these patients requires a different surgical strategy. It is extremely difficult to preoperatively predict malign and aggressive cases. FNAB is recommended in presence of suspicious or intermediate ultrasonographic findings.

Higher incidence of non-incidental diagnosis may be explained by the wide application of screening programs. Compared to results of other studies in the literature, malignancy and suspicious for malignancy findings were more commonly reported in our series due to the examination of the specimens by a single expert pathologist.

Pisanu et al. [22] showed that age and gender are not significant factors in terms of incidental or non-incidental diagnosis, whereas capsular invasion and lymph node metastasis were significantly more common in patients with non-incidental diagnosis (P<0.001 and P<0.001, respectively). Chow et al. [25] reported that age has no effect on the aggressiveness and metastasis of PTMC. Our study showed that age was an independent significant factor for non-incidental diagnosis. Non-incidental diagnosis of PTC patients ≤45 years were more common compared with the others.

Wang et al. [26] showed that microcalcification was present in nearly half of the PTMC nodules. Microcalcification in a nodule is associated with a higher probability of malignancy. It was observed in 45% of our patients and more frequently encountered in nodules of incidental group patients; however, preoperative FNAB was not definitive in this group.

Lombardi et al. [21] showed that lymph node metastasis and extra-thyroidal extension are significant factors for non-incidental diagnosis of the patients with PTC, with which our findings were consistent.

In a cross-sectional study, tumor size of non-incidentally diagnosed tumors was found significantly higher than incidentally diagnosed tumors (7.5mm vs. 4.2mm) [22]. Difference in tumor size remains one of the most important parameters. A consensus is yet to be reached. In our series, the rate of incidental diagnosis of PTMCs was higher than PTCs (76% vs 24%, respectively). Diameter of the tumor was a significant factor in our study.

Vlassopoula et al. [27] reported that tumor subtype (papillary or micropapillary) and/or capsular invasion was not effective on the course and outcome of the PTC. Our study showed that incidental diagnosis affects surgical strategy. Patients with non-incidentally diagnosed PTC more commonly underwent extended thyroidectomy with CLND, as expected. Total thyroidectomy was more commonly observed in patients with incidental PTC.

Limitations

Our study was conducted retrospectively on patients who were referred to our department for postoperative thyroid scintigraphy. Further prospective research involving a larger number of patients is needed.

Conclusions

Although the prognosis of PTC is generally excellent, some may behave aggressively, particularly those with a lymph node metastasis on first admission. The early, preoperative detection of potentially aggressive PTCs is crucial for formulating the appropriate surgical strategy. Unfavorable risk factors should be sought in all patients and if possible, all nodules should be evaluated with ultrasound-guided FNAB.

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Clinical evaluation of paroxysmal and permanent atrial fibrillation patients in cardiac inpatient unit: Cross-sectional study

Paroksismal ve sürekli atriyal fibrilasyon hastalarının kardiyoloji servisinde klinik olarak değerlendirilmesi: Kesitsel çalışma

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Abstract

Aim: Atrial fibrillation (AF), a supra-ventricular arrhythmia, is characterized by a rapid and irregular heart rate, for which electrocardiography is the diagnostic tool. Hypertension is the most common cause of AF. In this study, we aimed to evaluate the paroxysmal AF and permanent AF patients' symptoms, medical history, and clinical characteristics in the inpatient unit.

Methods: 115 patients (30 patients with paroxysmal AF and 85 patients with permanent AF) were enrolled in the study. All patients' detailed histories were taken; physical examination, routine biochemical tests, electrocardiographies, and transthoracic echocardiographies were performed. CHA2DS2-VASc (Congestive heart failure/left ventricular dysfunction, Hypertension, Age ≥75 years, Diabetes Mellitus, Stroke/transient ischemic attack/systemic embolism, Vascular Disease, Age 65-74 years, Sex Category) scores were recorded.

Results: Permanent AF patients were older (70.0 (10.5) vs 61.4 (15.8); *P*=0.01) and had a lower ejection fraction (41.0 (11.9) vs 53.3 (11.2); *P*=0.01) than paroxysmal AF patients. CHA2DS2-VASc scores were similar between the two groups (3.0 (1.5) vs 2.7 (1.3); *P*=0.24). In hematological analysis, prothrombin time (15.3(1.3-106.4) vs 13.6(11.0-75.5); *P*=0.03) and international normalized ratio (1.2(0.9-16.0) vs 1.1(0.9-6.0); *P*=0.01) values were higher in permanent AF patients compared to those with paroxysmal AF. Rhythm regulation was performed to paroxysmal AF patients. Rate regulation was performed significantly more frequently in permanent AF patients than paroxysmal AF patients (74(87%)) vs (12(40%)); *P*=0.01).

Conclusion: This study demonstrated that permanent AF patients had more comorbidities compared to paroxysmal AF patients. Rhythm control was the principal treatment strategy in paroxysmal AF, whereas rate control was the treatment of choice in permanent AF.

Keywords: Paroxysmal atrial fibrillation, Permanent atrial fibrillation, Anticoagulation

Öz

Amaç: Atriyal fibrilasyon (AF) hızlı ve düzensiz kalp atım hızı ile karakterize supra-ventriküler bir aritmidir. Elektrokardiyografi AF için tanı koyma aracıdır. Hipertansiyon AF'nin en sık nedenidir. Bu çalışmada, paroksismal AF ve sürekli AF hastalarının semptomlarını, tıbbi öykülerini ve klinik özelliklerini yatan hasta ünitesinde değerlendirmeyi amaçladık.

Yöntemler: Çalışmaya toplam 115 hasta (paroksismal AF'li 30 hasta ve sürekli AF'li 85 hasta) dahil edildi. Tüm hastalar ayrıntılı öykü, klinik muayene, rutin biyokimya, elektrokardiyografi ve transtorasik ekokardiyografi ile değerlendirildi. CHA2DS2-VASc (Konjestif kalp yetmezliği/sol ventrikül disfonksiyonu, Hipertansiyon, Yaş ≥75 yıl, Diabetes mellitus, İnme/geçici iskemik atak/sistemik emboli, Vasküler hastalık, 65-74 yaş, Cinsiyet kategorisi) skorları kaydedildi.

Bulgular: Sürekli AF hastaları paroksismal AF hastalarından daha yaşlıydı (70.0 (10.5) vs 61.4 (15.8); *P*=0.01) ve daha düşük ejeksiyon fraksiyonuna sahipti (41.0 (11.9) ve 53.3 (11.2); *P*=0.01). CHA2DS2-VASc skorları çalışma grupları arasında benzerdi (3.0 (1.5) vs 2.7 (1.3); *P*=0.24). Hematolojik analizde, protrombin zamanı (15.3(1.3-106.4) vs 13.6(11.0-75.5); *P*=0.03) ve uluslararası normalleştirilmiş oranı (1.2(0.9-16.0) vs 1.1(0.9-6.0); *P*=0.01) paroksismal AF ile karşılaştırıldığında sürekli AF hastalarında daha yüksekti. Paroksismal AF hastalarına ritim kontrolü yapıldı. Hız kontrolü sürekli AF hastalarında paroksismal AF hastalarından anlamlı derecede yüksekti (74 (87%)) ve (12 (40%)); *P*=0.01).

Sonuç: Bu çalışma sürekli AF hastalarının paroksismal AF hastalarıyla karşılaştırıldığında daha fazla komorbiditeye sahip olduğunu gösterdi. Ritim kontrolü paroksismal AF'de, hız kontrolü ise kalıcı AF'de esas tedavi stratejisiydi.

Anahtar kelimeler: Paroksismal atriyal fibrilasyon, Sürekli atriyal fibrilasyon, Antikoagülasyon

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Introduction

Atrial fibrillation (AF), a supraventricular arrhythmia characterized with a rapid and irregular heart rate, is associated with adverse events in the cardiac inpatient unit [1]. In electrocardiography (ECG), an RR interval with no discernible and distinct P wave is characteristic for AF. Hypertension is the most common cause of AF, and remodeling of the atrial tissue with inflammation and fibrosis is the primary pathophysiology in AF development [2,3]. Differentiating AF types is essential in clinical practice. Paroxysmal AF, which is the self-terminating form of arrhythmia, usually terminates within 48 hours. While AF paroxysms may last 7 days, the probability of spontaneous conversion to sinus rhythm is low after 48 hours. Cardioversion was not attempted in permanent AF, as this arrhythmia was not considered temporary by the physicians and the patients [4]. Paroxysmal AF patients are usually treated with anti-arrhythmic medications, while permanent AF patients receive rate control treatment.

The quality of life of an AF patient deteriorates due to AF-related symptoms such as shortness of breath, palpitations, and chest pain [7], which may lead to high rates of hospitalization [5,6]. These patients have the highest mortality rate in the first four months after diagnosis, for which sudden death, heart failure, and stroke are the primary reasons [8,9]. Oral anticoagulant reduces mortality and stroke risk in an AF patient [10]. Rate and rhythm control treatments improve AF-associated symptoms. Physicians should be aware of the diagnosis and treatment of AF types to decrease the mortality risk and hospitalization. In this study, we aimed to evaluate the paroxysmal and permanent AF patients' symptoms, medical history, and clinical characteristics in the cardiac inpatient unit.

Materials and methods

One-hundred and fifteen patients (30 paroxysmal and 85 permanent AF patients) who were admitted to our cardiac inpatient clinic between January 2014 and April 2015 were enrolled in this cross-sectional study. All patients' detailed histories were taken; physical examination, routine biochemical tests, ECG, and transthoracic echocardiographies were performed. All paroxysmal or permanent AF patients older than 18 years were included. Exclusion criteria included refusal to participate in the study, pericardial or pleural effusion on transthoracic echocardiography, severe hepatic dysfunction, defined by documented cirrhosis or serum amino-transferase levels at least five times the upper limit, known malignancy and bleeding disorders.

AF is divided into 5 types based on presentation, duration, and spontaneous termination: Newly diagnosed, paroxysmal, persistent, long-standing persistent, and permanent AF [4]. In the present study, we did not include patients with newly diagnosed, persistent, and long-standing persistent AF. Patients' CHA_2DS_2-VASc (Congestive heart failure/left ventricular dysfunction, Hypertension, Aged ≥ 75 years, Diabetes Mellitus, Stroke/transient ischemic attack/systemic embolism, Vascular Disease, Aged 65-74 years, Sex Category) scores were recorded [4].

Blood samples collected from the antecubital vein by an atraumatic needle were analyzed for white blood cell count, hemoglobin, platelet, mean platelet volume, prothrombin time, activated partial prothrombin time, international normalized ratio (INR), total cholesterol, triglyceride, low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL), aspartate transaminase, alanine transaminase, blood glucose and creatinine. Hematological parameters were analyzed with the LH 780 analyzer (Beckman Coulter Inc, Miami, Florida). In addition, patients' blood pressures, heart rates, 12-lead ECGs and smoking histories were recorded. Hypertension was defined with systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or requirement for antihypertensive medication. Hyperlipidemia was defined with total cholesterol >220 mg/dL or triglycerides ≥150 mg/dl. Type-2 Diabetes mellitus (T2DM) was diagnosed according to the American Diabetes Association criteria [11]. Smoking included active or previous (>10 pack-years) tobacco use.

Each patient underwent complete transthoracic echocardiography in accordance with the American Society of Echocardiography measurement guidelines [12]. Left ventricular end-systolic and end-diastolic diameters (LVESD, LVEDD) were measured from the parasternal long-axis view in M-mode. The thicknesses of the posterior wall (PW), inter-ventricular septum (IVS), ejection fraction (EF) and left atrium diameter were recorded. Rheumatic valvular disease diagnosis was based on echocardiographic characteristics. Informed consent was obtained from all patients prior to the study. This study was performed in accordance with the Declaration of Helsinki principles and approved by the local Ethics Committee of our Hospital (No: 2013-20).

Statistical analysis

Data were analyzed with SPSS software version 20.0 for Windows (SPSS Inc, Chicago, Illinois). Kolmogorov-Smirnov test was used to verify that continuous variables were normally distributed. Normally distributed variables were expressed as mean (standard deviation (SD)), while non-normally distributed variables as median with interquartile range (IQR). The categorical variables were presented as percentages. Differences between two groups were evaluated with Student's unpaired t-test or the Mann–Whitney U test for parameters with a normal or non-normal distribution. The frequencies of nominal variables were compared using Fisher's exact test or chi-square test. $P<0.05$ was deemed statistically significant.

Results

The demographic and clinical data of the patients are presented in Table 1. Age was higher in permanent AF group. Regarding the patients' history, chronic obstructive pulmonary disease (COPD) and heart failure were significantly higher in permanent AF group, whereas coronary artery disease (CAD) was higher in paroxysmal AF group. Urea levels were significantly higher in permanent AF group, unlike HDL levels, which were significantly higher in the paroxysmal AF group. Table 2 shows the hematological and echocardiographic data of the two groups. The white blood cell counts (WBC), platelet counts, and activated partial prothrombin times (aPTT) did not differ among the two groups. Hemoglobin levels were

significantly higher in the paroxysmal AF group, while prothrombin time (PT), INR and C-reactive protein were higher in the permanent AF group. Echocardiographic examination revealed that LVEDD and LVESD were significantly higher in the permanent AF group, and EF was significantly lower in permanent AF patients. Table 3 presents the rhythm and rate data of the AF patients. Paroxysmal AF patients received electrical or medical cardioversion, and rhythm regulation. The number of rate regulated patients was significantly higher in the permanent AF group. Figure 1 presents the mean CHA_2DS_2-VASc scores of the groups, which were not significantly different (P=0.24).

Table 1: The demographic and clinical data of the study population

	Paroxysmal AF	Permanent AF	*P*-value
Age (years)	61.4 (15.8)	70.0 (10.5)	0.01
Systolic blood pressure (mmHg)	119.3 (21.5)	121.0 (27.4)	0.76
Diastolic blood pressure (mmHg)	72.7 (9.8)	74.0 (16.3)	0.68
Heart rate (bpm)	117.0 (29.9)	110.8 (28.1)	0.31
Male n(%)	15(50%)	38(45%)	0.61
Smoking n(%)	8(26%)	27(32%)	0.60
Alcohol n(%)	3(10%)	2(2%)	0.07
Coronary artery disease n(%)	17(57%)	20(23%)	0.01
COPD n(%)	4(13%)	28(33%)	0.03
Diabetes mellitus n(%)	7(23%)	14(16%)	0.40
CVE n(%)	1(3%)	2(2%)	0.77
Hypertension n(%)	14(46%)	36(42%)	0.68
Hyperthyroidism n(%)	--	5(6%)	0.17
Heart failure n(%)	7(23%)	48(56%)	0.01
Valve operation n(%)	1(3%)	10(12%)	0.17
Rheumatic heart disease n(%)	2(7%)	6(7%)	0.94
Warfarin n(%)	5(17%)	25(29%)	0.17
ASA n(%)	26(86%)	73(86%)	0.91
Beta blocker n(%)	8(27%)	32(38%)	0.27
High density lipoprotein (mg/dl	42.1 (14.5)	33.6 (14.0)	0.01
Low density lipoprotein (mg/dl)	104.1 (32.4)	97.4 (36.1)	0.37
Triglyceride (mg/dl)	121.2 (50.9)	114.1 (49.7)	0.50
Total cholesterol (mg/dl)	169.1 (37.8)	153.1 (46.2)	0.09
Thyroxine 4 (µg/dL)	1.2 (0.3)	1.3 (0.3)	0.29
Thyroid stimulant hormone (mIU/L)	1.1(0.1-2.7)	1.1(0.1-8.4)	0.58
Serum glucose (mg/dl)	141.3 (64.0)	132.2 (51.7)	0.44
Sodium (mEq/L)	137.1 (4.5)	136.3 (5.1)	0.46
Potassium (mEq/L)	4.3 (0.9)	4.5 (0.7)	0.41
Calcium (mg/dL)	9.3 (0.5)	9.2 (0.9)	0.60
Urea (mg/dL)	20.5(12.0-94.0)	32.0(8.0-113.0)	0.01
Creatinine (mg/dl)	0.9(0.4-5.8)	1.1(0.5-4.5)	0.26
Aspartate transaminase (U/l)	21.5(9.0-111.0)	31.0(11.0-314.0)	0.18
Alanine transaminase (U/l)	22.0(10.0-95.0)	26.0(4.0-169.0)	0.52
CKMB (ng/ml)	27.0(13.0-313.0)	28.0(11.0-293.0)	0.48
Troponin (ng/ml)	0.01(0.01-10.7)	0.01(0.01-14.0)	0.26
CHA_2DS_2-VASc	2.7 (1.3)	3.0 (1.5)	0.24

AF: atrial fibrillation, COPD: chronic obstructive pulmonary disease, CVE: cerebrovascular event, ASA: acetylsalicylic acid, CKMB: Creatine Kinase-MB, CHADS-VASc: Congestive heart failure, Hypertension, Age, Diabetes mellitus, Stroke, Vascular disease, Sex

Table 2: The hematologic and echocardiographic data of the study population

	Paroxysmal AF	Permanent AF	*P*-value
White blood cell count (10^3/mm^3)	10.7 (3.7)	10.1 (5.7)	0.64
Hemoglobin (g/dL)	14.5 (1.8)	13.3 (2.1)	0.01
Hematocrit (%)	40.8 (8.8)	40.0 (6.3)	0.58
Platelet count (10^3/mm^3)	229.3 (67.5)	215.7 (85.3)	0.43
Mean platelet volume. fL	8.7 (1.2)	8.8 (1.2)	0.53
Prothrombin time (sec)	13.6(11.0-75.5)	15.3(1.3-106.4)	0.03
Activated partial prothrombin time (sec)	33.2 (6.1)	34.2 15.7	0.74
International normalized ratio	1.1(0.9-6.0)	1.2(0.9-16.0)	0.01
C-reactive protein (mg/L)	3.55(2.97-92.0)	13.6(2.0-170.0)	0.01
LVEDD (cm)	4.90 (0.70)	5.48 (0.73)	0.01
LVESD (cm)	3.3 (0.8)	4.3 (0.9)	0.01
Ejection Fraction (%)	53.3 (11.2)	41.0 (11.9)	0.01
Left atrial diameter (mm)	4.4 (0.8)	4.9 (0.8)	0.01
IVS (cm)	1.1 (0.1)	1.2 (0.2)	0.57
PWD (cm)	1.1 (0.1)	1.1 (0.1)	0.73

LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, IVS: interventricular septum, PWD: posterior wall diameter

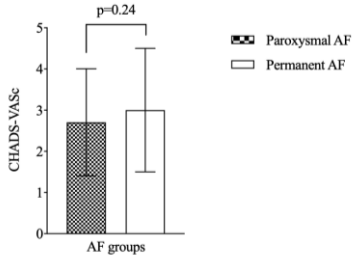

Figure 1: CHA_2DS_2-VASc scores between study groups. (CHA_2DS_2-VASc: Congestive heart failure, Hypertension, Age, Diabetes mellitus, Stroke, Vascular disease, Sex)

Table 3: Rhythm and rate data of the study population

	Paroxysmal AF	Permanent AF	*P*-value
Electrical cardioversion n(%)	4(13%)	--	0.01
Medical cardioversion n(%)	20(67%)	--	0.01
Rhythm regulation n(%)	16(53%)	--	0.01
Rate regulation n(%)	12(40%)	74(87%)	0.01
Exitus n(%)	2(7%)	11(13%)	0.35
Inpatient Duration (days)	4.0(1.0-12.0)	3.0(1.0-32.0)	0.11

Discussion

This study demonstrated three major findings in patients with paroxysmal and permanent AF hospitalized in the cardiac inpatient clinic: First, permanent AF patients were older than paroxysmal AF patients. Second, the ejection fractions of permanent AF patients were significantly lower. Third, CHA_2DS_2-VASc scores did not differ between the two groups. Management of AF patients depends on AF-associated symptoms and duration of AF. AF patients should undergo a detailed clinical evaluation that includes determination of AF type, stroke risk, AF-associated symptoms, and thromboembolism or left ventricular dysfunction assessment. AF patients exhibit a variety of symptoms, such as palpitations, dyspnea, and chest tightness [13]. Rhythm or rate control might be preferred as treatment. Symptoms should be taken into consideration, especially in older patients [4,14]. In AF treatment, rhythm control is not proven to exhibit better outcomes than rate control after five years [15]. However, rhythm control by antiarrhythmic drugs, electrical cardioversion, or ablation, better improves symptoms and functional status compared to rate control [16].

Evaluation of stroke risk and using an appropriate oral anticoagulant is more important than rhythm and rate control strategies in the management of AF patients [17]. Oral anticoagulant (OAC) should be started regardless of the final rhythm in AF patients with a high stroke risk score. OAC treatment could prevent most of the ischemic strokes and prolong life in AF patients [18]. According to the guidelines using the CHA_2DS_2-VASc risk score, a score of 2 needs OAC therapy [4,14]. The ESC guideline suggests administering direct OACs rather than vitamin K antagonist (e.g., warfarin), unlike the AHA guidelines, which recommend both [4,14]. Direct OACs include the direct thrombin inhibitor (dabigatran) and factor Xa inhibitors (apixaban, edoxaban, and rivaroxaban). Clinicians should use vitamin K antagonists in patients with a mechanical valve, rheumatic valve disease, or moderate to severe non-rheumatic mitral stenosis [4,14].

AF is commonly asymptomatic, and paroxysmal AF patients are rarely symptomatic [19]. Recent studies demonstrated that the risks of stroke and thromboembolism of paroxysmal AF patients are similar to that of non-paroxysmal AF patients [20,21]. However, some research showed that stroke and thromboembolic events were lower in paroxysmal AF patients compared to non-paroxysmal AF patients [22,23]. Similarly, Boriani et al. [24] reported that paroxysmal AF patients had a lower stroke risk than permanent AF patients in a meta-analysis, and that non-paroxysmal AF patients had worse outcomes for all-cause mortality than paroxysmal AF patients at one-year follow-up. Vanassche et al. [25] found that age ≥75 years, gender, history of stroke or transient ischemic attack (TIA), and AF patterns were independent predictors of stroke.

Hypertension is a major risk factor for the development of AF [26]. Blum et al. [27] demonstrated a higher prevalence of hypertension was related to a higher AF-progression rate. Also, Padfield et al. [28] reported that aging, mitral regurgitation, aortic stenosis, left ventricular hypertrophy, and left atrial dilatation were related to AF progression. AF patients generally develop sustained forms, and only a small proportion remains in paroxysmal AF during long-term follow-up [29]. A previous study revealed that patients with non-paroxysmal AF were older than paroxysmal AF patients [30]. Persistent AF patients had a larger left atrial volume index compared to paroxysmal AF patients. Structural and electrophysiological changes occur in atrial tissue with aging, which promotes AF progression.

In this study, we evaluated paroxysmal and permanent AF patients in the inpatient clinic. Similar to the studies in the literature, our permanent AF patients were older, had lower ejection fractions and higher COPD ratios compared to paroxysmal AF patients. These findings support the previous studies with respect to the relationship between permanent AF and comorbidities, such as heart failure. Also, left atrial diameters of permanent AF patients were higher, which correlated with permanent AF patients' reported echocardiographic findings in the literature. We showed that CHA_2DS_2-VASc scores were similar between study groups, due to higher CAD ratios in paroxysmal AF. In accordance with the guidelines, we managed rhythm control with electrical and pharmacological cardioversion in paroxysmal AF patients. In permanent AF patients, however, rate control was an essential treatment strategy. Before discharge, we treated almost all patients with oral anticoagulants and rhythm or rate control.

Limitations

This study has some limitations. First of all, this was a single-center study and based on a relatively small group of patients. The new OACs were not common at the time of the study in our country, so the lack of these drugs was another study limitation on AF management. Also, we did not evaluate the HAS-BLED (Hypertension, Abnormal liver/renal function, Stroke history, Bleeding history or predisposition, Labile INR, Elderly, Drug/alcohol usage) scores because of insufficient data associated with label INR levels. We managed the patients in inpatient clinical settings, and the results of this study may not apply to outpatient clinics or emergency settings.

Conclusion

This study demonstrates that permanent AF patients had more comorbidities compared with paroxysmal AF patients. We evaluated stroke risk and treated AF patients with oral anticoagulants according to guidelines. Rhythm control was an essential treatment strategy in paroxysmal AF, whereas rate control was preferred in permanent AF.

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Effects of mean platelet volume and platelet counts on peripheral biodegradable stent restenosis

Periferik biyoeriyebilir stent restenozu üzerine ortalama trombosit hacminin ve trombosit sayımının etkisi

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Abstract

Aim: Thrombocytes play a key role in atherosclerosis and acute vascular events. The purpose of this study is to investigate the association between mean platelet volume (MPV), platelet counts as well as other hematological parameters and biodegradable stent re-stenosis.

Methods: 53 patients who underwent percutaneous biodegradable peripheral stent placement procedure were included in this retrospective cohort study. Blood samples were collected a day before the operation. Patients were followed for a mean of 6 months and Doppler ultrasonography (DUSG) was performed for control examination. According to the DUSG results, patients were divided into two groups: Group 1 included patients with ≥50% in-stent re-stenosis and Group 2, with <50% in-stent re-stenosis.

Results: The mean age of the patients was 58.1 (6.7) years. 6 (11.3%) patients were female and 47 (88.7%) patients were male. Eight (15%) had iliac artery and 45 (84.9%) had superficial femoral artery stenosis. Doppler US reports showed that 11 (20.8%) patients had ≥50% in-stent re-stenosis (Group 1). Re-stenosis rates were higher at younger ages (P=0.020). There was no statistically difference between the groups in terms of gender (P=0.636), MPV (P=0.210), PLT counts (P=0.129) or any other hematologic parameters.

Conclusion: Several studies have shown that some blood parameters, especially MPV, are effective on coronary and peripheral stent stenosis. In our study, we found that none of the hematologic or common blood parameters can predict biodegradable stents re-stenosis.

Keywords: Biodegradable stent, Peripheral arterial disease, Platelet counts, Mean platelet volume

Öz

Amaç: Trombositlerin ateroskleroz ve akut vasküler olaylarda önemli bir rolü vardır. Bu çalışmanın amacı, ortalama trombosit hacmi (MPV), trombosit sayıları ve diğer hematolojik parametreler ile biyoeriyebilir stent stenozu arasında bir ilişki olup olmadığını araştırmaktır.

Yöntemler: Bu retrospektif kohort çalışmaya biyoeriyebilir periferik stentlerle perkütan müdahale yapılan 53 hasta dahil edildi. Kan örnekleri operasyondan bir gün önce alındı. Hastalar ortalama 6 ay takip edildi ve tüm hastalara kontrol amaçlı Doppler ultrasonografi (DUSG) yapıldı. Doppler ultrasonografi sonuçlarına göre hastalar iki gruba ayrıldı: Grup 1'deki hastalarda ≥%50 stentiçi restenoz ve Grup 2'deki hastalarda <%50 stentiçi restenoz mevcuttu.

Bulgular: Hastaların yaş ortalaması 58,1 (6,7) yıl olup, 6 (%11,3) hasta kadın, 47 (%88,7) hasta erkekti. Sekiz (%15) hastada iliak arter darlığı, 45 (%84,9) hastada yüzeyel femoral arter darlığı mevcuttu. Doppler US raporları, 11 (%20,8) hastada, ≥%50 stentiçi re-stenoz (Grup 1) olduğunu gösterdi. Re-stenoz oranları genç yaşta daha yüksekti (P=0,020). İki grup arasında cinsiyet (P=0,636), MPV (P=0,210), PLT sayısı (P=0,129) veya diğer hematolojik parametreler açısından istatistiksel olarak fark yoktu.

Sonuç: Birçok çalışmada özellikle MPV olmak üzere bazı kan parametrelerinin koroner ve periferik stent stenozu üzerinde etkili olduğu gösterilmiştir. Çalışmamızda, hematolojik veya kan parametrelerinin hiçbirinin biyobozunur stentlerin restenozunu öngöremediğini tespit ettik.

Anahtar kelimeler: Biyoeriyebilir stent, Periferik arter hastalığı, Trombosit sayıları, Ortalama trombosit hacmi

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Introduction

At common usage peripheral arterial disease (PAD) explains the stenosis of the abdominal aorta and the distal arteries of the abdominal aortic bifurcation. Men and women over 50 years of age are more frequently affected. The principle reason is atherosclerosis, and femoropopliteal section is mostly involved, but many vessels may have stenosed simultaneously [1]. Blood viscosity, collateral circulation, the level, and the severity of stenosis specify the clinical condition. Intermittent claudication (IC) is the main symptom and it due to the disturbed balance of muscle's blood demand and supply. Symptoms initiate with IC, and progress onto pain, palor, pulselessness, paresthesia, paralysis, necrosis, and gangrene.

Biodegradable stents (BDS) consist of non-metallic bio-absorbable materials, causing less intimal hyperplasia and acute thromboembolic events than other bare stents. Intravascular stent fracture is very rare and no residual materials are left after DBS resolves [2]. They are suitable for re-ballooning, re-stenting, or any other interventions due to their absorbable nature, which distinguishes BDS from other stents. Platelets are small cells which include various granules, a microtubular system and an active membrane [3]. The mean platelet volume (MPV) may play an important role in atherosclerotic and thrombotic pathways [4]. In this retrospective study, we aimed to investigate the association between preprocedural platelets as well as other hematologic parameters and in-stent re-stenosis of biodegradable stents.

Materials and methods

This retrospective study was conducted at University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, and approved by the Ethical Committee of the Uludag University Faculty of Medicine (Number: 2013-08/20). 53 patients who had iliac artery (IA) or superficial femoral artery (SFA) stenosis and were treated with BDS between January 2010 and January 2013 were included in the study.

All patients conformed to Fontain Classification II and III. Preoperative Ankle/Brachial Indexes (ABI) were measured. Those who had histories of peripheral artery operations, acute thrombosis after BDS treatment in the first 24 hours and were deceased within 6 months of the treatment were excluded.

All angiographic interventions were performed by Siemens 792AXA136160 Axiom Artis WEE at the angiography laboratory. Hemogram samples were collected to EDTA-containing tubes and processed with Beckman Coulter LH 750. Contralateral and ipsilateral femoral arteries of 8 and 45 patients were cannulated, respectively. 1 ml heparin (5000 IU) was administered intravenously before stent implantation. A loading dose of 3x75 mg clopidogrel was given orally at the end of the operation and maintained with 1x75 mg per day. Low molecular weight heparin (Enoxaparin Sodium, 1mg/kg once a day) was injected subcutaneously for 10 days following the procedure. Preprocedural hematologic parameters were noted from the patients' data. Arterial Doppler ultrasonography (DUSG) was performed at the 6th postoperative month. In patients with re-stenosis of 50% or more, clinical complaints tend to reoccur and may require re-intervention. Therefore, according to the DUSG reports, patients were categorized as those with ≥50% in-stent re-stenosis (Group 1, n=11) and with ≤50% in-stent re-stenosis (Group 2, n=42). The two groups were compared with respect to mean platelet volume (MPV), platelet count (PLT) and the other parameters.

Statistical analysis

Data was analyzed with the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013). Descriptive statistics were used to present continuous variables (mean, standard deviations, minimum, median, maximum). Chi-Square test was used to compare two groups of nominal variables. As for continuous variables, student t test was used for those with normal distribution and Mann Whitney U test was used for those without. $P<0.05$ was deemed statistically significant.

Results

Six patients (11.3%) were female and 47 (88.7%) were male. The mean age of the patients was 58.1 (6.7) years. Thirty-nine (73.5%) patients had hypertension, 28 (52.8%) had hyperlipidemia, and 16 (30.1%) had chronic obstructive pulmonary disease. Twelve (22.6%) patients underwent coronary bypass surgery or were diagnosed with coronary artery disease (Table 1).

The diagnosis of the arterial stenosis was made by conventional angiography. 36 (67.9%) patients had type A lesions, 11 (20.7%) had type B lesions, and 6 (11.3%) had type C lesions according to the Trans-Atlantic Inter-Society Consensus (TASC) classification. The ABI index scores of 32 (60.3%), 16 (30.1%) and 5 (9.4%) patients ranged between 0.7-0.9, 0.5-0.7 and 0.3-0.5, respectively (Table 2).

Preprocedural PLT, WBC counts and MPV values did not differ among the two groups (P=0.129, P=0.175 and P=0.210, respectively). The low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were similar in two groups, as well (P=0.756 and P=0.088, respectively) (Table 3).

The patients in Group 1 were younger than those in Group 2 (P=0.020).

Table 1: Baseline characteristics of the patients

	Total n=53	Group 1 n=11	Group 2 n=42	*P*-value
Age(years) (mean(SD))	58.1 (6.7)	52.1 (5.1)	59.7 (6.2)	0.020
Male / Female n (%)	47(88.7) / 6(11.3)	10(90.9) / 1(9.1)	37(88) / 5(12)	0.636
Hypertension, n (%)	39(73.5)	8(72.7)	31(73.8)	0.750
Hyperlipidemia, n (%)	28(52.8)	6(54.5)	22(52.3)	0.446
Diabetes mellitus, n (%)	18(33.9)	4(36.3)	14(33.3)	0.348
CAD, n (%)	12(22.6)	3(27.2)	9(21.4)	0.075
COPD, n (%)	16(30.1)	3(27.2)	13(30.9)	0.125
BMI> 30, n (%)	8(15)	2(18.1)	6(14.2)	0.240

SD: Standard deviation, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, BMI: Body mass index

Table 2: TransAtlantic Inter-Society Consensus (TASC) Classification and Baseline Ankle Brachial Index (ABI) measurements of the patients

	n=53	%
Ankle brachial index		
0.3-0.5	5	9.4
0.5-0.7	16	30.1
0.7-0.9	32	60.3
TASC type A lesion	36	67.9
TASC type B lesion	11	20.7
TASC type C lesion	6	11.3

Table 3: Preoperative hematologic and biochemical parameters of the patients

	Total Mean (SD)	Group 1 Mean (SD)	Group 2 Mean (SD)	*P*-value
Platelet (10^3/μL)	263.4 (108.8)	257.6 (100)	264.9 (112)	0.129
MPV	8.7 (1.9)	8.2 (0.9)	8.8 (2)	0.210
WBC (10^3/μL)	9.1 (2.9)	8.3 (2.2)	9.3 (3.1)	0.175
LDL-C (mg/dL)	118 (38.3)	116.8 (44.9)	119.2 (36.3)	0.756
HDL-C (mg/dL)	42.3 (8.8)	41.3 (7.2)	42.9 (9.1)	0.088
C-reactive protein, mg/dL	9.8 (10.8)	8.1 (11.5)	10.1 (10.4)	0.125
Albumin, g/dL	3.9 (0.7)	3.8 (0.7)	3.9 (0.6)	0.275
Creatinine, mg/dL	0.9 (0.5)	1.0 (0.3)	0.9 (0.6)	0.196
BUN, mg/dl	18.3 (9.1)	18.8 (6.7)	17.8 (9.4)	0.146
Total protein, g/dL	6.7 (0.7)	6.4 (0.9)	6.9 (0.6)	0.075

MPV: Mean platelet volume, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, BUN: Blood urea nitrogen

Discussion

Biodegradable stents are made of a poly-L-lactic acid (PLLA) polymer, which is absorbable by the vessels' endothelium. This material is mainly hydrolyzed. The final PLLA degredation products are eliminated by the Krebs cycle and excreted in the urine [5]. PLLA is also used in orthopedic implants, resorbable sutures, and soft-tissue implants [5,6].

The stents preserve their flexibility and radial strength during the first 6 months. According to a long-term follow-up report, 3 years are required for complete elimination of PLLA material from human coronary arteries [7]. Treatment of de novo lesions in peripheral arteries with biodegradable stents has similar outcomes with metal stents. Angiographic imaging data are comparable among metal stents and biodegradable stents in reaching a high patency rate at the first year [8].

Thrombocytes play a significant role in atherosclerosis and acute vascular events. Their activation capacity is directly proportional to their size, so MPV not only indicates their size, but also their activity [9]. Many studies report that elevated MPV values are related to coronary in-stent restenosis and are a risk factor for PAD [10,11]. Elevated MPV values accompany ischemic stroke, acute coronary syndrome, diabetes mellitus, and preeclampsia [12]. There are also studies showing that MPV is an early marker for peripheral bare metallic stent re-stenosis [13,14]. In contrast, Karauzum et al. [13] claim that low MPV levels have protective effects on in-stent re-stenosis. In our study, there was no statistically significant difference between the groups in terms of MPV values, and no association to in-stent re-stenosis. There are contradictions in the literature and more extensive research is needed to eliminate this complexity. For our own research, we believe that this result depends on the type of the stent used.

Smoking, diabetes mellitus, and hyperlipidemia are demonstrated predictors for PAD and in-stent re-stenosis [15]. Although taking precautions against these factors may decrease in-stent re-stenosis in the short term, stent obstructions cannot be prevented in the long term [16]. Therefore, all precautions should be taken to provide stent patency. In our study, DM, hyperlipidemia, hypertension, gender, or BMI was not proven to have a statistically significant relationship with in-stent re-stenosis. This finding is interesting, because it does not match the literature data.

Limitations

The main limitation of the study was the small number of patients and the retrospective design. In addition, restenosis was evaluated with DUSG, which is noninvasive and performer-dependent.

Conclusion

The purpose of this study was to compare the obstruction rates according to hematological parameters which could be simply measured and take precautions if needed. But none of the hematologic parameters were found to be associated with biodegradable stents' re-stenosis. This finding that contradicts with the literature may be due to the different raw materials of biodegradable stents. Further, larger scale studies are to support this opinion.

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A bibliometric analysis of *Bacillus anthracis* research published between 1975 and 2018

1975 ve 2018 yılları arasında yayınlanan *Bacillus anthracis* araştırmalarının bibliyometrik analizi

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Ethics Committee Approval: This study is not a clinical and experimental study. The data in this study were extracted from the Thomson Reuters Web of Science database (Thomson Reuters, New York, NY, USA) from 1975 to December 2018. Author declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki.
Etik Kurul Onayı: Bu çalışma klinik ve deneysel bir çalışma değildir. Bu çalışmadaki veriler, 1975'ten Aralık 2018'e kadar Thomson Reuters Bilim Web veri tabanından (Thomson Reuters, New York, NY, ABD) çıkarıldı. Yazar, araştırmanın Helsinki Dünya Tıp Birliği Deklarasyonu ilkelerine göre yapıldığını açıkladı.

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Abstract

Aim: Bibliometrics is a recent field and is performed to quantitatively assess the academic quality of journals or authors using statistical procedures such as citation rates, contents, authorship relations and productivity. Although anthrax still remains important globally, the scientific literature lacks a bibliometric assessment of the anthrax literature. In this study, it is aimed to perform the bibliometric analysis of anthrax.

Methods: The data in this study were extracted from the Thomson Reuters Web of Science database (Thomson Reuters, New York, NY, USA) from 1975 to December 2018. The database is accessible back to 1975 and we searched all documents using keywords [Bacillus anthracis OR anthrax] in the "Title" field.

Results: WoS database search recorded a total of 5557 publications. More than half of the publications were original articles (n=3828, 68.8%). The country with the greatest number of publications was the United States of America (n=3203), followed by England (n=301) and France (n=264). The Center for Disease Control and Prevention had the most published documents about anthrax with 210 papers and 3.7% of the total literature. The most productive authors are Leppla SH, Collier RJ, and Mock M (n=236, 124, 101 documents), respectively. The most productive journal was Infection and Immunity, which covered 4.3% of the publications with 241 manuscripts.

Conclusion: In this first bibliometric study in the literature about anthrax we found that nonendemic developed countries dominated the anthrax literature. This study will encourage further studies about the investigation of anthrax, which is endemic in some parts of the world.

Keywords: Bibliometrics, Citation analysis, Anthrax, Bacillus anthracis

Öz

Amaç: Bibliyometri, atıf oranları, içerik, yazar ilişkileri ve üretkenlik gibi istatistiksel prosedürleri kullanarak dergilerin ve yazarların akademik kalitesini kantitatif olarak değerlendiren bir alandır. Her ne kadar şarbon küresel olarak hala önemli olsa da, bilimsel literatürde şarbon literatürünün bibliyometrik bir değerlendirmesi yoktur. Bu çalışmada, şarbonun bibliyometrik analizinin yapılması amaçlanmıştır.

Yöntemler: Bu çalışmadaki veriler, 1975 - Aralık 2018 tarihleri arasında, Thomson Reuters Web of Science (Thomson Reuters, New York, NY, USA) very tabanından elde edildi. Veritabanında 1975 yılına kadar erişilebilir ve "Title" bölümünde [Bacillus anthracis veya anthrax] anahtar kelimeler kullanılarak tüm dökümanlarda aradık.

Bulgular: WoS (Web of Science) veritabanında toplam 5557 yayın bulundu. Yayınların yarısından fazlası orijinal makalelerdi (n=3828, %68,8). En fazla yayını olan ülke Amerika Birleşik Devletleri'ni (n=3203), İngiltere (n=301) ve Fransa (n=264) takip etti. The Center for Disease Control and Prevention, şarbon ile ilgili 210 yayın ve literatürün %3,7'si ile en fazla yayın yapan kurumdu. En üretken yazarlar sırasıyla Leppla SH, Collier RJ ve Mock M (n=236, 124, 101 yayın) oldu. En üretken dergi, 241 yazı ile yayınların %4,3'ünü kapsayan Infection and Immunity dergisiydi.

Sonuç: Şarbon ile ilgili literatürdeki bu ilk bibliyometrik çalışmada, endemik olmayan gelişmiş ülkelerin şarbon literatürüne hâkim olduğunu bulduk. Bu çalışma, dünyanın bazı bölgelerinde endemik olan şarbonun araştırılmasıyla ilgili ileri çalışmaları teşvik edecektir.

Anahtar kelimeler: Bibliyometri, Atıf analizi, Şarbon, Bacillus anthracis

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Introduction

Infectious diseases continue to be the biggest public health problem for centuries in all countries of the world [1]. Anthrax is a zoonotic infectious disease caused by the gram-positive bacteria *Bacillus anthracis* which forms endospores and produces exotoxins [2,3]. The disease occurs in humans, wild and domestic mammal species, especially in herbivores [4]. Anthrax cases in humans are classified in three forms based on clinical features and transmission routes; cutaneous form, comprising nearly 95% of all human cases reported in the world in general, gastrointestinal form, and pulmonary form [5]. There is no evidence of human-to-human transmission of *B. anthracis* and humans develop the disease due to direct contact with animals infected with anthrax or animal products contaminated with anthrax [6-8]. *B. anthracis* spores may remain viable for tens of years due to their resistance to extreme pH and temperature, drying and some chemical materials [8].

Although *B. anthracis* is generally an organism that is environmentally stable and found everywhere, it is known as a potential pathogen for use in biological weapons. Anthrax is observed in the world in generally, with lower rates in developed countries. It is endemic in Africa and Asia and WHO estimates the global incidence is from 2000 to 20,000 [9,10]. Due to animal and human epidemics in a variety of regions on earth, it has a potential for use as a biological weapon and it is very difficult to monitor information related to anthrax which is still endemic in some regions.

Bibliometrics is a recent field and is performed to quantitatively assess the academic quality of journals or authors using statistical procedures such as citation rates, contents, authorship relations and productivity. Bibliometrics is used in collaboration with the broader term "infometrics" [11-13], and the narrower term "scientometrics" [13-15]. Pritchard was the first author to suggest the term "statistical bibliography" in 1969 [16]. Scientometrics and bibliometrics often involve the scientific contribution of journals or specific works, citation analysis and a content analysis of words in titles, abstracts or the full text of journals. They also focus on authorship, social network analysis, co-word and keywords assigned to published articles. Nowadays, a number of tools have apparently made it much easier to produce these reports. Databases such as *Web of Science (WoS)*, *Scopus* or *Google Scholar* have added and incorporated reference handling features [17]. Bibliometrics could be considered knowledge of science because the scientific literature itself becomes the subject of analysis.

Bibliometric analysis is a commonly used tool to assess the productivity and growth of research in the health sciences. Bibliometric analyses have been performed and published in a variety of research areas like cancer [18], respiratory medicine [19], tuberculosis [20-22], and public health [23,24].

Though bibliometric studies in the health area are increasing with each passing day, there is no bibliometric study related to anthrax found in the literature.

Materials and methods

The data for this study were extracted from the Thomson Reuters Web of Science database (Thomson Reuters, New York, NY, USA) from 1975 to December 2018. The database is accessible back to 1975 and we searched all documents using keywords [Bacillus anthracis OR anthrax] in the "Title" field. We used VOSviewer software tool to arrange and set the bibliometric networks (VOSviewer 2018). Data were transferred from WoS in the "Full Record and Cited References" content pattern.

Results

Numbers of published items

The WoS database search recorded a total of 5557 publications between 1975 and December 2018. More than half of the publications were articles (3828, 68.8%), followed by meeting abstracts (522, 9.3%), news items (290, 5.2%) and reviews (251, 4.5%) (Table 1).

Table 1: Types of publications on anthrax literature [a]

Document types	Number	%
Article	3828	68.8
Meeting Abstract	522	9.3
News Item	290	5.2
Review	251	4.5
Editorial Material	212	3.8
Proceedings Paper	202	3.6
Letter	179	3.2
Note	54	0.9
Correction	51	0.9
Book Review	39	0.7
Reprint	34	0.6
Book Chapter	6	0.1
Discussion	2	0.03
Biographical Item	1	0.01
Poetry	1	0.01
Total	*5672*	*100*

[a] Total percentage may exceed 100% because certain items were included in more than one category

The number of publications between 2003 and 2012 varied between 250 and 290. There was a decrease in the number of publications after 2012. 2018 is the poorest year with only 137 publications. The most productive year was 2002 with 387 records.

The total number of articles cited in this field was 121,382 and the number is 56,878 if we exclude self-citations. Before the 2000s, the citation numbers were between 200 and 600, but after this time they clearly increased. After 2005, citation records are over 5,000, with the highest number of 9,446 in 2012. The number of publications and citations according to year for anthrax are shown in Figure 1.

The country with the greatest number of publications was the United States of America (n=3203), followed by England (n=301) and France (n=264). Publication density and distribution of the manuscripts is shown on the world map (Figure 2).

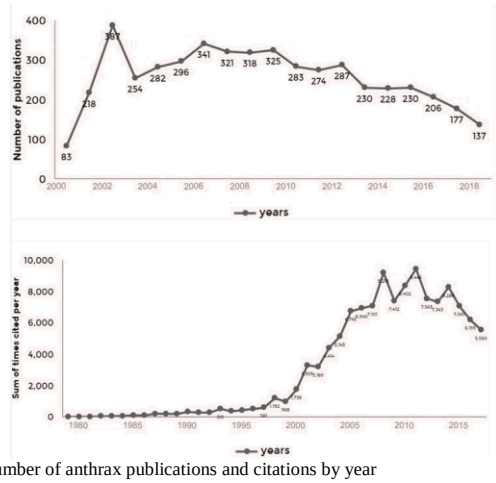

Figure 1: Number of anthrax publications and citations by year

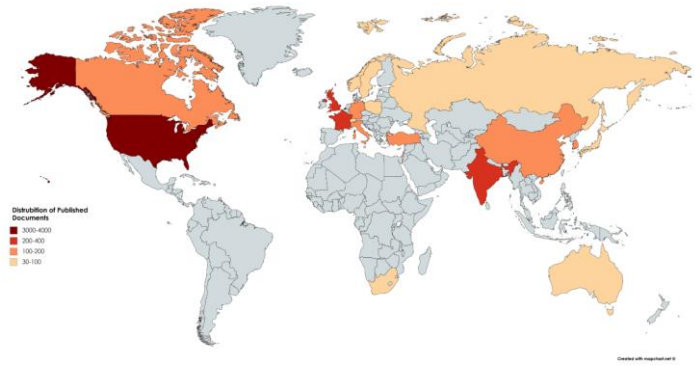

Figure 2: Distribution chart for documents published between 1975 and 2018

Authors and institutions

The co-authorship network for the countries in relation to anthrax was interrogated using the WoS database. The relationship network is described by at least 20 joint publications. In 130 countries, VOSviewer returned these criteria with 25 countries in 7 clusters. This network shows the productivity power of the countries with the size of the point and the connections between the countries and authors publishing together. Seven colored clusters mean that each color group worked with each other significantly. Connected countries with co-authorship relations were located closely in the same color as the clusters. The USA has the biggest point size and the highest number of publications about anthrax. For example, authors from USA worked with the authors from Australia, India, the People's Republic of China and South Korea. USA had 31 links with over 20 joint publications, while England had 20, France 18 and Germany 17 links (Figure 3).

We also created a citation network for these countries with VOSviewer. We defined the cut off boundary as 300 citations and found 26 countries in nine clusters. In this classification, the USA was again the most cited country (86,340) followed by France (10,497), England (7,079), Italy (3,363) and Germany (2,860).

The Centers for Disease Control and Prevention had the most published documents about anthrax with 210 papers and 3.7% of the total literature. This was followed by National Institute of Allergy and Infectious Diseases (186, 3.3%), Harvard University (163, 2.9%), Institut Pasteur (157, 2.8%), and the US Food and Drug Administration (96, 1.7%) (Table 2).

Co-authorship relations were identified between 2990 institutes, and with at least 40 as the minimum document number threshold this decreased to 28 centers. There are seven clusters and connections are located closely in the same color (Figure 4). For example, in the blue cluster the Centers for Disease Control and Prevention has 206 documents with other institutes and worked closely with Northern Arizona University, Emory University, University of California Berkeley, University of Maryland and United States Navy. The National Institute of Allergy and Infectious Diseases shown in red has 185 documents together and most of them clustered at the upper right side of the infographic.

The most productive authors are Leppla SH, Collier RJ, and Mock M (n=236, 124, 101 documents, respectively) (Table 3). The five most cited authors were Leppla SH, Mock M, Collier RJ, Friedlander AM, and Keim P (n=11707, 6966, 5968, 4087, 3711 citations respectively).

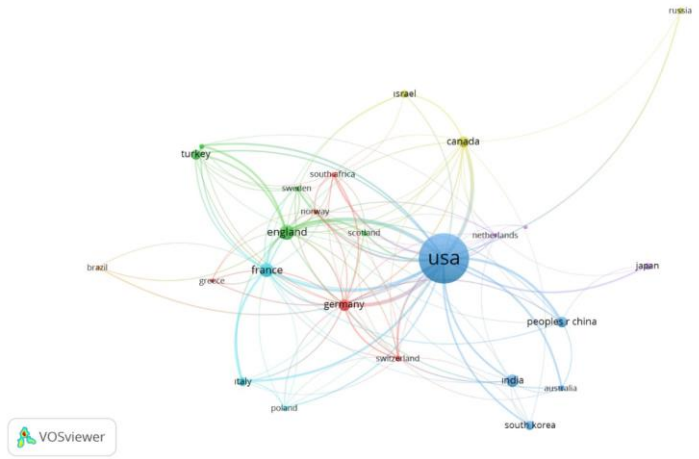

Figure 3: Network of collaborative countries in anthrax research

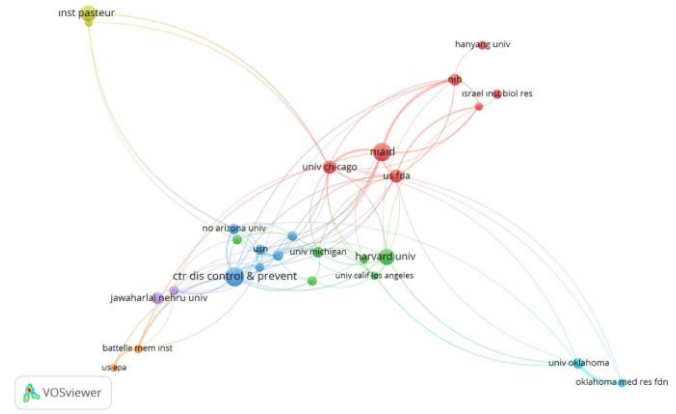

Figure 4: Co-authorship relations between institutions for anthrax

We also made a chart for the ten journal names with most published anthrax papers (Table 4). This showed 4.3% of the papers were printed in Infection and Immunity with 241 manuscripts, followed by Journal of Bacteriology (n=155, percentage 2.7%), Vaccine (n=143, percentage 2.5%), Plos One (n=138, percentage 2.4%), and Abstracts of Papers of the American Chemical Society (n=133, percentage 2.3%).

The most cited article is "Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor" published in 1998 by Duesbery et al. [25] in "Science". This is also the most-cited study with an average of 35.95 citations per year. The article "Anthrax Toxin Edema Factor - A Bacterial Adenylate-Cyclase That Increases Cyclic-Amp Concentrations In Eukaryotic Cells" is a relatively an old publication by Leppla [2] with an average citation per year of 19.63. This paper was published in 1982 in "Proceedings of the National Academy of Sciences of the United States of America" (Table 5).

Table 2: Most productive institutes in anthrax

Institute	Record Count	Country	% of 5557
Ctr Dis Control Prevent	210	USA	3.768
NIAID	186	USA	3.338
Harvard Univ	163	USA	2.925
Inst Pasteur	157	France	2.817
US FDA	96	USA	1.723
Univ Chicago	94	USA	1.687
NIH	77	USA	1.382
Jawaharlal Nehru Univ	76	India	1.364
Univ Maryland	75	USA	1.346
Univ Michigan	72	USA	1.292
Univ Oklahoma	71	USA	1.274
USN	64	USA	1.148
No Arizona Univ	60	USA	1.077
Univ Texas	58	USA	1.041
Israel Inst Biol Res	53	Israel	0.951

Ctr Dis Control Prevent: Centers for Disease Control and Prevention, NIAID: National Institute of Allergy and Infectious Diseases, NIH: National Institutes of Health, USN: United States Navy

Table 3: The most productive 15 authors [a]

Authors	Instution	Country	Records	%[a]
Leppla SH	NIAID	USA	236	4.235
Collier RJ	Harvard Medical School	USA	124	2.225
Mock M	Institut Pasteur	France	101	1.812
Moayeri M	NIAID	USA	82	1.471
Quinn CP	CDC	USA	82	1.471
Bhatnagar R	Jawaharlal Nehru University	India	77	1.312
Friedlander AM	USAMRIID	USA	72	1.292
Keim P	Northern Arizona University	USA	55	0.987
Koehler TM	The University of Texas	USA	50	0.897
Liu SH	Chaoyang University of Technology	Taiwan	47	0.843
Fouet A	Institut Pasteur	France	46	0.825
Little SF	USAMRIID	USA	44	0.790
Tang WJ	University of Chicago	USA	43	0.772
Hanna PC	University of Michigan	USA	42	0.754
Singh Y	University of Delhi	India	42	0.754

[a] Of total documents published in anthrax literature, NIAID: National Institute of Allergy and Infectious Diseases, USAMRIID: United States Army Medical Research Institute of Infectious Diseases Northern Arizona University, CDC: Centers for Disease Control and Prevention

Table 4: The top ten journal source according to the number of published documents

Journals	Records	% of 5557
Infection and Immunity	241	4.34
Journal of Bacteriology	155	2.79
Vaccine	143	2.57
Plos One	138	2.48
American Chemical Society	133	2.39
Journal of Biological Chemistry	113	2.03
Journal of Applied Microbiology	99	1.78
Proc Natl Acad Sci USA	95	1.71
Applied and Environmental Microbiology	86	1.55
Emerging Infectious Diseases	81	1.46

Proc Natl Acad Sci USA: Proceedings of the National Academy of Sciences of the United States of America

Table 5: The prominent 10 most cited articles

Article	Author	Publication year	Total citation	Average citations per year
Proteolytic inactivation of MAP-kinase- kinase by anthrax lethal factor	Duesbery NS, Webb CP, Leppla SH, et al.	1998	791	35.95
Anthrax toxin edema factor - a bacterial adenylate-cyclase that increases cyclic-amp concentrations in eukaryotic cells	Leppla SH	1982	746	19.63
Anthrax	Mock M, Fouet A	2001	723	38.05
Anthrax	Dixon TC, Meselson M, Guillemin J, et al.	1999	694	33.05
Anthrax as a biological weapon, 2002-Updated recommendations for management	Inglesby TV, O'Toole T, Henderson DA, et al.	2002	673	37.39
Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis - One species on the basis of genetic evidence	Helgason E, Okstad OA, Caugant DA, et al.	2000	660	33.00
Identification of the cellular receptor for anthrax toxin	Bradley KA, Mogridge J, Mourez M	2001	645	33.95
Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States	Shepard CW, et al.	2001	605	37.84
The genome sequence of Bacillus anthracis Ames and comparison to closely related bacteria	Read TD, Peterson SN, Tourasse N, et al	2003	595	35.00
Crystal structure of the anthrax toxin protective antigen	Petosa C, Collier RJ, Klimpel KR	1997	587	25.52

Keyword analysis

The total number of keywords was 4270, when we limited the minimum number of occurrences to15, 40 met the criteria (Figure 5). Most five commonly used keywords were “anthrax (715)”, “bacillus anthracis (705)”, “protective antigen (165)”, “lethal factor (104)”, and “vaccine(100)” . As seen in the infographic network, there are 5 clusters related to each other. For example, “anthrax” is mostly used with antibody, capsule, toxin, vaccine and virulence.

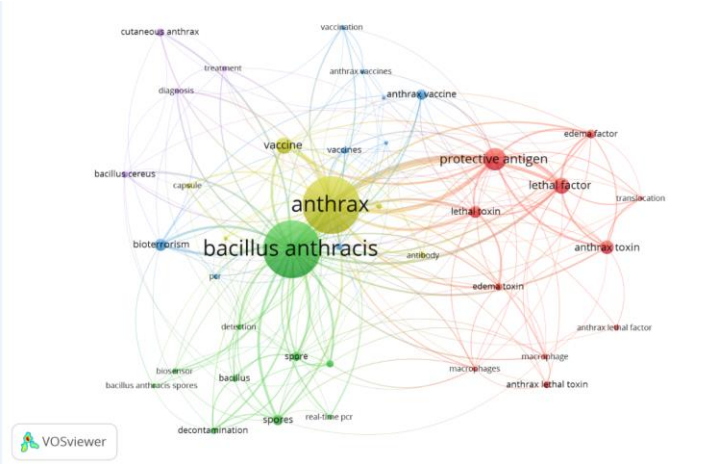

Figure 5: Keyword network for anthrax publications between 1975 and 2018

Discussion

Bibliometric studies provide qualitative and quantitative analysis of the scientific and academic literature and determine the most active and popular trends in a field [26]. Bibliometric analyses reveal the productivity of countries, authors and organizations and analyze the structure of publications [27]. Definition of anthrax disease begins in the antique period, with the oldest from the Roman poet Virgil. During the 19th century, anthrax was an infection involved in important medical developments. Robert Koch (1876) found the disease vector and Louis Pasteur (1881) created the first bacterial veterinary anthrax inoculation prepared containing weakened live organisms. Though it is one of the oldest diseases known in humans and animals, anthrax is still relevant today [28]. In recent times, anthrax has been used as a biological war agent both in the USA and abroad. In 1979, anthrax spores were mistakenly released in Sverdlovsk city in the Soviet Union after an accident in a biological weapons facility and 77 humans were infected with definite diagnosis. Of those infected, 66 died within 1 to 4 days after initial symptoms. In 1993, a group called Aum Shrinrikyo released anthrax in Tokyo during an attack. In 2001, a government agent with the US Army Research Institute for Infectious Disease deliberately distributed anthrax spores through the American postal service. Eleven people in contact with infected post had inhalation anthrax diagnosis and 5 of these patients died [29,30].

There is very limited research in the literature about bibliometric analysis of microbiological studies. There is no bibliometric analysis research in the literature about anthrax, causing serious diseases in both humans and animals, with very high transmission risk, used as biological weapon agent with worrying dimensions by some countries, and still endemic in some regions of the globe. Our study is the first international bibliometric assessment of papers published from 1975 to December 2018 about anthrax. The relatively low number of publications in this study is due to the lack of inclusion of publications from before 1975.

Though anthrax is not endemic in the United States of America, it is the most productive country for anthrax research comprising 57.64% of documents. Though they are endemic regions, there is no country from Africa or South America in the list of the top 25 countries, apart from South Africa. Of publications, 88% (4877/5557) were published after 2000. *B.*

anthracis spores may live in soil for many years and though the disease is endemic to Africa, Central Asia, Middle East and South America, it has spread around the world [31,32].

Our graph for anthrax research after the year 2000 shows highest number of publications were reached in 2002, with a continuous reduction in publication numbers until 2018. The promotion and common use of effective veterinary inoculations by the World Health Organization has reduced the incidence of anthrax in humans exposed to cattle, sheep, goat, camel, horses, and pigs and contaminated animal products. Control precautions in animal husbandry are key to low incidence and anthrax is reported to be one of the infectious diseases decreasing in the world [33]. In this context, the reason for the continuous reduction in publication numbers may be explained by the fall in disease incidence and researchers reducing interest in this disease.

The citation number for articles generally reach maximum levels 4-7 years after publication [34,35]. The citation numbers for anthrax articles were very low until the 2000s (200-600), with a rapid increase after the year 2000 to reach highest levels in 2012 with a continuous fall from 2012 to 2018. The reason for the increase in citation numbers is probably due to the citations of publications focusing from 2002-2009. Due to the fall in publication numbers after 2009, it is expected the number of citations will continue to fall in future years.

Analysis of the WoS database for the co-authorship network between countries found 25 countries with at least 20 common publications formed 7 clusters, with the USA the country with most common publications and 31 connections. As expected, the countries with most publications and common publication criteria of at least 20 or more were England, France and Germany with 20, 18 and 17 connected countries, respectively.

The WoS database revealed that most prolific country institutions cooperate mainly at national level and that international cooperation has risen intensely over the past 25 years as shown in this analysis. This finding simply shows only the publication relationships between countries, because research is only good if performed internationally.

With 12 institutions, American institutions led the published articles as expected. Apart from American institutions, the Pasteur Institute in France (157 articles, 4th place), the Jawaharlal Nehru University in India (76 articles, 8th place) and the Israel Biological Research Institute in Israel (53 articles, 15th place) were among the top 15 institutions. The Centers for Disease Control and Prevention was the institution with most publications at 210 (3.7%), with the unexpected inclusion of the military institution of the United States Navy in the top 15 institutions with 64 publications.

The most productive 15 authors were identified to be from the USA (n=10), France (n=2), India (n=2) and Taiwan (n=1). Lepla SH was the author with highest number of publications (236) and highest number of citations (11,707).

Among 10 journals publishing most articles about this topic, 9 were from the USA (Table 5). Of the thousands of journals, Infection and Immunity (n=241, 4.34%) and Journal of Bacteriology (n=155, 2.79%) were in the top 2 places, with an English journal Vaccine (n=241, 42.57%) in third place among the top 10 journals.

The article entitled “Proteolytic inactivation of MAP-kinase- kinase by anthrax lethal factor” published in 1998 by Duesbery et al. [25] was the article most cited, with a total of 791 citations and a mean 35.95 citations per year.

Bibliometric analysis is the scientific analysis of countries, organizations, authors, scientific cooperation, citations, key words, journals and time intervals and takes a snapshot of the science. It is a table that allows scientists who deal in details, and sometimes get bogged down in them, to look up and see the big picture. It is an analysis that summarizes the past and history of a scientific area and directs the science.

There are relatively few articles about bibliometric analysis of microbiologic diseases. It is probably due to the need for a sufficient volume of materials to be analyzed and for well-established databases. The emergence and widespread distribution of the internet also make data gathering easier [13].

Even though there is improved general awareness, increasing popularity of bibliometric studies, and the need for classification analysis and citation analysis, the number of reports about microbiological diseases is rather limited.

There are some limitations to the current study. We used only one internationally established database to search the literature of the Web of Science, because it is the most reliable scientific database for publications and citations [36], and one bibliometric tool “VOSviewer” to arrange and set networks [37,38]. Although the literature goes back to 1900s in PubMed, we could only reach 1975 by searching the WoS.

Conclusion

In this first bibliometric study in the literature about anthrax we found that nonendemic developed countries dominate the anthrax literature. This study will encourage further studies about the investigation of anthrax, which is endemic in some parts of the world. It may also be the beginning of a new field in the scientific literature for the evaluation of anthrax.

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Effect of growth hormone and somatomedin-C axis on fracture healing

Kırık iyileşmesinde büyüme hormonu somatomedin-C aksının etkisi

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Abstract

Aim: Many studies have examined the effects of different calciotropic hormones on fracture healing, whereas few studies focus on growth factors. Local detection of somatomedin C (IGF-1) in fracture callus, application of growth hormone (GH) and IGF-1 as non-union treatment, and low GH and IGF-1 levels in osteoporotic fractures indicate that these hormones are effective in fracture healing. However, most of these studies are based on post fracture GH and IGF-1 levels. GH and IGF-1 are also involved in acute phase response and can change due to trauma. The aim of this study is to investigate the change in GH and IGF-1 levels in patients treated with osteotomy, in which an iatrogenic fracture is created, and to evaluate the effect of these hormones on fracture healing by comparing the results before and after the fractures.

Methods: Patients who were diagnosed with developmental dysplasia of the hip and underwent surgery between 2014-2015 were prospectively followed for this cohort study. Forty-one patients were included, and two groups were formed. Patients who underwent open reduction and soft tissue release without osteotomy (n=20) were included in the first group. Patients who underwent pelvic osteotomy (n=21), in which iatrogenic fractures were created, were included in the second group. Blood samples were obtained from all patients pre-operatively and on the 1st and 28th postoperative days. Friedman and Mann-Whitney U tests were used for statistical analysis.

Results: Mean age of the first group, comprising 19 females (95%) and 1 male (5%), was 11.25 months (Range: 6-25 months). Mean age of the second group, including 17 females (85.7%) and 4 males (14.3%), was 74.4 months (Range: 24-120 months). While there was no significant difference between pre- and postoperative GH values in the first group (P=0.05), postoperative GH levels were significantly higher than preoperative GH levels in the second group (P<0.001). Postoperative IGF-1 levels were significantly lower than preoperative IGF-1 levels in both groups (P<0.001). When the difference of preoperative and postoperative 1st day GH and IGF-1 values were compared between two groups, GH changes were found significantly higher in the second group (P<0.001) whereas serum IGF-1 changes were significantly lower in the second group (P=0.043).

Conclusion: IGF-1 is inadequate in the investigation of fracture healing due to its short half-life and local production. On the other hand, GH plays an active role in fracture healing and increases significantly in comparison to pre-fracture values. Considering the GH increase during fracture healing, it may be beneficial to support patients with pathological fracture healing with growth hormone.

Keywords: Fracture healing, Iatrogenic fracture, Growth hormone, Somatomedin C

Öz

Amaç: Birçok çalışma, farklı kalsitropik hormonların kırık iyileşmesi üzerindeki etkilerini incelemiş, az sayıda çalışma ise büyüme faktörlerine odaklanmıştır. Kırık kallusunda lokal somatomedin C (IGF-1) tespiti, büyüme hormonu (GH) ve IGF-1'in kaynamama tedavisi olarak uygulanması ve osteoporotik kırıklarda saptanan düşük GH ve IGF-1 seviyeleri, bu hormonların kırık iyileşmesinde etkili olduğunu göstermektedir. Bununla birlikte, bu çalışmaların tümü kırık sonrası GH ve IGF-1 seviyelerine dayanır, ancak GH ve IGF-1 akut faz yanıtında rol oynar ve travma nedeniyle seviyeleri değişebilir. Bu çalışmanın amacı osteotomi ile tedavi edilen böylece bir iatrojenik kırık oluşturulan hastalarda GH ve IGF-1 seviyelerindeki değişimi araştırmak ve bu hormonların kırık iyileşmesi üzerindeki etkisini kırık öncesi ve sonrası sonuçları karşılaştırarak değerlendirmektir.

Yöntemler: Bu prospektif kohort çalışma için, 2014-2015 yılları arasında gelişimsel kalça çıkığı nedeniyle cerrahi olarak tedavi edilen hastalar tarandı. 41 hasta çalışmaya dahil edildi ve iki grup oluşturuldu. Sadece açık redüksiyon ve yumuşak doku gevşetmesi yapılan hastalar ilk grup olarak belirlendi. Pelvik osteotomi yapılan ve böylece bir iatrojenik kırık oluşturulan hastalar ikinci grup olarak belirlendi. Birinci gruba 20 hasta, ikinci gruba 21 hasta dahil edildi. Operasyon öncesi, postoperatif 1. gün ve 28. günde tüm hastalardan kan örneği alındı. İstatistiksel analizler için Friedman ve Mann-Whitney U testleri kullanıldı.

Bulgular: İlk grupta ortalama yaş 11,25 aydı (6-25 ay). Hastaların 19'u (95%) kadın, biri (5%) erkekti. İkinci grupta ortalama yaş 74,4 aydı (24-120 ay) ve 17 (85,7%) hasta kadın, dört (14,3%) hasta erkekti. İlk grupta pre-operatif ve postoperatif GH değerleri arasında anlamlı fark bulunmazken (P=0,05); ikinci grupta postoperatif GH değerleri daha yüksekti (P<0,001). Postoperatif IGF-1 düzeyleri her iki grupta anlamlı düşüş göstermekteydi (P<0,001). Pre-operatif ve postoperatif GH ve IGF-1 değerleri iki grup arasında karşılaştırıldığında, ikinci grupta postoperatif 1. günde GH değerleri anlamlı olarak daha yüksek seyrederken (P<0,001) aynı ölçümlerde serum IGF-1 düzeylerinde anlamlı düşüş saptandı (P=0,043).

Sonuç: IGF-1, kısa yarı ömrü ve yerel üretimi nedeniyle kırık iyileşmesinin araştırılmasında yetersizdir. Öte yandan, GH kırık iyileşmesinde aktif bir rol oynar ve kırık öncesi değerlere kıyasla önemli ölçüde artar. GH seviyesinin kırık iyileşmesi sırasında vücutta arttığı göz önüne alındığında, kırık iyileşmesinin patolojik olduğu düşünülen hastalarda GH desteği verilmesi yararlı olabilir.

Anahtar kelimeler: Kırık iyileşmesi, İatrojenik kırık, Büyüme hormonu, Somatomedin C

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Introduction

Many systemic and local factors are effective in fracture healing. In the literature, there are many studies examining the effects of different calciotropic hormones on fracture healing, whereas few studies focus on growth factors [1-5]. The secretion of growth hormone (GH) begins in fetal life and continues to be secreted during the whole life in decreasing amounts [6]. It regulates various metabolic processes throughout the body, effects protein, lipid and carbohydrate metabolism and has a major effect on longitudinal growth [6,7]. Somatomedin C (IGF-1), which is a small peptide bound to serum proteins, is responsible for the peripheral effects of GH [1]. It is secreted as a result of the autocrine and paracrine effects of cells in peripheral tissues such as bone [1-3]. Studies have demonstrated that the GH and IGF-1 axes affect the skeleton both directly and indirectly through steroids, parathyroid hormone, and vitamin d metabolites [8-10]. Local detection of IGF-1 in fracture callus, application of GH and IGF-1 as non-union treatment in different studies and low GH and IGF-1 levels in osteoporotic fractures indicate that these hormones are effective in fracture healing [8-13]. However, most of these studies are based on post fracture GH and IGF-1 levels, whereas GH and IGF-1 are also involved in acute phase response and their levels in serum change due to trauma [14].

The aim of this study is to investigate the change in GH and IGF-1 levels in patients treated with osteotomy, in which iatrogenic fractures are created, and to evaluate the effect of these hormones on fracture healing by comparing the pre-fracture and post fracture results.

Materials and methods

Following the approval of the ethics committee (Decision Date and Number: 14.04.2014; 161/2014), patients who were diagnosed with developmental dysplasia of the hip (DDH) and underwent surgery between 2014 and 2015 were prospectively followed. This study included 41 patients between the ages of six months and ten years, those within 25-97% percentile in terms of weight and height development and without additional systemic disease. Patients with syndromes, endocrine or metabolic diseases and growth and developmental delays were excluded from the study.

Evaluation

Blood samples from peripheral venous veins were obtained from all patients pre-operatively and on the 1st and 28th postoperative days. All blood samples were obtained in the morning, between 08:00-10:00, following a fasting of eight hours. IGF-1 and GH levels were measured with Radio Immune Assay and Electrochemiluminescence Immunoassay methods, respectively [15]. All results are presented as nanograms/ml.

GH and IGF-1 hormones are involved in both bone metabolism and acute phase response. Therefore, in order to distinguish whether the changes in the levels of these hormones were due to the response to surgical stress or fracture healing, two patient groups were formed [14]. Patients who underwent open reduction and soft tissue release without any osteotomy (n=20) constituted the first group. Patients who underwent pelvic osteotomy (n=21), in which iatrogenic fractures were created, were part of the second group.

Surgical technique

Open reduction and soft tissue loosening was performed via limited posteromedial approach to the patients in the first group, as this technique is our routine approach to DDH, and commonly used in our clinic. The limited posteromedial approach technique was previously discussed in our studies [16,17]. The posterior margin of tendineum adductor longus was incised 5 cm and layers were cut until tendon was reached. Following tenotomy of adductor longus tendon, the lesser trochanter was used as a guide to reach the iliopsoas tendon and incise it. 1 ml of contrast material was injected into the joint capsule and hip radiographs were filmed in human position. If the patient had grade one reduction based on Tönnis intraoperative grading system and/or an appropriate safe zone, surgery was concluded. On the other hand, if the patient had a grade two or three reduction and/or a narrow safe zone, arthrotomy was performed. The inferomedial capsule was opened and *ligamentum teres* and transverse acetabular ligament were incised. Reduction was confirmed with x-ray radiographies obtained in the human position, hip spica cast was applied and surgery was concluded. The patients in the second group received pelvic osteotomy through a bikini incision. The iliac wing apophysis was dissected and tilted medially and laterally. A tricortical graft was obtained from the iliac wing. Periacetabular osteotomy was performed through the sciatic notch with the help of gigli wire. Then, the tricortical graft was placed on the osteotomy line by tilting the acetabulum. After confirming the reduction by obtaining x-rays on human position and checking movements of the hip, long leg cast was applied, and surgery was concluded. All surgeries were performed by the same surgical team.

No additional treatment was administered to any of the patients during the postoperative period. The cast was removed at the postoperative third month and hip abduction orthosis was performed to patients of the first group. The cast was removed at one and a half-month follow-up in the second group. Nonunion or union delay was not observed in any patient.

Statistical analysis

Data were evaluated for normal distribution, and Friedman test was used to evaluate the difference in both groups separately. Mann-Whitney U test was used to evaluate the significance of difference between the two groups. The post-hoc power was calculated as 0.95, considering the correlation between two groups. $P<0.05$ was deemed statistically significant, and SPSS 11.5 package program was used for analysis.

Results

Mean age of the first group, comprising 19 females (95%) and 1 male (5%), was 11.25 months (Range: 6-25 months). Mean age of the second group, including 17 females (8.57%) and 4 males (14.3%), was 74.4 months (Range: 24-120 months). While there was no significant difference between pre- and postoperative GH values in the first group ($P=0.05$), postoperative GH levels were significantly higher than preoperative GH levels in the second group ($P<0.001$). GH

values of both groups are presented in Table 1. Postoperative IGF-1 levels were significantly lower than preoperative IGF-1 levels in both groups (P<0.001) (Table 2). When the difference of preoperative and postoperative 1st day GH and IGF-1 values were compared between two groups, GH changes were found significantly higher in the second group (P<0.001) whereas serum IGF-1 changes were significantly lower in the second group (P=0.043). Also, GH levels were significantly higher in the second group (P=0.027) and IGF-1 levels lower (P=0.01) on the 28th postoperative day compared to preoperative values. There was no significant difference between GH and IGF-1 values on the postoperative 1st and 28th days (P=0.419; P=0.285, respectively). The comparison of preoperative and postoperative GH and IGF-1 values between two groups is presented in Table 3.

Table 1: Comparison of preoperative and postoperative growth hormone values in both groups

	Group 1 (n=20)		Group 2 (n=21)	
	Mean (SD)	Median (min-max)	Mean (SD)	Median (min-max)
Preoperative	2.62 (2.21)	1.79 (0.49-8.80)	0.88 (1.95)	0.34 (0.07-8.80)
Postoperative 1st day	5.30 (4.35)	3.31 (0.41-14.30)	4.27 (5.96)	2.47 (0.46-26.13)
Postoperative 28th day	2.69 (2.15)	2.58 (0.40-10.50)	2.02 (2.58)	0.72 (0.15-9.40)
P-value	0.05		<0.001	

n: patient population, SD: standard deviation, min: minimum, max: maximum

Table 2: Comparison of preoperative and postoperative somatomedin C values in both groups

	Group 1 (n=20)		Group 2 (n=21)	
	Mean (SD)	Median (min-max)	Mean (SD)	Median (min-max)
Preoperative	34.39 (24.72)	25 (8.20-120)	160.63 (98.82)	136 (56.6-497)
Postoperative 1st day	30.80 (29.6)	25 (10.72-152)	124 (77.02)	110 (35-273)
Postoperative 28th day	43.55 (24.01)	31.34 (19.30-106.5)	156.01 (109.16)	122 (55.4-549)
P-value	<0.001		<0.001	

n: patient population, SD: standard deviation, min: minimum, max: maximum

Table 3: Comparison of preoperative and postoperative growth hormone and somatomedin C values in two groups

	Group 1 (n=20)		Group 2 (n=21)		
	Mean (SD)	Median (min-max)	Mean (SD)	Median (min-max)	*P*-value
GH change on preop. and postop. 1st day	124.09 (147.48)	114.7 (-57.3-535.04)	1817.1 (2922.6)	429.4 (-83-10878.9)	<0.001
IGF-1 change on preop. and postop. 1st day	-7.99 (28.59)	0 (-61.2-47.3)	-23.05 (26.26)	-18.82 (-58.1-25.27)	0.043
GH change on preop. and postop. 28th day	68.58 (253.8)	-2.79 (-83.7-1106.9)	528.01 (812.53)	102.35 (-93-2628.6)	0.027
IGF-1 change on preop. and postop. 28th day	45.57 (64.77)	16.9 (-32.8-176.8)	-2.69 (20.98)	-4.2 (-37.4-46.3)	0.010
GH change on postop. 1st and 28th day	-2.21 (114.34)	-43.9 (-94.0-348.7)	20.72 (154.03)	-61.49 (-99.2-368.9)	0.419
IGF-1 change on postop. 1st and 28th day	68.43 (80.82)	49.19 (-29.9-264.3)	46.09 (73.0)	17.95 (-34.1-226.45)	0.285

n: patient population, SD: standard deviation, min: minimum, max: maximum, preop: preoperative, postop: postoperative

Discussion

Understanding the physiological and biochemical interactions between cells in fracture healing enables the investigation of the factors that may affect this process [18]. Many studies, which were all performed by examining posttraumatic GH and IGF-1 levels only, confirm the systemic effect of GH-IGF axis on fracture healing [4,5,8-12]. However, it must not be forgotten that these hormones are also involved in acute phase response and their levels change due to traumas not involving fractures, as well [14]. Also, there are many metabolic factors that may affect GH and IGF-1 values. By using iatrogenic fractures instead of traumatic fractures in our study, we determined patients' pre-fracture GH and IGF-1 levels, which was necessary for comparison with post-fracture values and not reference ranges indicated in the literature. To exclude surgical stress, two groups were formed. We believe this prospective study is important because it includes the comparison of pre-fracture and post-fracture GH and IGF-1 values among two separate groups.

GH levels fluctuate during the day in normal individuals [19,20]. In our study, blood sampling was performed between 08.00-10.00 in the morning, following a fasting of eight hours, which minimized the factors affecting the release of GH and IGF-1.

We found that the difference in pre- and postoperative GH values were significant in patients with iatrogenic fractures. Similarly, when two groups were compared, GH levels were observed significantly higher in the osteotomy group. These results are consistent with the literature [8-12]. Weiss et al. [21], in their prospective study involving 186 patients, examined GH dependent acid labile subunit (ALS), IGF-1 and IGF binding protein (IGFBP-3). They found that GH-dependent ALS and IGFBP-3 levels were significantly lower in patients with nonunion. In their randomized, double blind, placebo-controlled clinical trial of 406 tibial fractures, Rarschke et al. [22] found that GH accelerated healing process significantly in closed tibial fractures. Tran et al. [23] stated that GH clearly demonstrates a positive effect on fracture healing.

Although IGF-1 is locally secreted and affects fracture callus, its systemic effects on fracture healing is controversial. Weiss et al. have examined the blood values of the two groups at the 1st and 8th posttraumatic weeks and found a significant difference between IGFBP-3 and GH-dependent ALS values. IGF-1 serum concentrations, however, were not significantly different. Similarly, Jeevenandam et al. [24] showed that IGFBP-3 and IGF-1 ratios did not change significantly in trauma patients. Weiss et al., based on both these results, reported that serum free IGF-1 level was not associated with fracture healing quality. On the other hand, Di Monaco et al. [25], in their study of 188 hip fractures, found that serum IGF-1 levels are significantly associated with ability to function after hip fracture. In our study, a significant difference was found between preoperative and postoperative IGF-1 values between the two groups. The significant difference in IGF-1 levels between preoperative and postoperative periods and the lower postoperative IGF-1 levels in the osteotomy group suggest that systemic IGF-1 levels may play a role in fracture healing. Various explanations exist for this result. First, unlike the studies in the literature, fractures in patients were iatrogenically formed in our study. There is no trauma other than surgical stress, which may affect serum IGF-1 levels. Secondly, IGF-1 has a noticeably short half-life, is secreted from numerous peripheral tissues [1], and its serum concentration may vary with factors such as hunger [26]. Its local release and effects may have affected the outcome of our study.

Limitations

There are some limitations in our study. First of all, the groups are not homogeneous in age. We tried to overcome this problem by comparing the patients' postoperative GH and IGF-1 levels with that of the preoperative period, and not the reference values; however, we could not control different factors affecting

GH and IGF-1 in children of different age groups. Also, we only evaluated the patients' blood test results and did not incorporate functional or radiological criteria into this study, which could have revealed the relationship between fracture healing and hormone values more clearly. Finally, the hormone levels were measured preoperatively, on the 1st and 28th postoperative days, and not later. Although repeating measurements after completion of fracture healing radiologically and clinically may be useful in examining the effects of GH-IGF-1 axis on fracture healing, we didn't measure the levels in late period because we observed fracture callus radiologically in nearly all of our patients in 28 days, and non-union is not typically a problem in children.

Conclusion

IGF-1 is inadequate in the investigation of fracture healing due to its short half-life and local production. On the other hand, GH plays an active role in fracture healing and increases significantly in comparison to pre-fracture values. Considering that the GH level increases in the body during fracture healing, it may be beneficial to give GH support to patients who exhibit pathological fracture healing.

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Evaluation of changes in perfusion index in patients with cytotoxic tissue damage after snake bite: A prospective cohort study

Yılan ısırması sonrası sitotoksik doku hasarı olan hastalarda perfüzyon indeks değerindeki değişimlerin değerlendirilmesi: Prospektif kohort çalışma

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Abstract

Aim: Pain, edema, and heat loss that develop and gradually increase in the extremities following a snake bite reduce microcirculation. The perfusion index indicates the intensity of the pulsatile signal relative to the nonpulsatile signal. The aim of this study was to measure the changes in perfusion index (PI) in the extremities of patients suffering from a snake bite who are treated in an intensive care unit, to detect early ischemia or necrosis in the affected organ, and to investigate the effectiveness of the administered antivenom.

Methods: Twenty patients admitted to our hospital with cytotoxic swelling of the upper or lower extremities after a snake bite were included in this prospective cohort study. Initial treatment was provided to the patients based on the snake bite treatment protocol. PI values of the affected extremity of each patient were measured for 24 hours using a finger probe and compared with the unaffected extremity in the same region. Patient age, gender, bites, antivenom administration times, and complications were also recorded and compared.

Results: Of all patients, 13 (65%) were male and 7 (35%) were female. The mean age of the patients was 37.5 (14.15) years. Eight patients (40%) were bitten in the lower extremity, and 12 patients (60%) were bitten in the upper extremity. PI values measured at the 19th, 23rd, and 24th hours were significantly higher in the affected extremity than in the unaffected extremity (P=0.043, P=0.049 and P=0.018, respectively). PI values measured at the 20th, 21st, and 22nd hours were insignificantly higher in the affected extremity than in the unaffected extremity (P=0.088, P=0.096 and P=0.085, respectively). An increase in the ratio between the PI of the unaffected extremity and that of the affected extremity was associated with a decrease in complications. One patient who had a snake bite in the right upper extremity developed necrosis and another patient developed compartment syndrome.

Conclusion: PI is a rapid, painless, and continuous measurement that provides clinicians with valuable information on both the effectiveness of the antivenom and perfusion of the extremity. Patients with local reactions such as swelling and bruising should be monitored for at least 24 hours, and clinicians should pay attention to the development of compartment syndrome or tissue necrosis in the following hours.

Keywords: Snakebite, Antivenom, Perfusion index, Venom

Öz

Amaç: Yılanın ısırması sonrası ekstremitede başlayan ve giderek artan ağrı, ödem ve ısı azalması mikrosirkülasyonu azaltmaktadır. Perfüzyon indeks pulsatil sinyalin pulsatil olmayana göre nabız şiddetini belirtir. Bu çalışmada Yoğun bakım ünitesinde takip edilen yılan ısırmasına maruz kalan hastaların ekstremitesinde perfüzyon indeksi (PI) değerindeki değişimlerin ölçülmesini; etkilenen organdaki erken dönem iskemi veya nekrozu tespit etmeyi ayrıca verilen antivenomun etkinliğini araştırmayı amaçladık.

Yöntemler: Hastanemize üst veya alt ekstremitelerinin yılan ısırığından sitotoksik şişmesi ile başvuran 20 hasta bu prospektif kohort çalışmasına dahil edildi. Hastalar yılan ısırması tedavi protokolü ile ilk tedavileri yapıldı. Her hastanın etkilenmiş ekstremitesi, parmak probu yardımıyla 24 saat boyunca PI ölçümleri yapıldı ve aynı bölgedeki etkilenmemiş ekstremite ile karşılaştırıldı. Hastaların yaşları, cinsiyetleri, ısırıkları, antivenin alma zamanları ve komplikasyonları kaydedildi ve karşılaştırıldı.

Bulgular: Hastaların 13'ü (%65) erkek, 7'si (%35) kadındı. Hastaların ortalama yaşları 37,5 (14,15) idi. 8 hasta (%40) alt ekstremiteden, 12 hasta (%60) ise üst ekstremiteden ısırığa maruz kalmıştı. 24 saat boyunca yapılan PI ölçümlerinde ve her iki ekstremite bölge ölçüm oranlarında sağlam ekstremite lehine olmak üzere 19., 23. ile 24. saatlerde istatiksel olarak anlamlı (Sırasıyla P=0,043, P=0,049 ve P=0,018); 20., 21. ve 22. saatte ise sınırda anlamlı sonuç bulunmuştur (Sırasıyla P=0,088 ve P=0,096, P=0,085). Etkilenmemiş ekstremitenin PI ile etkilenen ekstremitenin PI arasındaki orandaki bir artış, komplikasyonlardaki bir azalmayla ilişkilendirildi. Sağ üst ekstremite yılan ısırığına maruz kalan bir hastada nekroz, bir hastada da kompartman sendromu gelişti.

Sonuç: Klinisyene hem antivenomun etkinliği hem de ekstemite perfüzyonu konusunda değerli bilgiler sağlayan PI, hızlı, ağrısız ve sürekli ölçüm sağlayan bir ölçümdür. Şişlik ve morarma gibi lokal reaksiyonu olan hastalar en az 24 saat boyunca izlenmeli ilerleyen saatlerde kompartman sendromu veya doku nekrozu oluşmasına dikkat etmelidir.

Anahtar kelimeler: Yılan ısırması, Antivenom, Perfüzyon indeksi, Zehir

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Introduction

Bites of poisonous animals are a major cause of mortality and morbidity worldwide. Every year, more than five million people are bitten by snakes, resulting in approximately 20,000–25,000 deaths [1]. Because snake venom is a combination of many toxic proteins and enzymes, it is the most complex of all poisons. Snake venom contains low-molecular-weight peptides and numerous other components such as neurotoxins; cytotoxins; hemotoxins; glycoproteins; proteolytic, hydrolytic, and hyaluronidase enzymes; and metallic ions [2]. The enzymes present in the snake venom, which enter the human body after a snake bite, lead to the clinical picture associated with snake bite [3]. This clinical picture varies depending on individual factors and on whether the poison enters systemic circulation; and snake bite poisoning may be asymptomatic or may even result in mortality [4]. The main treatment modality for snake bite poisoning is systemic antivenom treatment. However, there are different opinions about the time of initiating the antivenom treatment and the dose to be administered. Although there is a consensus that the effectiveness of antivenom is sufficient against the systemic effects of the venom, its effectiveness against local effects is controversial [5].

Enzymes in snake venom cause edema and vasoconstriction. Ischemic damage starts in the bite area within the first 2 h due to the direct contact of the toxin with the tissues, leading to local tissue edema. Enzymes such as metalloproteinases cause capillary endothelial damage and cell apoptosis. Increased microvascular permeability, early leukocytosis, and increase in interleukin and tumor necrosis factor levels occur as part of the inflammatory response to this envenomation, all of which increase the painful swelling. Tissue necrosis may develop in approximately 10% of cases [6]. Myonecrosis may result from the toxic effects of snake venom proteins that directly cause necrosis without increasing compartment pressure. However, it may progress to compartment syndrome, which is a rare but fatal complication with symptoms such as severe pain, paresthesia, delayed capillary filling, pain in passive stretching, no pulse, and coldness [2].

Perfusion index (PI) is a continuous and non-invasive measurement of peripheral perfusion obtained using a pulse oximeter. PI indicates the percentage of the pulsatile signal relative to the nonpulsatile signal (pulse intensity). In other words, it enables the evaluation of the patient in terms of microcirculation by showing early real-time changes in blood flow [7].

The aim of this study was to measure the changes in PI in the extremities of patients suffering from a snake bite and being treated in an intensive care unit (ICU), to detect early ischemia or necrosis in the affected organ, and to investigate the effectiveness of the administered antivenom.

Materials and methods

The present study is a single-center, prospective, observational cohort study. The study was approved by the Harran University Faculty of Medicine Ethics Committee (13.07.2017, meeting number 07 and decision number 24) and conducted in the medical ICU of the Harran University Medical Faculty Hospital.

Patient selection

The study included 20 patients with upper or lower extremity swelling who were followed up in our ICU after a snake bite between August 2017 and August 2019. Written informed consents were obtained from all patients. Flow diagram of the study is shown in Figure 1.

Patients who refused to participate in the study, pregnant women, patients with minimal or no swelling in the affected extremity, patients who were bitten in anatomical sites other than extremities or had predominantly hemotoxic or neurotoxic envenomation, patients who were hospitalized for <1 day, patients with known peripheral vascular disease or hepatic cirrhosis, and patients aged <18 years were excluded from the study.

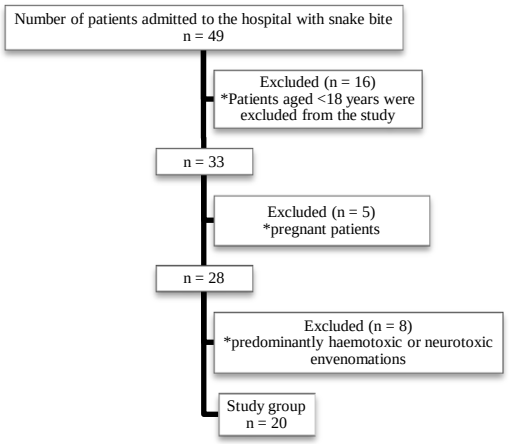

Figure 1: Flow diagram of the study

Measurement method

Twenty patients who were exposed to snake venom and followed up in the ICU were followed with 3-lead electrocardiography, peripheral O_2 saturation, and non-invasive blood pressure monitoring as part of standard evaluation.

All patients underwent routine blood tests and basic standard treatment such as the administration of analgesia, intravenous fluids, and tetanus toxoid. Bite wounds were washed with soap and water and the extremities immobilized. During this period, 10 mL Polysera snake anti-serum (Vetal Serum Manufacturing Limited, Adıyaman, Turkey) in 150 mL of saline, along with steroids and antihistamines, was administered intravenously on the basis of algorithms to ensure that one or two vials would last for 45 min [8].

After the initial treatment, finger probes were placed on the patients and the non-invasive Masimo Radical-7 Pulse co-oximeter (Masimo Corporation, Irvine, USA) measuring device was used to measure PI values both at the area bitten by the snake and at the corresponding unaffected area of the opposite extremity. Measurements were performed when the blood pressure cuff was not inflated. The ICU was air-conditioned, and room temperature was maintained at about 25°C. Patients were not provided additional oxygen therapy, and it was assumed that the fraction of inspired oxygen was 21%. Whenever the device issued a “low perfusion” alarm, the measurements were repeated on another finger for confirmation purposes. Measurements were recorded at 1-h intervals for a period of 24 h.

The study was initiated after recording the basal PI values of patients. In addition to the demographic data of the patients, we recorded the time elapsed between snake bite and antivenom administration, the extremity area that was bitten, the observed complications, and the ratio between hourly measurements of the PI of the unaffected extremity and that of the affected extremity.

Statistical analysis

Statistical analysis was performed using the IBM SPSS software (Armonk, NY, USA). Consistent factors were presented as mean (standard deviation) (SD) or median (interquartile range) and analyzed with Mann–Whitney U test. All factors were presented as frequency (percentage), and chi-square test or Fisher's exact test were used when necessary. Multiple logistic regression analysis was used to determine the independent predictors of achievement, and logistic regression was used to model independent predictors. All statistical tests were two-sided, and $P<0.05$ was considered as statistically significant.

Results

Patient characteristics

The present study comprised 20 patients [13 (65%) men and 7 (35%) women; mean age, 37.5 (14.15) years]. The mean age of female patients was 36.14 (15.35) years and that of male patients was 40.53 (13.99) years. Of all, 85% patients were farmers, whereas the rest belonged to different occupational groups. Eight patients (40%) were bitten in the lower extremity and 12 (60%) in the upper extremity. Three of the lower extremity bites were on the left side (37.5%), whereas five were on the right side (62.5%). Seven of the upper extremity bites were on the right side (58.3%), whereas five were on the left side (41.7%). One patient with snake bite in the right upper extremity developed necrosis and another patient developed compartment syndrome. Patients were brought to the emergency department in an average time of 1.5 (0.5–2.5) h. The mean time between snake bite and antivenom administration was 2 h.

Perfusion index values

PI values measured at the 19th, 23rd, and 24th hours were significantly higher in the affected extremity than in the unaffected extremity ($P=0.043$, $P=0.049$ and $P=0.018$, respectively). PI values measured at the 20th, 21st, and 22nd hours were insignificantly higher in the affected extremity than in the unaffected extremity ($P=0.088$, $P=0.096$ and $P=0.085$, respectively) (Table 1, Figure 2). On examining PI measurements at each time point, it was found that the PI value of 4.33 at the 1st hour in the unaffected extremity decreased to 3.98 at the 6th hour ($P=0.009$) and to 3.29 at the 24th hour ($P=0.05$). Furthermore, PI value significantly decreased from 5.12 at the 12th hour to 3.29 at the 24th hour ($P=0.02$). In the affected extremity, the PI value decreased from 5.84 at the 1st hour to 1.76 in 5 hours ($P=0.05$). The decrease in PI value from 4.39 at the 12th hour to 1.76 at the 24th hour was also significant ($P=0.012$) (Table 2).

Multiple logistic regression

In the regression analysis performed to investigate whether complications developed in the patients included in this study, complications were considered as a dependent variable. The results of the regression analysis are shown in Table 3.

Based on the regression analysis results, the likelihood of complications was found lower in males. There was a negative correlation between the affected extremity and complications. The likelihood of complications increased significantly when the upper right extremity was affected, and decreased when the upper left, lower left, and lower right extremities were affected. There was a positive correlation between the unaffected extremity/affected extremity (UE/AE) PI ratio and complications. As the ratio increased, the occurrence of complications decreased. We also investigated the effect of the time of antivenom administration on the likelihood of developing complications. As the time elapsed between snake bite and antivenom administration increased, the likelihood of complications increased.

Table 1: PI changes in the extremities within 24 hours after snake bite

Hour	Affected extremity		Unaffected extremity		Ratio	*P*-value
	Mean	SD	Mean	SD	Mean	0.204
1	5.84	3.64	4.33	2.62	2.04	0.204
2	5.34	3.27	3.92	3.06	1.90	0.326
3	3.39	2.87	3.80	2.25	1.66	0.453
4	4.73	2.52	4.52	4.09	1.94	0.326
5	5.67	2.38	3.80	2.51	1.86	0.057
6	4.19	2.79	3.98	2.67	1.20	0.885
7	3.52	2.71	3.13	1.26	1.16	0.602
8	4.19	2.73	5.34	3.8	1.52	0.326
9	4.41	2.3	4.58	2.51	2.21	0.954
10	4.18	2.75	4.46	2.48	1.14	0.470
11	3.16	2.58	4.12	2.56	0.98	0.729
12	4.39	3.23	5.12	4.01	1.03	0.603
13	4.02	2.14	4.31	2.59	1.30	0.954
14	3.27	1.62	5.04	2.4	0.86	0.094
15	4.40	2.22	4.63	2.61	1.11	0.908
16	2.95	0.88	3.13	1.27	1.39	0.795
17	3.24	0.71	3.37	2.01	1.64	0.312
18	2.74	0.69	3.62	1.82	1.09	0.224
19	2.51	0.35	2.92	1.19	1.42	0.043
20	3.03	1.44	3.26	1.28	1.15	0.088
21	2.74	1.01	2.99	1.36	1.13	0.096
22	2.48	1.00	2.89	1.21	1.08	0.085
23	1.99	0.87	3.21	1.46	0.73	0.049
24	1.76	0.81	3.29	1.67	0.75	0.018
P-value	0.002		0.012		0.02	

SD: Standard deviation

Table 2: PI changes in the extremities between two time points

	Unaffected extremity *P*-value	Affected extremity *P*-value
6th h PI−1st h PI	0.009	0.875
12th h PI−1st h PI	0.059	0.366
24th h PI−1st h PI	0.002	0.050
12th h PI−6th h PI	0.937	0.070
24th h PI−12th h PI	0.020	0.012

h: hour, PI: Perfusion Index

Table 3: Multiple Logistic Regression Results

Variable	Coefficient	SD	t-Statistic	*P*-value
Complication	0.9846	0.1235	7.9718	<0.001
Gender	−0.7062	0.0063	−11.1682	0.0002
Affected Extremity	−0.0405	0.0219	−1.8478	0.0662
Patient	0.0313	0.0153	2.0520	0.0416
UE/AE	0.0290	0.0157	1.8502	0.0659
Antivenom Time	0.4160	0.0526	7.9023	0.0004
Age	0.0186	0.0043	4.2885	0.0003
Complication Time	0.0010	0.0029	0.3363	0.7370
R-squared	0.6099			
Adjusted R-squared	0.5951			
F-statistic	41.1036			
P-value	<0.001			

SD: Standard deviation, UE/AE; Unaffected extremity/Affected extremity

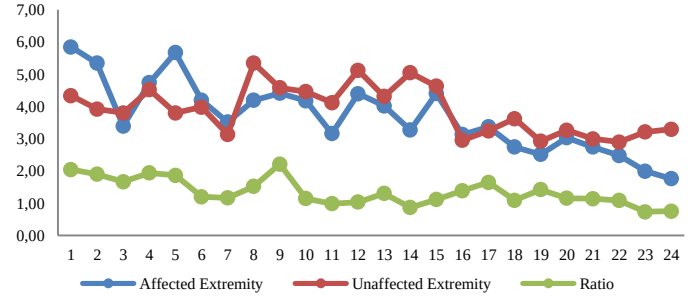

Figure 2: Changes in PI over time (affected extremity, unaffected extremity, ratio*)

Discussion

This is a single-center, prospective, observational cohort study that is the first to evaluate the changes in PI, the presence of early ischemia and necrosis in the affected organ, and the effectiveness of the administered antivenom among patients with increasingly painful swellings in the upper and lower extremities following snake bites, for which antivenom treatment was administered. Our results indicate that PI measurements can be used to evaluate the severity of snake bite in the extremities. PI values in the bitten extremity decreased, especially after 19 hours, compared with those in the contralateral extremity.

Snake bites in Turkey are mostly encountered in the Southern and Southeastern Anatolia regions of the country and represent an important cause of morbidity and mortality [4]. The venom of the snakes belonging to the Viperidae family, most commonly implicated in snake bites in Turkey, is known to be both cardiotoxic and myotoxic and contains hyaluronidase, phospholipase A, and various proteases that lead to tissue disruption [9]. When a snake of the Viperidae family bites, hyaluronidase in the venom accelerates the spread of the venom in the tissues. Myonecrosis is mainly caused by the effect of myotoxic phospholipase A2. Phospholipase A2 induces hemolysis by converting lecithin to lysolecithin, and hemorrhagin causes rapid hemorrhagic edema by destroying the inner endothelium that covers the blood vessels in the bite area. In addition to myonecrosis, blood vessel integrity is affected. Metalloproteinases cause basal hydrolysis of the basal membrane of capillaries, particularly of type IV collagen, and weaken the mechanical stability of microvessels [3,10].

Systemic antivenom treatment is the main treatment modality for patients with snake bite envenomation. There are different opinions regarding the time of initiation of antivenom treatment and the dose to be administered. In a series in which Viperidae bite cases were treated, it was reported that the complication rates and severity increased when antivenom treatment was inadequate [11-13]. Although complication rate is lower when the appropriate antivenom treatment is applied, it should be kept in mind that various complications can be encountered in envenomation cases associated with snake bites. The most common complication is necrosis, with a reported rate of 10% [3]. On the other hand, as the venom activity increases, the mediators of vasodilatation increase as well, and the increase in vasodilatation and vascular permeability increase the pressure inside and outside the compartment. Consequently, even if the necrotic period is not triggered due to insufficient circulation alone, the lysis and degradation caused by the venom increase and may trigger compartment syndrome. Therefore, strict follow-up is necessary for compartment syndrome that may occur in patients with snake bites on the extremities, and fasciotomy should be performed to achieve full functional recovery when compartment syndrome is clinically suspected [2].

To date, no rapid, painless, and objective methods have been developed that can help keep track of the complex clinical picture observed in the extremities following a snake bite and that can provide valuable information to the clinician about the extent of soft tissue damage. The only current method that has been proposed for these purposes is ultrasound evaluation [14,15]. In relevant studies, the exposed extremity of patients was evaluated at the point of maximum swelling, and the thickness of the tissue structures was compared between the affected (subcutaneous tissue or muscle compartment) and unaffected extremities. Imaging was focused on identifying the depth and location of tissue edema, the presence of fluid accumulation, the evidence of muscle fasciculation, and fascia and tendon lesions. However, ultrasound evaluation was performed only once, after admission, for the patients included in these studies. Repeat ultrasound measurements were not performed. As the findings depend on personal interpretations, it is possible to overlook or misinterpret fine sonographic findings. Considering all these findings and the circulatory mechanisms mentioned above and taking into account the needs of the clinicians, PI measurement may be a preferable method for evaluating snake bite cases because it is a relatively new predictor of blood pulsatility in the extremities that is calculated using the infrared spectrum within the scope of the plethysmography waveform procedure. It is a simple, cost-effective, and non-invasive method for evaluating peripheral perfusion determined using the percentage of pulsatile-to-nonpulsatile blood flow in the extremities. PI reflects the state of microcirculation, which is intensely stimulated by sympathetic nerves, and is thus influenced by factors that cause vasodilatation or vasoconstriction of the microvascular system [16]. There are reports showing that PI measurement in ICU is effective in evaluating different patient groups of different ages [17-19].

In this study, we performed 24 hour-PI monitoring of the affected area of the extremity that was exposed to the snake bite and the corresponding unaffected area of the opposite extremity. In our follow-up results, we observed a significant decrease in PI values at the end of 24 hours compared with those at the 1st hour in both areas. Although PI measurements showed variability in the first 12 hours, we found a significant decrease in PI values in both extremities. PI was insignificantly higher in the unaffected extremity between the 12th and 19th hours. On the other hand, PI was significantly higher in the unaffected extremity in the next three measurements, and a limited significant change was observed in the last three measurements. Although there is no definitive information in the literature regarding the duration of antivenom activity, there is information indicating that the local effectiveness of antivenom, such as in the extremities, is minimal [5]. We attributed the decrease and increase in PI values within the first 12 hours to the positive effective of the administered antivenom. We believe that the changes observed after 12 hours are due to decreased effectiveness of the antivenom.

UE/AE PI ratio decreased significantly at the end of the 24th hour, compared with that at the 1st hour. The decrease was especially prominent after the 19th hour. There is a positive correlation between the UE/AE PI ratio and complications. As this ratio increases, the complications decrease. One patient developed necrosis on the second day of hospitalization, and another patient developed compartment syndrome on the third day.

Limitations

One of the limitations of the present study is that PI measurements were obtained only in the first 24 hours, despite

the time of occurrence and duration of the complications. Another limitation is that the PI values obtained in the present study are valid only for the venom of the snakes observed in Turkey. We could not determine how PI measurements would change in response to the venom of other snake species.

Conclusion

We propose that PI measurements be used to monitor the extremities with snake bite and the resulting swelling. It is a rapid, painless, and continuous measurement that provides the clinician with valuable information on both the effectiveness of the antivenom and perfusion of the extremity. Patients with local reactions such as swelling and bruising should be monitored for at least 24 h, and clinicians should be careful against the occurrence of compartment syndrome or tissue necrosis.

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Preoperative detection of lymph node metastasis in endometrial cancer: The role of 18-FDG PET/CT

Endometrial kanserde preoperatif lenf nodu metastazi tespiti: Endometrial kanserde 18-FDG PET/BT'nin rolü

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Abstract

Aim: Fluorine-18 2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET)/CT imaging technique combines the advantages of localization and functional imaging, and thus has become a preferred method for detection of metastases to the lymph nodes (LN) of patients with endometrial cancer. The aim of this study was to evaluate the efficacy of 18F-FDG PET/CT imaging results in the detection of metastasis of endometrial cancer to lymph nodes.

Methods: This is a retrospective cohort study conducted between 2009 and 2015. The study group consisted of females who were admitted to our clinic and diagnosed with endometrial cancer (n=135). We included patients who underwent LN dissection with pre-operative 18F-FDG PET / CT imaging, and evaluated the capability of this method in terms of its effectiveness in determining metastases to the lymph nodes. Assessments and comparisons were performed with gold-standard pathological methods.

Results: The 18F-FDG PET/CT method for identification of lymph node metastases had a sensitivity of 76%, a specificity of 80%, a positive predictive value of 46.34% and a negative predictive value of 93.62%. The overall diagnostic accuracy was 79.26%.

Conclusion: Our analysis suggests that 18F-FDG PET/CT has a high negative predictive value with regards to lymph node metastasis in endometrial cancer and may be used effectively in evaluating lymph node metastasis in this cohort.

Keywords: Endometrial cancer, Lymph node metastasis, 18F-FDG PET/CT, Prognosis

Öz

Amaç: Fluorine-18 2-floro-2-deoksi-D-glukoz (FDG) pozitron emisyon tomografisi (PET)/ BT görüntüleme tekniği, lokalizasyonun ve fonksiyonel görüntülemenin avantajlarını birleştirdiğinden, endometrial kanserli hastalarda lenf nodu (LN) metastazlarının tespiti için tercih edilen bir yöntem haline gelmiştir. Bu çalışmada 18F-FDG PET/BT görüntüleme sonuçlarının, endometrial kanserin lenf nodu metastazının belirlenmesindeki etkinliğini araştırmayı amaçladık.

Yöntemler: Bu çalışma 2009-2015 yılları arasında yapılan retrospektif kohort bir çalışmadır. Çalışma grubu kliniğimize başvuran ve endometriyal kanser tanısı alan tüm kadınlardan (n=135) oluşmaktaydı. Çalışmaya, ameliyat öncesi 18F-FDG PET/BT çekilen ve LN diseksiyonu yapılan hastaları dahil edildi. Bu yöntemin lend nodu metastazını belirlemede etkinliği değerlendirildi. Değerlendirmeler ve karşılaştırmalar altın standard patolojik tanı yöntemleriyle yapıldı.

Bulgular: Lenf nodu metastazlarının tanımlanmasında 18F-FDG PET/BT yönteminin %76 duyarlılık, %80 özgüllük, %46.34 pozitif prediktif değeri ve %93.62 negatif prediktif değeri gösterdiği bulundu. Genel tanısal doğruluk ise %79,26 idi.

Sonuç: Analizlerimiz, 18F-FDG PET/BT'nin, endometriyal kanser hastalarında lenf nodu metastazı açısından yüksek negatif prediktif değere sahip olduğunu ve bu hastalarda lenf nodu metastazı araştırmada uygulanabilir bir görüntüleme yöntemi olduğunu göstermiştir.

Anahtar kelimeler: Endometrial kanser, Lenf nodu metastazı, 18F-FDG-PET/BT, Prognoz

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Introduction

Endometrial cancer (corpus uteri cancer) is a particularly important type of cancer in women, due to its high frequency [1]. Fortunately, it is easily diagnosed, and the progression is slow [2]. Therefore, many cases are at the early stage at time of diagnosis and have good prognoses. Most studies have shown that survival rates of up to 90% are achievable with the classical surgical methods of total hysterectomy and bilateral salpingo-oophorectomy [3]. Endometrial cancer is known to be associated with several factors that contribute to poor prognosis: advanced staging with FIGO criteria, non-endometrioid histological subtype, higher level grade, presence of >50% myometrial invasion, and lymph node metastasis (LNM) [4]. As such, it is crucial to accurately identify the extent of the disease diagnosis to implement appropriate treatment and management.

In early cases of endometrial cancer, pelvic lymph node metastases are mostly present in extrauterine regions, but performing lymphadenectomy is greatly disputed [5,6]. Most of the trials have demonstrated better staging (surgical) and prognostic evaluation when lymphadenectomy is performed, with no positive effect on survival [7,8]. Current treatment recommendations do not include lymphadenectomy as a routine practice.

Generally, magnetic resonance imaging (MRI) is the imaging of choice in endometrial cancer [9,10]. However, MRI has some limitations, such as of low accuracy and reproducibility [11]. At this point, positron emission tomography (PET), which accrues data via glucose-metabolism levels, has emerged as an option to MRI as a modality that provides functional data [12], but this method is also hampered by sensitivity-related problems and cannot localize tumors. Fluorine-18 2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET)/CT imaging technique combines the advantages of localization and functional imaging, and thus has become a preferred method for detection of metastases to the lymph nodes of patients with endometrial cancer [13-15]. Despite the advantages brought by 18F-FDG PET/CT [16], the studies in this field demonstrate contrasting results and many have concluded that further studies are necessary. The current aim of this study was to analyze the predictive capabilities of 18F-FDG PET/CT in detecting the absence or presence of metastasis in lymph nodes of women scheduled for surgery due to endometrial cancer.

Materials and methods

Patients

This is a retrospective study conducted between 2009 and 2015. The study group consisted of females who were admitted to our clinic and diagnosed with endometrial cancer (n=135). We included patients who underwent LN dissection with pre-operative 18F-FDG PET/CT imaging. Each of these patients had undergone lymph node dissection involving the paraaortic region of the pelvis (after imaging via 18F-FDG PET/CT was performed), and all procedures had been performed by an experienced gynecological oncologist at the Obstetrics and Gynecology Department of Acibadem Kayseri Hospital. Stage determination in the clinical aspect was made according to results and reports obtained from surgical specimens. Two independent experienced gynecological pathologists assessed the specimen in accordance with the "International Federation of Gynecology and Obstetrics" (FIGO) 2009 system. The study group included 86 patients with stage IA, 15 patients with stage IB, 4 patients with stage II, and 30 patients with III/IV cancer (Table 1). Histological grade was assessed per WHO classification (Table 1). The Ethical Committee of Acibadem Mehmet Ali Aydinlar University approved this study protocol (decision No. KB 7/14/2016). All participants provided written informed consent for the procedures and their inclusion in the study.

Imaging procedure

The imaging protocol used in our study was largely based on the study of Dolanbay et al. [17] Briefly put, 18F-FDG PET/CT investigations of patients were performed by the utilization of a high-resolution PET/CT scanner integrated with a computerized tomography device that was capable of 16-slice multidetector imaging CT (Philips Healthcare, The Netherlands). The hydration of all patients was achieved orally or through IV physiologic serum infusions before injection of fluorodeoxyglucose (FDG), and blood glucose levels were measured. Patients were administered intravenous 296-703 (MBq) FDG if their serum glucose levels were under 200 mg/dL. Following injection, patients lay or sit in a secluded room. After urination, they were directed with appropriate positioning to the CT scanner. All patients were positioned in the regular head-first supine position and moved to just above the first scanning position of the CT, after which imaging was performed. PET/CT images were analyzed in two separate sessions by one independent reader with 10 years of experience in the field of nuclear medicine and oncology who was blinded to patient history and pathological findings. The state of the 'sentinel' lymph nodes was defined as either metastatic or reactive.

Statistical analysis

Data was entered into the SPSS version 15.0 computer software for Windows operating system, in which all statistical analysis was performed (IBM, NY, USA). The mean and standard deviation results of continuous variables were determined. Categorical variables were presented as frequency (count) and percentage. ROC analyses were performed, and sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) were calculated.

Results

Patient characteristics

Mean (min-max) age of the study group was 60.59 (36-81) years. The stages of the patients according to surgical FIGO staging were as follows: Stage IA in 63.7% (86/135, <50% myometrial invasion), stage IB in 11.1% (15/135; >50% myometrial invasion), stage II in 2.96% (4/135; cervical stromal invasion), stage IIIA in 2.96% (4/135; local or regional spread), stage IVA in 1.48% (2/135) and stage IVB in 0.74% (1/135) of patients. Data for grade were available in 113 cases. Among these, 29.6% (40/113) were grade 1, 37% (50/113) were grade 2, and 17% (23/113) were grade 3. Histological evaluation revealed adenocarcinoma in 83.7% (113/135), serous histology in 9.62%

(13/135), mixed histology in 5.92% (8/135), small cell histology in 0.74% (1/135) (Table 1).

Results of 18F-FDG PET/CT in preoperative staging

Diagnostic capability of the 18F-FDG PET/CT modality was evaluated with ROC analysis according to 'gold standard' pathological findings. Overall accuracy was determined as 79.26% and the AUC was found to be 0.780 (0.054) (Table 2).

Figure 1 depicts a sample case of a 72-year-old female patient for demonstrative purposes. Her complaint was severe vaginal bleeding. After endometrial sampling, the pathological finding was adenocarcinoma of endometrium. 18F-FDG PET CT whole body findings were as follows: Increased 18F-FDG uptake of a iso-hypodense-hypermetabolic lesion was detected on corpus uteri (SUVmax 17.0). There was intense 18F-FDG uptake in both parauterine and paracervical lymph nodes (SUVmax 8.9). Obturator lymph nodes, especially those on the left, and bilateral iliac lymph nodes (SUVmax 8.2) showed increased 18F-FDG.

Table 1: Baseline characteristics of study participants

Parameters	All patients		
Age	Mean: 60.58 years (min-max: 36-81 years)		
FIGO stage	IA	86	63.7%
	IB	15	11.1%
	II	4	2.96%
	IIIA	4	2.96%
	IIIB	0	0
	IIIC	23	17%
	IVA	3	1.5%
	IVB	1	0.74%
Grading	1	40	29.6%
	2	50	37%
	3	23	17%
Histological type n (%)	Adenocarcinoma	113	83.7%
	Serous	13	9.62%
	Mixed	8	5.92%
	Small cell	1	0.74%

Table 2: The diagnostic efficacy of 18F-FDG PET/CT for detecting lymph node metastasis

		Metastasis		
		Present	Absent	Total
PET	Positive	19	22	41
	Negative	6	88	94
	Total	25	110	135
Sensitivity	76.00%		PPV	46.34%
Specificity	80.00%		NPV	93.62%
FNR	24.00%		LR (+)	3.80
FPR	20.00%		LR (-)	0.30
Accuracy	79.26%		AUC	0.780 (0.054)
			P-value	<0.001

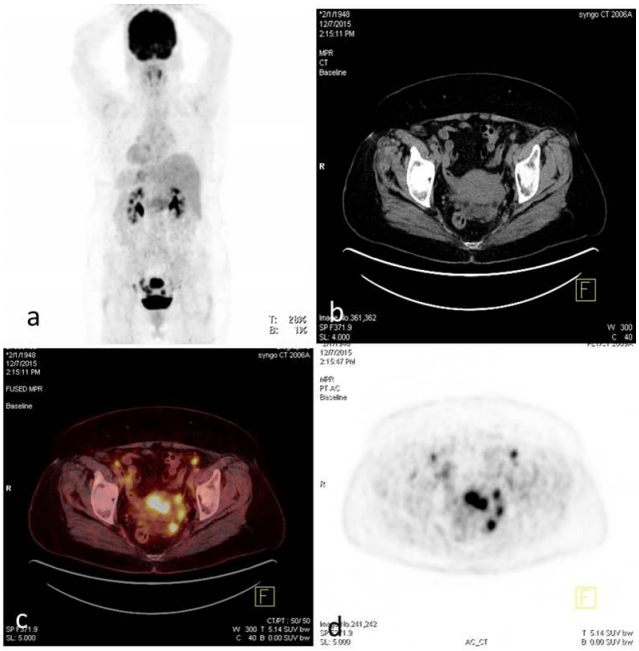

Figure 1: 18F-FGD PET/CT a) 18F-FDG MIP image, b) Axial images CT, c) 18F-FDG PET CT fusion d) 18F-FDG PET

Discussion

The current study aimed to determine the performance of 18F-FDG PET/CT in detecting metastases to the lymph nodes in endometrial cancer. Although we found a sensitivity value of 76%, a specificity percentage of 80% and a relatively low PPV of 46.34%, we believe the most important parameter was NPV which was found to be 93.62%. This result shows that this imaging method may have most practical use in determining patients without LNM (due to higher NPV and specificity) in endometrial cancer, which may prove critical in the management of endometrial cancer by restricting lymphadenectomies in low-risk patients.

Precise preoperative identification of lymph node metastases in patients diagnosed with endometrial cancer is of utmost concern as surgical approach somewhat relies on this assessment. Various studies have shown that metastases in endometrial cancer are often present in various lymph nodes, i.e., the parametrial, interiliac and common iliac [16-18]. Therefore, the data that can be drawn from the imaging of these nodes (and others) could be extremely valuable for physicians. In 2012, Chang performed a systematic review of 7 studies (243 patients) utilizing the 18F-FDG PET or PET/CT methods for this purpose and reported high specificity of both modalities [19]. Even though they could not determine which of the methods was superior to the other, they concluded that both methods were unable to replace lymphadenectomy. Furthermore, they could not identify with the available data whether these two modalities had superiority to each other. However, they supported the use of hybrid methods in endometrial cancer. In a more recent systematic review which included 378 patients from 8 studies, the sensitivity and specificity of 18F-FDG PET/CT in the preoperative detection of LNM were 72% (95% CI: 63–80) and 94% (95% CI: 93–96), respectively. The authors also noted that this combined method may be particularly beneficial to investigate patients that were deemed at high risk in terms of disease spread, which is a common suggestion in various similar studies [12].

There are numerous studies in which the 18F-FDG PET/CT modality was evaluated for detection of nodal involvement in patients with endometrial cancer and various other cancers. A multicenter study by Atri et al. [20] gathered 215 patients from 22 institutions and after rigorous inclusion/exclusion criteria, analyzed 23 LNM-positive and 26 negative cases. They concluded that 18F-FDG PET/CT had significantly higher diagnostic capability compared with CT in the detection of pelvic lymph node metastasis (AUC: 0.82 vs. 0.75, *P*=0.02). Kitajima similarly suggested that 18F-FDG PET/CT was superior to other conventional methods of imaging in endometrial cancer; however, the identification of LNM was moderate [16]. A very similar study was then performed by Lee et al. [21] who determined a sensitivity of 53.3% and a specificity of 97.8% for LNM detection and suggested that future studies should focus on patients with higher grade tumors. In another study, Picchio et al. [18] investigated 18F-FDG PET/CT for staging high-risk endometrial cancer patients and found that it is helpful to determine lymph nodes in the abdomen and extra-abdominal regions. Furthermore, in a study which focused on

high-risk cases, the sensitivity, specificity, PPV, NPV and accuracy of 18F-FDG PET/CT in identifying lymph node metastases were reported as 78.6%, 98.4%, 91.7%, 95.3% and 94.7%, respectively. The authors also found that LNM was associated with SUVmax values [22]. Another study which also focused on patients with higher risk reported 77.8% sensitivity and a remarkable 100% specificity in pelvic LNM detection [23]. The higher specificity values obtained in such patients support the thesis that this imaging method is especially useful in high-risk patients. However, the current study reports that the 18F-FDG PET/CT imaging modality yielded a respectable level of accuracy (79.26%) in determining lymph node metastases among patients with various risk levels. Therefore, we believe our findings support (and add to) previous studies in showing that this method is highly accurate in determining patients without disease as demonstrated by relatively higher specificity and NPV values. Even though studies with extremely high sensitivity levels can be found in the literature [24,25], our results are supported by the majority of studies which have reported high specificity in the presence of varying lower levels of sensitivity for LNM identification [26-28]. Aside from endometrial cancer, this combined imaging method provides highly conclusive data for lymph nodes in vulvar [17], cervical [29,30] and ovarian cancer [31].

To summarize, PET-CT is used in various types of cancer for preoperative evaluation; however, the method is hampered by various limitations for LNM identification in many cancers. We designed this study to evaluate the LNM detection accuracy of 18F-FDG PET/CT in patients with endometrial cancer, therefore, we limited our study to these patients. The low number of cases in several FIGO stages and histological types could limit the generalization of our findings to these respective groups, which is a limitation of the study. Furthermore, we only assessed prediction of LNM; accuracy for other tumor characteristics, such as the malignancy of the primary tumor or distant metastases, require novel studies.

Conclusion

Our analysis demonstrates that 18F-FDG PET/CT is an effective method for the determination of the absence of lymph node metastases. Our results contribute to current data and provide further evidence that this combined imaging modality can enable the restriction of lymphadenectomy procedures in endometrial cancer.

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Evaluation of the platelet volume index as a prognostic factor after aneurysmal subarachnoid hemorrhage

Anevrizmatik subaraknoid kanama sonrası prognostik faktör olarak platelet volüm indeksinin değerlendirilmesi

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Abstract

Aim: Subarachnoid Hemorrhage (SAH) originating from an intracranial aneurysm is a severe and life-threatening disease, witnessed by physicians in Emergency Departments (ED). Despite the improvements in diagnostic and therapeutic techniques, morbidity and mortality rates of SAH remain high. Several parameters based on biochemical analysis and imaging techniques are used to evaluate the prognosis. However, efforts for identifying an ideal marker have not been successful. For many years, Fisher Grading System, which is based on computerized tomography (CT), has been used as a reliable predictive scale to identify SAH. Besides, the immune response plays an active role in determining neuro-damage after SAH. Recently, Mean platelet volume (MPV) to platelet count (PLT) ratio has become a trend indicator to anticipate the outcome of a patient suffering from SAH. This study aims to determine whether the comparison of the immune response marker [Platelet Volume Index (PVI)] with the Fisher Grading System could be used as a prognostic factor in subarachnoid hemorrhages with ruptured intracranial aneurysms.

Methods: In this retrospective cohort study, 52 patients diagnosed with spontaneous SAH in the ED were included. The patients' ages, genders, Fisher grades, locations of the ruptured aneurysm, PVI and Glasgow Coma Scale (GCS) scores were recorded. MPV:PLT ratio was defined as MPV value(fL) x100 /PLT (per 1000). Each patient's GCS at the time of their admission to the ED was noted. An experienced radiologist graded their initial CT scans immediately according to the Fisher Grading System. Patients' GCS scores were noted by the investigators and PVI was calculated. A retrospective review was carried out regarding medical records of age, sex, and other conditions. The Pearson Correlation Coefficient was used in the analysis of the interrelationship.

Results: The correlation among the PVI with GCS and Fisher Grade test was found to be positive statistically correlation. Relevant literature establishes the same result. Additionally, analyses established a significant positive correlation between the Fisher Grading Scale and the PVI among the data of ruptured aneurysms.

Conclusion: The PVI can be used as a prognostic, predictive factor for SAH. Nevertheless, further studies concerning the prognosis of SAH are needed to confirm this hypothesis.

Keywords: Platelet volume index, Mean platelet volume, Inflammatory markers, Subarachnoid hemorrhage

Öz

Amaç: Anevrizmatik subaraknoid kanama (SAK), acil servislerde görülen şiddetli ve ölümcül bir hastalıktır. Tanı ve tedavi tekniklerinde ki tüm gelişmelere rağmen halen yüksek morbidite ve mortalite oranına sahiptir. Biyokimyasal ve görüntüleme analizlerini içeren bir çok prognostik yöntemler olmasına rağmen, halen ideal bir belirteç mevcut değildir. Anevrizmatik subaraknoid kanamalarda Fisher skalası, beyin bilgisayarlı tomografisi (BT) görüntülerinden elde edilen ve uzun yıllardır güvenle kullanılan bir prognoz göstergesidir. Diğer yandan immün yanıt ise nöronal hasarın dolayısı ile prognozun ana belirleyicisidir. Ortalama platelet hacminin platalet sayımına oranı olan platelet hacim indeksini (PHİ), immün yanıt göstergesi olarak kullanan çalışma sayısı gittikçe artmaktadır. Biz de çalışmamızda immün yanıtın göstergesi olan platalet hacim indeksini, güvenilir bir yöntem olan Fisher skalası ile karşılaştırarak prognoz belirteci olma olasılığını araştırdık.

Yöntemler: Bu retrospektif kohort çalışmaya, acil servise başvuran 52 hasta çalışmaya dahil edildi. Hasta yaşı, cinsiyet, Fisher değeri, rüptüre anevrizmanın lokalizasyonu, PHİ, Glasgow koma değeri (GKS) ile çalışma kartı oluşturuldu. PHİ'i ortalama platalet volümü (fL) x100 / platalet sayısı (per 1000) olarak belirlendi. Tecrübeli nöroradyolojist tarafından, başvuru anındaki BT'e göre Fisher değeri hesaplandı, beyin cerrahisi ve acil tıp doktorları tarafından GKS değeri kayıt edildi. Pearson korelasyon testi, veri analizi amacıyla kullanıldı.

Bulgular: PHİ'i ile GKS ve Fisher değerleri arasında istatiksel olarak anlamlı ilişki bulundu. Bu sonuç literatür ile uyumluydu. Dahası, PHİ ile Fisher skala değeri arasında istatiksel olarak anlamlı, pozitif yönde bir korelasyon olduğu görüldü.

Sonuç: PHİ'i SAK'larda prognostik bir belirteç olabilir. Fakat daha fazla hasta sayısı ile yapılan çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Platalet hacim indeksi, Ortalama platalet hacmi, İnflamatuar belirteç, Subaraknoid kanama

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Introduction

Subarachnoid hemorrhage (SAH) following a ruptured intracranial aneurysm is a severe and life-threatening disease [1,2]. The term "ruptured intracranial aneurysm" includes consequent bleeding in the subarachnoid, intraventricular or subdural spaces. Despite improvements in diagnostic and therapeutic techniques, SAH has a high morbidity and mortality rate. Moreover, only 25% of patients are able to live without assistance [1,3,4].

When present in the subarachnoid space, blood components act as potent activators for neuroinflammation. Following SAH, the immune system recognizes the blood components and irritated brain tissue as well as the antigens to which they are exposed. This triggers a robust neuroinflammatory response. The mechanism of the immune response is still not well understood. Many factors contribute to the activation of neuroinflammation after SAH, one of the components being platelet functions [5,6]. The role of activated platelets in thrombosis and neuroinflammation after SAH have been well studied [7]. Platelets play an active role in the regeneration of injured vessel wall and regulation of immune response. Mean Platelet volume (MPV) is one of the parameters analyzed in a CBC. Platelet count (PLT) and mean platelet volume (MPV) are readily available in the first complete blood count (CBC) in the ED [2]. Platelets' activation and functions are positively related to the MPV. Larger mean platelet volume is an indicator of rapid reaction to thrombosis, with inflammation and activation. Therefore, an increase in the MPV values indicates activated inflammation [8]. In summary, the stage of inflammation affects the size of platelets and consequently, MPV. An increase in platelet volume is associated with several conditions such as severe preeclampsia, active or chronic infection, ischemic stroke [7,8].

Recent studies are increasingly examining the ratio of MPV to platelet count detected in the CBC as a more specific index to predict platelet-related immune response [7]. On the other hand, computerized cranial tomography (CCT) is frequently made use of as the initial imaging modality while diagnosing SAH [9]. CCT clearly demonstrates density differences between hyperdense acute hemorrhage and the surrounding parenchyma. Many studies have demonstrated the close relationship between initial CCT and clinical outcome. The primary CCT evaluation has a strong relationship with the development of neurological deficits. To this end, several criteria are used to evaluate neurological prognosis after SAH. One of these is the Fisher scale (FS). FS is the initial and best-known classifying system for subarachnoid hemorrhage on CT scans. This scale is useful in predicting the mortality, morbidity and/or vasospasm. FS as well as some other image related variant scales have been widely used as reliable predictive scales for many years. The Fisher scale evaluates the amount of blood seen on computed tomography (CT) and predicts the prognosis of the clinical outcome. The significance of the Fisher Scale is evident in identifying patients at higher risk of increased morbidity or mortality. Therefore, it indicates the need for a closer and more aggressive observation [10].

Several bio-markers have frequently used certain parameters based on biochemical analysis, but perfect markers have not yet been identified. This study aims to determine whether the comparison of immune response marker [Platelet volume index (PVI)] with Fisher grading system could be used as a prognostic factor in subarachnoid hemorrhages with ruptured intracranial aneurysms.

Materials and methods

Patient population and data collection

The institutional ethical board approved the study (2018.876). We obtained the data of all patients admitted to the Emergency Department with SAH between November 1, 2016 and February 31, 2019. The study involved a retrospective chart review with no risk to the patients.

All patients admitted to the Department of Emergency Medicine with SAH underwent a CBC and CCT upon initial admission. CBC included the MPV and PLT values. Neuro-imaging and other records are available for all patients. The assessment of all initial CCT scans were carried out by the same examiner who has ten years of experience in neuroradiology. The images were graded according to the FS (Table 1). Neurological examinations were performed using the Glasgow coma scale (GCS) by the same specialists.

All patients had undergone treatment (clipping or endovascular alternatives) within 3-24 hours after the onset of SAH at the Department of Neurosurgery. Patients who suffered from coagulation abnormalities, malignancies, renal or liver dysfunction, severe myocardial dysfunction, any active or chronic infection findings, and any other immune deficiency syndrome (AIDS, leukemia) were excluded from this study, as well as patients admitted later than 48 hours from the onset of symptoms, since MPV and/or PLT may show progressive change following acute inflammatory disorders [19,20]. Patients who were included in the study were those experiencing SAH confirmed by a CCT without trauma, accompanied by a cerebral angiogram confirming an intracranial aneurysm.

Calculation of PVI

PVI was described as $\frac{MPV\ value(fL)}{PLT\ per\ 1000}$ x100. Investigators who calculated the platelet volume index were blinded to patient's clinical condition and outcome. PVI value of each patient was noted.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) software 24.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The normality of distribution was assessed with Kolmogorov Smirnov test. Pearson correlation test was used for correlation analyses. *P*-values <0.05 were considered statistically significant. According to the power analysis based on platelet count, a sample size of 40 patients was required for 80% power and %5 conventional two-sided type 1 error.

Table 1: Fisher scale

Grade	CT finding
I	No subarachnoid (SAH) or intraventricular hemorrhage (IVH) detected
II	Diffuse thin (<1 mm) SAH, no clots
III	Localized clots and/or layers of blood >1 mm in thickness, no IVH
IV	Diffuse or no SAH, CH or IVH present

Results

We studied 52 patients diagnosed with SAH who had ruptured aneurysms. 30 (57.7%) of them were female, while the other 22 (42.3%) were male. Mean age of the cohort was 52.73 (13.23) (min=24, max=89).

Patients' ages, genders, the Fisher grades, the locations of the ruptured aneurysms, PLT index and GCS scores were noted (Table 2). A moderately to highly significant negative correlation was found between FS and GCS (r=-0.476, *P*=0.001) (Table 3, Figure 1 and 2). Between FS and PVI, there was a positive, strong and highly significant (r=0.731, *P*=0.001) (Table 3, Figure 3).

Table 2: Baseline demographic and clinical characteristics of patients

Characteristics	n (%)
Gender	
Female	30 (57.7)
Male	22 (42.3)
	mean (SD) (min, max)
Age	52.73 (13.23) (24, 89)
GCS	13.54 (2.42) (6, 15)
Fisher Grade	2.96 (1.23) (1, 4)
PVI	3.91 (1.55) (1, 8.39)
Location of aneurysm	n (%)
Anterior circulation	48 (92.3)
Posterior circulation	4 (7.7)
Clinical Presentation	n (%)
Headache	21 (40.4)
Lethargy	5 (9.6)
Seizure	2 (3.8)
Hemiplegia	7 (13.7)
Headache, Stupor	12 (23)
Headache, Ptosis	1 (1.9)
Headache, Vomiting	4 (7.6)

SD: Standard deviation, PVI: Platelet volume index

Table 3: The Pearson correlation coefficients were used to determine the relationship between either PVI or FS and GCS

		FS	PVI	GCS
FS	Pearson Correlation	1	0.731	-0.476
	P-value		<0.001	<0.001
	n	52	52	52
PVI	Pearson Correlation	0.731	1	-0.237
	P-value	<0.001		0.090
	n	52	52	52
GCS	Pearson Correlation	-0.476	-0.237	1
	P-value	<0.001	0.090	
	n	52	52	52

PVI: Platelet volume index, FS: Fisher scale, GCS: Glasgow coma scale

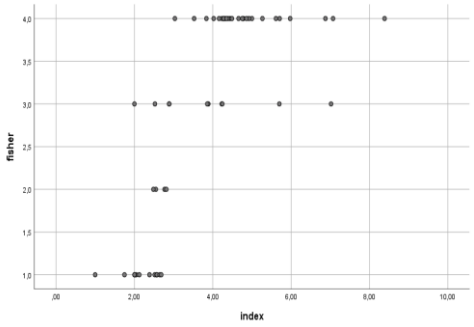

Figure 1: The correlation between the platelet volume index and Fisher scale (r -0.731, *P*-value <0.001)

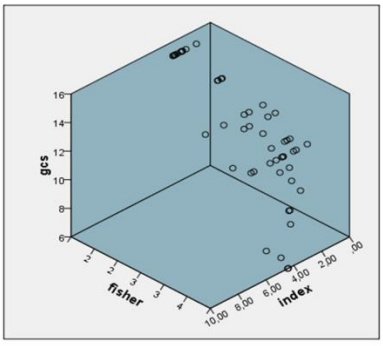

Figure 2: The correlation between platelet volume index, Glasgow coma scale and Fisher scale with the box pyramid table

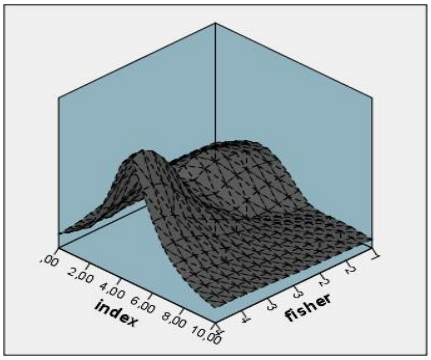

Figure 3: The correlation between platelet volume index and Fisher scale with distribution diagram

Discussion

SAH resulting from ruptured aneurysm is associated with significant morbidity and mortality rates. The mortality rate ranges from 32% to 67%. Approximately 10% to 16% of patients die before hospital admission, and around 25% patients remain dependent on assistance [8]. Clinical symptoms of SAH include varying degrees of neurological disorders, from headache and aphasia to hemiplegia or coma [3,4]. The most common clinical symptom in our study was headaches (Table 2). Although extensive studies have been performed about neuro-degeneration after SAH, factors causing poor clinical outcome have not yet been clearly established [11,12]. Rebleeding, hydrocephalus, seizures, delayed cerebral ischemia, elevated intracranial pressure and several systemic complications contribute to serious neurodegeneration following intracranial aneurysm rupture [8]. The intensity of bleeding observed in the subarachnoid space on CT has a strong relationship with the prognosis [10]. The Fisher grading scale, the initial and most established classifying system in subarachnoid hemorrhages, relies on brain CT scans [12]. Several other scales that have been proposed include various parameters, and have been helpful in predicting mortality, morbidity and/or vasospasm. The Fisher scale is still more widely preferred [14,15]. Fishers scale is described by four grades based on the amount of blood in the intracranial area. This grading scale is rather practical and useful for prognosis and prediction of patient outcomes in SAH. Score increases in this grading have been associated with poor prognosis in numerous studies, with the exception of the risk of clinical vasospasm, which is higher Fisher grade 3 than grade 4. In accordance with the existing literature, our study found a negative correlation between FS and GCS that is significant at a moderate-to-high level. Higher grades in this scale indicate the need for more aggressive and serious observation of the patient. Its importance for higher risk of developing cerebral damage, especially vasospasm, is evident [15,16].

Uncontrolled inflammation that is observed has been attributed to primary neuro-damage. According to this hypothesis, uncontrolled inflammation is a driving factor for poor outcomes following SAH [17]. Extravasated blood cell breakdown components in the subarachnoid space trigger the inflammatory cascade [18]. Platelet activation is also associated with worsening neuro-injury. Recently, studies have demonstrated that several cytokines, growth factors and platelet-derived molecules are elevated after SAH. Platelet activation causes provocation in such a way as to complement the spread of

inflammation and feeds the cycle of injury expansion (18). The mean platelet volume, one of parameters of complete blood count (CBC), is associated with platelet function and activation. When the size of platelets is increased, cytokines, chemokines or coagulation factors become activated. Increased MPV values indicate activated platelet functions and are associated with neuro-inflammation [8,11]. Platelet size changes during megakaryocytopoiesis. This activation is regulated by thrombopoietin, several growth factors and cytokines. This organization is the response of the bone marrow to inflammation [20]. After all, neuro-inflammation and prothrombotic agents, which alter the microenvironment of platelets, cause increases in platelet volume, changing the discoid platelet shape to spherical. The purpose of platelet activation is to restrict the damage of brain tissue [8,21,22]. In several studies, platelet size has been reported as a prognostic factor in acute ischemic stroke, but more studies are needed regarding SAH. The aim of this study was to compare the PLT index with the Fisher grading scale which is a well-established prognostic factor for SAH. Intracranial vasospasm is the most serious complication resulting from SAH. Treating or preventing the progression of vasospasm after SAH is highly challenging. The main cause of intracranial vasospasm (and therefore, of poor prognosis) is the immune response to blood fragments in either the subarachnoid space or the intraparenchymal area [7,16]. In our study, we investigated a possible correlation between the thrombocyte index as an immune response marker and the Fisher grading system, and concluded that there is a positive, strong correlation between FS and PVI that is moderately-to-highly significant.

Limitations

We believe the wide spectrum of PVI distribution (ranging between 1 and 8.39) is most likely due to the differences in the timing of patients' hospital admissions. It seems that the shortcoming of the study was the lack of standardization in terms of admission time due to the low number of patients included in the study.

Conclusion

This study has demonstrated a statistically significant correlation between the Fisher grading scale and PLT index among the study group. Therefore, we suggest that PLT index can be used as a prognostic, predictive factor for SAH. Nevertheless, further research studying the prognosis of SAH is needed to support this hypothesis.

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Impact of leg elevation added to a 15˚ left lateral incline on maternal hypotension and neonatal outcomes in cesarean section: A randomized clinical study

Sezaryen seksiyolarda 15˚ sol lateral eğime eklenen bacak elevasyonunun maternal hipotansiyon ve yenidoğan sonuçları üzerine etkisi: Randomize klinik çalışma

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Abstract

Aim: The supine position can cause maternofetal complications by exacerbating hypotension resulting from spinal anesthesia (SA). The aim of this study was to investigate and compare the effect of positioning the patient with a 15˚ left lateral incline and both positioning and elevating both legs on SA-induced hypotension.

Methods: This randomized clinical study was conducted on 200 pregnant women who underwent cesarean section in a university hospital between November 1, 2016 and April 15, 2017.

Pregnant women were separated into 2 groups as the 15˚ left lateral incline (Group 1) and the 15˚ left lateral incline and leg elevation group (Group 2). Mean arterial blood pressure (MAP) and heart rate (HR) values, ephedrine use, neonatal APGAR scores and umbilical cord vein blood gas analysis samples were compared at varying times. After administration of SA, MAP and HR were recorded at the second, fourth, sixth, eighth and tenth minutes until delivery of the infant, every five minutes after delivery until the 30th postpartum minute, and at the end of the surgery.

Results: Two and four minutes after the administration of SA and 20 minutes after delivery, mean HR values of group 2 were significantly higher than that of group 1 (P=0.002, P=0.005, P=0.006, respectively). MAP values were similar in both groups at all time points, however, a greater number of patients in group 1 had MAP <60 mmHg and HR <60 bpm at two and six minutes after the administration of SA.

Conclusion: Positioning the table at a 15˚ incline and leg elevation during caesarean section may provide maternofetal benefits by reducing the frequency of post-spinal hypotension in pregnant women.

Keywords: Pregnancy, Cesarean section, Spinal anesthesia, Hypotension

Öz

Amaç: Sırtüstü pozisyon, spinal anesteziden (SA) kaynaklanan hipotansiyonu arttırarak maternofetal komplikasyonlara neden olabilir. Bu çalışmanın amacı, SA uygulanan sezaryenlerde 15˚ sol lateral eğim ve bacak elevasyonu yöntemlerinin, SA'nın oluşturduğu hipotansiyon üzerine etkilerini araştırmaktır.

Yöntemler: Randomize klinik bu çalışma 1 Kasım 2016 ile 15 Nisan 2017 tarihleri arasında bir üniversite hastanesinde sezaryen uygulanan 200 gebede yapıldı.

Gebeler, 15˚ sol lateral eğim (grup 1) ve 15˚ sol lateral eğim + bacak elevasyon grubu (grup 2) olarak 2 gruba ayrıldı. Hastaların değişik zamanlardaki ortalama arter kan basıncı (OAB) ve kalp atım hızı (KAH) değerleri, efedrin kullanımları ile neonatal APGAR skorları ve umblikal kord veni kan gazı analiz örnekleri karşılaştırıldı. SA uygulamasından sonra OAB ve KAH değerleri, bebeğin doğumuna kadar 2, 4, 6, 8, 10. dakikalar ve doğumdan sonra her 5 dakikada bir kaydedildi. Benzer şekilde, bu değerler doğum sonrası 5, 10, 15, 20, 25, 30. dakikalar ve cerrahi bitiminde değerlendirildi.

Bulgular: SA uygulamasından 2-4 dakika sonra, KAH değerlerinin grup 2'de anlamlı derecede yüksek olduğu belirlendi (sırasıyla; P=0,002, P=0,005). OAB değerleri her iki grupta tüm zaman noktalarında benzer olup, doğumdan 20 dakika sonraki KAH değerleri grup 2'de yüksekti (P=0,006). Bebeğin doğumundan önce SA'den 2-6. dakikalar sonrasındaki, OAB <60 mmHg ve KAH <60 atım olan hasta sayısı grup 1'de daha fazla idi.

Sonuç: Masanın 15˚'lik bir eğimde konumlandırması ve bacakların elevasyonu, gebelerde postspinal hipotansiyon sıklığını azaltarak maternofetal faydalar sağlayabilir.

Anahtar kelimeler: Gebelik, Sezaryen, Spinal anestezi, Hipotansiyon

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Introduction

General or regional anesthesia administered during cesarean sections (CS) may affect maternofetal morbidity at varying rates. The necessity to provide safety for both the mother and the fetus increases the importance of anesthesia techniques preferred in these operations [1,2].

The administration of general anesthesia to pregnant women carries risks, such as difficult intubation and anesthesia-induced respiratory depression in the neonate, which reduce with regional anesthesia. Spinal anesthesia is more widely used due to easy administration and rapid onset of effect. The amount of anesthetic used is decreased, and it is not passed to the fetus. [3]. Nevertheless, with the occurrence of sympathetic block and peripheral venodilatation in spinal anesthesia, cardiac fluid reduces due to decreased venous return. Thus, hypotension is a frequent complication [4]. The incidence of hypotension following spinal anesthesia in pregnant women was reported as 7-80 % [5,6].

Hypotension may be exacerbated with the pressure of the uterus, which has grown during pregnancy, on Vena Cava Inferior in supine position. Therefore, the prevention of hypotension is of high importance. If it develops despite precautions, rapid treatment must be administered to protect uteroplacental perfusion and avoid fetal hypoxia [7].

In pregnant women receiving spinal anesthesia, in addition to fluid loading, oxygen support and vasoconstrictor administration, tilting the patient to the left while bandaging lower extremities or passive leg elevation may prevent the development of hypotension, and contribute to central circulation by rechanneling the blood pooled in lower extremities in supine position [8].

The aim of this study was to investigate SA-induced hypotension as well as vasoconstrictor use and compare the effects of positioning the patient with a 15˚ left lateral incline and leg elevation in addition to positioning on SA-induced hypotension and the neonate in cesarean section.

Materials and methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

This randomized clinical study was approved by the Scientific Research Ethics Committee of Kahramanmaras Sütcü Imam University (approval number-date: 215-27.07.2016) and included pregnant women who underwent caesarean section with spinal anesthesia between 1 November 2016 and 15 April 2017.

Informed consent was obtained from all the study participants pre-operatively. All patients (n=200) were between 18-45 years-of-age, more than or equal to 156 cm tall, more than 37 weeks pregnant and conformed to American Society of Anesthesiologists (ASA) risk score I-II. Patients with age <18 years or >45 years, ASA>II, multiple pregnancies, pregnancy-related diseases (pre-eclampsia-eclampsia, placental anomaly etc.), previous comorbid diseases, contra-indicative conditions for SA and those who refused were excluded from the study.

20 minutes before the operation, patients were admitted to the preoperative preparation room and non-invasive blood pressure (NIBP), heart rate (HR) and SpO_2 were monitored. 10 ml/kg Hartmann solution was administered intravenously; hemodynamic values were measured and recorded. Before the administration of spinal anesthesia, 200 patients were divided into two groups, with 100 patients in each group: Group 1 consisted of patients who were inclined 15^o to their left and Group 2 comprised patients whose both legs were elevated after inclination.

All patients were monitored with a 3-lead electrocardiogram (ECG) for HR, NIBP and SpO_2, and values were recorded. Following local sterilization with the patient in the sitting position, lumbar region was punctured at the L3-L4 level or the L4-L5 level with a 25G, 90 mm pencil point, spinal atraumatic needle (Egemen International, Turkey). After visualization of cerebral spinal fluid flow, 10 mg, 0.5% bupivacaine (Marcaine® Spinal Heavy 0.5% ampule, AstraZeneca, UK) was administered for spinal anesthesia.

The patients were then immediately given the supine position and the operating table was adjusted to a 15º left lateral incline using a Digital Inclinometer® for patients in Group 1. In addition to positioning, the legs of patients in Group 2 were elevated with a standard 25 cm-high cushion positioned below the legs. After the administration of spinal anesthesia, the sensory motor block level was evaluated with the pinprick test from the mid-clavicular line, and as the block reached T4 level, the surgical team was notified so they could begin the operation. The time taken for the sensory block to reach T4 level was recorded.

Mean arterial pressure (MAP) and HR were measured and recorded immediately after the administration of spinal anesthesia and at the second, fourth, sixth, eighth, tenth minutes of the surgical procedure until delivery of the infant. Following delivery, the operating table and legs were brought to the neutral position. The above-mentioned values were recorded every five minutes. The placenta was sent for venous blood gas analysis immediately after the delivery. The Apgar scores of the neonate were recorded at the first and fifth minutes post-delivery.

MAP falling below 60 mmHg during surgery was considered severe hypotension and 10 mg ephedrine was administered to these patients. The amount of vasopressor used and total operating time was recorded for each patient.

Statistical analysis

The power of this study, obtained with comparing the findings of the two groups, was determined as 0.93. Statistical Package for the Social Sciences (SPSS) 22.0 software was used for statistical analysis of the data. Descriptive statistics were presented as number (n), percentage (%), and mean (standard deviation) (SD). Conformity of the data to normal distribution was assessed with the Kolmogorov Smirnov test. The Independent Samples t-test was used in comparing the data of the groups, and Chi-square and Fisher's exact tests were utilized in the comparison of categorical data. A value of $P<0.05$ was considered statistically significant.

Results

There was no statistically significant difference between the groups with respect to age, height, weight, body mass index (BMI) and gestational week (P=0.658, P=0.098, P=0.888, P=0.489, P=0.117, respectively) (Table 1). The number of ASA I and ASA II patients in Groups 1 and 2 were 65, 35 and 77 and 23, respectively. The time sensory block took to reach the T4 dermatome level after the administration of spinal anesthesia was 4.81 (1.01) minutes in group 1 and 4.82 (0.72) minutes in group 2. Total operating times were 41.74 (8.63) and 42.81 (9.06) minutes in groups 1 and 2, respectively. The two groups were similar with regards to these two parameters (P=0.936, P=0.394, respectively).

The HR and MAP values of groups 1 and 2 were compared preoperatively, before, immediately after and two, four, six, eight, ten minutes following the administration of spinal anesthesia and immediately after the delivery of the infant. The HR values at two and four minutes after spinal anesthesia were significantly higher in group 2, and that at the tenth minute was significantly higher in group 1 (P=0.002, P=0.005, P=0.014, respectively). With respect to MAP values, there was no statistically significant difference between the groups at any point in time (Table 2). The changes in the MAP and HR rates in both groups before delivery are shown in Figure 1.

After neutralization of position and delivery, the above-mentioned values were measured every five minutes until the 30th minute and at the end of the surgery

With the equalization of the position of the patients after delivery of the infant, these values were recorded at 5, 10, 15, 20, 25, 30th minutes and at the end of surgery. The HR values were significantly high in group 2 at 20 minutes after delivery (P=0.006) and no difference was found at any other time. The MAP values were similar in both groups at all periods in time (Table 3).

For better interpretation of the parameters, the HR and MAP values at the defined times before delivery of the infant were evaluated as <60 and >60. Accordingly, although no statistically significant difference was determined between the groups with respect to HR and MAP values at all the time points, there was a greater number of patients with MAP <60 mmHg in group 1 at two minutes after the administration of SA (P=0.043). HRs of 6 patients in group 1 were <60 bpm at six minutes following SA, and all patients in group 2 had HR >60 bpm (P=0.007).

The mean amount of ephedrine used after spinal anesthesia was 5.20 (8.10) mg (36% of patients) in group 1 and 6.20 (9.40) mg (40% of patients) in group 2, which were statistically similar (P=0.421).

PH and lactate values of umbilical vein blood gases obtained immediately after delivery were 7.31 (0.05), 1.90 (0.87) mmol/l and 7.32 (0.04), 1.77 (0.62) mmol/l in groups 1 and 2, respectively, with no significant difference (P=0.059, P=0.214).

Apgar scores of neonates in Groups 1 and 2 were 8.56 (1.26), 8.73 (1.01) at the first minute and 9.61 (0.85), 9.54 (0.59) at the fifth minute, respectively. There was no statistically significant difference between the groups (P=0.294, P=0.501, respectively).

Table 1: The demographic data of the patients

	Group 1 Mean (SD)	Group 2 Mean (SD)	t	*P*-value
Age (y)	29.73 (5.53)	29.37 (5.95)	0.443	0.658
Height (cm)	162.73 (4.61)	161.72 (3.95)	1.663	0.098
Weight (kg)	78.36 (12.85)	78.62 (13.19)	0.141	0.888
BMI (kg/m^2)	29.59 (4.71)	30.06 (4.95)	0.693	0.489
Gestational week	38.42 (0.67)	38.27 (0.66)	1.575	0.117

Independent samples t test, α: 0.05

Table 2: Comparison of HR and MAP values at birth

	Group 1 Mean (SD)	Group 2 Mean (SD)	t	*P*-value
HR pre-op	96.54 (13.57)	94.90 (13.98)	0.842	0.401
HR before spinal	97.10 (13.40)	99.88 (18.53)	1.216	0.226
HR after spinal	96.35 (18.33)	99.16 (18.17)	1.089	0.277
HR second minute	88.93 (21.98)	98.11 (19.65)	3.114	0.002*
HR fourth minute	86.60 (21.28)	94.95 (18.00)	2.884	0.005*
HR sixth minute	87.71 (21.03)	90.75 (17.39)	0.884	0.378
HR eighth minute	93.04 (17.14)	86.77 (15.00)	1.484	0.143
HR tenth minute	96.25 (13.67)	81.38 (14.30)	2.652	0.014*
HR baby birth	92.41 (15.75)	92.09 (15.56)	0.145	0.885
MAP (mmHg)	Mean (SD)	Mean (SD)	t	*P*-value
MAP pre-op	91.24 (14.54)	90.48 (15.58)	0,357	0.722
MAP before spinal	101.69 (14.16)	102.38 (13.04)	0,358	0.720
MAP after spinal	86.15(17.71)	88.17 (17.70)	0,807	0.421
MAP second minute	76.71 (18.60)	79.29 (18.05)	0,996	0.321
MAP fourth minute	77.73 (18.52)	78.00 (16.27)	0,105	0.916
MAP sixth minute	79.05 (17.95)	76.78 (15.20)	0,768	0.444
MAP eighth minute	78.30 (11.86)	81.03 (15.73)	0,739	0.463
MAP tenth minute	80.33 (10.91)	80.77 (15.83)	0,079	0.937
MAP baby birth	87.16 (13.47)	85.64 (13.71)	0,791	0.430

Independent samples t test, α: 0.05, * the difference was statistically significant, HR: heart rate, MAP: mean arterial pressure

Table 3: Comparison of HR and MAP values according to groups after the time of baby emergence

	Group 1 Mean (SD)	Group 2 Mean (SD)	t	*P*-value
HR fifth minute	96.41 (16.30)	98.15 (15.42)	0.775	0.439
HR tenth minute	95.97 (14.36)	97.01 (15.58)	0.491	0.624
HR fifteenth minute	95.58 (13.32)	98.01 (15.52)	1.186	0.237
HR twentieth minute	94.30 (13.32)	100.03 (14.64)	2.801	0.006*
HR twenty-fifth minute	94.52 (13.62)	96.52 (15.54)	0.874	0.383
HR thirtieth minute	93.04 (13.37)	94.79 (14.91)	0.685	0.495
HR surgical end	89.00 (12.58)	91.44 (14.19)	1.287	0.200
MAP (mmHg)	Mean (SD)	Mean (SD)	t	*P*-value
MAP fifth minute	85.08 (12.82)	83.00 (14.33)	1.082	0.281
MAP tenth minute	83.40 (12.02)	83.84 (14.04)	0.238	0.812
MAP fifteenth minute	83.00 (11.88)	80.41 (13.06)	1.463	0.145
MAP twentieth minute	81.54 (11.47)	81.39 (13.25)	0.087	0.931
MAP twenty-fifth minute	83.77 (10.88)	83.51 (12.84)	0.140	0.889
MAP thirtieth minute	85.02 (10.17)	84.66 (10.75)	0.191	0.849
MAP surgical end	90.87 (11.22)	88.34 (11.44)	1.579	0.116

Independent samples t test, α: 0.05, * the difference was statistically significant, HR: heart rate, MAP: mean arterial pressure

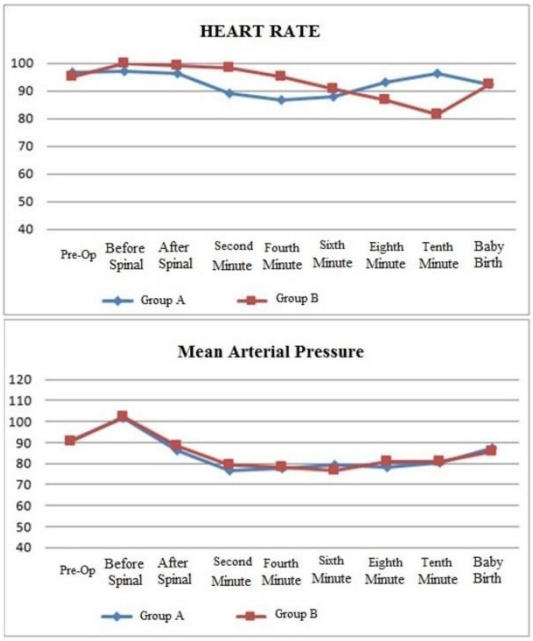

Figure 1: The changes in the mean arterial pressure and heart rates before delivery

Discussion

In this study examining the effects of positioning with a 15º left lateral incline and leg elevation in addition to positioning

on the neonate and SA-induced maternal hypotension in caesarean section, HR values were found significantly high at the second and fourth minutes following SA administration in leg elevation patients. MAP values were similar at all time points before and after delivery, and HR values 20 minutes after delivery was significantly higher in the leg elevation group. More patients in the positioning only group were observed to have MAP <60 and HR <60 two and six minutes after the administration of SA.

SA administered during caesarean section has various benefits for both the mother and the infant, beginning with decreased hemorrhage rate compared to general anesthesia. This can be associated with reduced venous return and a drop in central venous pressure following vasodilatation, which develops as a result of sympathetic blockage [9,10]. However, venous pooling and reduction in vascular resistance due to sympathetic blockage, along with the sensory block effect of local anesthetics can lead to severe hypotension. The incidence of hypotension in spinal anesthesia is reported as 83% [11]. Hypotension can be exacerbated by dehydration, pressure exerted on the uterus by Vena Cava Inferior in the supine position and vagal activation during the operation. Although this effect is desired in some situations, various complications may develop as severity increases. Numerous studies have been conducted for the prevention of deep hypotension in patients who were administered regional anesthesia. In a study by Singh et al. [12], the effect of tying the legs on hemodynamic changes was evaluated in cesarean section performed with spinal anesthesia. The frequency of hypotension was reported as 10% in the group with tied legs and 43.3% in the group with untied legs. MAP values were recorded at the fourth, sixth, eighth minutes post-SA administration and statistically significantly higher in the group with tied legs. It was concluded that tying the legs could prevent post-spinal anesthetic hypotension by increasing cardiac output through cardiac venous return. In another study, pregnant patients' legs were elevated by 30 cm after spinal anesthesia administration and its effect on post-spinal anesthetic hypotension was compared with a control group. Leg elevation was found to significantly lower SA-induced hypotension [13].

In our study, which was similar to the above-mentioned study in terms of leg elevation, MAP values between the two groups were found similar. This could be due to the use of lower elevation, 25 cm, in this present study, which was not increased to avoid difficulty in manipulation of the surgeon by the surgical team. Unlike the previous study, we did not include a control group with supine positioning, which could be another reason for the lack of significant difference between MAP values of the two groups. The reason we did not include a supine, neutral position group is that we always tilt the operation table to the left to reduce the pressure on Vena Cava Inferior and never prefer the supine position in pregnant women during caesarean section.

A review of the literature showed that no tilt angles were specifically reported by the studies. Left lateral inclines at different angles can create different aortocaval pressures in pregnant patients and it has been reported that non-invasive arterial pressure, cardiac output, stroke volume and systemic vascular resistance are least affected in patients in a 15º left inclined position [14]. As cardiac output was found to increase by more than 5% in the 15º left inclined position and higher angles have not provided patient comfort and safety, this angle was selected in the current study.

Although no significant difference was determined between the two groups with respect to MAP values at all the time points, a more detailed subgroup analysis showed that two minutes after spinal anesthesia, a greater number of patients in Group 1 had MAP <60 mmHg. Similarly, HR values of <60 bpm at two and six minutes post-SA were detected in more patients in group 1, whose legs were not elevated. As a primary outcome of this study, our data shows that leg elevation has positive effects on hemodynamics in the early stage after spinal anesthesia with insignificant effects.

In prophylaxis and treatment of maternal hypotension, the use of vasopressors and crystalloid-colloid fluids is important along with physical maneuvers that increase venous return. Different effects have been reported on central venous pressure, heart rate, systemic vascular resistance for each method used [15]. In studies that have investigated the effect of non-invasive manipulations on maternal hypotension, both leg elevation and tying the legs reduce the use of vasopressors [12,13]. We found no significant differences between the two groups in terms of ephedrine use, which can be attributed to reasons previously discussed, such as not preferring the supine position and tilting the patients in both groups, thereby preventing a significant hemodynamic difference between the groups.

Maternal hypotension that can develop due to decreased systemic arterial pressure, which decreases increased uterine perfusion pressure, can cause a reduction in uterine blood flow and impairment in fetal oxygenation. Fetal metabolic acidosis is reported at high rates in spinal anesthesia-administered cases, which is consequential of hypotension and related use of high doses of ephedrine [16].

With the aim of determining maternal hypotension and its possible fetal effects, the umbilical vein blood gas pH and lactate values obtained immediately after birth were evaluated together with the first and fifth minute Apgar scores, which yielded similar results in both groups. This result was not surprising as the pregnant patients operated on and the infants had no previously known pathology and the manipulations did not cause any differences between the groups with respect to evident hemodynamic changes or the use of ephedrine.

Limitations

Limitations of the study include that the sitting position was preferred during the administration of SA even though fewer hemodynamic changes have been reported in pregnant women when SA is administered in the left lateral position [17]. Several pregnancy-associated changes in the vertebral anatomy, the presence of lumbar lordosis, and more importantly, the severe discomfort formed in the lateral position in pregnant women played a role in this preference.

Conclusion

The methods of positioning the operating table at a 15º incline and leg elevation, applied on their own or together, to pregnant women receiving spinal anesthesia, insignificantly reduced the frequency and depth of SA-induced maternal hypotension. It could provide maternofetal benefits with respect to less use of vasopressors. Furthermore, despite all these

positive effects, appropriate conditions must be provided with preoperative fluid resuscitation and pharmacological support during the operation together with these methods for pregnant women undergoing caesarean section, and necessary interventions must not be delayed.

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Anatomical and radiological evaluation of modiolus anguli oris in facial anatomy

Yüzde modiolus anguli oris'in anatomik ve radyolojik değerlendirilmesi

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Abstract

Aim: Modiolus is a dense, mobile fibromuscular structure lateral to the mouth corner. It is of great importance in aesthetic and reconstructive surgery. The aim of this study was to enlighten the structural changes in modiolus with demographic variables such as age and gender in living individuals.

Methods: This retrospective cohort study was conducted on MR images of healthy individuals. Age and sex-related changes in modiolus level and volume were retrospectively analyzed in 64 patients [37M; 27F; mean age 48.2(16.3)] who underwent head and neck magnetic resonance imaging. ROC analysis was performed to determine the cut off values for age of modiolus. Correlation analysis (Pearson and point biserial correlation) was used to determine whether there was a significant correlation between age and volume. Significance of the differences between the volumes of right and left modiolus of the same individual were evaluated by dependent t tests.

Results: The mean volume of the modiolus was calculated as 0.51(0.26) mm^3. The volumes of right and left modioli decreased by aging. Ninety-one percent of the patients with modioli located inferior to horizontal line were over 49 years old. Right and left modioli relocated inferior to horizontal line with age. Modiolus volume was prominently less in women and the downward displacement of modiolus in women was 3.3 times higher than in men. Gender and age had significant effects on modiolus level. The right and left modiolus volumes were similar (P=0.975).

Conclusion: Surgeon's knowledge on modiolus and its relations will provide benefit, not only for the procedures such as face lifting and botulinum toxin injection, but also for the surgeries of facial paralysis and trauma patients.

Keywords: Modiolus, Magnetic resonance imaging, Radiologic anatomy, Senile changes, Aging

Öz

Amaç: Modiolus, ağız köşesinin lateralinde yoğun, hareketli, fibromüsküler bir yapıdır. Estetik ve rekonstrüktif cerrahide büyük öneme sahiptir. Çalışmanın amacı; yaş ve cinsiyet gibi demografik değişkenlere bağlı olarak modiolusta oluşan yapısal değişikleri göstermektir.

Yöntemler: Bu çalışma sağlıklı bireylerin MR görüntülerinden retrospektif kohort çalışması olarak tasarlanmıştır. Modiolus düzeyinde ve hacminde yaş ve cinsiyete bağlı oluşan değişiklikler, baş ve boyun manyetik rezonans görüntülemesi yapılan 64 hastada [37E; 27K; ort. yaş 48,2(16,3)] retrospektif olarak incelendi. Yaşta cut off değerini belirlemek için ROC analizi yapıldı. Yaş ve hacim arasında anlamlı bir korelasyon olup olmadığı korelasyon analizi (Pearson ve point biserial korelasyon) ile değerlendirildi. Aynı bireyin sağ ve sol modiolus hacimleri arasındaki önemli farklar dependent t testi ile yapıldı.

Bulgular: Modiolusun ortalama hacmi 0,51(0,26) mm^3 bulundu. Sağ ve sol modiolus hacimleri yaş ile azalmıştır. Modiolus seviyesi horizontal hattın altında olan hastaların %91'i 49 yaşın üzerindeydi. Sağ ve sol modiolus, yaşlanma ile birlikte horizontal çizginin altına yer değiştirdi. Kadınlarda modiolus hacmi belirgin olarak daha azdı ve kadınlarda modiolusun aşağı doğru yer değiştirmesi, erkeklerden 3,3 kat daha yüksekti. Cinsiyet ve yaşın modiolus seviyesi üzerinde önemli etkisi vardı. Sağ ve sol hacim ölçümleri arasında istatistiksel fark bulunamadı (P=0,975).

Sonuç: Cerrahın modiolus ve komşulukları hakkındaki bilgisi sadece yüz germe ve botulinum toksin enjeksiyonu gibi prosedürler için değil ayrıca facial paralizi ve travma hastalarının ameliyatları için de fayda sağlayacaktır.

Anahtar kelimeler: Modiolus, Manyetik rezonans görüntüleme, Radyolojik anatomi, Yaşlılık değişiklikleri, Yaşlanma

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Introduction

Modiolus is a dense, mobile fibromuscular structure that is found on the lateral border of the corner of the mouth [1-3]. It is composed of engaging muscle fibers around the mouth. These fibers converge towards or diverge from this decussation, which can be palpated easily. The fibers attaching to modiolus form spirals and then separate into two or more fiber bundles, each bundle coursing in separate ways. It extends between the orbicularis oris muscle and labial tractor muscles ending at the angle of the mouth [2,3] (Figure 1). In general, nine muscles attach to modiolus: Buccinator, risorius, orbicularis oris, depressor anguli oris, depressor labii inferioris, zygomaticus major, platysma pars modiolaris, levator anguli oris and mentalis. Most of these muscles have dermal terminations [4]. Patients with a prominent nasolabial fold, which is considered one of the principle landmarks of lower face aging, have weak trophic modioli [2,5].

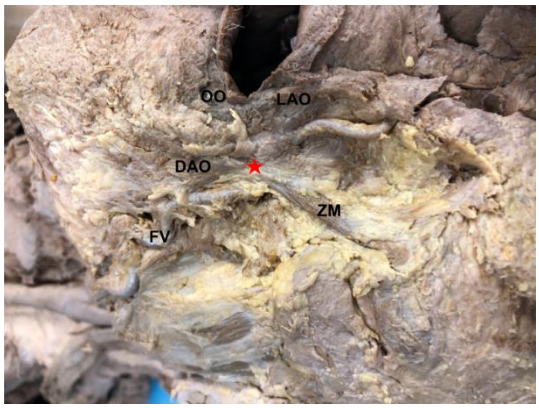

Figure 1: Localization of modiolus anguli oris and the muscles attached to it in cadaver (OO: Orbicularis oris, LAO: Levator anguli oris, ZM: Zygomaticus minor, DAO: Depressor anguli oris, FV: Facial vein, Star: Modiolus)

In the literature, there are microscopic studies examining its histo-morphological structure [2,5], and macroscopic cadaveric studies indicating the relationship of modiolus with the vessels and neighboring expression muscles [4]. Location of the modiolus was also studied in living individuals by palpation [6]. The three-dimensional structure of modiolus is very complex and hard to evaluate. The mobility of modiolus effects the movement of the lips and cheeks and it is active during talking, chewing, eating and drinking. Directly related to all the functions of the mouth corner, it is also highly important in facial expression. [4].

Modiolus has a key role in the formation of nasolabial fold, which is why it is particularly important in aesthetic and reconstructive surgery [2]. The modiolus can be regarded as an angular corner stone because the shape of the nasolabial fold is in direct relation with it [7]. It is considered critical for the beauty of the lower third of the face and prevents the appearance of facial aging [3,4,8].

In this study, we aimed to define the location, level and volume of the modiolus in living individuals based on objective data of Magnetic resonance (MR) images (Figure 2 and 3), and evaluate the changes related to age and gender. It is hypothesized that modiolus displaces below the horizontal line passing through the angle of the mouth, and its volume decreases with aging.

The results of this study will be especially useful in plastic surgery for the treatment of the angle of the mouth.

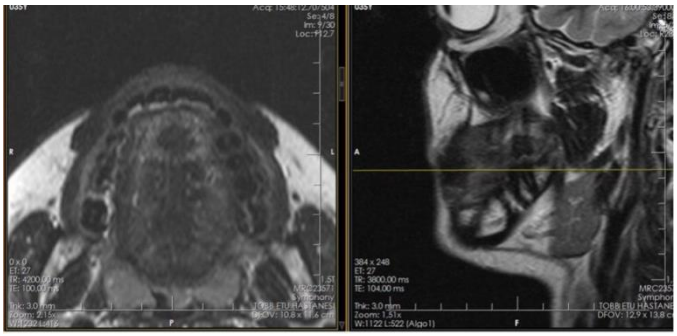

Figure 2: Magnetic resonance images of modiolus anguli oris at the corner of the mouth

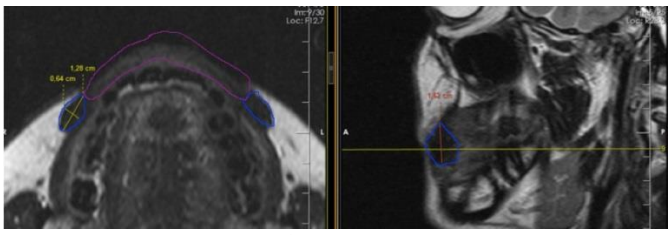

Figure 3: Magnetic resonance images of modiolus anguli oris labelled with blue lines at the corner of the mouth

Materials and methods

Ethics committee approval was received by TOBB ETU Faculty of Medicine Clinical Research Ethics Committee, 16.01.2019, Number: KAEK 118/029.

Magnetic Resonance (MR) images of 64 patients obtained for head and neck pathologies between 2012 and 2015 were evaluated retrospectively. MR imaging was performed with 1.5 T Siemens Magnetom Symphony device. Coronal, sagittal and axial T2-weighted images passing from the angle of the mouth were used. Anatomical structures around modiolus were determined at each interpretation to identify the modiolus correctly. A horizontal line passing from the angle of the mouth was defined to indicate the level of modiolus (Figure 2 and 3). Volume measurements were made automatically with the software by measuring anteroposterior, transverse and craniocaudal axis lengths for each side. The age and gender-related changes of modiolus were examined by the same radiologist, certified with five years' experience at head and neck imaging.

Statistical analysis

The statistical analysis was performed by IBM SPSS Statistics Version 21 and STATA version 13.0. The variables were described by using minimum-maximum values, mean, standard deviation, frequency and percent statistics. 95% confidence interval for mean is used to estimate the mean value for volume of right and left sided modioli in the study population. ROC analysis was used to determine cut off values for age of modiolus. Correlation analysis (Pearson and point biserial correlation) determined whether there was a significant correlation between age and volume. Significant differences between the volumes of right and left modiolus of the same individual were evaluated by dependent t test. Volumes and levels of the right and left modioli of the same individual were assumed correlate. Due to suspicion of the clustered data structure we needed to calculate intra-cluster correlation coefficient for left and right side of the same patient by using multilevel (two-level) linear and logistic regression models. Factors effecting level and volume of modiolus were determined by these multi-level models.

Results

Sixty-four patients who were admitted to the hospital from 2012 to 2015 were subsequently enrolled to the study. Among these, 37 were male and 27 were female. Mean age of all patients were 48.2(16.3) years (range: 17-88 years). Mean volume of the modiolus was 0.51(0.26) mm^3 (range: 0.07-1.17 mm^3). The mean volume of right and left modioli were 0.51(0.29) and 0.51(0.26) mm^3, respectively. The mean difference between the volumes of right and left modioli was 0.00047 mm^3, which was not significant. The volumes of the right and left modioli superior and inferior to the horizontal line passing through the angle of the mouth are presented in Table 1. The related r and p values are presented in Table 2.

Table 1: The volumes of the right and left modioli superior and inferior to the horizontal line passing through the angle of the mouth

	Min(mm^3)	Max(mm^3)	Mean (SD)(mm^3)	95% CI for Mean(mm^3)	
RV					
Inferior to HI	0.07	0.66	0.35 (0.17)	0.28	0.42
Superior to HI	0.14	1.65	0.61 (0.31)	0.51	0.71
LV					
Inferior to HI	0.07	0.71	0.36 (0.17)	0.29	0.43
Superior to HI	0.20	1.17	0.61 (0.27)	0.53	0.70

RV: volume of the right modioli, LV: volume of the left modioli, SD: standard deviation, CI: confidence interval, HI: horizontal line

Table 2: *P*- and r values of correlation between age and volume

Age-Location	RV	RV	SV	SV
R Inferior to HI	r=-0.54	*P*=0.008		
R Superior to HI	r=-0.07	*P*=0.663		
L Inferior to HI			r=-0.37	*P*=0.073
L Inferior to HI			r=-0.15	*P*=0.353

RV: volume of the right modioli, LV: volume of the left modioli, HI: horizontal line, R: Right, L: Left

It was hypothesized that modiolus displaces below the horizontal line passing through the angle of the mouth and its volume decreases with aging. According to the results, twenty-three patients' (35.9%) right modioli were localized inferior and 41 patients' (64.1%) right modioli were localized superior to the horizontal line passing from the angle of mouth. Twenty-four patients' (37.5%) left modioli were localized inferior and 40 patients' (62.5%) left modioli were localized superior to the horizontal line. There was a negative correlation between age and the volumes of right and left modiolus (Figure 4). There was a significant negative correlation between gender and volume. Volume of modiolus in women was prominently less than that in men.

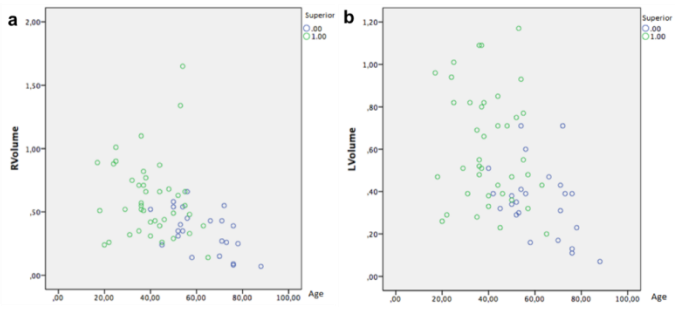

Figure 4: Scatter plot graphic showing the relationship between the volume of (a) right modiolus, (b) left modiolus and age (RVolume: volume of the right modioli, LVolume: volume of the left modioli)

In our study, 91% of the individuals whose modioli were located inferior to horizontal line were over 49 years old. The right and left modioli of 66% of the patients older than 49 years were located inferior to the horizontal line, while this was true for only 6% and 9% of the patients younger than 49 years, respectively. Age was a significant factor in the downward displacement of bilateral modioli.

Logistic regression analysis showed that the risk of downward displacement of modiolus in women was 3.3 times higher than in men [Odds ratio (OR): 3.3, 95% confidence interval (CI) for OR (1.3-8.7)].

In multilevel logistic regression analysis, gender (reference category: male) and age (reference category: less than 49) were found to have significant effects on the level of modiolus.

Discussion

The shape and the dimensions of the modiolus differ according to the structure, age, gender and ethnicity. The modiolus is a tortuous, cone-like structure that extends vertically from the buccal mucosa to the dermis of the skin with a base that is adherent to mucosa [9]. The modiolus does not have precise boundaries, it extends vertically 20 mm above, below, and lateral to a horizontal line that passes through the buccal angle. The apex of this conical structure is about 12 mm lateral to the buccal angle [4,10]. Considering the shape and localization of modiolus, in our study, a horizontal line passing from the angle of the mouth was used to indicate the level of the modiolus. This level was considered a landmark that separates the upper and lower localizations of modiolus.

Nine muscles in different planes attach to the modiolus, as their stems spiralize and separate into many bundles. These fibers course in different ways, and due to this complicated structure, analyzing the three-dimensional (3D) organization of modiolus is difficult [9]. In this study, we aimed to define the location, level and volume of the modiolus in living individuals on MR images, which may help understand its 3D structure [4,10]. Studies in the literature are generally related to the muscular attachments, histology, location of modiolus and its relationship with neurovascular structures.

To the best of our knowledge, the volume, age and gender related changes of modiolus are discussed for the first time. Here, the mean volume of the modiolus was calculated about 0.51 mm^3 with no significant differences between the volumes of the right and left modiolus.

We hypothesized that the volume of the modiolus decreases with aging. Accordingly, there was a negative correlation between the age and volume of right and left modioli. It was also found that the volume of modiolus was prominently less in women than in men.

It was also hypothesized that modiolus displaced below the horizontal line passing through the angle of the mouth with aging. In our study, 35.9% of the right modioli and 37.5% of the left modioli were placed inferiorly and 64.1% of the right modioli and 62.5% of the left modioli were localized superior to the horizontal line passing from the angle of the mouth. Ninety-one percent of the individuals who had modiolus located inferior to horizontal line were older than 49 years. Right and left modioli were observed to significantly displace inferior to horizontal line with aging, as observed on retrospective MR images. Despite the studies in the literature indicating the level of the modiolus in vertical position, the influence of gravity on modiolus was excluded due to the normal positioning procedure of patients undergoing MR imaging [6]. Positioning the patients

in such a way to exclude gravity effect on modiolus carried the results of this study to a more constant ground.

In the literature, there are studies reporting the relationship between hyperactivity of Depressor anguli oris muscle and dropped mouth corner. The appearance of the aging in the perioral region has many reasons, one of which is the drooping of the corner of the mouth, related to Depressor anguli oris muscle. Dropped mouth corner may lead to a sad and tired expression which is a common complaint for the patients referred for facial rejuvenation [11]. Botulinum toxin injection into Depressor anguli oris muscle is the current treatment modality for elevation of mouth corner. The target of the botulinum toxin is depressor anguli oris muscle but the neighboring muscles at mouth corner can also be affected. Unwanted changes in the lower third of the face can be a problem after the injection. Besides, botulinum toxin injection is a temporary treatment. Depressor anguli oris is one of the muscles attaching to modiolus anguli oris. Perioral wrinkles are also corrected by dermal fillers including hyaluronic acid which is also a temporary treatment [12]. The enhancement of modiolus anguli oris, which is a key point for the anatomical features of lower face, may be a long-lasting safe option for treatment [7].

It was found that the facial artery traversed around the modiolar region and was closely associated with the perioral musculature. During plastic and reconstructive surgery procedures of the face, such as the superficial muscular aponeurotic system (SMAS) facelift and facial artery musculo-mucosal flap, the relationship between the facial artery and the muscles attached to modiolus gains great importance [13].

The main purposes of the reconstruction of the labial commissure are the maintenance of functions and, the preservation of symmetry and facial appearance. Knowledge of the anatomy of the modiolus and the muscles attaching to this structure will assure a proper reconstruction technique [10,14,15].

The modiolus plays a fundamental role in facial actions because from therein is provided the force for closure, eversion, and opening of the mouth. The increased knowledge of the surgeon regarding modiolus and its relations will provide benefit not only for the procedures such as face lifting and botulinum toxin injection, but also for the surgeries of facial paralysis and trauma patients. A better comprehension of surgical anatomy and understanding of how facial anatomical relationships change with aging will lead to advances in plastic and reconstructive surgery [2,3,7,10,14,15].

Limitations

This study is a retrospective cohort study, which is its limitation. A prospective study with long-time follow up of patients is needed for further enlightenment on this subject. Also, further studies in large series are required to investigate whether modiolus is a hormone-dependent tissue. To clarify the hormone effects on modiolus, comparative studies between the pre- and post-menopausal states of women should be conducted. Microscopic studies analyzing the hormone receptors of modiolus may lead to a new overview for treatment of lower face aging. Understanding the histo-morphological changes in modiolus and the components of the connective tissue may provide insight to improve its structure by injectable treatment modalities like platelet rich plasma (PRP) treatment. We suggest prospective comparative studies in large series to determine the long-term effects of treatments strengthening the modioli.

Conclusion

In our study, age was a significant factor in the downward displacement of modiolus. Modioli were more prominently displaces downwards in women compared to men. Morphometry and age-related changes in modiolus are important for aesthetic interventions, radiologists, and anatomists.

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Factors affecting the hospitalization of female patients with asthma

Astımlı kadın hastaların hastaneye yatışlarını etkileyen faktörler

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Abstract

Aim: Asthma is one of the most common chronic respiratory diseases and an important public health problem leading to mortality. The aim of this study was to evaluate the hospitalization rates of female patients with asthma.

Methods: This cross-sectional study was conducted with the approval of a university hospital ethics committee between September 2018 and January 2019. The study included a total of 183 females: 56 patients with exacerbation were included in Group 1 and 127 patients with stable asthma constituted Group 2. Demographic data, pulmonary function test results, number of hospital admissions, hospitalization rates and laboratory findings were evaluated. All statistical calculations were performed with SPSS 23 for Windows.

Results: The mean age, number of hospitalizations and admissions to the emergency room were significantly higher in Group 1 (P=0.003, P<0.001 and P<0.001, respectively). Patients in Group 1 had lower oxygen saturations, FEV1, FVC, FEV1/FVC values, higher respiratory and heart rates, blood glucose, lactate dehydrogenase, c-reactive protein, lactate, phosphorus, high-density lipoprotein, calcium levels, white blood cell and neutrophil counts. Increased eosinophil count and decreased basophil count were associated with increased emergency room admission rates (P=0.043).

Conclusion: Women admitted to the hospital with asthma exacerbation were older and had an increased number of hospitalization rates. Higher eosinophil blood levels were associated with recurrent hospital admissions.

Keywords: Asthma, Hospitalization, Female

Öz

Amaç: Astım en yaygın kronik solunum hastalıklarından biridir ve mortaliteye yol açan önemli bir halk sağlığı problemidir. Bu çalışmanın amacı astımlı kadın hastaların hastaneye yatış oranlarını değerlendirmektir.

Yöntemler: Bu kesitsel çalışma bir üniversite hastanesi etik kurulundan onay alınarak Eylül 2018 ve Ocak 2019 tarihleri arasında yapıldı. Çalışmaya 183 kadın hasta dahil edildi (alevlenme olan 56 hasta Grup 1 ve stabil astım olan 127 hasta Grup 2 olarak adlandırıldı). Demografik özellikler, akciğer fonksiyon testleri, hastaneye başvuru sayıları, hastaneye yatış oranları ve laboratuvar bulguları değerlendirildi. Tüm istatistik hesaplamaları SPPS 23 Windows ile yapıldı.

Bulgular: Grup 1'deki hastaların ortalama yaşı daha büyüktü (P=0,003). Grup 1'de hastaneye yatış ve acil servise başvuru sayıları önemli derecede yüksekti (P<0,001). Grup 1'deki hastalar daha düşük oksijen satürasyonu, FEV1, FVC, FEV1/FVC'ye ve daha yüksek solunum sayısı ve kalp atım sayısına sahipti. Bu grupta, hastalar daha yüksek kan glukozu, beyaz küre, nötrofil sayısına ve daha düşük fosfor, yüksek dansiteli lipoprotein ve kalsiyum düzeylerine sahipti. Artmış eozinofil sayısı ve azalmış bazofil sayısı acil servis başvuru oranları ile ilişkili idi (P=0,043).

Sonuç: Sonuç olarak, astım alevlenme ile hastaneye başvuran kadınlar daha yaşlıydı ve hastaneye yatış oranları daha fazlaydı. Yüksek eozinofil kan seviyeleri tekrarlayan hastaneye başvurular ile ilişkilidir.

Anahtar kelimeler: Astım, Hastaneye yatış, Kadın

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Introduction

Asthma is one of the most prevalent chronic airway diseases and an important public health problem leading to morbidity, mortality, and worsened quality of life globally, affecting people of all genders, ethnicities, and ages [1]. Nowadays, around 300 million people suffer from asthma disease in the world and asthma is responsible for 180.000 deaths per year, with a prevalence rate higher than 10% [2].

The incidence, prevalence, severity, and hospitalization rates of asthma vary with gender. The disease is more severe, and exacerbation rates, hospital admissions, hospitalization rates, morbidity and mortality rates are higher among women. The reasons for gender inequality may be linked to hormonal and immunological factors, as well as environmental and occupational exposures [3].

Asthma-related hospitalization rates differ among countries, genders and age groups [2]. Asthma is more common among women, and therefore it is estimated that women have higher rates of hospital admission. The main purpose of this study was to evaluate the hospitalization rates of female patients with asthma, determine the patients' demographic profile and analyze blood parameters.

Materials and methods

Design and assessment

This cross-sectional study was conducted with the approval of Kafkas University ethics committee, between September 2018 and January 2019 in Kars, Turkey. It included randomly selected 56 asthma patients with acute exacerbation who were referred to the emergency room (ER) (Group 1) and 127 stable asthma patients who were admitted to the Pulmonology department (Group 2) (n_{total}=183). Group 2 patients were stable with no acute exacerbation of the disease for at least one month prior to admission and they were receiving regular treatment. Age, smoking status (smoker, ex-smoker, never a smoker), exposure to passive smoke, type of asthma (allergic, non-allergic), occupation, educational status, comorbidity, pulmonary function tests, number of hospitalizations in Pulmonology Clinic (NHPC), number of admissions to emergency room (NAER) and number of hospitalizations in the intensive care unit (NHIC) within the last year due to asthma and complications, respiratory rate, oxygen saturation in room air (%), pulse rate per minute, complete blood counts, blood gas analysis, renal-liver function tests, lipid profile, electrolytes, blood glucose and vitamin D level of patients were evaluated. According to Global Strategy for Asthma Management and Prevention 2018 report, the NAER and NHPC of Group 2 patients were not considered as asthma exacerbation. Pulmonary function tests were measured at baseline using a spirometer (Spirolab III-MIR, Italy).

Blood samples

An alcohol swab was used to clean the skin and a band was used to tie for arm of patients. All blood samples were drawn from the veins in the forearm and collected into separate tubes with ethylenediamine tetraacetic acid (EDTA) and acid citrate dextrose. The samples were analyzed with Pentra DF Nexus, Horiba Medical, Japan with Automated Cell Counter Methodology and Cobas C 702 Module, Roche, Switzerland. The complete blood count samples, which were stable for 24 hours at room temperature, and 36 hours at 2 – 8 ^{o}C, were stabilized optimally when run within in 4 hours of collection. The tube with acid citrate dextrose was centrifuged for 8-10 minutes at 3500-4000 revolution per minute (rpm) and serum, which remained stable for 8 hours at 2 – 8 ^{o}C, was separated.

Statistical analysis

All statistical calculations were performed with SPSS 23.0 (SPSS for Windows, Chicago, IL, SA). All continuous variables were expressed as mean (standard deviation), and categoric variables were defined as percentages (%). The categorical parameters were compared with Chi Square test and Fischer's exact test. The normal distribution was determined by histogram and Kolmogorov-Smirnov test. Mean values of continuous variables were compared between the groups using Mann-Whitney U test. The statistical significance level was $P<0.05$. The power of the test was calculated with P_{005} (power analysis) program. A sample size of 86 people, 43 in each group, was needed for 80% power and 0.05 type-1 error at 95% confidence interval.

Results

Clinical features, number of hospitalizations and referrals, respiratory findings and blood parameters of all patients included in this study are presented in **Table 1**. The mean age of the patients in Group 1 was higher than that of Group 2 (P=0.003). Smoking status, type of asthma, occupation, educational status and comorbidities did not differ between the two groups.

The NHPC and NAER rates within the last year due to asthma and its complications in Group 1 patients were significantly higher than that of Group 2 (P<0.001). None of the patients in Group 2 were hospitalized in the intensive care unit during the last year for asthma and its complications. Patients in Group 1 had lower oxygen saturations, FEV1, FVC, FEV1/FVC values, higher respiratory and heart rates, blood glucose, lactate dehydrogenase, c-reactive protein, lactate, phosphorus, high-density lipoprotein, calcium levels, white blood cell and neutrophil counts. Patients in Group 2 had higher red cell distribution width (RDW) and lower albumin, protein and plateletcrit (PCT) values. Eosinophil count increased, and basophil count decreased with increasing number of ER admissions (P=0.043 and P=0.043, respectively). Clinical features, number of hospitalizations and admissions, respiratory findings, and blood parameters of the two groups are presented in Table 1.

Table 1: Clinical features, number of hospitalizations and admissions, respiratory findings, blood parameters of two groups

	Group 1 mean(SD) / n (%)	Group 2 mean(SD) / n (%)	*P*-value
Age	51.1(14.2)	44.8(12.3)	0.003
Smoking status			
Ex-smoker	8 (14.3%)	23 (18.4%)	0.399
Smoker	9 (16.1%)	24 (19.2%)	
Never smoker	39 (69.6%)	77 (61.6%)	
Exposure to passive smoke	-	1 (0.8%)	
Type of asthma			
Allergic	28 (50.9%)	52 (41.9%)	0.328
Non allergic	27 (49.1%)	72 (58.1%)	
Occupation			
Housewife	49 (87.5%)	113 (89%)	0.246
University staff	-	1 (0.8%)	
Student	-	6 (4.7%)	
Engineer	-	1 (0.8%)	
Worker	3 (5.4%)	1 (0.8%)	
Teacher	1 (1.8%)	3 (2.4%)	
Officer	3 (5.4%)	2 (1.6%)	
Educational status			
Illiterate	22 (39.3%)	39 (30.7%)	
Primary school graduate	20 (35.7%)	58 (45.7%)	0.209
Secondary school graduate	9 (16.1%)	8 (6.3%)	
High school graduate	3 (5.4%)	6 (4.7%)	
University graduate	2 (3.6%)	16 (12.6%)	
Comorbidity			
Hypertension	13 (23.2%)	25 (19.7%)	0.693
Diabetes Mellitus	4 (7.1%)	7 (5.5%)	0.739
Hyperlipidemia	2 (3.6%)	4 (3.1%)	0.596
Panic disorder	-	1 (0.8%)	0.694
Coronary Artery Disease	2 (3.6%)	5 (3.9%)	0.634
Hypothyroidism	1 (1.8%)	3 (2.4%)	0.641
Depression	1 (1.8%)	3 (2.4%)	0.641
Number of hospitalizations in Pulmonology Department (NHPC) in the last year due to asthma and complications			
None	43 (76.8%)	122 (96.1%)	<0.001
One time	8 (14.3%)	2 (1.6%)	
Two times or more	5 (8.9%)	3 (2.4%)	
Number of admissions to emergency room (NAER) in the last year due to asthma and complications			
None	29 (51.8%)	96 (75.6%)	<0.001
One time	7 (12.5%)	17 (13.4%)	
Two times	10 (17.9%)	12 (9.4%)	
Three times	5 (8.9%)	-	
Four times or more	5 (8.9%)	2 (1.6%)	
Number of hospitalizations in intensive care unit (NHIC) in the last year due to asthma and complications			
None	52 (92.9%)	127 (100%)	0.003
Once	1 (1.8%)	-	
Two times or more	3 (5.4%)	-	
Respiratory findings			
Oxygen saturation (%)	89.1(9)	94.5(2.2)	<0.001
Respiratory rate (rpm)	19.9(2.5)	15.8(1.1)	<0.001
Heart rate (rpm)	99.2(17.9)	88(10.4)	<0.001
FEV1, L	2.43(0.45)	2.65(0.46)	0.005
FVC, L	2.97(0.49)	3.15(0.52)	0.045
FEV1/FVC, %	79.8(2.8)	81(2.6)	0.006
Biochemical parameters			
Glucose, mg/dL	116.7(64.4)	104.8(44.7)	0.001
Blood Urea Nitrogen, mg/dL	31.5(17.4)	28.4(8.6)	0.658
Creatinine, mg/dL	0.7(0.22)	0.88(2.82)	0.120
Uric acid, mg/dL	4.52(1.33)	4.23(1.19)	0.209
Phosphore, mg/dL	3.39(0.6)	3.6(0.57)	0.029
Aspartate aminotransferase, U/L	19.5(6.9)	18.6(5.4)	0.552
Alanine aminotransferase, U/L	18.3(8.1)	16.8(8.9)	0.170
Lactate dehydrogenase, U/L	244.4(75.7)	210.6(45.2)	0.001
Albumin, g/dL	24.4(19.57)	13.02(16.33)	0.014
Protein, g/dL	41.44(33.39)	21.76(27.42)	0.002
Triglyceride, mg/dL	127.2(76)	125.4(71.2)	0.817
High density lipoprotein, mg/dL	50.5(15.9)	56.1(13.8)	0.009
C-reactive protein, mg/dL	11.65(27.27)	2.12(4.98)	<0.001
Very low density lipoprotein	25.1(15.1)	25.2(14.1)	0.638
Calcium, mg/dL	9.36(0.42)	9.51(0.38)	0.024
Sodium, mmol/L	139(3)	137.4(17)	0.470
Potassium, mmol/L	4.38(0.34)	4.32(0.49)	0.094
Magnesium, mg/dL	1.98(0.2)	1.93(0.15)	0.132
Vitamin D	12.94(6.21)	13.2(5.56)	0.688
Blood gas analysis			
pH	7.39(0.03)	7.39(0.02)	0.831
pCO2, mmHg	42.7(6.8)	45.1(30.8)	0.972
pO2, mmHg	33.1(12.8)	33(15)	0.787
HCO3, mmol/L	24.5(2.3)	24(1.8)	0.327
Lactate, mmol/L	1.3(0.5)	1.12(0.43)	0.013
Complete blood count			
White blood cell, 10*3/mm^3	9.3(3.5)	7.8(2.2)	0.011
Hemoglobin, g/dL	14.31(1.5)	14.03(1.31)	0.078
Plateletcrit, %	0.25(0.06)	0.24(0.05)	0.409
Platelet count, 10*3/mm^3	286.3(80.8)	278.4(69.4)	0.996
Red cell distribution width, %	14.8(2)	15.6(2.6)	0.023
Basophil count, 10*3/mm^3	0.04(0.03)	0.04(0.03)	0.289
Eosinophil count ,10*3/mm^3	0.29(0.29)	0.22(0.2)	0.640
Neutrophil count, 10*3/mm^3	6.98(6.67)	4.75(1.74)	0.002
Lymphocyte count, 10*3/mm^3	3.26(5.37)	2.53(3)	0.924

SD: Standard deviation

Discussion

Asthma exacerbation is characterized by the progression of symptoms such as shortness of breath, cough, wheezing or chest tightness and decreasing lung functions [4]. This study found that higher age was associated with exacerbation in female patients with asthma. Kang et al. [5] also reported that the risk of exacerbation was associated with age older than 45 years in mild and moderate asthma. In the study of Sekiya et al. [6] the mean age of inpatients with severe asthma was similar to that of Group 1 in our study. Asthma is a chronic inflammatory disease which is related to age and characterized by unregulated inflammation with low-grade, chronic and systemic response that causes aging [1, 7]. The aging of patients can be a risk factor for inflamed exacerbation episodes.

An asthma exacerbation episode comprises worsening of respiratory symptoms, decreased lung function tests that requires systemic steroid treatment, increased ER visits and hospitalization rates [8]. As expected in the study, patients with exacerbation had higher NHMRS, NAER, NHIC, respiratory and heart rates, lower oxygen saturations and pulmonary function tests.

In this study, we observed that patients with exacerbation had higher blood glucose, LDH, CRP, lactate levels, WBC and neutrophil counts, and lower serum phosphorus, HDL, and calcium levels. The increase in eosinophil count and decrease in basophil count during the exacerbation episode were associated with increasing number of ER admissions. Increased or decreased serum biomarker levels during this period may be due to drug side effects or inflammatory status. The association of eosinophil count with recurrent admissions may provide information regarding prognosis. Steroid therapy, used in asthma attacks, is hyperglycemic. Stress hormones such as catecholamines and cortisol may also induce hyperglycemia [9]. CRP, WBC and neutrophil counts are well-known airway-blood inflammatory and infection markers commonly used by physicians in clinical practice [10-12]. Corticosteroid therapy may cause corticosteroid-induced neutrophilic leukocytosis [13]. Beta-agonist agents used for asthma act by decreasing intracellular free Ca+ ion concentration, thus relaxing smooth muscle, which may cause hypocalcemia [14]. In addition, short-acting beta agonists may decrease blood phosphate and increase lactic acid levels [13].

LDH, an intracellular cytoplasmic enzyme, is indicative of cellular integrity. The inflammatory cells are derived from LDH and the increment of LDH is related to vascular permeability and epithelial tissue necrosis. During the acute period, it is expected to increase with inflammation [15]. Surfactant is a lipid-protein composite that reduces surface tension and alveolar distance, thus effecting respiratory immune response. Low density lipoproteins induce surfactant lipid synthesis, and the composition and function of surfactant can be damaged with inflammatory diseases such as asthma. Similar to this study, it was reported that LDH and HDL levels increased during an asthma attack [15].

Severe eosinophilic asthma or eosinophilic refractory asthma is a common phenotype of asthma disease. Higher blood eosinophil count is a well-established risk factor for asthma exacerbation episodes and eosinophil count may decrease after inhaled steroid treatment [16]. Some studies demonstrated that blood eosinophil count is a substantial indicator of economic burden, management and hospitalization rates for asthma [17, 18]. Basophil cells are activated by IgE and responsible for aggravation of inflammation by releasing histamine and

leukotriene C4, but the exact function of basophils in the pathogenesis of asthma remains a myth [19].

Limitations

This cross-sectional study, comparing women with asthma in acute and stable periods, was designed with the information obtained from the file records of the patients. The retrospective nature, lack of more specific biomarkers of inflammation, lack of mortality data and the short follow up period were the limitations of our study. More studies including these parameters are needed in the future.

Conclusion

In conclusion, women admitted to hospital with asthma exacerbation were older and had increased number of hospitalization rates. Higher eosinophil blood levels were associated with recurrent hospital admissions.

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Antibacterial efficacy of mesenchymal stem cell administration in diabetic rats infected with MRSA: An experimental study

Mezenkimal kök hücre uygulamasının MRSA ile enfekte olmuş diyabetik ratlardaki antibakteriyel etkinliği: Deneysel çalışma

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Abstract

Aim: The antibacterial effects of mesenchymal stem cell (MSC) administration and vancomycin, tigecycline and daptomycin treatment were compared in a diabetic rat model with wound infection due to methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: Experiment, negative and positive control groups were created using 60 rats. The antibacterial efficacy of MSC, vancomycin, tigecycline and daptomycin were compared. All rats had diabetes induced with streptozotocin. They were shown to be hyperglycemic with fasting blood glucose monitoring. During surgery, subdermal pouches were created. Group 0 (negative control group) was not infected or treated. All other groups were infected with MRSA. Group 1 (positive control group) was infected but not treated. The other 4 groups were determined as treatment groups: Group MSC was treated with MSC, Group Van treated with vancomycin, Group Tig treated with tigecycline and Group Dap treated with daptomycin. After one week of treatment, samples were collected following euthanasia. Tissue samples were evaluated after histopathologic staining with hematoxylin/eosin. The presence of MSC in the wound region was shown by immunofluorescent staining. Bacterial colony counts were identified quantitatively. TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels in blood samples were measured with the ELISA method.

Results: While bacterial colonization was not observed in Group 0, a clear colonization was identified in Group 1. Full eradication was achieved in Group Tig and Group Dap. Eradication could not be achieved for 1 rat in Group Van and 4 rats in Group MSC. The uncontaminated negative control group rats (Group 0) had minimal inflammation, while the most severe inflammation was observed in infected and untreated rats (Group 1) (P<0.001). Group-MSC, Group-Van, Group-Dap and Group-Tig had moderate levels of inflammation and edema. The MSC group showed significant increase in vascularity (P=0.001). Adhesion and fibrosis were observed significantly less in the negative control group and MSC groups, similarly (P<0.001, P<0.001).

Conclusion: MSC may exert antibacterial-like effects for MRSA-induced wound infection treatment in diabetic rats, and limit the inflammation in and around the wound. Further clinical studies researching the synergistic effects of MSC with antibiotherapy for treatment of diabetic wound infections are needed.

Keywords: Mesenchymal stem cell, Vancomycin, Tigecycline, Daptomycin, Methicillin-resistant Staphylococcus aureus, Diabetes mellitus

Öz

Amaç: Mezenkimal kök hücre (MSC) uygulamasının Metisiline dirençli *Staphylococcus aureus* (MRSA) kaynaklı yara enfeksiyonu üzerindeki antibakteriyel etkileri, diyabetik rat modelinde, vankomisin, tigesiklin ve daptomisin tedavisi ile karşılaştırıldı.

Yöntemler: 60 rat ile oluşturulan deney ve negatif-pozitif kontrol grupları oluşturuldu. MSC, vankomisin, tigesiklinin ve daptomisinin antibakteriyel etkinlikleri karşılaştırıldı. Ratların tümü streptozotosin ile diyabetik hale getirildi. Açlık kan glukozu takibiyle hiperglisemik oldukları gösterildi. Cerrahi olarak cilt altına keseler oluşturuldu. Grup-0 (negatif kontrol grubu) enfekte ve tedavi edilmedi. Diğer tüm gruplar MRSA ile enfekte edildi. Grup-1 (pozitif kontrol grubu) enfekte edildi fakat tedavi edilmedi. Diğer 4 grup tedavi grupları olarak belirlendi. Grup-MSC; MSC ile, Grup-Van; vankomisin ile, Grup-Tig; tigesiklin ile, Grup-Dap; daptomisin ile tedavi edildi. Bir haftalık tedavi sonrası ötenazi ile numuneler toplandı. Doku örnekleri histopatolojik olarak hematoksilen/eosin ile boyanarak değerlendirildi. Yara bölgesinde MSC varlığı immun floresan boyama ile kanıtlandı. Kantitatif olarak bakteri koloni sayıları tespit edildi. Alınan kan numunelerinden TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF ve Kaspaz-3 seviyeleri ELISA yöntemiyle ölçüldü.

Bulgular: Grup-0'da bakteri kolonizasyonu gözlenmez iken, Grup-1'de belirgin kolonizasyon saptandı. Grup-Tig ve Grup-Dap'ta tam eradikasyon sağlandı ve Grup-Van'da 1, Grup-MSC'de 4 ratta eradikasyon sağlanamadı. Kontamine edilmeyen negatif kontrol grubunda (Grup-0) minimal inflamasyon düzeyi izlenirken, en şiddetli inflamasyon enfekte edilmiş ve tedavi verilmemiş ratlarda (Grup-1) gözlendi (P<0,001). MSC, Vankomisin, Daptomisin ve Tigesiklin gruplarında orta düzeyde inflamasyon ve ödem gözlendi. MSC grubunda önemli vaskülarite artışı görüldü (P=0,001). Negatif kontrol grubunda ve MSC grubunda benzer oranda anlamlı olarak daha az adezyon artışı ve fibrozis görüldü (P<0,001, P<0,001).

Sonuç: Bu çalışma, diyabetik ratlarda MRSA kaynaklı yara enfeksiyonu tedavisinde MSC'nin antibakteriyel etkinliği sağlayabileceğini düşündürmektedir. Buna ilaveten MSC sayesinde yara bölgesinde sınırlandırılmış bir inflamasyon sağlanabileceği düşünülmüştür. Diyabetik yara enfeksiyonlarının tedavisinde antibiyoterapiyle birlikte MSC kullanımının sinerjistik etkisinin araştırılacağı klinik çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Mezenkimal kök hücre, Vankomisin, Tigesiklin, Daptomisin, Metisiline dirençli Staphylococcus aureus, Diabetes mellitus

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Introduction

Mesenchymal stem cells (MSC) are obtained from non-hematopoietic bone marrow cells which can theoretically renew themselves and differentiate into a variety of cells [1,2]. According to their source, they may release characteristic active mediators and antimicrobial peptides [3]. It is reported in a variety of studies that these secretions strengthen the natural immune response against bacterial infection [4]. 3 stages defined in injured tissue repair begin with hemostasis after the inflammatory period ends in 24-48 hours and continues with proliferative and maturation stages. During the inflammatory period, the infection shield is strengthened by neutrophil and macrophage migration, which may be equivalent to the rate of angiogenesis, for the foundations of tissue repair laid in this stage [5]. In addition to fibroblastic proliferation and differentiation, macrophages stimulate angiogenesis by collagen production and secretion of transforming growth factor beta (TGF-β), platelet derived growth factor (PDGF), interleukin 1 (IL-1), platelet activated growth factor (PAGF), transforming growth factor alpha (TGF-α), tumor necrosis factor (TNF-α), fibroblast growth factor (FGF), and epidermal growth factor (EGF) [6].

Wound infections are a significant problem for many types of surgery, especially vascular surgery, in the short and medium term. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens and is resistant to many antibiotics [7,8]. Methicillin-resistant *Staphylococcus* strains which are resistant to glycopeptides have been reported [9]. Additionally, wound infections that don't respond to antibiotherapy cause high morbidity and mortality [10]. New generation broad-spectrum tetracyclines such as vancomycin and daptomycin-tigecycline are the strongest treatment choices for *Staphylococcus* infections with excessive resistance [11].

In this study, the antibacterial efficacy of MSC obtained from rat fatty tissue was compared with strong antibiotics with known efficacy for MRSA treatment, namely, daptomycin, vancomycin and tigecycline.

Materials and methods

Groups: Sixty rats were divided into a total of 6 groups of 4 treatment groups and negative and positive control groups (age>6 months, weight: 300-350 g).

Diabetic rats: Rats were given streptozotocin-induced diabetes (STZ; Sigma-Aldrich, St Louis, MO), (IP, 60 mg/kg, single dose). One week later, fasting blood sugar obtained from the tail vein was shown to be higher than 200 mg/dL [12].

MRSA: MRSA strains were isolated from the tissue culture of a patient treated due to surgical wound site infection in Kırıkkale University Faculty of Medicine Hospital. A sterilized colony was obtained from a single wound infection with gram staining, catalase reaction, tube coagulation test and API-staph test (BioMérieux, Lyon, France). Methicillin resistance was analyzed using the Kirby-Bauer disk diffusion method.

Surgery and contamination: All rats were anesthetized with ketamine hydrochloride (Pfizer, Lüleburgaz, Turkey) and xylazine hydrochloride (Bayer AG, Leverkusen, Germany). They were shaven and cleaned with povidone-iodine. Under sterile conditions, all rats, except the negative control group, had bacterial seeding of MRSA $2x10^7$cfu/ml using a tuberculin injection in created pouches [10].

MSC: Commercially sold rat adipose tissue-derived GFP-labeled mesenchymal stem cells (MLP laboratory, Istanbul, Turkey) were brought to the laboratory in accordance with cold chain rules and centrifuged after a rapid defrost technique with 37 °C water bath. The supernatant was removed, and the pellet was resuspended. Cell counts and viability were measured (Countess®, Invitrogen, San Diego, CA, USA). For each rat, $1x10^6$ cells per injection were prepared [13].

Treatment: Group MSC was treated with mesenchymal stem cells (administered locally to the wound site, $1x10^6$ MSC, single dose); Group Van was treated with vancomycin (IP, 15 mg/kg, 2 times per day) [14]; Group Dap was treated with daptomycin (IP, 3 mg/kg, 2 times per day) [15]; and Group Tig was treated with tigecycline (IP, 10 mg/kg, 2 times per day) [16]. The negative and positive control groups received no treatment.

During the experiments, rats were housed in fives per cage. They were kept under standard environmental conditions (12 hours light/dark cycle, temperature ~ 21 ºC) and fed with standard rat feed and water ad libitum.

Euthanasia: One week after surgery, rats were euthanized with high-dose anesthesia and tissue and blood samples were taken.

Histopathological Evaluation: Perigraft and skin subdermal samples were collected and fixated in formalin for 2 days, dipped in ethanol and xylene bath, submerged in paraffin, divided and stained with hematoxylin/eosin. Inflammation severity was graded histopathologically: Grade 0 showed no neutrophils, Grade 1 showed some neutrophils, Grade 2 showed moderate amount of neutrophils, and neutrophils were commonly observed in Grade 3 [17].

Immunofluorescent Antibody (IFA) imaging: The presence of GFP-labeled MSCs in the wound region was shown with a fluorescent antibody microscope.

Infection Evaluation: Tissue samples were fragmented and cultured with agar, and colonies were quantitatively counted.

ELISA: TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels of blood samples were measured with ELISA and recorded.

Statistical analysis

SPSS 20.0 (SPSS; Chicago, IL, USA) software was used for statistical analysis. Continuous data were presented as mean (standard deviation) and non-normal categoric data were presented as median with 25-75% interval. The chi-square test was used to compare categoric variables. The Mann-Whitney U (MWU) and Kruskal Wallis tests (two-way comparisons of groups) were used to compare continuous data with non-normal distribution. $P<0.05$ was considered statistically significant.

Results

All groups were examined macroscopically first. MRSA colonization was observed in all rats in the positive control group (10/10; 4.7 $x10^9$CFU/mL), whereas no colonization was observed in the negative control group. In the VAN group, one (1/10) rat was colonized. Full eradication was achieved in DAP and TIG groups, and not achieved in 4 rats (4/10) in the MSC

treatment group. During the study, no animal showed clinical symptoms due to antibiotherapy or MSC.

Histopathological evaluation: Histopathological evaluation was performed semiquantitatively using a conventional microscope (Nikon Eclipse E600, Nikon AG Instruments, Switzerland). Cases were grouped as absent, mild, moderate and prominent, and scored with 0, 1, 2, and 3, respectively [17]. The final score was obtained by summing the obtained values.

First, sections were examined to see the distribution differences of inflammation using a x10 objective (4.9 mm^2). One section, where inflammation was observed homogenously, was chosen in each rat and evaluated for areas bordered with inflammatory cells, which showed intense inflammation. Following H&E staining of these intensely inflamed areas, all specimen were examined semiquantitatively by an experienced pathologist under 20x magnification (0.785 mm^2). All rats were then classified according to the above-mentioned scoring system.

H&E evaluation: No contamination was noted histopathologically and minimal inflammation was observed in Group 0. Group 1 and Group DAP showed significantly more severe inflammation compared to other groups. The histiocytic response and fibrosis parameters, used to measure the level of inflammation, were the highest among all groups. The findings observed in MSC, tigecycline and vancomycin groups were similar to each other: Histopathologic findings showed mild-to-moderate inflammation and edema. Significantly increased vascularity was observed in the MSC group. The increase in fibrosis was minimal in Group 0 and MSC groups.

IFA evaluation: GFP-labeled MSCs were localized at the site of surgery injected with IFA. Especially in the MSC group, there was no significant difference in the intensity of inflammation as noted above, but a marked increase in vascularity was observed where stem cells had concentrated.

Biochemical evaluation: TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels, measured with ELISA, were markedly increased in Group 1 and Group DAP, consistently with histopathological evaluation. The increases in the other groups, particularly the MSC group, were less compared to these two. There was no increase in the negative control group.

ELISA: Along with histopathological assessment, level of inflammation markers were researched in blood and similar increases at moderate levels were observed in all antibiotherapy groups. There was no increase in Group 0. In terms of TGF-β, Group 0 and Group 1 were similar.

FGF, IL-1, TNFα, and PDGF levels of Group MSC were similar to all treatment groups and lower levels of Caspase-3 were detected. Comparisons between the groups for inflammation parameters measured with ELISA are comparatively shown in table 1. During the study, no side effects linked with antibiotherapy or MSC administration were observed in any of the animals. The multiple comparison of inflammation parameters and pathological evaluations of the groups is presented in table 2 and table 3, respectively.

Table 1: Comparison of inflammation parameters among the groups

Variables		n	Median	Minimum	Maximum	*P*-value
FGF (pg/mL)	Group-0	10	165.00	105.47	199.23	<0.001
	Group-1	10	330.68	249.52	602.46	
	Group-Van	10	374.34	218.58	532.51	
	Group-Dap	10	176.33	145.44	258.75	
	Group-Tig	10	205.50	133.50	778.30	
	Group-MSC	10	173.24	30.05	281.52	
TGF-β (pg/mL)	Group-0	10	49.08	18.59	70.42	0.010
	Group-1	10	87.19	40.44	177.14	
	Group-Van	10	107.26	49.21	137.50	
	Group-Dap	10	71.48	41.33	92.60	
	Group-Tig	10	67.37	51.33	91.78	
	Group-MSC	10	49.08	16.11	92.62	
IL-1 (pg/mL)	Group-0	10	22.66	12.84	32.48	<0.001
	Group-1	10	155.22	107.76	251.77	
	Group-Van	10	124.99	109.07	146.49	
	Group-Dap	10	61.44	25.94	73.22	
	Group-Tig	10	69.35	22.66	141.49	
	Group-MSC	10	71.90	24.90	237.30	
VEGF (pg/mL)	Group-0	10	204.64	79.91	259.14	<0.001
	Group-1	10	558.42	248.31	676.62	
	Group-Van	10	412.73	101.53	648.59	
	Group-Dap	10	353.75	268.95	518.98	
	Group-Tig	10	294.29	184.21	544.54	
	Group-MSC	10	254.69	41.71	418.81	
TNF-α (pg/mL)	Group-0	10	67.70	46.98	96.31	0.001
	Group-1	10	244.49	120.10	576.87	
	Group-Van	10	258.93	81.97	730.95	
	Group-Dap	10	190.66	37.02	318.43	
	Group-Tig	10	96.90	71.98	334.30	
	Group-MSC	10	204.97	120.10	929.27	
PDGF (pg/mL)	Group-0	10	4.22	2.15	6.14	<0.001
	Group-1	10	6.66	6.24	7.89	
	Group-Van	10	6.39	5.05	6.89	
	Group-Dap	10	6.54	5.40	7.09	
	Group-Tig	10	5.83	3.89	8.51	
	Group-MSC	10	5.90	4.33	6.99	
Caspase-3 (pg/mL)	Group-0	10	6.75	4.08	8.86	<0.001
	Group-1	10	12.63	11.24	13.74	
	Group-Van	10	12.32	10.66	18.54	
	Group-Dap	10	13.00	10.84	20.41	
	Group-Tig	10	11.92	10.56	14.64	
	Group-MSC	10	8.63	6.72	12.16	

P-values were determined by Kruskal-Wallis H test and $P<0.05$ was considered statistically significant.

Table 2: Multiple comparison of inflammation parameters (*P*-values)

FGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.096	0.028	0.650
Group-1		0.364	0.001*	0.059	0.001*
Group-Van			0.001*	0.049	0.001*
Group-Dap				0.256	0.545
Group-Tig					0.131
TGF-β (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.021	0.008	0.068	0.067	0.894
Group-1		0.880	0.174	0.111	0.023
Group-Van			0.070	0.072	0.008*
Group-Dap				0.935	0.307
Group-Tig					0.178
IL-1 (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.003*	0.001*
Group-1		0.005*	0.001*	0.001*	0.023
Group-Van			0.001*	0.002*	0.104
Group-Dap				0.496	0.174
Group-Tig					0.406
VEGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.076	0.001*	0.010	0.248
Group-1		0.019	0.010	0.004*	0.001*
Group-Van			0.705	0.450	0.151
Group-Dap				0.257	0.049
Group-Tig					0.290
TNF-α (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.033	0.033	0.003*	0.001*
Group-1		0.791	0.186	0.003*	0.762
Group-Van			0.364	0.070	0.940
Group-Dap				0.130	0.427
Group-Tig					0.005
PDGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.018	0.008*
Group-1		0.016	0.162	0.096	0.002*
Group-Van			0.520	0.545	0.151
Group-Dap				0.406	0.112
Group-Tig					0.940
Caspase-3 (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.001*	0.021
Group-1		0.762	0.406	0.762	0.001*
Group-Van			0.705	0.406	0.001*
Group-Dap				0.001*	0.345
Group-Tig					0.002*

P-values were determined with the Mann-Whitney U test.* $P<0.010$, For post hoc multiple comparisons, statistical significance was assessed at $P<0.010$ levels for 6 groups.

Table 3: Pathological evaluations and comparison of groups (*P*-values)

Fibrosis	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.146	0.001*	0.146	0.146
Group-1		0.001*	0.010	0.001*	0.001*
Group-Van			0.001*	1.000	1.000
Group-Dap				0.001*	0.001*
Group-Tig					1.000
Histiocytic response	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.542	0.001*	0.342	0.615
Group-1		0.001*	0.055	0.003*	0.002*
Group-Van			0.001*	0.131	0.276
Group-Dap				0.001*	0.001*
Group-Tig					0.648
Vascularization	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.374	0.001*	0.615	0.648
Group-1		0.001*	0.021	0.001*	0.001*
Group-Van			0.001*	0.170	0.661
Group-Dap				0.001*	0.001*
Group-Tig					0.342
Granulocytic response	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.029	0.001*	0.004*	0.317
Group-1		0.001*	0.028	0.001*	0.001*
Group-Van			0.001*	0.383	0.131
Group-Dap				0.001*	0.001*
Group-Tig					0.022

P-values were determined with the Mann-Whitney U test.* $P<0.010$, For post hoc multiple comparisons, statistical significance was assessed at $P<0.010$ levels for 6 groups.

Discussion

We did not investigate whether MSC obtained from rat fatty tissue had any antibacterial effects on MRSA. Group 0 had no colonization and Group 1 had proliferation shown with CFU counts. The presence of GFP-labeled MSC in the administration area was shown with fluorescent microscopy. A variety of in vivo studies showed that MSC prevented bacterial sepsis and supported bacterial scavenging [3]. However, we unfortunately did not have the same level of success in our study of MRSA treatment in diabetic rats. During evaluation of this outcome, it should be kept in mind that DM makes wound healing particularly difficult [18]. Eradication was not achieved in 4 rats in the MSC group and 1 rat in the vancomycin group. In a study of Meisnel et al. [19], human MSC and MSC-released IFN-α and TNF-α were shown to successfully inhibit *S. Epidermis* proliferation. Contrary to this study, Guerra et al. [20] found that bone marrow-derived MSC ($1x10^6$ cells) did not inhibit the colony-forming capability of biofilm-related Staphylococcus. A mouse study by Qian et al. [21] showed that MSC exerted strong antibacterial and anti-inflammatory effects on S. aureus infection. Although the species of the subjects was different, the basic difference from our study is the diabetic condition of the rats, which further reduces the success rates for resistant strains. In our study, the more pronounced vascular structure formation in the MSC group shows that MSCs were effective at a cellular level, albeit insufficiently compared to antibiotherapy. One may conclude that MSC and antibiotherapy combination may achieve more effective infection control. Alcayaga-Miranda et al. [3] researched the synergistic interaction of antibiotherapy and MSC in an in vivo mouse model and reported that survival rates may increase while inflammation reduces. They strongly recommended combined treatment to prevent sepsis. In this study, diabetic rats were not used, which may have rendered more realistic results. Future studies should be planned to measure the success of combined treatment in a diabetic rat study. TGF-β was the only parameter which was similar in negative and positive control groups. It may be concluded that comparison of this parameter will not contribute to evaluation, at least in this setup. Apart from this parameter, the levels of all parameters measured with ELISA were similar among the treatment groups. MSC caused less histopathological fibrosis compared to all treatment groups, and we believe that similarity of results between Group MSC and antibiotherapy groups should be noted. All treatment groups were significantly different from the untreated positive control group (Group 1) in terms of inflammatory parameters and this is not associated with eradication only. Prevention of inflammation is thought to stem from the immunomodulatory effects of MSC. Additionally, while daptomycin is the strongest known antibiotic against MRSA, it increased all inflammation parameters, especially fibrosis. This may be due to a possible side effect apart from the healing process. The significantly lower fibrosis in the MSC group may be considered an additional positive effect achieved by limiting inflammation. In our current study, Caspase-3 levels were negative among all MSC groups with no similarity to the treatment groups. The systemic effects of MSC may have contributed to this result. In the literature, a variety of studies mention the anti-inflammatory or inflammation-limiting properties of MSC, whose response to pathogens is akin to that of the natural immune cells [22]. MSC migrate to the injury site and exert paracrine effects by secreting a range of soluble mediators, which simulate angiogenesis, remodeling and immune cell activation [23]. At the same time, they actively contribute to bactericidal activity [24]. This reaction initially begins with a series of reactions like receptor identification, signal conduction and specific inflammation. The immune process involves suppression of T cells, macrophage activation, neutrophil aggregation, collagen synthesis, fibroblast proliferation, platelet activation, fibrinolysis and angiogenesis regulation [25]. All these processes speed up infection healing and improve clinical status accompanying the wound healing process. In the literature, there are various studies similar to ours. Kong et al. [26] added MSC administration to linezolid treatment in a rabbit model of MRSA-induced pneumonia and showed that MSC-linezolid combination treatment was superior to linezolid treatment only. In addition, inflammatory markers such as IL-8, IL-6, TNF, CRP, and IL-10 were found to decrease dramatically in MSC-linezolid treated group of animals compared to the linezolid group. Extensive in vitro studies showed that MSCs can suppress proliferation of T and B cells by inhibiting cell division [27]. This immunomodulatory potential of MSCs is associated with cell-to-cell interactions and a number of soluble factors, such as NO in T cells (28). Although the systemic immunosuppressive characteristics of MSCs in humans and animals was reported in various disease models, these immunomodulatory effects have not been observed in vivo. Therefore, further studies in animals and humans are needed to evaluate the use of MSC as immunotherapy. Although this study was not as effective as the others suggesting MSCs may be an alternative to antibiotherapy, this is one of the first studies providing data on wound infection and antibacterial efficacy of MSC, especially in diabetic rats. The increased vascularity and tissue repair, more limited inflammation, and 60% (6/10 rats) eradication success in MSC-treated rats compared to other treatment options should not be overlooked. The results are associated with many variables, such as infection control, wound healing, inflammation regulation, and different processes in diabetic tissue. Data obtained in our study are considered to positively contribute to the final outcome about the use of MSC.

Limitations

This is an experimental preliminary study and was carried out in the laboratory. It is not a clinical trial. New clinical advanced phase studies are needed. We investigated the efficacy of stem cell administration for MRSA in diabetic rats only. The effect of mesenchymal stem cell administration on non-diabetic rats can be investigated. In addition, research on the effectiveness of mesenchymal stem cell administration on other pathogenic bacteria are needed.

Conclusion

Even though we did not achieve the same level of success with the other, similar studies investigating MSC treatment as a new alternative to antibiotherapy, it should be kept in mind that this study involved wound infection in diabetic rats. Inflammation was notably limited. This in vivo study of a rat infection model is a preliminary study and further, more comprehensive phase 1 and 2 studies to determine dosing and administration methods are needed.

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Does the combination of myo-inositol improve pregnancy outcomes in obese polycystic ovary syndrome women undergoing ovarian stimulation with clomiphene citrate?

Klomifen sitrat ile over stimülasyonu yapılan yüksek kilolu polikistik over sendromlu hastalarda myo-inositol kombinasyonu gebelik sonuçlarını iyileştirir mi?

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Abstract

Aim: Recent evidence has shown that Myoinositol (MI), a nutrient belonging to vitamin B family, may improve hormone profile, and the metabolic disorders accompanying polycystic ovary syndrome (PCOS), probably through the amelioration of preexisting insulin resistance. This study aimed to compare the ovulation and pregnancy outcomes of clomiphene citrate (CC) and its combination with MI in obese PCOS women with infertility.

Methods: Data concerning 80 obese, PCOS women with infertility who had undergone ovarian induction were retrieved from the institutional digital database. Controlled ovarian stimulation (OS) was performed using CC 100 mg or CC (100mg) + MI (4 g). The primary outcome measure of this study was the difference in the ovulation and the pregnancy rates of the women receiving CC (CC group) or CC+MI (combination group) for ovarian stimulation. There were 40 patients in each group.

Results: Endometrial thickness was significantly higher in the combination group than in the CC group (8.4 (1.1) mm vs. 7.7 (1.2) mm, *P*=0.006) and the number of the follicles>17 mm following OS was significantly higher in the combination group compared to that of the CC group (1.6 (0.5) vs. 1.4 (0.5), *P*=0.036). However, the rate of ovulation following OS [37 (92%) vs. 37 (92%), *P*=1.000] and the rate of the pregnancy were similar in CC and combination groups [6 (15%) vs. 11 (27.5%), *P*=0.172, respectively].

Conclusions: Compared to ovarian stimulation with CC alone, the combination provides a beneficial effect on endometrial thickness and the number of mature follicles. However, ovulation rates are similar with the two regimens. Although not statistically significant, there was a trend towards higher rates of pregnancy on CC+MI combination compared to CC alone. Further prospective and randomized trials are required to clearly address the role of the MI in management of the PCOS women with infertility.

Keywords: Polycystic ovary syndrome, Ovarian stimulation, Clomiphene citrate, Myo-inositol

Öz

Amaç: Güncel kanıtlar, B vitamini ailesine ait bir besin maddesi olan miyoinositolün (MI), muhtemelen önceden var olan insülin direncinin iyileştirilmesi yoluyla hormon profilini ve polikistik over sendromuna (PKOS) eşlik eden metabolik bozuklukları düzenleyebileceği gösterilmiştir. Bu çalışmada klomifensitrat (CC) ile CC ve MI kombinasyonunun infertil obez PKOS'lu kadınlarda ovülasyon ve gebelik sonuçları üzerine etkisinin karşılaştırılması amaçlandı.

Yöntemler: Obez ve infertil 80 PCOS hastası kadın dijital veri tabanı aracılığıyla çalışmaya alındı. Kontrollü over stimülasyonu (OS), CC (100 mg) veya CC (100mg) + MI (4 g) kullanılarak yapıldı. Bu çalışmanın birincil sonuç ölçütü, ovülasyondaki fark ve overstimülasyonu için CC (CC grubu) veya CC + MI (kombinasyon grubu) alan kadınların gebelik oranları olarak alındı. Her bir grupta 40'ar hasta bulunmaktaydı.

Bulgular: Kombinasyon grubunda endometriyal kalınlığın (8,4 (1,1) mm ve 7,7 (1,2) mm, *P*=0,006) ve 17 mm'den büyük folikül sayısının (1,6 (0,5) vs. 1,4 (0,5), *P*=0,036) CC grubundan anlamlı olarak daha yüksek olduğu görüldü. Bununla birlikte, OS'yi takiben yumurtlama oranı [37 (%92) vs. 37 (%92), *P*=1,000] ve hamilelik oranı [6 (%15) vs. 11 (%27,5), sırasıyla *P*=0,172] açısından CC ve kombinasyon grupları benzerdi.

Sonuç: Sadece CC ile overstimülasyonu ile karşılaştırıldığında, CC + MI kombinasyonunun endometriyal kalınlık ve olgun folikül sayısı üzerinde olumlu etkileri olduğu görüldü. Ancak, yumurtlama oranları her iki rejimde birbirine benzerdi. İstatistiksel olarak anlamlı olmamakla birlikte, CC + MI kombinasyonunda sadece CC ile karşılaştırıldığında daha yüksek gebelik oranları yönünde bir eğilim vardı. İnfertilitesi olan PKOS'lu kadınların yönetiminde MI'nın rolünü açıkça ele almak için ileriye dönük ve randomize çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Polikistik over sendromu, Over stimülasyonu, Klomifensitrat, Miyoinositol

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Introduction

Polycystic ovary syndrome (PCOS) affects 4% to 18% of women of reproductive age and is one of the leading causes of women infertility [1]. PCOS is characterized by anovulation, hyperandrogenism, hirsutism and polycystic ovaries on ultrasonographic examination. This syndrome presents with several manifestations, including reproductive disorders, metabolic features, and psychological squeals [2]. Dyslipidemia and insulin resistance are common in women with PCOS, and it has been shown that these women are at a higher risk of developing type-2 diabetes than their age and weight-matched counterparts without PCOS [3]. Compensatory hyperinsulinemia resulting from the insulin resistance plays a critical role in the pathophysiology of PCOS [4]. The synergistic action of the insulin with luteinizing hormone (LH) increases androgen production from theca cells, which in turn leads to masculine phenotypic changes [5]. Male phenotypic changes existing in women with PCOS eventually affect psychological health. Anxiety, depression, and negative body image are frequent in PCOS women.

Obesity and insulin resistance also contribute to anovulation and menstrual irregularities in women with PCOS [6]. This syndrome is currently recognized as the leading cause of anovulatory infertility accounting for >80% of all cases [7]. It has been reported that 90% to 95% of the women referred for infertility treatment have PCOS [8]. In addition, the time to conception is increased in women with PCOS. The response to assisted reproductive technologies are also reduced in women with PCOS [9,10]. Obesity is observed in almost 90% of the infertile PCOS patients and reduces the success of infertility treatment [8].

Clomiphene citrate (CC) is a nonsteroidal selective estrogen receptor modulator and has long been assumed as the standard first-line agent in ovulation induction of PCOS patients due to the satisfying ovulation rates of 85% and pregnancy rates exceeding 35% [11]. CC is also used for ovulation induction in many other infertility problems [12]. Recent evidence has shown that myoinositol (MI), which is a nutrient belonging to vitamin B family, may improve hormone profile, and the metabolic disorders accompanying PCOS, probably through the amelioration of preexisting insulin resistance [13,14]. MI has also been shown to increase estradiol and thus can be used to eliminate the symptoms arising from decreased estrogen in PCOS [15]. A number of trials have revealed that D-chiro-inositol, which is a derivate of MI, increases the ovulation frequency and suppresses hyperandrogenism [16].

We hypothesized that implementation of MI in conjunction with CC might improve the outcomes expected from ovulation induction compared to CC alone. The present study, therefore, aimed to compare ovulation induction with CC alone or in combination with MI in obese PCOS patients.

Materials and methods

Subjects

Data concerning 80 obese, PCOS patients with infertility undergoing with ovarian stimulation (OS) in gynecology department of a tertiary center, between January 2016 and January 2019, were retrieved from the institutional digital database. Inclusion criteria for the current retrospective cohort study were as follows: age between 18 and 40 years, presence of PCOS according to Rotterdam criteria, ovarian stimulation in conjunction with either CC or CC + MI combination, and having a body mass index (BMI) ≥30 kg/m^2. The diagnosis of PCOS was based on the Rotterdam criteria, and accordingly patients having the two of the following three features: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) polycystic ovaries compatible with PCOS [17].

Sample size calculation was performed for two independent groups. We used the mean values of endometrial thickness which was reported in a study conducted by Nakamura et al. [18]. According to power analysis results, with 0.05 alpha error and 80% power level, each group had to contain at least 37 patients.

The total of 40 patients who received CC alone were defined as the "CC group" and the remaining 40 patients who received CC + MI combination were defined as the "combination group".

Written informed consents for inclusion into the study were obtained from all patients. The study protocol was approved by the Acibadem Mehmet Ali Aydınlar University Ethical Committee (2019-15/1) and the study was performed in accordance with the most recent version of the Helsinki Declaration guidelines.

Detailed medical history was obtained from all patients, and each patient underwent a gynecologic examination. Initial workup, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, prolactin, and thyroid-stimulating hormone (TSH) levels was conducted the 3rd day of the menstrual cycle. Hysterosalpingography was performed to confirm the tubal patency.

Ovulation stimulation

Controlled ovarian stimulation was performed using CC (Clomen®; KocakFarma, Turkey) 100 mg (CC group) or CC (100mg) + MI (4 g) (combination group) from days 3 to 7 aiming at least one mature follicule > 17mm in diameter. Follicular growth and endometrial thickness were evaluated with transvaginal ultrasound during stimulation. When the presence of at least one mature follicle was confirmed, 250 μg of recombinant human chorionic gonadotropin (Ovitrelle®; Merck-Serono, Italy) was administered to trigger ovulation.

The primary outcome measure of this study was the difference in the ovulation and the pregnancy rates of the women receiving CC or CC+MI for ovarian stimulation.

Statistical analysis

All analyses were performed with SPSS v21. Shapiro-Wilk test was used for evaluation of normality. Data are given as mean (standard deviation) or median (minimum-maximum) for continuous variables regarding normality. Categorical variables are presented as frequency and percentage. Comparison of the normally distributed variables (endometrial thickness and >17 m follicle count) was performed using the Student's t-test. Non-normally distributed variables (age, BMI, infertility duration, FSH, LH, prolactin, and TSH) were compared using the Mann Whitney U test. Chi-square test was used to compare the

categorical variables. A *P*-value <0.05 was considered statistically significant.

Results

A total of 80 women with PCOS [median age 25 (19-34) years] and obesity [median BMI 32 (30-37) kg/m^2] were enrolled in this retrospective cohort study. The median duration of infertility was 15 (8-32) months. Forty women received CC, and other forty received CC+MI for ovarian stimulation. Demographic characteristics and laboratory measurements of the study groups are presented in Table 1. There were no significant differences between the two groups with respect to age, BMI, duration of infertility, and laboratory measurements. Anovulation before ovarian stimulation was recorded in 24 (60%) women in the CC group and in 22 (55%) women in the combination group (*P*=0.651).

As shown in Table 2, endometrial thickness was significantly higher in the combination group than in the CC group (8.4 (1.1) mm vs. 7.7 (1.2) mm, *P*=0.006) and the number of follicles >17 mm following OS was significantly higher in the combination group compared to that in the CC group (1.6 (0.5) vs. 1.4 (0.5), *P*=0.036). However, the rate of the ovulation following OS [37 (92%) vs. 37 (92%), *P*=1.000] and the rate of the pregnancy were similar in CC and combination groups [6 (15%) vs. 11 (27.5%), *P*=0.172, respectively].

Correlation analysis revealed that there was no significant relationship between endometrial thickness and presence of the pregnancy (*P*=0.265). However, the number of the follicles >17 mm following OS was significantly correlated with the presence of the pregnancy (*P*=0.001).

Table 1: Demographic features and the laboratory measurements of the study group

	OS with CC n=40	OS with CC+MYO n=40	*P*-value
Age, years	25 (21-32)	25 (19-34)	0.755
Body mass index, kg/m2	32.2 (30.3-37.0)	32.0 (30.0-36.7)	0.129
Infertility duration, months	15 (8-32)	15 (9-24)	0.845
FSH, mIU/ml	5.4 (3.4-9.9)	5.6 (3.4-9.8)	0.478
LH, mIU/ml	9.7 (6.0-16.0)	10.4 (5.6-17.0)	0.112
Estradiol, pg/ml	43.5 (21.4-72.2)	43.4 (22.1-67.0)	0.689
Prolactin, ng/ml	11.1 (5.5-18.7)	10.4 (6.0-18.7)	0.857
TSH, uIU/ml	3.2 (0.9-5.2)	2.6 (0.9-5.2)	0.901
Pretreatment anovulation, n	24 (60%)	22 (55%)	0.651

Data given as mean (standard deviation) or median (minimum - maximum) for continuous variables regarding normality and frequency (percentage) for categorical variables, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, OS: Ovarian stimulation, TSH: Thyroid-stimulating hormone

Table 2: Comparison of the outcomes of the ovarian stimulation in the two groups

	OS with CC n=40	OS with CC+MYO n=40	*P*-value
Endometrial thickness , mm	7.7 (1.2)	8.4 (1.1)	0.006
Follicles >17 mm, n	1.4 (0.5)	1.6 (0.5)	0.039
Ovulation following OS, n	37 (92%)	37 (92%)	1.000
Pregnancy, n	6 (15%)	11 (27.5%)	0.172

Data given as mean (standard deviation) and frequency (percentage) for categorical variables, OS: Ovarian stimulation

Discussion

This study was based on the hypothesis that implementation of MI in conjunction with CC, would improve the outcomes expected from ovulation induction compared to CC alone. Our findings demonstrate that compared to ovarian stimulation with CC alone, CC+MI combination provides a beneficial effect on endometrial thickness and the number of the mature follicles. However, ovulation rates are similar with the two regimens. Although not statistically significant, there was a trend towards higher rates of pregnancy on CC+MI combination compared to CC alone.

Oligo or anovulation is one of the cardinal features of the PCOS. Consequently, menstrual irregularities also accompany PCOS frequently; however, they can be masked by the use of oral contraceptive drugs. The endocrine and metabolic abnormalities existing in patients with PCOS may also deteriorate uterine function and lead to abnormal endometrial cellular proliferation which in turn causes challenges in implantation. PCOS accounts for >80% of all cases with anovulatory infertility and eugonadotrophic hypogonadism. Pregnancy usually takes longer than expected in PCOS women. However, population-based studies have revealed that lifetime fertility is likely not impaired in these women [19].

Weight loss and drugs that induce monofollicular ovulation are the primary treatment of infertility related to PCOS. CC followed by the exogenous gonadotropins in conjunction with intrauterine insemination (IUI) is the most common treatment in PCOS related infertility [20]. Assisted reproductive techniques, including in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are reserved for women in whom IUI has failed [21]. Controlled stimulation is challenging in PCOS and resistance to stimulation is more frequent [22]. Oocyte quality and maturity, which complicates the adoption of assisted reproductive techniques, may also be compromised in PCOS [23].

Clomiphene citrate has selective estrogen receptor modulator properties and it is by far the most commonly used drug for infertility worldwide. While ovulation rates with CC are about 85%, pregnancy rates range between 35% to 40% [24]. The divergence between the ovulation rates and the pregnancy rates is attributed to the peripheral anti-oestrogenic actions of CC on endometrial development and cervical mucus [18, 25]. A recent randomized study conducted by Ozay et al. [26] revealed that MI has improved pregnancy rates in PCOS patients undergoing controlled ovarian hyperstimulation with recombinant FSH and IUI. Another trial by Benelli et al. [15] has documented that combination therapy with MI and D-chiro inositol in young overweight PCOS women leads to a significant reduction in LH, free testosterone, fasting insulin, and HOMA index and increases 17-beta-Estradiol levels. With this in mind, we hypothesized that combining MI with CC could ameliorate the pregnancy outcomes obtained with CC alone, through the suppression of the peripheral anti-estrogenic actions of the CC.

This retrospective cohort study, which investigated the impact of the CC + MI combination on ovulation and the pregnancy outcome in obese PCOS women, demonstrates that the addition of MI to CC for ovulation induction improves endometrial thickness and the number of the mature follicles. Ovulation rate, however, was similar in the two regimens. Although not statistically significant, adding MI to CC also led to a favorable trend in pregnancy rate. Nevertheless, despite the combination of MI with CC, the pregnancy rate was still lower than those indicated in previous data [27]. A possible explanation for this might be that we enrolled only the first cycles after the induction with CC and CC+MI. Findings of the present study also demonstrated a significant correlation between the mature follicle count and presence of the pregnancy. As follicle count reflects the normalization of the hormone levels, the close relationship between the follicle count and the pregnancy rate

observed in this study may be explained by the improvement in the hormone profile, which is essential for both ovulation and implantation [28-30]. Despite the lack of the statistical significance the trend towards a higher pregnancy rate in the combination group might be explained by the increase of mature follicle count of this group. We consider that prospective data, including more cycles of the ovulation induction with CC+MI combination and a larger sample size may provide additional evidence concerning the benefits of this combination, not only in mature follicle count but also in the ovulation and the pregnancy rates.

Limitations

There are some limitations concerning the presents study. First, we analyzed the retrospective data of the post-induction period and therefore could not provide the causal relationships. Second, we could not present data regarding the degree of the insulin resistance, HOMA-IR and the androgen levels. We suggest that prospective, randomized trials with larger sample size are required to clearly address the role of the MI in PCOS women with infertility.

Conclusions

This retrospective analysis demonstrates that compared to CC alone, CC+MI combination improves endometrial thickness and oocyte maturation in obese PCOS women undergoing ovarian induction. However, our findings failed to show any additional benefit of MI in terms of the ovulation rate and the pregnancy rate, although there was a trend towards a higher rate of pregnancy in the combination group. Further prospective and randomized trials are required to clearly address the role of the MI in management of the PCOS women with infertility.

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Evaluation of changes in meibomian glands in polycystic ovary syndrome by noncontact infrared meibography

Polikistik over sendromu'nda meibomian bezlerdeki değişikliklerin kontakt olmayan kızılötesi meibografi ile değerlendirilmesi

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Abstract

Aim: The human meibomian gland contains several overexpressed genes that are related to lipid dynamics and glandular structure. Meibomian gland dysfunction (MGD) was found to be associated with significant changes in these genes. The aim of this study was to compare the changes in meibomian glands in patients with polycystic ovary syndrome (PCOS) and healthy young women by non-contact meibography for the first time.

Methods: A total of 58 right eyes belonging to 28 patients with PCOS and 30 healthy women in the control group were included in this case-control study. The ocular surface and eyelid margins were evaluated with slit lamp examinations. Participants were asked about dry eye symptoms. Schirmer and tear film break time (TBUT) tests were performed consecutively. The morphology of the meibomian glands was observed with non-contact meibography and scored with the meiboscore.

Results: Dry eye symptoms were more prevalent and average TBUT was shorter in PCOS patients (P=0.001, P=0.02, respectively). However, Schirmer test results did not differ among the PCOS and control groups (P=0.47). The meiboscores for upper eyelids and total eyelids were significantly higher in the PCOS group (P=0.001 and P=0.004, respectively), suggesting that PCOS is accompanied by meibomian gland dropout.

Conclusion: In this study, we found that the morphological status of the meibomian gland and the ocular surface were in worse condition in PCOS patients than in the normal controls, and we successfully observed the loss of meibomian gland in PCOS patients through non-contact meibography.

Keywords: Dry eye, Meibography, Meibomian gland dysfunction, Polycystic ovarian syndrome

Öz

Amaç: İnsan meibomian bezi lipid dinamikleri ve glandüler yapı ile bağlantılı, aşırı eksprese edilmiş birkaç gen içerir; ve meibomian bez disfonksiyonunun (MBD) bu genlerdeki önemli değişikliklerle ilişkili olduğu bulunmuştur. Bu çalışmanın amacı, ilk kez temassız meibografi ile polikistik over sendromlu (PKOS) hastalarda ve sağlıklı genç kadınlarda meibomian bezlerindeki değişiklikleri karşılaştırmaktır.

Yöntemler: Bu olgu-kontrol çalışmasına PKOS'lu 28 hasta ve kontrol grubundaki 30 sağlıklı kadının toplam 58 sağ gözü dahil edildi. Oküler yüzey ve göz kapağı kenarları yarık lamba incelemeleri ile yapıldı. Katılımcılara kuru göz semptomları hakkında sorular soruldu. Schirmer ve gözyaşı kırılma zamanı (GKZ) testleri sırasıyla tamamlanmıştır. Meibomian bezlerinin morfolojisi temassız meibografi ile gözlendi ve meiboscore kullanılarak skorlandı.

Bulgular: PKOS'da kuru göz semptomları daha sık görülmüştür (P=0,001). PKOS'da ortalama GKZ daha kısaydı (P=0,02). Bununla birlikte Schirmer test sonuçları PKOS ve kontrol grubu arasında farklı değildi (P=0,47). Ek olarak, PKOS'un meibomian bez kaybı ile birlikte olduğunu düşündürecek şekilde, üst göz kapakları ve toplam göz kapakları için meibomian bez skorları, PKOS'da kontrol grubundan anlamlı olarak daha yüksekti (sırasıyla, P=0,001 ve P=0,004).

Sonuç: Bu çalışmada, PKOS'lu hastalarda meibomian bezinin morfolojik durumunun ve oküler yüzey durumunun normal kontrollerden daha kötü olduğunu ve PKOS'lu hastalarda temassız meibografi ile meibomian bezinin kaybını başarıyla gözlemledik.

Anahtar kelimeler: Kuru göz, Meibografi, Meibomian bezi disfonksiyonu, Polikistik over sendromu

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Introduction

Sex hormones have been proven to influence tear production and function [1,2]. Hormone studies have shown that the effect of sex hormones on tear production and goblet cell density and on meibomian gland functions and structures may cause dry eyes [3]. Supporting this hypothesis, androgen receptors have been identified on the surface of the meibomian glands [4]. Lipid, produced by the meibomian glands, is an essential component of the tear film and prevents evaporation of the aqueous layer while reducing surface tension of the tear film, and ensuring stability of the lacrimal layer [5].

Although previous studies have shown a correlation between meibomian gland dysfunction (MGD) and polycystic ovarian syndrome (PCOS), little is known regarding the meibomian gland morphology in this syndrome. The recent development of noninvasive meibography and scoring systems for meibomian glands has provided important insight into meibomian gland structure and function. The objective of this study was to evaluate, for the first time, the meibomian glands of PCOS patients with meibography and to compare the results of meibography analysis in normal subjects and patients with PCOS.

Materials and methods

Ethics committee approval (Kafkas university faculty of medicine, 2015/80576354-050-99/120) was obtained prior to the study. We included all patients over 18 years of age who were treated for PCOS between November 2015 and December 2016. All subjects were given informed consent forms to participate in this case-control study.

Participants

Twenty-eight female patients monitored for PCOS at Kafkas University Medical Faculty Obstetrics and Gynecology clinic were selected as the study group, while 30 healthy women volunteers examined by the eye clinic were included as the control group (n_{Total}=58). Patients who were referred to Kafkas University Medical Faculty Obstetrics and Gynecology clinic were diagnosed with PCOS in accordance with the Rotterdam 2003 criteria, in which at least two of the following criteria were needed: oligo-amenorrhea (more than 45 days between menses or eight or fewer menses per year), presence of hyperandrogenism clinical hirsutism (hirsutism, acne, acanthosis nigricans, androgenic alopecia) or elevated androgens in laboratory tests (increased levels of serum total and free testosterone), and ultrasonographic polycystic ovarian appearance (2-9 mm diameter, 12 or more follicles and/or increased ovarian volume (>10 ml) [6].

All patients had their luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio, prolactin levels, thyroid functions, dehydroepiandrosterone sulfate, 17 hydroxyprogesterone and total testosterone levels analyzed and patients with hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease, Cushing syndrome, and those who used hormonal medications, glucocorticoids, ovulation inducing agents, and antiandrogens in the past six months were excluded from the study. Additionally, patients who used contact lenses, diuretics, steroids or antidepressants for systemic or ocular treatment, those with systemic diseases such as diabetes mellitus or thyroid disease, history of ocular or refractive surgery, blockage of the nasolacrimal channel or ocular surface problems were excluded from the study.

Study protocol

In addition to the study group, a detailed ophthalmologic examination was meticulously performed to the control group subjects by a masked observer (HC) between the third and fifth day of the menstrual cycle, which included the following: Assessment of lid margin abnormalities, the Schirmer 1 test, tear film break-up time (TBUT), and grading of meibomian gland status. Each participant completed an ocular surface disease index (OSDI) questionnaire with 12 questions to evaluate ocular surface symptoms. An interval of at least 10 minutes was assured between TBUT and the Schirmer test. The MGD was characterized based on the existence of anterior or posterior displacement relative to the mucocutaneous junction, irregular lid margin, vascular engorgement and obstruction of the meibomian gland orifices [7]. Meibomian glands were evaluated by a noncontact meibography system (Sirius Scheimpflug Camera, Schwind, Germany), and images were captured by noncontact meibography system after inverting both upper and lower of eyelids. Partial or complete loss of the meibomian glands for each eyelid were graded with meiboscores as previously reported: Grade 0 (no loss of meibomian glands), grade 1 (loss of 1/3 of the total area of meibomian glands), grade 2 (meibomian glands loss between 1/3 and 2/3), and grade 3 (meibomian glands loss ≥2/3). Meiboscores for the upper and lower eyelids were summed up to make up a total of 0 to 6 scores [8].

Statistical analysis

The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) program version 10.0 (SPSS Inc., Chicago, IL, USA). Independent samples t-test and Chi-square tests were used for statistical analysis. Pearson's correlation test was used for correlation between parameters. *P*-value <0.05 was considered statistically significant.

Results

A total of 58 right eyes belonging to 28 PCOS patients and 30 control group subjects were included in this study. The average age of patients in the PCOS group was 25.0 years (minimum 19, maximum 41), while the average age of individuals in the control group was 26.4 years (minimum 19, maximum 31). There was no statistically significant difference between PCOS group and control group in terms of age (*P*=0.32). The demographic and metabolic parameters in the groups are given in Table 1.

The OSDI scores between PCOS patients and normal individuals was found significantly different (*P*=0.001). The incidence of MGD was 22.6% in the PCOS group and 10% in the control group (*P*=0.04). The Schirmer test was measured as 20.1 mm in the study group and 17.7 mm in the control group. The average Schirmer measurements were similar in both groups (*P*=0.47). TBUT was 12.1s in the PCOS group and 14.6 in the control group, the difference was statistically significant (*P*=0.02). The average meiboscore of the upper lids, the lower lids and total (upper eyelid plus lower eyelid) lids in PCOS were

1.3 (range, 0–3), 0.6 (range, 0–2), and 1.1 (range, 0–2.5), respectively. The average meiboscore of the upper lids, the lower lids and total (upper eyelid plus lower eyelid) lids in healthy subjects were 0.5 (range, 0–1), 0.4 (range, 0–2), and 0.4 (range, 0–1.5), respectively. The meiboscores for upper eyelids and total eyelids were significantly higher in patients with PCOS (P=0.001 and P=0.004, respectively) (Table 2). The degree of each image is noted at the upper left. Representative images of meibomian glands in the upper and lower eyelids are seen in patients obtained by no-contact meibography from PCOS (Figure 1A, 1B) and control group (Figure 1C, 1D). Dropout, distortion, and shortening of meibomian gland ducts are apparent in the upper and lower eyelids in patients from with PCOS.

Table 1: Demographic and metabolic parameters

Variable	PCOS Group (n=28)	Control Group (n=30)	*P*-value
Age, years	26.4 (5.8)	25.0 (4.2)	0.32
BMI, kg/m^2	25.4 (7.3)	23.5 (2.6)	0.21
VA (Snellen)	1,0	1,0	0.49
SE (diopter)	0.9 (1.1)	1.1 (0.9)	0.46
Plasma glucose (mg/dl)	89.7 (11.6)	87.1 (5.7)	0.56
FSH (IU/L)	6.0 (1.4)	6.4 (1.8)	0.33
LH (IU/L)	10.9 (8.0)	4.9 (2.1)	0.001*
E2 (pmol/L)	58.8 (47.2)	58.5 (20.1)	0.17
DHEAS (µmol/L)	218 (113)	209 (104)	0.65
Total testosterone (ng/mL)	55.6 (2.4)	28.1 (1.2)	0.001*

PCOS: Polycystic Ovary Syndrome, BMI: Body Mass Index, VA: Visual Acuity, SE: Spherical Equivalent, DHEAS: Dehydroepiandrosterone sulfate, E2: Estradiol, FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, *P<0.05 was considered statistically significant

Table 2: Ocular surface and meibomian gland clinical test results

Variable	Control Group (n=30)	PCOS Group (n=28)	*P*-value
OSDI	10.5 (9.4)	28.1 (18.5)	0.001*
Schirmer test score (mm)	17.7 (3.4)	20.1 (7.4)	0.47
TBUT (s)	14.6 (3.2)	12.1 (3.5)	0.02*
The incidence of MGD (%)	10.0	22.6	0.04*
Meiboscore (Upper eyelid)	0.5 (0.5)	1.3 (1.1)	0.001*
Meiboscore (Lower eyelid)	0.4 (0.6)	0.6 (0.6)	0.33
Meiboscore (Total)	0.4 (0.5)	1.1 (0.6)	0.004*

PCOS: Polycystic Ovary Syndrome, MGD: Meibomian Gland Dysfunction, OSDI: Ocular Surface Disease Index, TBUT: Tear Break-up Time, *P<0.05 was considered statistically significant

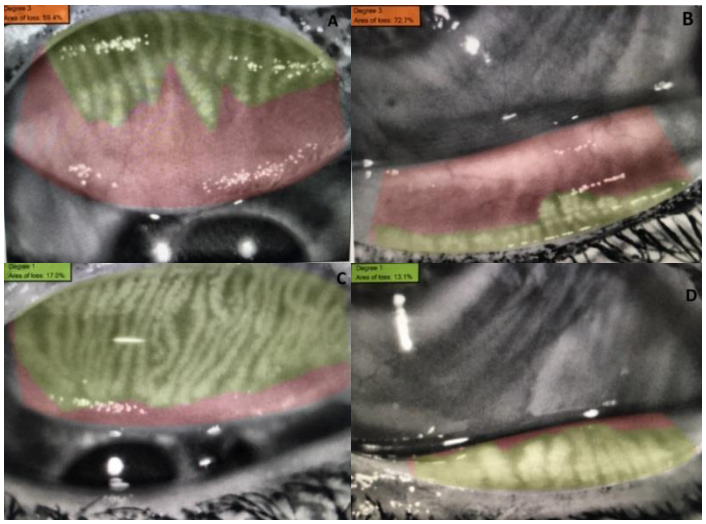

Figure 1: Representative images of meibomian glands in the upper and lower eyelids in patients from PCOS (A, B) and control group (C, D) obtained by non-contact meibography.

Discussion

Dry eye disease, a multifactorial disease with the potential to cause injury to the ocular surface, is characterized by visual impairment, feeling of discomfort and variability of the tear film according to the DEWS classification reported in 2007 [9]. The meibomian glands are vertically placed large sebaceous glands within the rim of the upper and lower lids and dysfunction of these glands is present in nearly 2/3 of all dry eye cases. Increased viscosity of meibum secretions and increased keratinization of ductal epithelium causes MGD [10]. Meibography allows observation of meibomian gland morphology in silhouette through illumination of the eyelids from the skin side, thereby identifying morphological abnormalities. This procedure shows narrowing or blockage of the gland orifice, distention of the glands, dilatation of the channels or loss of the gland structures in MGD [11]. Previous studies have shown correlations between contact lens use, phlyctenular keratitis, rosacea, diabetes and chronic smoking with variations in meibomian gland morphology [11-14]. To the best our knowledge, this is the first study to appraise the lid margin and meibomian glands, particularly through the use of a noncontact meibography technique in PCOS patients.

It is well-known that hormonal regulation has a significant effect on meibomian gland biology [10]. Previous studies identified expression of androgen receptors, estrogen receptors, and progesterone receptors in human meibomian gland epithelial cells [15]. Androgens primarily act on acinar epithelial cells in sebaceous glands, which contain both androgen receptor mRNA and protein in their nuclei. Acinar cells respond to androgens by increasing the transcription of multiple genes and synthesizing proteins that enhance the secretion of lipids [16]. Additionally, insulin, essential for the desired sebaceous gland activity, is known to induce glandular cell proliferation and lipid secretion [12]. Although changes in the meibomian glands have been shown with dry eye tests in PCOS patients, to the best of our knowledge, the meibomian gland morphology in PCOS has not been investigated with meibography [17,18]. However, a recent study has showed that the human meibomian gland comprises many highly expressed genes associated with lipid dynamics and glandular structure, and MGD was found to be associated with significant alterations in these genes [19].

PCOS, characterized by hyperandrogenic chronic anovulation, is a complex metabolic and endocrine disorder. The U.S. Department of Health and Human Services anticipated that between 1 in 10 and 1 in 20 women of childbearing age suffer from PCOS [20]. Laboratory investigation of patients yielded hyperandrogenemia characterized by increases in ovarian and adrenal sourced androgenic hormones in addition to increased LH levels and LH/FSH ratio. Nearly 25-60% of cases have hyperinsulinemia and insulin resistance, and 15-20% of PCOS patients may have mildly elevated prolactin levels without functional prolactinoma [21,22]. Clinically, PCOS is generally diagnosed after cosmetic complaints such as hirsutism, or menstrual dysfunction and infertility. Dry eye symptoms are triggered by hormonal imbalance encountered at considerable rates [23]. Although meibomian gland function is shown to be significantly disrupted in PCOS patients, the full underlying mechanism is still unknown [17,18].

Previous studies reported controversial results. Bonini et al. [23] showed that TBUT was significantly reduced in PCOS patients, with a contrary increase in the density of conjunctival goblet cells. They proposed that the itchy-dry eye symptoms encountered in PCOS were a separate clinical entity sharing many characteristics of dry eye and ocular allergy diseases. A series by Yavaş et al. [17] found TBUT significantly reduced, with no significant difference in terms of Shirmer test results, goblet cell count and rose Bengal score between the control and

PCOS patient groups. Baser et al. [18] showed that TBUT was significantly low in PCOS, while the incidence of pathologic TBUT values (<10s) was similar among PCOS and control groups. The differences in these results may be related to personal differences between patients like age and insulin resistance, and/or differences in androgen and estradiol levels. In this study, by using noncontact meibography, the morphology of the meibomian glands were compared in eyelids between patients with PCOS and healthy young adults. We found that PCOS is accompanied by significant loss of meibomian gland tissue, defined by reduced meibomian gland area and increased meibomian gland distortion.

Postmortem studies have shown differentiation of meibocytes and reduced cell cycles in aging meibomian glands, with a clear correlation between meibomian gland atrophy and age [24,25]. However, when males and females with similar advanced age are assessed, the incidence of abnormal eyelid and gland dropout is found higher in males compared to females. The proposed cause is hormonal differences [26]. A study by Mizoguchi et al. [27] assessed the morphology and function of meibomian glands in 15-year old middle school students and found that significant sex differences were apparent in the meiboscore (males:3.3, females:2.4). Machalińska et al. [25] proved that postmenopausal hormone treatment was among the independent predictors of meibomian gland loss in the healthy population.

In this study, MGD and OSDI scores were significantly higher and TBUT was significantly lower in PCOS patients than in the control women. When the morphology of meibomian glands was examined with the noncontact meibography system, it was found that changes to the meibomian gland morphology were significantly higher in patients with PCOS. These results indicate that MGD and tear lipid layer deficiency associated with structural changes may be one of the mechanisms underlying the chronic itchy-dry eye associated with PCOS.

Limitations

We believe that there are some limitations in our study. The most important of these is the limited number of cases and another one is the lack of long-term follow-up. It would be useful to extend and follow-up the study with more patients.

Conclusion

This study showed that ocular surface condition in PCOS patients was worse than those in normal controls and remarkable loss of meibomian gland was objectively indicated with meibography. In light of these results, we conclude that MGD and the loss of meibomian gland evaluation is important in female patients with advanced and persistent dry eyes and PCOS should be considered. The severity and mechanisms of chronic tear film dysfunction can vary between different diseases and conditions such as diabetes, rosacea, age or smoking. These differences should be kept in mind during patient management. Further prospective research is needed to prove the provocative impact of PCOS on the meibomian gland morphology.

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