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EDITORIAL

This year we are excited and proud to publish our 2nd issue with 20 articles. In this issue, there are 15 research articles, 2 reviews, and 3 case reports. As getting to know of our journal, we continue to receive articles from other countries. This journal appeals to all fields of health sciences and medicine. For this reason, we also accept articles from different branches of nursing, nutrition and dietetics, child development, health management, dentistry and health. The majority of our articles is in English and there are also articles in Turkish. With the next year, we will plan to publish all articles in English. In this way, we aim to both get more citations and get the ISI (Web of Science) index, which will open the way to enter indices such as SCI and SCI-E. Thanks for the authors for submitting articles that contribute to quality and science. Our goal is to reach better points with your support day by day. Sincerely yours.

Assoc. Prof. Dr. Aydın ÇİFCİ

Editor in Chief

EDİTÖRDEN

Bu yıl 2. sayımızı da 20 makale ile çıkartmanın heyecanı ve gururunu yaşıyoruz. Bu sayımızda 15 araştırma makalesi, 2 derleme, 3 olgu sunumu var. Dergimizin tanınırlığı arttıkça yurtdışından da makale almaya devam ediyoruz. Sağlık bilimleri ve tıbbın her alanına hitap eden bir dergidir. Bu nedenle dönem dönem hemşirelik, beslenme ve diyetetik, çocuk gelişimi, sağlık yönetimi, diş hekimliği ve sağlıkla ilgili farklı branşlardan da makaleler kabul etmekteyiz. Makalelerin çoğunluğu İngilizce, ayrıca Türkçe makaleler de var. Önümüzdeki yıl ile birlikte tüm makaleleri İngilizce yayımlamayı planlıyoruz. Bu sayede hem daha çok atıf almayı hem de SCI ve SCI-E gibi indekslere girmenin yolunu açacak ISI (Web of Science) indeksine girmeyi hedefliyoruz. Kaliteli ve bilime katkısı olan makaleleri gönderdikleri için yazarlara teşekkür ediyorum. Hedefimiz sizlerin de desteği ile her geçen gün daha iyi noktalara ulaşabilmek. Saygılarımla.

Doç. Dr. Aydın ÇİFCİ

Baş Editör

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JOURNAL OF HEALTH SCIENCES AND MEDICINE

Sağlık Bilimleri ve Tıp Dergisi

J Health Sci Med 2020; 3(2): 92-96

Research Article / Araştırma Makalesi

The relationship between serum homocysteine levels and development of coronary collateral circulation in patients with acute coronary syndrome

Akut koroner sendromlu hastalarda serum homosistein düzeyleri ile koroner kollateral dolaşım gelişimi arasındaki ilişki

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ABSTRACT

Aim: Homocysteine is an amino acid that plays a role in folate metabolism and inhibits endothelial cell proliferation which is important for angiogenesis. In this study, we aimed to investigate the relationship between serum homocysteine levels and coronary collateral development.

Material and Method: Consecutive 176 patients, with acute coronary syndrome and chronic total occlusion, were divided into two groups according to coronary collateral development. Rentrop 0 and 1 were regarded as group I and Rentrop 2 and 3 as group II.

Results: Plasma homocysteine levels were 18.2±7.0 µmol/L in the group I and 15.7±5.1 µmol/L in the group II. Univariate logistic regression analysis showed that mean platelet volume and homocysteine were associated with poor coronary collateral. Multivariate logistic regression analysis showed that homocysteine level was independently associated with poor coronary collateral circulation (OR 1.069 [95% CI 1.012-1.130]; p=0.018).

Conclusion: In this study clearly demonstrates that high serum homocysteine level is associated with poor collateral development in patients with acute coronary syndrome.

Keywords: Coronary artery disease, coronary collateral circulation, homocysteine

ÖZ

Amaç: Homosistein, folat metabolizmasında rol oynayan, anjiyogenez için önemli olan endotelyal hücre proliferasyonunu inhibe eden bir aminoasittir. Bu çalışmada serum homosistein düzeyiyle koroner kollateral gelişimi arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Kronik total oklüzyonu olan 176 hasta koroner kollateral gelişimine göre 2 gruba ayrıldı. Rentrop 0 and 1 olanlar grup I Rentrop 2 ve 3 olanlar grup II olarak kabul edildi.

Sonuç: Plazma homosistein düzeyleri grup I'de 18,2±7,0 µmol / L ve grup II'de 15,7±5,1 µmol / L olarak saptandı. Tek değişkenli lojistik regresyon analizi ortalama trombosit hacmi ve homosisteinin zayıf koroner kollateral ile ilişkili olduğunu gösterdi. Çok değişkenli lojistik regresyon analizi homosistein düzeyinin bağımsız olarak zayıf koroner kollateral dolaşım ile ilişkili olduğunu göstermiştir (OR 1,069 [%95 CI 1,012-1,130]; p=0,018).

Sonuç: Bu çalışmada yüksek serum homosistein düzeyinin akut koroner sendromlu hastalarda zayıf kollateral gelişim ile ilişkili olduğu açıkça görülmektedir.

Anahtar Kelimeler: Koroner arter hastalığı, koroner kollateral dolaşım, homosistein

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INTRODUCTION

Coronary collateral circulation (CCC) is a potential vessel that develops between different coronary arteries or sections of the same coronary artery to provide blood flow to the ischemic area in order to maintain the viability of the myocardium when a severe stenosis or total occlusion occurs that reduces blood flow in the coronary artery (1). Development of CCC plays a significant role in decreasing cardiovascular events and angina symptoms and preserving the myocardium from the ischemia (2-4). Coronary collateral vessels provide feeding of the ischemic myocardium by increasing blood supply when stenosis or complete occlusion of the coronary artery occurs. They play a crucial role in maintaining systolic function of the left ventricle in case of complete obstruction. Also previous studies have shown that collateral arteries also occur in patients without coronary artery disease (5). CCC does not develop in more than half of patients with coronary artery disease. Although the exact cause is unknown, genetic factors are thought to be responsible (6).

Homocysteine is an amino acid comprises of sulfide that plays a major role in folate metabolism and is produced by the demethylation of methionine (7). Homocysteine inhibits endothelial cell proliferation, which is important for angiogenesis. Nagai et al. (8) reported that hyperhomocysteinemia inhibited endothelial proliferation in vitro and angiogenesis in vivo. Therefore, we investigated that the relationship between homocysteine levels and the development of coronary collateral circulation in patients with acute coronary syndrome.

MATERIAL AND METHOD

The study population consisted of 176 consecutive patients with acute coronary syndrome and chronic total occlusion (CTO) in at least one major coronary artery detected during coronary angiography between June 2019 and December 2013. Coronary collateral status were graded from 0 to 3 according to the Rentrop's classification. Grade 0 = no visible filling of any collateral, grade 1= filling of the side branches of the artery, grade 2= partial filling of the epicardial vessel by way of collateral, grade 3= complete collateral filling of the epicardial vessel (9). Patients were divided into two groups according to coronary collateral development. Rentrop 0 and 1 were regarded as group I (poor collateral) and Rentrop 2 and 3 as group II (good collateral). In cases of discrepancy between the 2 reviewers on the collateral status, a third cardiologist was called in for a decision.

The exclusion criteria were the presence of any of the followings: acute/chronic renal failure, chronic obstructive lung disease, any malignancy, evidence of ongoing infection or inflammation, liver failure, previous history of heart failure, previous history of revascularization (percutaneous or surgical), moderate or severe valvular heart disease, peripheral arterial disease, hematological disorders, aortic dissection, pulmonary embolism, any thyroid and rheumatological disease.

Hemoglobin, white blood cell count, platelets, glucose, glycated hemoglobin (HbA1C), creatinine, cholesterol levels, and homocysteine were assessed. The homocysteine level was determined using a commercially available kit (Chromsystems Instruments & Chemicals GmbH Am Haag 12, 82166 Gräfelfing, Germany) by high-pressure liquid chromatography and fluorometric methods in blood samples with ethylenediaminetetraacetic acid.

Judkins technique was used for coronary angiography using a digital angiographic system (Siemens Axiom Artis zee 2011; Siemens Healthcare, Erlangen, Germany). All coronary arteries were visualized in at least two different projections. At least 50% stenosis was accepted as significant CAD. In cases of disagreement between the two calculations, a senior interventional cardiologist was consulted and a common consensus was obtained from the three operators.

Hypertension; defined as systolic blood pressure ≥140 mmHg and / or diastolic blood pressure ≥90 mmHg or use of antihypertensive drugs in at least two measurements. Diabetes mellitus; defined as fasting blood sugar ≥126 mg / dL or use of antidiabetic treatment. Hyperlipidemia; defined as fasting total cholesterol level ≥200 mg / dL or triglyceride level ≥150 mg / dL or use of lipid-lowering drugs. Smoking has been accepted as a smoker in the last 6 months.

Angiographic images were examined from coronary angiography unit records. Coronary angiographic records were examined by interventional cardiologists and stent restenosis; defined as a stenosis of more than 50% in the previously deployed stent or 5mm proximal or distal.

Transthoracic echocardiography (TTE) was performed for all patients within 48 hours after hospitalization (Vivid 9; GE Medical System, Horten, Norway). Left ventricular ejection fraction (LVEF) was measured using the Simpson method.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL). The chi-square test was used for comparison of categorical variables. Quantitative variables are expressed as the mean value ± SD for parametric variables, and median and quartiles for non-parametric variables. Continuous variables were analyzed for normal distribution using the Kolmogorov-Smirnov test and analyzed for homogeneity using the Levene tests. Comparisons of parametric values among groups were performed by the Student t-test and Mann-Whitney U test for non-parametric values. Categorical variables were compared with the chi-square test. Multiple logistic regression analysis was used to determine the effects of parameters on the development of collaterals. A two-tailed $p<0.05$ was considered significant.

Ethical Declaration

The study was carried out subsequent to receiving permission for its conduct from the presiding local Ethics Committee (Permission Granted 21.08.2019, Decision No. 2019.08.10).

RESULTS

A total of 176 patients, 120 in group I and 56 in group II,

were included in the study. The mean age of the patients were 62.7±13.2 years in first group and 66.7 ± 11.1 years in second group. There was no difference between the 2 groups in terms of body mass index, sex, hypertension, diabetes mellitus, smoking, dyslipidemia, blood pressure, and left ventricular ejection fraction. The mean age of the patients was higher in group II than group I (p= 0.04) (**Table 1**).

Table 1. Main clinical and angiographic features of the study population

	Group I (n=120)	Group II (n=56)	p
Age (years, mean ± SD)	62,7 ± 13,2	66,7 ± 11,1	**0,04**
Body mass index	26,1 ± 4,4	30,5 ± 2,5	0,16
Male sex	76 (63,3%)	37 (67,3%)	0,61
Hypertension	65 (55,1%)	27 (49,1%)	0,46
Diabetes mellitus	45 (38,1%)	18 (32,7%)	0,49
Smoking	50 (42,4%)	19 (34,5%)	0,32
Dyslipidemia	32 (27,1%)	22 (40,0%)	0,08
Systolic blood pressure, mm Hg	132 ± 26	125 ± 21	0,11
Diastolic blood pressure, mmHg	77 ± 15	76 ± 13	0,48
Ejection fraction (%)	48 ± 10	46 ± 9	0,36

SD: Standart deviation

The hematological and biochemical parameters of the groups are listed in Table 2. White blood cell (WBC) count, hemoglobin, platelets were similar in both groups. Mean platelet volume (MPV) was higher in the group I. There was no difference between the groups in terms of biochemical parameters except homocysteine. Plasma homocysteine levels were 18.2 ± 7.0 µmol/L in the group I and 15.7 ± 5.1 µmol/L in the group II. Homocysteine levels were higher in the group I compared to the group II (p= 0.02).

Tablo 2. Comparision of baseline blood features in the groups

	Group I (n=120)	Group II (n=56)	p
White blood cells (×10³ µL)	9.9±3.2	10.0±3.1	0.91
Hemoglobin (gr/dl)	13.7±1.9	13.4±1.9	0.28
Mean platelet volume (fL)	9.0±1.1	8.6±1.0	**0.03**
Platelets (×10³ µL)	245±81	225±80	0.13
Glucose (mg/dl)	153 (98-184)	142 (97-158)	0.57
Creatinine (mg/dl)	1.1±0.3	1.1±0.3	0.21
Üric acid (mg/dl)	5.6±1.4	5.8±1.6	0.34
Albumin (g/dl)	3.8±0.4	3.7±0.5	0.29
Total cholesterol (mg/dl)	194±60	179±60	0.14
LDL-C (mg/dl)	122±44	109±43	0.07
HDL-C (mg/dl)	40±11	39±9	0.62
Triglyceride (mg/dl)	176 (98-189)	138 (83-181)	0.16
Homocysteine (µmol/L)	18.2±7.0	15.7±.1	**0.02**
Fibrinogen (mg/dl)	427±134	419±106	0.67
Hs-CRP (mg/L)	6.9 (2.9-0.6)	7.4 (2.9-0.9)	0.24

LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, Hs-CRP: High sensitive C-reactive protein

Univariate logistic regression analysis showed that MPV and homocysteine were associated with poor coronary collateral (**Table 3**). Multivariate logistic regression analysis showed that homocysteine level was independently associated with poor coronary collateral circulation (OR 1.069 [95% CI 1.012-1.130]; P= 0.018) (**Table 3**).

Table 3. Logistic regression analysis

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age	1.026 (0.999-1.052)	0.055		
Dyslipidemia	1.792 (0.912-3.519)	0.090		
Glucose	0.998 (0.994-1.003)	0.424		
LDL-C	0.994 (0.986-1.001)	0.103		
MPV	1.374 (1.028-1.838)	**0.032**	1.335 (0.993-1.795)	0.056
Hs-CRP	1.031 (0.947-1.122)	0.482		
Homocysteine	1.074 (1.017-1.134)	**0.010**	1.069 (1.012-1.130)	**0.018**

MPV: mean platelet volume, LDL: low density lipoprotein cholesterol, Hs-CRP: high sensitive C-reactive protein, OR: odds ratio, CI: confidence interval

DISCUSSION

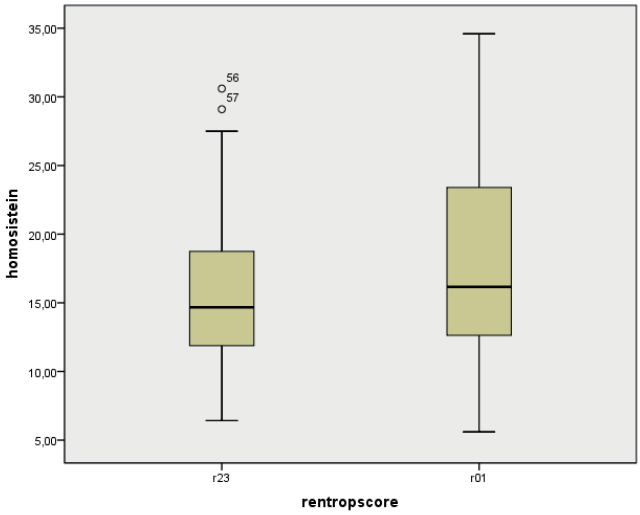

Figure 1. Comparison of serum homocysteine levels among Rentrop collateral grades

In the present study, we investigated the relationship between serum homocysteine levels and coronary collateral development in patients with chronic total occlusion. Our findings showed that serum homocysteine levels were higher in the poor collateral group than the good collateral group in patients with acute coronary syndrome and CTO. In addition, higher homocysteine levels were found as an independent predictor of poor collateral circulation.

Coronary collateral circulation plays an important role in maintaining systolic function of the left ventricle when stenosis or complete occlusion of the coronary artery occurs (10). The development of good collateral circulation may reduce the infarct area after total occlusion of the coronary artery and prevent systolic function of the left ventricle. Many clinical studies have shown that chronic hypoxia

trigger the activation of angiogenesis and arteriogenesis (8,11). The most important triggers for angiogenesis is shear stress at the endothelium. When the development of obstruction of a major artery, a steep pressure gradient develops across the collateral anastomoses and this pressure gradient is the driving force for an enhancement in blood flow through the collateral arterioles, leading to an augmented fluid shear stress that increases the collateral arteriolar endothelium (11). Some of the factors involved in the development of CCC have been studied in many studies. Several hematological and biochemical factors have been revealed to be associated with the degree of the collateral development.

Sayar et al. (12) found no correlation between plasma homocysteine concentration and coronary collateral development. But Nagai et al. (13) revealed that hyperhomocysteinemia negatively affected the development of coronary collaterals. And Yang et al. (14) found that serum level of homocysteine is independently and negatively associated with the development of collateral circulation in severe coronary artery stenosis. In this study, hyperhomocysteinemia was found to negatively affect the collateral development in accordance with the other two studies. Previous studies have shown that homocysteine stimulates vascular smooth muscle proliferation and decreases nitric oxide levels through inhibiting nitric oxide synthetase enzyme (15-17). As homocysteine inhibits endothelial cell proliferation, which plays an important role in angiogenesis, its elevation is expected to inhibit collateral development.

In the current study, we found that good collateral circulation with increasing age. Sahin et al. (18) have found that the age in the good collateral was higher than in the poor collateral. Shen et al. (19) reported that age was significantly higher in poor collateralization group.

The larger platelets, produced from megakaryocytes in the bone marrow, have greater prothrombotic potential than smaller (20). Previous studies have reported that MPV levels were significantly higher in the poor CC group compared with the good CC group (21,22). In our study, MPV was found to be higher in poor CC group, similar to other studies. Because of prothrombotic agents released from larger platelets may increase endothelial dysfunction more.

There are some limitations of our study. The major limitation is that the angiographically visualized collaterals are only part of the total collateral circulation. Because collateral vessels less than 100μm in diameter cannot be evaluated. The second this a single-centered study. The third the study was the small sample size so further studies with larger number of patients were needed.

CONCLUSION

In this study, we found that high serum homocysteine level is associated with poor collateral development in patients with acute coronary syndrome. Homocysteine treatment is not routinely applied for patients who have high homocysteine levels so we suggest that it is important to treat hyperhomocysteinemia in CAD patients, especially in those with poorly developed collaterals.

DECLARATION OF CONFLICTING INTERESTS

The author declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Research Article / Araştırma Makalesi

Effects of tryptophan, a precursor for melatonin, on IVF outcomes and Doppler parameters

Melatoninin kaynağı triptofanın IVF hastalarının sonuçlarına ve Doppler parametrelerine etkisi

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ABSTRACT

Aims: Melatonin is the most powerful antioxidant and protects sperm, oocyte, and embryo against oxidative stress. The effect of tryptophan, which is the building block of melatonin, on follicular melatonin levels and IVF outcomes is unknown. The objective of this study was to investigate the effect of tryptophan administration, a precursor for melatonin, on the levels of intrafollicular melatonin with the aim to reveal the correlation between tryptophan and the total number and quality of oocytes as well as clinical pregnancy rates. In addition, we aimed to examine the effect of melatonin increased by tryptophan on uterine and ovarian blood flow.

Material and Method: Out of 103 patients who applied to Ondokuz Mayıs University Hospital, IVF clinic for IVF treatment, 51 patients were administered a 100 mg dose of tryptophan orally (Group A) and 50 control patients who were randomly selected did not receive tryptophan (Group B). Firstly, follicular melatonin levels were compared between Group A receiving tryptophan and Group B without tryptophan. Both groups were also compared according to the oocyte count, oocyte count, fertilized oocyte count, embryo count, and pregnancy rates, ultimately. In addition, all patients were measured for uterine and ovarian artery blood flow by vaginal ultrasound on the day of OPU.

Results: There were no differences in age (32.16±3.82 years vs 33.06±4.44 years) (p=0.276), BMI (28.45±2.82 kg/m)^2 vs 28.15±3.03kg/m2 (p=0.602) and peak estradiol levels (2451.69±469.75 pg/ml vs 2420.26±443.71 pg/ml) (p=0.73) between the groups. Group A exhibited high levels of melatonin in the follicular fluid with a mean value of 259.8 pg/ml, whereas Group B had 91.3 pg/ml (p<0.001). There were found significant differences in the oocyte count (9.08±3.22 vs 7.66±1.89) (p=0,008), mature oocyte count (7.2±2.8 vs 6.1±1.8) (p=0,021) and fertilized oocyte count (6.35±2.44 vs 5.28±1.69) between group A and group B. Pregnancy rates were higher in group A (35.3%). The pregnancy rate (30%) was lower in Group B, which did not receive tryptophan and had low melatonin levels in follicular fluid. However, there was no statistically significant difference. Uterine, ovarian artery systolic and diastolic blood flows of Group A were significantly lower than Group B (p<0.001).

Conclusions: Administration of tryptophan to IVF patients significantly increases the level of melatonin in follicular fluid. The results demonstrate that high levels of melatonin in follicular fluid may increase oocyte count and quality although they do not significantly improve clinical pregnancy rates.

Keywords: Tryptophan, melatonin, pregnancy, IVF-ICSI

ÖZ

Amaç: Melatonin bilinen güçlü bir antioksidandır ve sperm, oosit ve embrioyu oksidatif strese karşı korur. Melatoninin yapı taşı olan triptofanın foliküler melatonin seviyesine ve IVF sonuçlarına etkisini bilinmemektedir. Bu çalışmada IVF tedavisinde melatonin prekürsörü olan triptofan desteğinin intrafolliküler melatonin seviyesine etkisini incelemek ve bunun oosit sayısı, oosit kalitesi nihayetinde klinik gebelik oranlarında yaptığı farklılığı araştırmak amaçlandı. Ayrıca triptofan desteği ile artan melatoninin uterin ve overiyan kan akımına etkisi amaçlandı.

Gereç ve Yöntem: IVF tedavisi için Ondokuz Mayıs Üniversitesi Hastanesi IVF Kliniği'ne başvuran 103 hastadan 51 hastaya triptofan oral 100 mg verilirken (Grup A); randomize seçilen 50 kontrol hastasına verilmedi (Grup B). Öncelikle triptofan alan Grup A ve almayan Grup B arasında foliküler melatonin seviyesi kıyaslandı. Yine her iki grup oosit sayısı, matür oosit sayısı, fertilize oosit ve embrio sayıları nihayetinde gebelik oranları açısından karşılaştırıldı. Ayrıca tüm hastaların OPU yapıldığı gün uterin ve overiyan arter kan akımları vajinal ultrasonografi ile ölçülüp kayıt edildi.

Bulgular: Gruplar arasında yaş (32,16±3,82 yıl vs 33,06±4,44 yıl) (p=0,276), BMI (28,45±2,82 kg/m^2 vs. 28,15±3,03 kg/m^2) (p=0,602), pik östradiol (2451,69±469,75 pg/ml vs 2420,26±443,71 pg/ml) (p=0,73) değerleri için fark yoktu. Grup A'da folikül sıvısındaki melatonin düzeyi yüksek olup ortalama değeri 259,8 pg/ml; Grup B'de 91,3 pg/ml olarak elde edilmişti (p<0,001). Oosit sayısı(9,08±3,22 vs 7,66±1,89) (p=0,008), matür oosit sayısı (7,2±2,8 vs 6,1±1,8) (p=0,021), fertilize oosit sayısı (6,35±2,44 vs 5,28±1,69) (p=0,012) için grup A ve B arasında anlamlı farklılık görüldü. Yine Grup A'da gebelik oranları (%35,3) daha fazlaydı. Triptofan kullanmayan ve folikül sıvısında melatonin düzeyi düşük olan Grup B'de ise daha az gebelik(%30) elde edilmişti. Ancak bu fark istatistiksel olarak anlamlı değildi (p=0,723). Grup A'nın uterin, ovariyan arter sistolik ve diyastolik kan akımları Grup B'ye göre anlamlı derecede düşük izlendi (p<0,001).

Sonuç: IVF hastalarına triptofan verilmesi follikül sıvısında melatonin seviyelerini önemli ölçüde arttırmaktadır. Bu sonuçlara göre foliküler sıvıdaki yüksek melatonin düzeyleri klinik gebelik oranlarını anlamlı ölçüde arttırmasa da oosit sayısı ve kalitesinin gelişmine olumlu destek sağlayabilir.

Anahtar Kelimeler: Triptofan, melatonin, gebelik, IVF-ICSI

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INTRODUCTION

Many couples who are unable to conceive spontaneously resort to assisted reproductive techniques such as in-vitro fertilization (IVF). Although these techniques help infertile couples, the rate of pregnancy remains around 29% per cycle (1). Research and studies on infertility prioritize to improve IVF outcomes. There are ongoing research and studies regarding novel adjuvant therapies to meet expectations and reduce health costs. Recently, there has been an increasing interest in the effects of oxidative stress on IVF outcomes.

Standard IVF procedures such as superovulation, oocyte cryopreservation, and embryo freezing lead to the accumulation of reactive oxygen species (ROS). Thus, oocytes and embryos are exposed to high concentrations of ROS. Excess ROS in follicular fluid is considered to impair oocyte quality by inducing apoptosis of oocyte and granulosa cells (2). It has been suggested that anti-oxidant therapy reduces the harmful effects of ROS and therefore improves success rates (3).

Melatonin is a potent free radical scavenger and a broad-spectrum antioxidant. Melatonin alleviates oxidative stress by neutralizing ROS. It modulates the physiology and molecular biology of the cells through different mechanisms. Many human and animal studies support the use of melatonin in the treatment of infertility due to its antioxidant properties (4).

The biosynthesis of melatonin is derived from tryptophan via the pineal gland within the brain. Tryptophan, a precursor for melatonin synthesis, is an essential amino acid and needs to be obtained through the foods that naturally contain it. Tryptophan is removed from plasma via the pineal gland and then hydroxylated with tryptophan hydroxylase in pinealocytes into 5-hydroxytryptophan (5-HTP) and then 5-HTP is converted into serotonin. Serotonin is then converted into N-acetyl 5-methoxy tryptamine, i.e. melatonin, via the enzyme NAT (N-acetyltransferase). All substances involved in the biosynthesis of melatonin by tryptophan exhibit a certain level of antioxidant activity. 5-HTP has been reported to be a more potent radical scavenger compared to melatonin and vitamin C (5).

In this study, the melatonin level in the follicular fluid was measured upon the administration of tryptophan, a melatonin precursor, in IVF patients with the aim to investigate the effects on IVF outcomes. At the same time, we investigated the correlation between the levels of melatonin in the follicular fluid on the day of oocyte pickup (OPU) and oocyte count, embryo quality and clinical pregnancy. In addition, we measured and compared uterine, ovarian artery systolic and diastolic blood flows on OPU day.

MATERIAL AND METHOD

A total of 103 patients who applied to Medical Faculty of Ondokuz Mayıs University, IVF Center were included in the study. The study was designed as a randomized controlled single-blind study.

Ethical Declaration

All authors and the study protocol have complied with the World Medical Association Declaration of Helsinki regarding ethical issues and principles in research involving human subjects. Local ethics committee approval was obtained for the study (OMU TAEK 20117-339) and written informed consent was obtained from the subjects who participated in the study.

The study included a total of 103 women undergoing IVF cycles. 2 patients discontinued treatment and the study was carried out with the remaining 101 patients. A total of 51 patients included in the study were given a dose of 100 mg tryptophan daily (group A), whereas the remaining 50 patients (group B) did not receive tryptophan. The two groups were randomized using a 1: 1 randomization ratio. Embryologists were "blind" as they did not know which group received tryptophan (**Figure 1**).

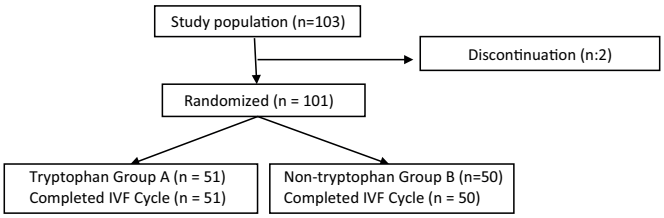

Figure 1. Distribution of patients

Inclusion Criteria

Our study included patients with a history of at least 1-year infertility, regular ovulation and menstruation in addition to normal spermiogram results and tubal patency confirmed by HSG. The patients were selected based on the criteria of the American College of Obstetricians and Gynecologists (ACOG) guidelines. Unremarkable spermiogram results according to ACOG criteria, presence of ovulation, normal tubal patency and uterine cavity by hysterosalpingogram, normal ovarian reserve and diagnosis of infertility by diagnostic laparoscopy were among the inclusion criteria.

Exclusion Criteria

Patients with male factor infertility, poor ovarian reserve, tubal factor infertility and those with any other causes of infertility were excluded. In addition, patients with systemic chronic diseases, uterine fibroids or polyps, endometriosis, polycystic ovary syndrome, endocrine disorders, patients using melatonin-interacting drugs (hypnotics, antidepressants, antiepileptics), continuous medication use, patients undergoing IVF-ICSI due to low ovarian reserve, male factor and tubal factor infertility, patients with BMI under 25, anovulation and those working night shifts were excluded from the study.

Primary outcome: Clinical pregnancy rate.

Secondary outcome: Oocyte count, mature oocyte count, fertilized oocyte count and uterine, ovarian artery systolic and diastolic blood flows.

Tryptophan Administration

The study group (group A) receiving Tryptophan (Lifetime Q-5-Hydroxy Tryptophan) was administered 100 mg tablets orally every day at 22:00 from the day of ovarian stimulation injections (cycle day 2-3). The last capsule was collected at 22:00 the night before oocyte retrieval.

Ovulation Induction

All patients underwent ovulation induction with the standard antagonist protocol. Oocyte pick-up (OPU) was performed 36 hours following the HCG treatment and intracytoplasmic sperm injection (ICSI) was performed 4-6 hours after that. Embryo transfer was performed 3 days after oocyte retrieval. Starting from the day of OPU, progesterone was administered intramuscularly (progestin 50 mg; Koçak, Turkey) and estrogen (estrofem 2 mg; NovoNordisk, Denmark) was administered orally as luteal support. Pregnancy was diagnosed in patients with positive bHCG 14 days after transfer. The diagnosis of clinical pregnancy was confirmed upon the presence of fetal heartbeat.

Sample Collection

Serum samples were taken from the patients to determine the level of melatonin on the second day of menstruation. Transvaginal ultrasound-guided ovum pick up (OPU) was performed under general anesthesia. The levels of melatonin in serum (MSOpu) and Follicular (MFolOpu) fluid were measured on OPU day. The follicular fluid was aspirated from the first dominant follicle without adding any diluent or contaminating with blood. All aspiration procedures were performed between 9-11 am. After oocyte isolation, the follicular fluid samples were centrifuged at 3000 rpm for 10 minutes to separate the supernatants from the tubes containing the follicle fluid and all samples were stored at -80 ° C until the day of OPU. In addition, uterine and ovarian artery Doppler blood flows were measured and recorded by vaginal ultrasound by the same specialist (FDB) on the day of OPU. Doppler ultrasonography was performed using GE LOGIQ P5 3.75 mHz convex probe ultrasound device while the patient was on the supine and lithotomy positions.

Laboratory Analysis

Melatonin levels in serum and follicular fluid samples were examined by Enzyme-linked Immunosorbent Assay (ELISA) method using commercial kits of Melatonin ELISA kit (USCN Life Science Inc., Wuhan, China, Lot. No.E90908Hu) in Research Laboratories of Faculty of Medicine, Ondokuz Mayıs University, Department of Biochemistry Research Laboratories. The samples with high concentration were repeated.

Statistical Method

The data were analyzed using IBM SPSS V23. Kolmogorov-Smirnov test was used to examine the normal distribution. The independent t-test was used for comparing normally distributed data, whereas the Mann Whitney U test was used for comparing non-normally distributed data. The Chi-square test was used for the comparison of categorical data. Normally distributed data were presented as mean±-standard deviation while non-normally distributed data were presented as median (min-max). The categorical data were presented as frequency (percentage). The significance level was accepted as $p<0.05$.

RESULTS

There was no significant difference between the mean age of the patients (32.16-33.06 years) ($p = 0.276$). The mean BMI was (28.45 kg/m^2) in the tryptophan group, which was the same in the non-tryptophan group (28.15 kg/m^2) ($p=0.602$). There was no difference in the mean estradiol values according to the administration of tryptophan ($p=0.73$). The mean value of Group A was 2451,69 pg/ml, whereas the mean value of non-tryptophan group was 2420,26 pg/ml.

The mean values of serum melatonin levels (MSD2) on cycle day 2 did not show significant difference according to the administration of tryptophan ($p=0.429$). The mean value of tryptophan group was 29.28, whereas it was 28.04 in non-tryptophan group. There was no difference in serum melatonin levels (MSOPU) on OPU day according to the administration of tryptophan ($p=0.3307$). The mean value of patients receiving tryptophan was 52.41 while it was 44.98 in the non-tryptophan group, however, there was no significant difference.

The level of melatonin (MFolOPU) in aspirated follicular fluid during OPU on the day of OPU revealed significant differences according to the administration of tryptophan ($p<0.001$). The mean value of Mfol OPU was 259.8 in patients receiving tryptophan, whereas the level of Mfol OPU was 91.3 in those who did not receive tryptophan. It was noted that the administration of tryptophan significantly increased the melatonin level in the follicular fluid.

On the day of OPU, uterine artery blood flow was measured by Doppler. There was found a significant difference between the subjects who received tryptophan and had low follicular melatonin levels and the subjects who did not receive tryptophan and had low follicular melatonin levels ($p<0.001$).

The mean value of uterine Arterial Doppler Pulsatile Index (DoppUa PI) was 1.78 in Group A and 1.83 in Group B.

The mean value of Uterine Arterial Doppler Resistance Index (DoppUa RI) was 0.83 in the tryptophan group and 0.88 in the non-tryptophan group ($p <0.001$).

There was a highly significant difference in the evaluation of ovarian artery blood flow by Doppler between those with low melatonin levels in both the tryptophan and non-tryptophan groups ($p<0.001$).

The mean value of Doppler ovarian artery pulsatile index (DoppOa PI) was as low as 1.07 in patients receiving tryptophan, whereas it was 1.32 in the non-tryptophan group.

The mean values of Doppler overian artery resistance index (DoppOa RI) were also significantly different according to tryptophan administration (p<0.001). The mean value was 0.83 in the tryptophan group, whereas it was 1.01 in the non-tryptophan group (**Table 1**).

Table 1. Comparison of parameters according to the administration of tryptophan

	Group A (n = 51)	Group B (n = 50)	P
Age	32.16 ± 3.82	33.06 ± 4.44	0.276
BMI	28.45 ± 2.82	28.15 ± 3.03	0.602
Peak Oestradiol Level	2451.69 ± 469.75	2420.26 ± 443.71	0.730
MS D2 (serum on day 2)	29.28 ± 7.58	28.04 ± 8.07	0.429
MS Opu (serum on OPU day)	52.41 ± 50.56	44.98 ± 10.48	0.307
M FolOpu	259.8 ± 64.31	91.3 ± 19.83	<0.001
DoppUa PI	1.78 ± 0.03	1.83 ± 0.04	<0.001
DoppUa RI	0.83 ± 0.03	0.88 ± 0.05	<0.001
DoppOa PI	1.07 ± 0.19	1.32 ± 0.14	<0.001
DoppOa RI	0.83 ± 0.21	1.01 ± 0.15	<0.001
Oocyte Count	9.08 ± 3.22	7.66 ± 1.89	0.008
Mature Oocyte Count	7.2 ± 2.8	6.1 ± 1.8	0.021
Fertilized Oocyte Count	6.35 ± 2.44	5.28 ± 1.69	0.012
G1 Embryo	5.41 ± 2.22	4.04 ± 1.67	0.001

The mean values of total oocytes retrieved were significantly different with respect to the levels of melatonin in the follicular fluid according to the administration of tryptophan (p= 0.008). The mean value was 9.08 in Group A and 7.66 in Group B, respectively. Similarly, Mature Oocyte Count (p=0.021), Fertilized Oocyte Count (p=0.012) and G1 embryo count (p=0.001) were also significantly higher in Group A (**Table 2, Figure 2-3**)

Table 2. Comparison of pregnancy rates between the groups

Pregnancy Rate	Positive	Negative
Group A	18 (35.3)	33 (64.7)
(Group B)	15 (30)	35 (70)

p = 0.723 (for positive)

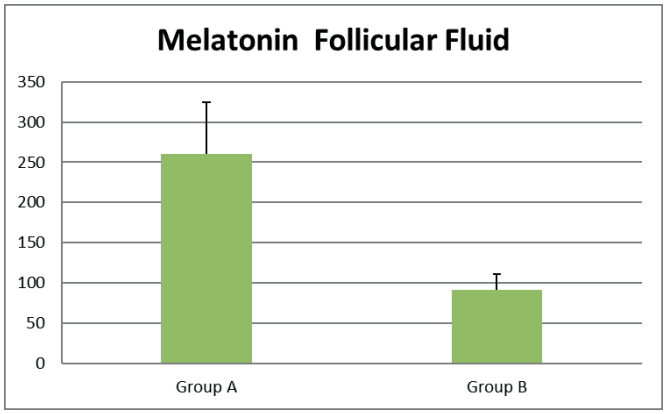

Figure 2. Comparison of melatonin in follicular fluid between the groups

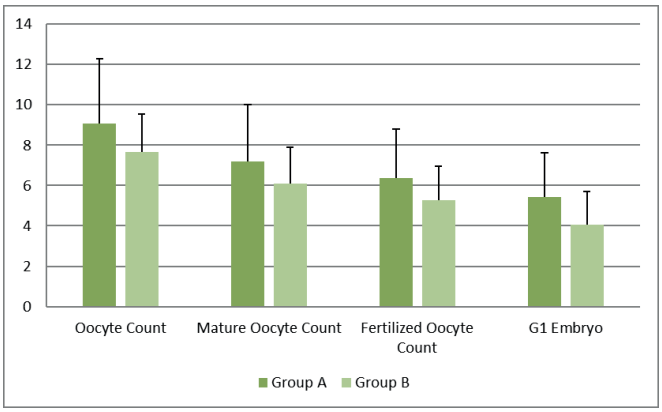

Figure 3. Schematic representation of the oocyte count, mature oocyte count, fertilized oocyte count and G1 embryo count between the groups

Pregnancy rates were higher in Group A (35.3%). In Group B, lower rates of pregnancy (30%) were achieved. However, there was no statistically significant difference in pregnancy rates between the groups (p=0.723).

DISCUSSION

Melatonin is a very powerful antioxidant produced via the pineal gland. Unlike other antioxidants, melatonin can exert its antioxidant effects both directly or through MT1 and MT2 receptors (6-8). Melatonin has an amphiphilic molecular character, which allows it to easily pass through the cell membrane and dispense to the nucleus (7,9,10). Most importantly, the metabolites of melatonin also show antioxidant effects without leading to oxidative stress (8). This leads to the formation of a cascade. Melatonin increases the effect of other antioxidants such as glutathione peroxidase and superoxide dismutase (11). Melatonin produced in the reproductive organs is thought to play a role in the regulation of many reproductive processes. Granulosa cells contain melatonin receptors. (12). In addition, large follicles have more melatonin than small ones (13). Again, it was observed that melatonin supplementation lead to a higher increase in serum levels compared to follicular fluid levels (14). These results suggest that melatonin may be effective in oocyte maturation. Free oxygen radicals are formed in each stage of IVF treatment. Melatonin has therefore been investigated in many studies due to its antioxidant effects in adjuvant therapy. In their study, Tamura et al. (15) found that the level of melatonin decreased in parallel with increasing levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a marker of oxidative stress in follicular fluid. Rizzo et al. (16) carried out a study and compared two groups receiving myo-inositol plus folic acid plus melatonin and myo-inositol plus folic acid revealing that the number of mature oocytes was higher in the melatonin group. Tamura et al. (17) studied patients undergoing unsuccessful IVF, divided the patients into two groups and treated the first group with orally administered melatonin. It was found that fertilization rates were higher in the group receiving melatonin. However, Fernando et al. (18) divided the patients into four groups in the pilot double blind study. They found that there were no differences

in pregnancy rates or the oocyte and embryo parameters other than the level of melatonin in follicular fluid between the patients who received placebo or 2mg, 4mg and 8mg melatonin replacement twice daily. Tong et al. (19) showed that the level of melatonin in follicular fluid was a marker of oocyte count and quality. In addition, it was revealed that there was a correlation between melatonin levels in follicular fluid and AMH levels as a marker of ovarian reserve. Again, it was found that the level of melatonin in follicular fluid was correlated with IVF outcomes, which was consistent with our study. The patients with high melatonin levels exhibited a higher number of oocytes, retrieved, more fertilized oocytes and higher rates of blastocyst. In this study, we investigated the effect of tryptophan, which is a precursor for melatonin and an essential amino acid, on IVF outcomes and found that the group treated with tryptophan exhibited a higher number of oocytes, mature oocytes and fertilized oocytes although there was no statistical difference in clinical pregnancy rates. The study also examined the effect of tryptophan on Doppler parameters in IVF patients. Although melatonin altered blood flow in many vascular beds, Fernando et al. (20) demonstrated that administration of melatonin in IVF patients did not alter uterine and ovarian Dopplers. We found higher levels of uterine and ovarian Doppler flow in the tryptophan group. Our study was significant in respect to including patients only with unexplained infertility as a homogenous group, besides, the patients were examined for both serum and follicular fluid. A greater number of patients should be examined in order to present more accurate results. Melatonin is a highly safe preparation when used as an antioxidant. No serious side effects were observed in any of the studies (21). No teratogenic effects were observed in the pregnant or infertile patient population (22). However, there are several authors who argue that it can aggravate diseases such as rheumatoid arthritis or multiple sclerosis as it causes immunostimulation. There can be found some authors reporting autoimmune hepatitis in the literature (23-25). In this study, we did not encounter any side effects associated with the administration of tryptophan.

In conclusion, tryptophan, a melatonin precursor, did not lead to a statistically significant difference in pregnancy rates but it increased the number and quality of oocytes in IVF patients. Prospective studies with greater number of patients are needed to obtain more accurate results.

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No

CONFLICT OF INTEREST DISCLOSURE

The authors have stated explicitly that there are no conflicts of interest in connection with this article

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Sağlık Bilimleri ve Tıp Dergisi

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Research Article / Araştırma Makalesi

Factors influencing study of physiotherapy and preferred choice of specialization among final year physiotherapy students in Nigeria

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ABSTRACT

Introduction: Over the years increasing number of students are seeking admission to study Physiotherapy in the universities leading to increased preference for specialization as compared with general practice. The aim of this study was to identify factors influencing the study of physiotherapy and preferred choice of specialization.

Material and Method: The research was a cross-sectional survey involving final year physiotherapy students in three training institutions in South-West, Nigeria. The questionnaire is in three sections (section A sought responses on Socio-demographic characteristics, section B on factors influencing the study of Physiotherapy and section C on preferred choice of specialization). Content validation was carried out to eliminate ambiguity and ensure that all items of the questionnaire are relevant and well understood. Descriptive statistics of percentages, bar chart and mean were used to summarize data on age, factors influencing study of physiotherapy, the level of influence of each factor on preferred specialization areas of each participant and the factors influencing the choice of specialization.

Result: There was a response rate of 72.2% and the mean age was 22.69±2.32 years. Out of the eight factors influencing study of physiotherapy, 'Desire to help people optimize their physical health and 'Personal interest' had the greatest influence with mean scores of 3.24 and 3.18 respectively. Ninety-eight participants (94.26%) were interested in pursuing postgraduate specialization in Physiotherapy. Orthopedics and Musculoskeletal physiotherapy is the most preferred specialization while Geriatrics is the least preferred specialization. Male students have preference for Sports while female have preference for Paediatric specialty.

Conclusion: Exposure to specialties in Physiotherapy like Geriatrics and Cardio-pulmonary may help improve interest in those areas and hence increased preference for those specialties.

Keywords: Physiotherapy study; choice of specialization; physiotherapy students

INTRODUCTION

Physiotherapy is concerned with identifying and maximizing quality of life and movement potential within the spheres of promotion, prevention, treatment/intervention, habilitation and rehabilitation (1). It constitutes one of the oldest and most prestigious components of a group of professionals referred to as Allied Health professionals (2). Dating as far back as 3000 BC (3), the practice of physiotherapy has changed greatly. There has been a shift from the traditional emphasis on practice based on the opinion of authorities to an emphasis on data-based clinically relevant studies and research (4). Now universally acknowledged as an autonomous profession, physiotherapy enjoys a positive reputation and is generally well regarded by other health professionals and the general public (5).

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Generally, Physiotherapy in Nigeria has undergone tremendous change since its introduction though it is not quite at par with the rest of the world yet (6). In the first three decades, it was viewed as a sub-profession with limited advancement opportunities in the civil service [6]. With the increased autonomy now associated with physiotherapy and broader knowledge of what it entails, the circumstances have improved greatly with physiotherapists serving greater roles in the health care sector.

In the area of physiotherapy education in Nigeria, what began as a 3-year program in 1966 at the University of Ibadan transitioned to a 5 year Bachelor of Physiotherapy degree program following curriculum upgrades in 1998 (7) with the possibility of obtaining a Master's degree at the postgraduate level and even a further doctoral degree in any of the available specialties. These specialties according to the Nigeria Society of Physiotherapy are Musculoskeletal and Orthopedic, Cardiorespiratory, Neurology, and Neurosurgery, Sports, Paediatrics and Women's Health. Also, with the introduction of the National Postgraduate Physiotherapy College of Nigeria, physiotherapists can now undergo residency training thus providing an opportunity for post-professional training along specialty lines.

Maduagwu et al. (8) in an article published in 2015 identified that a total of nine institutions offered physiotherapy, yet the number of practicing physiotherapists in Nigeria is still grossly inadequate to serve the enormous population. The Nigerian population is currently estimated to be about 178.5 million (9). Statistics from the Health Workforce Country Profile for Nigeria put the number of registered physiotherapists practicing within Nigeria whether in private or the public setting at 1473 (10). Compared to the global ratio of physiotherapists to patients which stands at 1:4000, this figure shows that there is still a gross deficiency in the number of physiotherapists graduating from the training institutions even though entrants into the profession have increased with the establishment of more training institutions.

While there is no existing literature detailing the areas of specialization of practicing physiotherapists in Nigeria based on post-graduate qualification, Ibikunle et al. (11) in a study carried out among physiotherapists found out that only 40% of the participants were interested in pursuing post-graduate specialization in physiotherapy. The choice of a career is a complex and multifaceted phenomenon influenced by both individual and contextual factors of structure and culture which enhance or construct one's social world (12). Given the current strength of physiotherapy manpower in Nigeria, it became necessary that an insight into the factors that influence the decision of students to study physiotherapy as well as select their preferred area of specialization to be researched into. This may help identify individual and contextual factors of structure and culture influencing the study of physiotherapy and specialization in it so that policymakers, health educationist and other stakeholders can make the right decisions to ensure that manpower development for both basic and specialized physiotherapy care is continuously achieved.

MATERIAL AND METHOD

This research is a cross-sectional survey and a purposive sampling technique was used to recruit participants for this study.

Participants

Participants for this study were consenting final year physiotherapy students in all physiotherapy training institutions in the South Western region of Nigeria namely;

a. University of Ibadan, Ibadan. Oyo State

b. Obafemi Awolowo University, Ile Ife. Osun State

c. University of Lagos, Lagos

Instruments

The questionnaire titled "Factors Affecting the Study of Physiotherapy and Preferred Choice of Specialisation among Final Year Physiotherapy Students in South-Western Nigeria" was used for data collection in this study. It was adapted from an earlier study by Folayan et al. (13). Domains relevant to the present study were identified in the original questionnaire and used for developing the questionnaire for this study. Adaptations made included a change of all terms referring to dentistry to that relevant to physiotherapy in the domains selected for development of this new questionnaire. The new questionnaire was given to 5 experts to ascertain that the content was adequate and relevant to this new study. Also, socio-demographic data on age were grouped and factors relevant to physiotherapy study have also been included. Five students were given the questionnaire to complete to be able to identify and correct ambiguity and ensure that all items of the questionnaire are relevant and well understood. The final questionnaire had two sections as shown below:

Reasons for studying physiotherapy were assessed. Participants were asked to rate from 0-5 each of the 8 alternatives to the degree to which any of the alternatives may have had an impact on their decision to study Physiotherapy. The alternatives were; Failure to be admitted to another program, perso-

Section A: This has 4 questions about the biodata of the participants, gender, age, profession of parent and institution.

SECTION A: Personal information

1. Sex: **Male** ☐ **Female** ☐

2. Age (Years): **18-21** ☐ **22-25** ☐ **26-29** ☐ **30 and above** ☐

3. Is any of your close relative a Physiotherapist? **YES** ☐ **NO** ☐

IF YES, state which: **Mother** ☐ **Father** ☐ **Sibling** ☐

Uncle ☐ **Aunt** ☐ **Other** ☐

4. Training Institution

University of Ibadan ☐

University of Lagos ☐

Obafemi Awolowo University ☐

This is the end of the Questionnaire.

Thank you for completing it

Section B: This part explored factors that may have contributed to the decision of individuals to or not to study physiotherapy.

SECTION B: Factors Influencing the Study of Physiotherapy

The following factors may have positively influenced your decision to study physiotherapy. Give a score from 1 to 5 to each of the factors, where 1represents the factor that had the least influence on your decision and 5 represents a very influential factor (two or more alternatives can have same scores). Where not applicable, please tick under N/A.

S/N		N/A	1	2	3	4	5
5	Failure to be admitted to other programme						
6	Personal interest						
7	Parent's recommendation						
8	Friend's or relative's recommendation						
9	Possibility of receiving high remuneration						
10	Desire to help people optimise their physical health						
11	Opportunity of working in a hospital setting						
12	Opportunity to travel and work outside Nigeria						
If any other, please state and rate and rate in the space provided below							
13							
14							
15							

Section C: This section explored the area of specialization they would prefer in the future and the factors that influenced their decisions.

SECTION C: Preferred Choice of Specialization

16. Would you like to pursue further specialization in Physiotherapy in the form of

Post-graduate studies. YES ☐ NO ☐

If Yes, please select the area you are willing to specialize in by giving a score from 1 to 5,

Where 1 = least interest and 5= Highly interested. Please tick under NI if you have no interest in the specialty.

S/N	Specialty	NI	1	2	3	4	5
17	Cardiopulmonary						
18	Geriatrics						
19	Neurology and Neurosurgery						
20	Orthopedics and Musculoskeletal						
21	Paediatrics						
22	Sports						
23	Women's Health						
If any other, please state and rate and rate in the space provided below							
24							
25							
26							

nal interest, Parent's recommendation, Friends' or relatives' recommendation, Possibility of receiving high remuneration, Desire to help people optimize their physical health, Opportunity of working in a hospital setting, Opportunity to travel and work outside Nigeria. They were also asked to specify other factors that may have influenced their decision but which were not stated and to rank them. The mean score for each alternative was calculated to rank the motives. To facilitate comparison between sub-groups, scores 0 and 1 were categorized as no influence, 2 and 3 as minimal influence while 4 and 5 were categorized as strong influence.

A Yes/No question was posed to participants about their willingness to pursue further specialization in physiotherapy in the form of postgraduate studies. Participants who replied in the affirmative were then asked to indicate their level of interest in the 7 available fields by giving a score from 0-5. They were also asked to specify other fields they may be interested in but which were not stated. Scores 0 and 1 were classified as no interest, 2 and 3 as little interest while 4 and 5 were classified as high interest.

A further question was then posed on the most preferred choice of specialization. Participants who selected a particular field were then asked to rate the factors that may have influenced their decision by giving a score from 0-5 to each of the 7 alternatives to the degree to which any alternative may have had an impact. Room was given for participants to identify other factors that may have influenced their decision but which were not listed and to rank them. Scores 0, 1 and 2 were classified as low while 3, 4 and 5 were categorized as strong for each of the motives.

Ethical Declaration and Procedure for Data Collection

Ethical approval was obtained from the University of Ibadan/ University College Hospital (UI/UCH) Health Research Ethics Committee before the commencement of the study with number UI/EC/16/0207. Informed consent was obtained from prospective participants. Participants were informed about the confidentiality of their responses and their right to withdraw from the study should they decide such. Questionnaires were sent to contact persons in each of the participating schools after their approval had been obtained. The contact persons following due training proceeded to collect the data and sent back the completed.

Out of 144 questionnaires distributed to participants, 104 (72.2%) were returned. The questionnaires were distributed to students in their classrooms and collected back after completing the questionnaires. The objective of study and voluntariness of participation was clearly stated in the informed consent form which was attached to the questionnaire.

Statistical Analysis

Descriptive statistics of mean and standard deviation were used to summarize data on age. Descriptive statistics of percentages, frequencies were used to present data on gender, institutions, factors influencing the study of physiotherapy, the level of influence of each motive, preferred areas of specialization of each participant and the factors influencing the choice of specialization.

RESULTS

One hundred and four students of the 144 eligible students filled and returned the questionnaire giving a response rate of 72.2% (104). There were 38 respondents (36.5%) from Obafemi Awolowo University, 23 (22.1%) from the University of Ibadan and 43 (41.3%) from the University of Lagos.

Socio-demographic Characteristics of Participants

Fifty-five participants were male (52.9%) while 49 were female (47.1%). The mean age of participants is 22.69±2.32 years. Thirty participants (28.8%) were aged 18-21, 65(62.5%) were aged 22-25, while 9(8.7%) participants were within age group 26-29. In the University of Ibadan, 9 (39.1%) participants were aged 18-21years, 13 (56.5%) were aged 22-25years while only 1 (4.3%) was aged 26-29years. 47.82% were males while 52.18% were females. In the University of Lagos, 15(34.9%) were aged 18-21, 60.5% were aged 22-25years while 2 (4.7%) were aged 26-29%. 39.53% were males while 60.47% were females. In Obafemi Awolowo University 71.5% of participants were males while

28.5% were females. 6 (15.8%) were aged 18-21, 26(68.4%) were aged 22-25years while 6 (15.8%) were aged 26-29years.

Fifteen (14.4%) participants had family or close relatives who are physiotherapists. Out of these 15, 4 had siblings who are physiotherapists, 4 had uncles who are physiotherapists, 3 had aunties who are physiotherapists, 1 had a mother who is a physiotherapist while 3 had one of the other relatives as a physiotherapist.

Factors Influencing the Study of Physiotherapy

Table 1 shows the distribution of factors that influence the choice of Physiotherapy as a course. The Desire to help people optimize their physical health had the strongest influence with a mean score of 3.24, followed by Personal interest with a mean score of 3.18 and the least motivating factor is Parents' recommendation with a mean score of 1.73.

For the male and female participants, the desire to help people optimize their physical health had the strongest influence on choosing Physiotherapy as a course while parents recommendation had the weakest influence in the decision to study Physiotherapy as shown in **Table 2**. For females, personal interest had the strongest influence on their choice of studying Physiotherapy. For participants aged 18-21, failure to be admitted had the strongest influence on their choice of Physiotherapy as a course. For the ages 22-25 and 26-29, personal interest had the strongest influence in their choice of Physiotherapy as a course as shown in **Table 1,2.**

Table 1. Factors influencing the study of physiotherapy

Statements	Mean Scores	Frequency in percentage					
	0	1	2	3	4	5	
Failure to be admitted into other program	2.27	23.1	25.0	7.7	10.6	13.5	20.2
Personal Interest	3.18	7.7	12.5	10.6	17.3	26.9	25
Parents' Recommendation	1.73	26.0	28.8	13.5	14.4	12.5	4.8
Friend or Relatives recommendation	2.13	18.3	26.0	12.5	20.2	13.5	9.6
Possibility of receiving high remuneration	2.19	23.1	19.2	9.6	19.2	21.2	7.7
Desire to help people optimise their physical health	3.24	11.5	6.7	5.8	25.0	24.0	26.9
Opportunity of working in a hospital setting	2.71	19.2	8.7	11.5	18.3	26.9	15.4
Opportunity to travel and work outside Nigeria	2.57	17.3	21.2	6.7	16.3	19.2	19.2

Table 2. Distribution of factors influencing the study of physiotherapy based on sex and age

Statements	Mean Scores				
	SEX		AGE		
	Male	Female	18-21	22-25	26-29
Failure to be admitted into other program	2.42	2.1	3.17	1.91	1.81
Personal Interest	3.02	3.37	2.47	3.42	3.89
Parents' Recommendation	1.65	1.82	2.1	1.58	1.56
Friend or Relatives Recommendation	2.27	1.98	1.6	2.4	2.8
Possibility of receiving high remuneration	2.24	2.14	1.97	2.26	2.44
Desire to help people optimize their physical health	3.24	3.24	2.97	3.25	4.11
Opportunity of working in a hospital setting	2.71	2.71	2.67	2.69	3.00
Opportunity to travel and work outside Nigeria	2.73	2.39	2.47	2.6	2.67

Post-Graduate Specialization

Out of 104 students, only 6 (5.77%) were not interested in pursuing post-graduate specialization. **Table 3** shows the different areas participants are willing to specialize in and the extent to which they are interested in the specialties. In order of preference, Orthopedic and Musculoskeletal physiotherapy is the most preferred with a mean score of 3.35 followed by Sports physiotherapy. The least preferred area of specialty is Geriatrics with mean scores of 1.18.

In order of interest, male participants were willing to specialize in Sports (3.65), Orthopedics (3.33), Neurology and Neurosurgery (2.65), Cardiopulmonary (2.35), Paediatrics

Table 3. Preferred specialty areas and level of interest

Specialty	Mean	Level of Interest (in %)					
	0	1	2	3	4	5	
Cardiopulmonary	1.88	37.5	13.5	8.7	18.3	7.7	14.4
Geriatrics	1.18	44.2	21.2	14.4	14.4	3.8	1.9
Neurology and Neurosugery	2.39	26.9	11.5	9.6	16.3	18.3	17.3
Orthopedics and Musculoskeletal	3.35	14.4	5.8	4.8	18.3	19.2	37.5
Paediatrics	2.06	28.8	21.2	7.7	13.5	15.4	13.5
Sports	3.27	17.3	9.6	3.8	9.6	17.3	42.3
Women's Health	1.55	39.4	23.1	6.7	16.3	2.9	11.5

Keys
0 – No Interest
1 – Hardly interested
2 – Just interested
3 – Moderately interested
4 - Very interested
5 - Totally interested

Table 4. Distribution showing preferred specialty areas and level of interest based on age and sex

Statements	MEAN SCORE				
	SEX		AGE		
	Male	Female	18-21	22-25	26-29
Cardiopulmonary	2.35	1.37	1.91	1.74	2.67
Geriatrics	1.38	0.96	1.22	1.11	1.67
Neurology and Neurosu rgery	2.65	2.10	2.67	2.28	2.33
Orthopedics and Musculo skeletal	3.33	3.37	3.47	3.34	3.0
Paediatrics	1.6	2.57	1.93	2.06	2.44
Sports	3.65	2.84	3.23	3.42	2.33
Women's Health	1.22	1.97	1.5	1.46	2.33

(1.6), Geriatrics (1.38) and Women's Health (1.22). For female participants, their choices in order were Orthopedics (3.37), Sports (2.84), Paediatrics (2.57), Neurology and Neurosurgery (2.10), Women's Health (1.97), Cardiopulmonary (1.37) and Geriatrics (0.98) as shown in Table 4. For participants aged 18-21, the choice of specialization in order of interest was as follows; Orthopedics and musculoskeletal (3.47) with the highest mean score and geriatrics (1.2) is with the lowest mean score. For those aged 22-25, they were Sports (3.42), Orthopedics (3.34), Neurology and Neurosurgery (2.28), Paediatrics (2.06), Cardiopulmonary (1.74), Women's Health (1.46) and Geriatrics (1.11). For those aged 22-25, Orthopedics ranked highest with a mean score of 3.0 followed by Cardiopulmonary (2.67), Paediatrics (2.44), Sports, Women's Health and Neurology and Neurosurgery (all 2.33) and Geriatrics with 1.67 (**Table 4**).

Preferred Choice of specialization

8 participants (7.7%) chose Cardiopulmonary, 13(12.5%) chose Neurology and Neurosurgery, 34 (32.7%) chose Orthopedics, 9 (8.7%) chose Paediatrics, 14(13.5%) chose Sports physiotherapy while 5(4.8%) chose Women's Health as their preferred area of specialization. 19(18.3%) were undecided while 2(1.9%) identified some other unspecified area as their preferred choice of specialization as shown.

DISCUSSION

This study evaluated the motivations and career choices among final year physiotherapy undergraduates in different training institutions in the South Western part of Nigeria. Final year students were selected because previous reports have demonstrated that senior students provide more realistic answers regarding their motivation in choosing their course of study (14). Also, it is expected that final year students would have had enough exposure to the practice of physiotherapy to be able to make informed decisions about current and future prospects. A considerable number of studies have previously evaluated factors that led to studying medicine, dentistry, nursing, pharmacy and other health-related professions around the world.

In line with Odebiyi and Adegoke (15), it was observed that generally more males than females will be graduated from the University of Ibadan, Lagos and Obafemi Awolowo University thus leading to a higher proportion of males in the profession. Findings about age distribution were considerably lower than the 25.9±13.21 years identified by Ibikunle et al. (11) in a similar study. This may be due to the difference in the population of studies as the previous study was conducted in a South Eastern university in Nigeria.

The first aim of the study was to identify the factors influencing the study of physiotherapy among students in their final year of study in physiotherapy schools in South-Western Nigeria. The results showed that the three top reasons for the study of Physiotherapy in South-Western Nigeria are 'Desire to help people optimize their physical health', 'Personal interest', 'Opportunity to work in a hospital setting'. These findings were partly in agreement with those of Mulnar et al. (16) and Puljak et al. (17) who reported that motivations and personal interest are the major factors that drive students toward medicine. Recommendation by parents, friends' or relatives who were physiotherapists did not play a major role. This is a variant with another finding by Ibikunle et al. (11) that the majority of those studying physiotherapy were influenced to do so by physiotherapists.

The outcome of identifying preferred specialization areas of physiotherapy by students in their final year of study in physiotherapy schools in South Western Nigeria showed that majority of the participants (94.26%) were interested in pursuing post-graduate specialization which is in line with findings throughout the literature (18-20). As regards age, the older final year physiotherapy students' choice was more influenced by personal interest showing that they are actually more mature. There was however a difference with regards to gender. Females were more willing to specialize in Paediatrics than their male counterparts. This is consistent with findings by Nancy et al. (21) that more females than males tend to be interested in Paediatrics related practice.

The most preferred specialization across schools is Orthopedics and Musculoskeletal Physiotherapy. This is in line with findings in several literatures that the majority of physiotherapy students wished to specialize in Musculoskeletal physiotherapy. Students were least interested in Geriatrics. This mirrors findings in a study by Fitzgerald et al, (22) that medical students generally exhibit low interest towards geriatric medicine. This has often been associated with limited knowledge about aging and older adults and limited exposure to caring for them. One way to improve this would be to provide more of such experiences to care for the older ones during training.

CONCLUSION

Based on the outcomes of the study, one can conclude that more males than females will graduate and join the existing physiotherapy workforce thus continuing the trend of a male dominated profession. Factors influencing the study of physiotherapy were more work-related than personal hence anything done to improve the prestige of the profession and also increase awareness about the profession will likely culminate in an increase in entrants into the profession. Interest in a specialty like cardiopulmonary and geriatrics can be stimulated by increasing knowledge and allowing students more exposure to the specialties during their clinical training program.

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Research Article / Araştırma Makalesi

Monocyte to HDL ratio as an indicator of subclinical atherosclerosis in diabetic retinopathy

Diyabetik retinopatide subklinik aterosklerozun bir göstergesi olarak monosit /HDL oranı

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ABSTRACT

Aim: Incidence of cardiovascular diseases is gradually increasing in patients with diabetic retinopathy (DR). MHR (Monocyte/HDL ratio), is a novel marker related to cardiovascular and cerebrovascular diseases. The aim of this study was to investigate the relationship between a subclinical atherosclerosis marker, carotid intima media thickness (CIMT), and MHR in diabetic retinopathy patients without an apparent cardiovascular disease.

Material and Method: 106 diabetic patients without an apparent cardiovascular disease and 35 healthy controls matched for age, gender and body mass index (BMI) were included in this study. The patients were separated into four groups which were proliferative diabetic retinopathy (PDR, n=30), nonproliferative diabetic retinopathy (NPDR, n=35), diabetic patients without retinopathy (n=41) and control group (n=35). Anthropometric, biochemical parameters and CIMT were measured. Correlation and regression analysis were done to assess the relation between MHR and CIMT.

Results: MHR was significantly different between groups and significantly higher in PDR group ($p<0.001$). CIMT, a marker for atherosclerosis, significantly differed between groups ($p<0.001$). CIMT levels were significantly higher in PDR while similar values were found in other than groups. In PDR group, a significant correlation was found between MHR and CIMT ($r=0.96$; $p<0.001$). According to binary logistic regression analysis, MHR had a significant effect on CIMT [β=0.206, (%95 CI: 1.004-1.505), p=0.046].

Conclusion: This study showed that in patients with diabetic retinopathy, high levels of MHR which is a non-invasive, simple and inexpensive marker, might be useful for determination of subclinical cardiovascular risk. This study which is the first in literature that investigated the relation between MHR and CIMT in diabetic retinopathy might have a benefit on early detection of cardiac risk in diabetic patients without an apparent cardiovascular disease.

Keywords: Diabetic retinopathy, subclinical atherosclerosis, monocyte to HDL ratio

ÖZ

Amaç: Diyabetik retinopatisi olan hastalarda kardiyovasküler hastalıkların artan sıklığı bilinmektedir. MHO (Monosit/HDL oranı), kardiyovasküler ve serebrovasküler hastalıklarla ilişkisi yeni saptanmış bir belirteçtir. Bu çalışmanın amacı aşikar kardiyovasküler hastalık bulgusu olmayan diyabetik retinopatisi olan hastalarda subklinik ateroskleroz risk göstergesi karotis intima media kalınlığı (KİMK) ile MHO ilşkisini araştırmaktır.

Gereç ve Yöntem: Aşikar kardiyovasküler hastalığı olmayan 106 diyabetik hasta ve 35 yaş, cinsiyet ve BMI (body mass index) ile uyumlu sağlıklı kontrol alındı. Hastalar proliferatif diyabetik retinopati (PDR, n=30), nonproliferatif diyabetik retinopati (NPDR, n=35), retinopati olmayan diyabet hastaları (n=41) ve kontrol grubu (n=35) olmak üzere dört gruba ayrıldı. Antropometrik, biyokimyasal parametreler ve KİMK ölçüldü. MHO ile KİMK arasındaki ilişkiyi değerlendirmek için korelasyon ve regresyon analizi yapıldı.

Bulgular: MHO değerleri gruplar arasında anlamlı farklılığa sahip ve PDR grubunda anlamlı düzeyde daha yüksekti ($p<0.001$). Ateroskleroz göstergesi olan KİMK gruplar arasında anlamlı farklılık gösterdi ($p<0.001$). KİMK değeri PDR grubunda anlamlı düzeyde yüksek iken diğer gruplarda birbirine yakın değerler ölçüldü. PDR grubunda MHO ile KİMK arasında önemli düzeyde anlamlı korelasyon bulundu (r=0,96; $p<0.001$). Binary logistic regression analizinde MHO'nin KİMK üzerine anlamlı etkisi bulunmaktaydı [β=0,206, (%95 CI: 1,004-1,505), p=0,046].

Sonuç: Bu çalışma, yüksek MHO'nın diyabetik retinopatisi olan hastalarda subklinik kardiyovasküler riski belirlemede non-invaziv, basit ve maliyeti düşük bir marker olarak kullanılabileceğini göstermektedir. Diyabetik retinopatide MHO ve KİMK arasındaki ilişkiyi inceleyen literatürdeki ilk çalışma olan bu çalışma, aşikar kardiyovasküler hastalık bulgusu olmayan diyabetik hastalardaki kardiyak riski erken belirlemede fayda sağlayabilir.

Anahtar Kelimeler: Diyabetik retinopati, subklinik ateroskleroz, monosit /HDL oranı

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INTRODUCTION

Besides oxidative stress and inflammation, monocyte/HDL cholesterol ratio (MHR) is recently defined as a marker related to negative cardiovascular outcomes (1). In patients with chronic renal disease, it is reported to have a relation with cardiovascular events (2) and in patients with infective endocarditis and normal left ventricular function, it is shown to be related to in-hospital and long term mortality (3).

CIMT is considered an indicator of subclinical atherosclerosis. In many studies, value of CIMT in predicting cardiac and cerebrovascular events has been proven (4,5).

In several studies, diabetic retinopathy has been reported as an independent predictor of all cause mortality and cardiovascular events in patients with type 1 and 2 diabetes (6). Many potential mechanisms including oxidative and glycemic stress, chronic inflammation and defective vascular tissue repair mechanisms are suggested to clarify the pathophysiological relation between diabetic micro- and macroangiopathy (7).

The aim of this study in patients with diabetic retinopathy and without apparent cardiovascular disease is to investigate the relationship between subclinical atherosclerosis risk factors (CIMT) and MHR as well as to assess the value of MHR as a marker in determination of cardiovascular risk.

MATERIAL AND METHOD

Selection of Cases for the Study Population

During the assessment of complications of diabetic patients who admitted to Süleyman Demirel University Endocrinology Clinic, the patients with retinopathy were included in this study. The patients were separated into four groups which were proliferative diabetic retinopathy (PDR, n=30), nonproliferative diabetic retinopathy (NPDR, n=35), diabetes without a retinopathy (n=41) and control group (n=35). Age and BMI values did not differ between groups. MHR sample size was determined due to the MHR data obtained during pilot study. Inter-group effect size was calculated as 0,40 according to mean and variance values. Sample size was calculated as 80, according to 85% potency and 5% error margin. However, to increase potency, more data (141 patients) was included in pre-determined time interval.

The patients with diseases that might cause inflammation (acute infectious diseases, rheumatic diseases, malignancies, etc), pregnancy, chronic renal disease, acute and chronic liver diseases, who were using glucocorticoid and/or nonsteroidal anti-inflammatory drugs, who smoked and who had cardiovascular diseases were excluded.

Anthropometric Measurements and Biochemical Tests

In initial assessment, systolic and diastolic blood pressures were measured in all patients; Body weights and heights were measured and body mass index was calculated (BMI: body weight (kg) /height (cm^2)). From all patients, brachial venous blood samples were taken for biochemical analysis, in the morning, after 12 hours fasting. The blood samples were analyzed for plasma fasting glucose, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). All the analyses were performed with Symex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan) according to manufacturer's instruction. MHR was calculated as ratio of monocyte count to HDL level.

Measurement of Carotid İntima-Media Thickness

CIMT was measured in each patient with Shimadzu (2200 X plus, Kyoto, Japan) device using a 7,5-13,5 MHz multifrequency lineer array probe. All ultrasound examinations were performed by the same investigator in a silent environment after the patient rested for 15 minutes. For carotid artery imaging, the patient stayed in supine position. Measurements were done from three different places of 1 cm distal of right and left anterior carotid artery. Mean of right and left carotid artery measurements was calculated and recorded as CIMT.

Statistical Analysis

Statistical analyses were performed by SPSS 20.0 software (IBM Inc., Chicago, IL, USA). Numerical variables were expressed as mean±SD ((median; IQR) where necessary) and the gender was expressed as frequency (percentage) in tables. Continuous variables were checked for normality by Kolmogorov-Smirnov test. Comparison of independent groups was done by One-Way ANOVA with Tukey HSD post-hoc test. The relation between numerical variables was performed by Pearson Correlation Analysis. The sample size was calculated by GPower 3.1.9.2 software. Binary logistic regression model of CIMT was created. In all analyses, $p<0.05$ was considered as statistically significant with 5% type-I error.

Ethical Declaration

This study was approved by Süleyman Demirel University Medical Faculty Ethics Committee. All patients planned to be included have been informed both verbally and written, and an informed consent was taken (Date 05/03/2019, Decision No: 32).

RESULTS

The groups did not differ for demographic features such as age (p=0.79), gender (p= 0.96) and BMI (p= 0.41). MHR calculated according to monocyte and HDL levels was significantly different between groups, and was higher in PDR group ($p<0.001$). In other groups the results were close to each other. CIMT which is an indicator of atherosclerosis significantly differed between groups ($p<.001$). CIMT was

Table 1. Patient demographics and clinical features

	PDR (n=30)	NPDR (n=35)	DM+No DR (n=41)	Control (n=35)	
	Mean±SD (median, IQR)				p
Gender Male N(%)	18 (56.3)	18 (58.1)	19 (57.6)	18 (56.3)	0.991
Female N(%)	14 (43.8)	13 (41.9)	14 (42.4)	14 (43.8)	
Age (yrs)	61.66±5.24	60.51±5.04	61.29±4.85	60.82±4.97	0.798
BMI (kg/m^2)	28.73±1.73	28.42±2.65	29.19±2.69	29.32±2.71	0.413
Body weight (kg)	79.10±5.20	78.34±6.34	79.43±5.95	80.05±5.54	0.661
Height (cm)	165.93±4.65	166.14±4.39	165.14±5.61	165.45±5.72	0.838
SBP (mmHg)	122.16±9.79	122.85±10.72	122.56±10.84	123.0±10.65	0.990
DBP (mmHg)	72.33±6.26	71.71±7.56	71.82±7.56	72.14±7.79	0.937
DM duration (years)	11.53±1.73^{a}	11.14±1.35^{b}	7.29±1.05^{a,b}	N/A	< 0.001*
Neutr (10^9/L)	6.87±0.70 ^{a,b}	5.37±0.29 ^{a,c}	4.42±0.42 ^{b,c}	4.41±0.41 ^{b,c}	< 0.001*
Lymp (10^9/L)	1.89±0.36	1.90±0.39	1.94±0.31	1.93±0.30	0.900
Mono (10^9/L)	725.0±96.26^{a,b,c}	501.42±60.83 ^{a}	503.41±57.68 ^{b}	501.42±60.83 ^{c}	< 0.001*
HDL (mg/dl)	37.63±1.69 ^{a,b,c}	48.20±1.95 ^{a}	48.19±1.90 ^{b}	48.31±2.08 ^{c}	< 0.001*
LDL (mg/dl)	132.80±34.06 ^{a,b}	133.02±28.32 ^{c,d}	111.21±21.18 ^{a,c}	110.48±20.49 ^{b,d}	< 0.001*
Cholesterol (mg/dl)	204.80±32.15	209.85±32.00	196.29±24.78	196.0±23.40	0.105
TG (mg/dl)	197.83±46.56 ^{a,b}	198.68±46.96 ^{c,d}	177.14±20.53 ^{a,c}	178.71±20.92 ^{b,d}	0.010*
Glucose (mg/dl)	182.03±52.78 ^{a,b}	181.0±48.62 ^{c,d}	87.21±9.08 ^{a,c}	87.20±9.63 ^{b,d}	< 0.001*
HbA1C (%)	8.09±1.09 ^{a,b,c}	7.93±1.10 ^{a}	7.21±0,48 ^{b}	5.35±0.31 ^{c}	< 0.001*
CRP (mg/L)	5.53±1.30 ^{a,b}	5.00±1.30 ^{c,d}	2.09±1.01 ^{a,c}	1.88±0,83 ^{b,d}	< 0.001*
CIMT (mm)	0.91±0.04^{a,b,c}	0.63±0.08^{a}	0.62±0.11^{b}	0.63±0.11^{c}	< 0.001*
Fibrinogen (g/L)	534.47±39.12 ^{a,b}	419.72±71.65 ^{c,d}	370.72±53.16 ^{a,c}	361.26±48.06 ^{b,d}	< 0.001*
MHR	19.41±3.47 ^{a,b,c}	10.46±1.65 ^{a}	10.50±1.57 ^{b}	10.44±1.66 ^{c}	< 0.001*
NLR	3.78±0.92 ^{a,b,c}	2.95±0.67 ^{a}	2.35±0.55 ^{b}	2.35±0.53 ^{c}	< 0.001*

*: significant at p< 0.05 level according to One-way ANOVA
a,b,c,d: Same superscript letters denote the significant pairwise groups

significantly high in PDR groups but similar to each other in the remaining groups (**Table 1**).

Correlation coefficients between measurements and MHR values were calculated in each group. In PDR group, there was a highly significant correlation between MHR and CIMT (r=0,96; p<.001). In addition, a low correlation was found with neutrophil (r=0,39) and a moderate correlation, with lymphocyte (r=0,53). A high and positive correlation was found between diabetes duration and MHR (r=0,62; p= 0.002) besides a significant and positive relation, with LDL (r=0.36; p= .034) and glucose (r=0.33; p= 0.047). In NPDR group, a significant relation has existed between MHR and CIMT and only a moderate positive correlation was detected with diabetes duration (R=0.51; p= .013) (**Table 2**).

In the group in which the patients had only diabetes, patients' body weight and BMI were low and positively correlated with MHR. In control group, MHR was not correlated with atherosclerotic parameters (**Figure 1**).

Figure 1. Correlation values between CIMT and MHR in DR groups

Results of CIMT measurement were divided into two groups as median below and above 0.64. The values below 0.64 were considered as reference group and a binary logistic regression model was developed. To overcome the multiple correlation problem, a model was formed with forwarding LR stepwise method and adjustment results were significant (Nagelkerke R^2=0.361; -2LL=138.99 and Hosmer-Lemeshow Chi-square=12.74 (p=.121)). Four factors affected CIMT and all of them positively contributed to the model. Contribution of fibrinogen was significant and MHR, HbA1C, and CRP had obvious contributions (**Table 3**).

DISCUSSION

Standard risk factors of cardiovascular disease (CVD) do not sufficiently explain the high cardiovascular risk in diabetes. Regarding the poor prognosis of CVD in diabetes, detection of novel subclinical atherosclerotic markers such

Table 2. Correlation values between MHR and other biochemical measures in DM and proliferation groups

MHR	PDR		NPDR		DM+No DR		Kontrol	
	r	p	r	p	r	p	r	p
NLR	-0.327	0.078	-0.038	0.827	-0.229	0.150	-0.256	0.138
SBP	-0.131	0.489	0.164	0.348	-0.038	0.815	0.011	0.949
DBP	-0.239	0.204	-0.277	0.107	-0.005	0.974	-0.041	0.813
DM Duration	0.628[R]	0.002*	0.517	0.013*	0.231	0.084	N/A	N/A
NEUT	0.397	0.030*	0.248	0.151	-0.104	0.520	-0.109	0.532
LYMP	0.532	0.002*	0.099	0.573	0.203	0.203	0.221	0.203
MONO	0.999	<0.001*	0.995	**<0.001***	0.992	<0.001*	0.996	<0.001*
HDL	-0.996	<0.001*	-0.991	**<0.001***	-0.988	<0.001*	-0.994	<0.001*
LDL	-0.112	0.556	0.359	**0.034***	-0.193	0.226	-0.257	0.135
CHOLESTEROL	-0.178	0.347	-0.241	0.164	-0.229	0.150	-0.302	0.078
TG	-0.154	0.416	-0.029	0.867	-0.037	0.817	-0.105	0.547
GLUCOSE	0.084	0.661	0.338	0.047*	0.054	0.739	0.059	0.735
HBA1C	-0.039	0.837	-0.236	0.173	-0.108	0.500	-0.083	0.634
CRP	-0.094	0.623	-0.135	0.441	-0.076	0.637	-0.171	0.326
CIMT	0.968	<0.001*	0.233	0.177	0.142	0.376	-0.404	0.016
FIBRINOGEN	-0.234	0.213	-0.155	0.375	-0.003	0.984	0.008	0.962
AGE	0.042	0.826	-0.048	0.786	0.010	0.952	-0.037	0.832
BODY WEIGHT	0.028	0.883	0.183	0.292	0.388	**0.012***	0.411	0.014*
HEIGHT	0.192	0.308	-0.096	0.585	0.004	0.982	0.034	0.848
BMI	-0.135	0.477	0.219	0.205	0.322	**0.040***	0.291	0.089

r: Pearson Correlation Coefficient ; R: Spearman's Rho Correlation Coefficient ; *: 0.05 significance level

Table 3. Binary logistic regression model presenting factors that affect CIMT

Factors	Beta	p	OR	95% CI
Fibrinogen	0.013	< 0.001*	1.013	(1.007-1.019)
MHR	0.206	0.046*	1.229	(1.004-1.505)
Age	0.598	.439		
Gender	0.006	0.936		
HbA1C	0.292	0.037*	1.356	(1.010-1.618)
CRP	0.326	0.032*	1.488	(1.116-1.734)
BMI	0.005	0.941		
NLR	1.837	0.175		

OR: Odds ratio; CI: Confidence interval; *: 0.05 significance level

as coronary artery calcium (CAC) and CIMT for early diagnosis and prevention of CVD in diabetes is important (8). Structural and functional alterations in microvascular circulation are related to cardiac, retinal and renal atherosclerosis (9,10). In type 2 diabetic patients, diabetic retinopathy as a marker of microvascular disease is suggested to be related to subclinical atherosclerosis and CVD (11).

Some previous studies showed that DR had a relation with noninvasive subclinical atherosclerotic measures such as CIMT, carotid plaque and arterial stiffness (12). In Chennai Urban Rural Epidemiology Study (CURES-2), a relation between DR and CIMT was reported (13). Jang-Won Son et al. (12), in Korean newly diagnosed type 2 diabetes patients reported that DR was an independent risk marker for subclinical atherosclerosis. Lian-Xi Li et al. (14) found retinal microvascular abnormalities related to independently increased CIMT in Chinese hospitalized patients. In our study, parallel to other studies, CIMT was high in PDR group.

As important markers for inflammatory response, WBC count and its subtypes are related to CVD (15). As well, platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR) and neutrophil/lymphocyte ratio (NLR) are potential biomarkers of inflammatory response. In many studies, conventional inflammatory markers had positive correlation with PLR and NLR. Besides, in many studies, in especially DM patients, PLR and NLR had predictive effects on acute coronary syndrome (16,17). In addition, only one study has been performed that included MLR in diabetic retinopathy as an immune marker. (18). Our study is the only one in literature investigating the effect of MHR in diabetic retinopathy.

MHR is investigated as a novel inflammatory marker and suggested to be superior to WBC subtypes in patients with cardiovascular and cerebrovascular diseases (19,20). Monocytes are inflammatory reaction markers responsible of release of proinflammatory and prooxidant cytokins (21). Monocytes previously have been shown to be related to diabetic micro-and macrovascular complications (22).

Matsumura et al. (23) reported that monocyte amount was positively correlated with CIMT in T2DM patients. On the other hand, HDL cholesterol can decrease macrophage accumulation, prevents monocyte migration, increases nitric oxide synthase expression in endothelial tissues and has antioxidant and anti-inflammatory effects on endothelial cells (24). MHR seems like a novel and useful marker related to pro-inflammatory and anti-inflammatory processes. In our study, we showed that MHR had a significant role in PDR patients for prediction of subclinical atherosclerosis. In PDR patient group, there was a strong correlation between CIMT and MHR. Therefore, MHR might be considered a good predictor for vascular structural alteration in PDR patients. However, diabetic patients without a complication and nondiabetic patients had a moderate imbalance between pro- and anti-inflammatory mechanisms. This might be because MHR was related to CIMT in PDR group but not in other groups. Previously, Kanbay et al. (2) reported that in chronic renal disease patients, MHR behaved like an independent marker for cardiovascular events. In patients with Behçet disease, MHR was correlated with brachial artery flow-mediated dilatation (FMD) (25). All this evidence suggests that predictive value of MHR for CVD is higher in chronic inflammatory diseases such as complicated diabetes.

In literature, there are two studies that aim to evaluate MHR in ocular diseases. In the first study, Mirza et al. (26) found high MHR values in glaucoma patients. In the second one, MHR level was high in patients with retinal venous occlusion (27). Thus, our study is the first one that investigates the relation between diabetic retinopathy and MHR. In especially PDR patients, MHR might be a promising marker for cardiovascular risk.

As reported in current literature, this study had 4 major results. First of all, MHR and CIMT were significantly higher in proliferative diabetic retinopathy group. Secondly, in PDR patient group, MHR, and a subclinical atherosclerotic marker, CIMT had a very significant correlation. Thirdly, there was a significant positive correlation between classical atherosclerotic risk factor LDL and MHR, in PDR group. And lastly, MHR and diabetes duration were high and positively correlated in both PDR and NPDR groups. There was an increased cardiovascular risk in patients with diabetic retinopathy. In these patients, CIMT was an important non-invasive method to determine subclinical cardiovascular risk (6,11). Also in our study, CIMT was significantly high in PDR group. Studies suggest that NLR could be used as a marker for subclinical CVD (28). Till now, no study has investigated the relationship between diabetic retinopathy and MHR. In our study, MHR was significantly high in PDR group and was significantly correlated with subclinical cardiovascular risk marker, CIMT. According to binary logistic regression analysis, MHR had a significant effect on CIMT [β=0.206, (%95 CI: 1.004-1.505), P=0.046] (Table 3). So, MHR might be used as a non-invasive, simple and inexpensive marker on determination of subclinical cardiovascular risk in patients with diabetic retinopathy.

This study has several limitations. First of all, this is a cross sectional study; The relation between DR and early atherosclerosis is not evaluated as a cause-result relation and prospective studies are required. Secondly, in our study, though a correlation is found between MHR and CIMT, MHR effect on CIMT progression is not examined since it is a cross-sectional study. Therefore, in the future large prospective studies should be planned. Thirdly, oxidative stress, inflammation, and endothelial dysfunction have important role in pathogenesis of DR and subclinical atherosclerosis (29,30). However, in our study, lack of oxidative stress and endothelial dysfunction markers limited the relation between MHR and CIMT in PDR group. However, regression analysis showed that; hyperglycemia, inflammation (CRP, MHR), and coagulation defect (Fibrinogen) responsible for etiopathogenesis of diabetic retinopathy had also an effect on atherosclerosis (Table 3). All of these findings suggest that a common pathophysiological process might be responsible for both retinopathy and atherosclerosis for which endothelial damage might be the reason. Further studies are required to clarify this suggestion.

CONCLUSION

According to these findings and especially to the results of prospective studies, it is concluded that biomarkers of inflammation, endothelial dysfunction and coagulation might help to determine subjects that are under risk for diabetes, diabetic microangiopathy, and CVD. Although disease progression will be slowed down or delayed with most of the interventions used for diabetes treatment, future dilemma is finding new biomarkers as CRP with proven clinical diagnostic and therapeutical value. Increasing burden of diabetes demands such brand approaches.

As a conclusion, this study shows that high MHR is an appropriate and effective method to predict subclinical atherosclerosis and its progression in patients with diabetic retinopathy.

CONFLICT OF INTEREST

No conflict of interest among authors.

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Sağlık Bilimleri ve Tıp Dergisi

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Research Article / Araştırma Makalesi

The significance of ankle-brachial index in determining peripheral artery disease in patients with type 2 diabetes mellitus over 40 years of age and the relationship of peripheral artery disease with chronic complications of diabetes

Kırk yaş üzeri tip 2 diabetes mellituslu hastalarda ayak bileği kol indeksinin periferik arter hastalığını saptamadaki değeri ve periferik arter hastalığının diyabetin kronik komplikasyonları ile ilişkisi

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ABSTRACT

Introduction: Peripheral artery disease (PAD) acts as an important predictor of mortality and morbidity in cerebrovascular and cardiovascular diseases. The incidence of PAD was reported to be 2 to 4 times higher in diabetic patients compared with non-diabetic patients. Ankle-brachial index (ABI) is an easily applicable method for the diagnosis of PAD. The aim of this study is to determine the prevalence of PAD by using ABI in patients with Type 2 diabetes mellitus (DM) over 40 years of age, compare the results with lower extremity arterial Doppler ultrasonography (USG) and to reveal the relationship between chronic complications of DM and PAD.

Material and Method: The study included 111 DM patients over 40 years of age. ABI; was calculated by dividing the higher systolic blood pressures (SBP) taken from both ankle levels to the higher SBPs measured in both arms and defined as "ABI-1". ABI-2 was calculated by dividing the lower SBPs taken from both ankle levels to the higher SBPs measured from both arms. ABI values calculated by both methods were divided into 3 groups according to cut off values. ABI values of 0.9 and less in Group 1, between 0.9 and 1.30 in Group 2, between 0.9 and 1.40 in Group 3 were interpreted in favor of PAD.

Results: The prevalence of PAD was 19.8%. The most specific group for detecting PAD was ABI-2G1, and the most sensitive groups were ABI-1G2 and ABI-2G2. A significant relationship was found between PAD and clopidogrel use, decreased vibration sensation, age, duration of DM, insulin resistance, glomerular filtration rate, albuminuria, homocysteine, and uric acid levels.

Conclusion: ABI is a sensitive method for detecting PAD. The superior side of our study compared to the other studies is that the ABI is calculated by 2 methods and ABI values are divided into 3 groups according to cut off values (≤0.9; ≤0.9- >1.30; ≤0.9->1.40).

Keywords: Peripheral artery disease, ankle-brachial index, diabetes mellitus

ÖZ

Giriş: Periferik arter hastalığı (PAH); serebrovasküler ve kardiyovasküler hastalıklarda mortalite ve morbiditenin önemli bir prediktörüdür. Diyabetik ve diyabetik olmayan hastalar karşılaştırıldığında diyabetik hastalarda PAH insidansının 2 ila 4 kat daha fazla olduğu bildirilmiştir. Ayak bileği-kol indeksi (ABI), PAH tanısında kullanılan kolay uygulanabilir bir yöntemdir. Çalışmamızda merkezimizde takip edilen 40 yaş üzeri Tip 2 diabetes mellitus (DM) hastalarında ABI ile PAH prevalansını saptamak, alt ekstremite arteriyel doppler ultrasonografi (USG) bulgularıyla kıyaslamak ve tip 2 DM'nin kronik komplikasyonları ile PAH arasındaki ilişkileri ortaya koymak amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 40 yaş üzeri 111 tip 2 DM hastası alındı. ABI; her iki ayak bileği seviyesinden alınan sistolik kan basınçlarından (SKB) daha büyük olanının, her 2 koldan ölçülen SKB'larından daha büyük olanına bölünmesiyle hesaplandı ve "ABI-1" olarak tanımlandı. Her iki ayak bileği seviyesinden alınan SKB'larından daha DÜŞÜK olanının, her iki koldan ölçülen SKB'larından daha büyük olanına bölünmesi ile de ABI-2 hesaplandı. Daha sonra her iki yöntemle de hesaplanan ABI değerleri, aralık değerlerine göre üç gruba ayrıldı. Grup 1'de 0,9 ve altı ABI değerleri, Grup 2'de 0,9 ve 1,30 arası dışındaki, Grup 3'te 0,9 ve 1,40 arası dışındaki değerler PAH lehine yorumlandı.

Bulgular: Çalışmamızdaki 40 yaş üzeri diyabetik hastalarda PAH prevalansı %19,8 olarak bulundu. PAH'ı saptamada en spesifik ABI-2G1 grubu, en sensitif ise ABI-1G2 ve ABI-2G2 grupları oldu. PAH ile klopidogrel kullanımı, azalmış vibrasyon hissi, yaş, DM süresi, insülin direnci, glomerüler filtrasyon hızı, albüminüri, homosistein ve ürik asit düzeyleri arasında anlamlı bir ilişki bulundu.

Sonuç: ABI, DM hastalarında PAH tanısı için sensitif bir yöntemdir. Çalışmamızın diğer çalışmalara göre üstün tarafı ABI'nın her iki yöntemle de hesaplanması ve ABI değerlerinin kesim noktalarına göre üçer gruba (≤0,9 ; ≤0,9 - >1,30 ; ≤0,9 - >1,40) ayrılmasıdır. Diyabetik hastalarda PAH tanısını koymak için ABI'nın özellikle ikinci hesaplama yöntemiyle (ABI-2) değerlendirilmesi, gelecekte oluşması beklenen komplikasyonların önüne geçilmesi açısından önem taşır.

Anahtar Kelimeler: Periferik arter hastalığı, ayak bileği-kol indeksi, diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease that requires constant medical care in which the organism can not make sufficient use of carbohydrates, fats, and proteins due to insulin deficiency or insulin-effect defects (1). Macrovascular complications of DM include cerebrovascular disease (CVD), coronary artery disease (CAD) and peripheral artery disease (PAD). Cardiovascular disease is the primary cause of death in 55% of patients with Type 2 DM (2). PAD is an important sign of atherosclerosis and acts as an important predictor of mortality and morbidity in CVD and CAD (3).

Arteriography is the gold standard method in the diagnosis of PAD and shows the localization, severity, and extent of the disease (4). Because it is an invasive procedure, the use of arteriography is limited. Therefore, many non-invasive tests have been developed for the diagnosis of PAD. The ankle-brachial index (ABI) is the simplest and cheapest of these tests (5). ABI is the ratio of systolic blood pressure (SBP) measured at ankle level to brachial artery SBP. According to the American Diabetes Association (ADA), ABI measurement should be performed in all diabetic patients over the age of 50 (6).

The aim of this study was to determine the prevalence of PAD by ABI in Type 2 DM patients over 40 years of age and to reveal the relationship between chronic complications of type 2 DM and PAD.

MATERIAL AND METHOD

This study was conducted prospectively between December 2010 and November 2013 in endocrinology, metabolism and nutrition department of our center. 111 patients over 40 years of age diagnosed with type 2 DM according to ADA criteria were included in the study.

Ethical Declaration

The study was approved by the Ethics Committee of Eskişehir Osmangazi University (Year: 29.11.2013, Number/Decision No: 80558721/40).

ABI was measured by Hadeco Bidop ES-100V3 Doppler device by the same physician in all patients. SBPs were measured from both brachial arteries, tibialis posterior and dorsalis pedis arteries while the patients were in the supine position. ABI was calculated by dividing the higher SBPs taken from both ankle levels by the higher SBPs measured from both arms and defined as "ABI-1". ABI-2 was calculated by dividing the lower of the SBPs taken from both ankle levels by the higher SBPs measured from both arms. After calculating both right and left ABI values of each patient, the lower ABI value was taken as the patient's overall ABI value. Then, ABI values calculated by both methods were divided into three groups according to their cut off values (**Table 1**).

Table 1. Groups formed according to ABI, lower extremity Doppler ultrasound and cut off values used for the diagnosis of PAD in patients

	Measurement method for the diagnosis of PAD	Cut off value
ABİ-1G1	**Higher** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9
ABİ-1G2	**Higher** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9 and >1,30
ABİ-1G3	**Higher** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9 and >1,40
ABİ-2G1	**Lower** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9
ABİ-2G2	**Lower** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9 and >1,30
ABİ-2G3	**Lower** SBP measured from the ankle / **Higher** brachial artery SBP	≤0,9 and >1,40
Doppler USG Group	Lower extremity doppler USG performed by a radiologist	≥%50 stenosis

ABI: Ankle Brachial Index, USG: Ultrasound
Group 1 (G1): ABI values of 0.9 or less were interpreted in favor of PAD.
Group 2 (G2): Normal between 0.9 and 1.30, other values were interpreted in favor of PAD.
Group 3 (G3): Normal between 0.9 and 1.40, other values interpreted in favor of PAD.

Microalbumin excretion in 24-hour urine (mg) was calculated by the "microalbumin in 24-hour urine (mg) × 24-hour urine volume (ml)/1000" formula. Albuminuria levels of 30 mg/day and below were evaluated as normoalbuminuria, 30-300 mg/day as microalbuminuria and more than 300 mg/day as macroalbuminuria. Urine creatinine (mg/dl)×daily urine volume (ml)/serum creatinine (mg/dl)×1440 formula was used to calculate GFR (glomerular filtration rate). Chronic renal failure (CRF) was considered as creatinine elevation for at least 3 months, or as GFR being below 60 ml/min.

To determine parasympathetic autonomic neuropathy, 30/15 ratio test and heart rate response to deep breathing tests were performed. For the 30/15 ratio test, after patients were stood up with unipolar derivations of the electrocardiography (ECG), the R-R interval of their 30th heartbeat was divided by the R-R interval of their 15th heartbeat. Values of 1.04 and above were considered normal, values of 1 and below were considered abnormal, and values between the limits were considered to be borderline. Heart rate differences were found in sitting position while deep breathing 6 times in 1 minute to determine heart rate response to deep breathing. The maximum and minimum values of 6 respiratory cycles were found and their mean values were calculated. The minimum mean was subtracted from the maximum mean and recorded as beats/min. Values of 15 and above were considered normal, values of 10 and below were abnormal, and values between were considered as borderline (**Table 2**). In order to detect sympathetic autonomic neuropathy, the presence of postural hypotension was investigated in each patient. SBP changes of 30 mmHg or more when standing up were defined as postural hypo-

Table 2. Normal, borderline and abnormal values in 30/15 ratio, heart rate response to deep breathing tests

Cardiac Autonomic Neuropathy Tests	Normal	Borderline	Abnormal
30/15 ratio test	≥1.04	1.01-1.03	≤1
Heart rate response to deep breathing test	≥15	11-14	≤10

tension.For peripheral neuropathy, patients were evaluated by vibration tests on physical examination. 15 seconds or more was considered normal and less than 15 seconds was considered abnormal.

Electroneuromyography (EMG) was performed in all patients. According to EMG results, it was recorded whether the patients had sensory, motor or sensorimotor neuropathy. Insulin resistance (HOMA-IR) was calculated using the Homeostasis Model Assessment method by using the formula [(Fasting plasma glucose (mmol / L) X Fasting plasma insulin (microU / L)) / 405] (7).

HbA1c level was studied by high-performance liquid chromatography (HPLC) method. The collected data were evaluated with SPSS 15.0 program. Normality test in order to determine whether the variables were distributed normally, t-test for comparison of quantitative data showing normal distribution, Mann Whitney U test for cases that could not show normal distribution, and Chi-Square test for comparison of qualitative data were employed. A p-value of ≤0.05 was considered significant.

RESULTS

Of the 111 patients included in the study, 72 were female and 39 were male. Coexisting with DM, 87 patients had hypertension (HT), 27 had CAD, 8 had CVD, 57 had hyperlipidemia, 3 had CRF and 24 had hypothyroidism.

Table 3. Specificity and sensitivity of the groups in the diagnosis of PAD

Groups	Sensitivity	Specifity
ABI-1G1	96	81.4
ABI-1G2	100**	76
ABI-1G3	96	79.1
ABI-2G1	96	82.6**
ABI-2G2	100**	77.9
ABI-3G3	96	80.2

The prevalence of PAD was 19.8% in diabetic patients older than 40 years based on bilateral lower extremity arterial Doppler USG. Based on Doppler USG results; the sensitivity of dorsalis pedis artery palpation in detecting PAD was 100%, specificity was 82%, and kappa value was 0.644. The most specific group for detecting PAD was ABI-2G1 and the most sensitive groups were ABI-1G2 and ABI-2G2 (**Table 3**).

Relationship between PAD and age, DM duration, HOMA-IR, GFR, body mass index (BMI), albuminuria, homocysteine, phosphorus, high-density lipid (HDL), low-density lipid (LDL), triglyceride, HbA1c and uric acid levels in diabetic patients were analyzed in each group (**Table 4**).

The relationship between PAD and gender, concomitant diseases (HT, CAD, CVD, hyperlipidemia, CRF, hypothyroidism) and drugs used (metformin, sulfonylurea, acarbose, glinide, pioglitazone, dipeptidyl peptidase 4 inhibitors, basal-bolus insulin, angiotensin-converting enzyme inhibitor, calcium channel blocker, diuretic, beta-blocker, angiotensin receptor blockers, alpha antagonist, statin, fenofibrate, acetylsalicylic acid, clopidogrel, pregabalin, alpha-lipoic acid, gabapentin) in each groups were shown in **Table 5**.

Table 4. Factors related to PAD

	doppler	ABI1G1	ABI1G2	ABI1G3	ABI2G1	ABI2G2	ABI2G3
Age (years)	**0,001***	**0,009***	**0,010*****	**0,006*****	**0,007*****	**0,008*****	**0,005*****
DM duration (years)	**0,026***	0,200	0,104	0,136	0,172	0,061	0,116
BMI (kg/m2)	0,052	0,099	**0,014*****	0,072	0,097	**0,011*****	0,072
GFR (ml/min)	**0,046***	0,610	0,933	0,521	0,704	0,872	0,425
Albuminuria(mg/day)	**0,046***	0,883	0,931	0,711	0,718	0,904	0,871
Homocystein (umol/L)	**p<0,001***	**0,002***	**0,009*****	**0,005*****	**0,001*****	**0,009*****	**0,005*****
Phosphorus (mg/dL)	0,113	0,243	0,065	0,131	0,167	0,081	0,160
HDL (mg/dL)	0,865	0,740	0,931	0,733	0,908	0,898	0,901
LDL (mg/dL)	0,461	0,543	0,313	0,496	0,464	0,202	0,901
TG (mg/dL)	0,474	0,893	0,785	0,872	0,872	0,765	0,852
HbA1c (%)	0,091	0,749	0,954	0,607	0,408	0,816	0,417
Uric acid (mg/dL)	**p<0,001***	**<0,001***	0,397	0,240	**<0,001*****	**<0,001*****	0,216
HOMA-IR	**p<0,001***	**<0,001***	**<0,001*****	**<0,001***	**<0,001*****	**<0,001*****	**<0,001*****

BMI: body mass index, GFR: glomerular filtration rate, HDL: high density lipid, LDL; low density lipid, TG: triglyceride, HOMA-IR: insulin resistance calculated by HOMA method

Table 5. The relationship of PAD with gender, concomitant diseases and drugs used in each groups.

	Doppler USG	ABI1 G1	ABI1 G2	ABI1 G3	ABI2 G1	ABI2 G2	ABI2 G3
Gender	0,077	0,154	0,276	0,261	0,114	0,217	0,202
HT	0,109	0,302	0,295	0,220	0,351	0,342	0,259
CAD	0,019	0,082	0,250	0,134	0,063	0,206	0,106
CVD	0,014	0,134	0,266	0,152	0,126	0,261	0,143
HPL	0,226	0,242	0,355	0,251	0,167	0,259	0,175
CRF	0,539	p>0,05	p>0,05	>0,05	p>0,05	p>0,05	p>0,05
Hypothyroidim	p>0,05	0,582	0,564	0,452	0,652	0,633	0,514
Hepatosteatosis	0,352	0,502	0,557	0,842	1,00	0,550	0,690
MEDICATION							
Metformin	0,180	0,532	0,572	0,282	0,691	0,733	0,394
Sulfonylurea	0,691	0,491	0,523	0,499	0,317	0,514	0,494
Acarbose	0,986	0,669	0,910	0,833	0,755	p>0,05	0,924
Nateglinide	0,654	0,701	p>0,05	>0,05	0,695	p>0,05	0,708
DPPA4 inh	0,741	p>0,05	0,742	0,920	p>0,05	0,800	0,981
Basal insulin	0,851	0,962	0,897	0,914	p>0,05	0,794	0,809
Bolus insulin	0,513	0,579	0,436	0,571	0,455	0,333	0,449
ACEI	0,452	0,415	0,484	0,298	0,639	0,718	0,484
Calcium channel blockers	0,816	p>0,05	p>0,05	>0,05	p>0,05	p>0,05	p>0,05
Diuretic	0,246	0,519	0,431	0,432	0,432	0,355	0,355
Beta blocker	0,551	0,910	0,653	0,681	p>0,05	0,762	0,793
ARB	0,202	0,558	0,405	0,440	0,476	0,339	0,369
Alpha antagonist	0,654	p>0,05	p>0,05	>0,05	p>0,05	p>0,05	0,708
Statin	0,985	0,578	0,557	0,293	0,475	0,459	0,226
Fenofibrate	p>0,05	0,484	0,736	0,479	0,490	p>0,05	0,481
ASA	0,733	0,853	p>0,05	0,761	0,740	0,987	0,652
Clopidogrel	**0,002**	**0,025***	0,060	0,051	**0,022****	0,056	**0,049****
Pregabalin	p>0,05	0,480	0,866	0,626	0,414	0,784	0,551
Alfalipoic acid	0,539	p>0,05	p>0,05	>0,05	p>0,05	p>0,05	p>0,05
Gabapentin	p>0,05	0,418	0,699	0,250	0,418	0,701	0,257

HT: Hypertension; CAD: Coronary Artery Disease, CVD: Cerebrovascular Disease, HPL: Hyperlipidemia; CRF: Chronic Renal Failure; DPPA4 inh: dipeptidyl peptidase 4 inhibitors, ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blockers, ASA: acetylsalicylic acid

The relationship between PAD and neuropathy tests (orthostatic hypotension, decreased vibration sensation, neuropathy detected in EMG, 30/15 ratio or parasympathetic autonomic neuropathy determined by heart rate response to deep breathing, retinopathy diabetic retinopathy, hypertensive retinopathy, and nephropathy in all groups was given in **Table 6**.

DISCUSSION

When diabetic and non-diabetic patients were compared, the incidence of PAD was reported to be 2 to 4 times higher in diabetic patients (8). In China, the prevalence of PAD was 24.1% in type 2 DM patients over 60 years of age (9). In our study, the prevalence of PAD was found 19.8% in diabetic patients over 40 years of age by lower extremity peripheral Doppler USG.

In various studies, it was shown that ABI-2 method was more sensitive but less specific and less positive predictive than ABI-1 method (10). In our study, the most specific group in detecting PAD was ABI2G1 group and the most sensitive groups were ABI1-G2 and ABI2-G2 groups.

In many previous studies, a significant relationship was found between the prevalence of low ABI and age (11,12). Similarly, in our study, a significant relationship was found between PAD and age in all groups. In a study, it was found that PAD was more common in males than in females independently of age (13). Similarly, although many studies found a significant relationship between ABI prevalence and gender, Bozkurt et al. (14) found no significant dif-

Table 6. The relationship of PAD with neuropathy tests, retinopathy and nephropathy in all groups

	Doppler USG	ABI1 G1	ABI1 G2	ABI1 G3	ABI2 G1	ABI2 G2	ABI2 G3
NEUROPATHY TESTS							
Vibration test	**<0,001**	**0,028***	**0,003***	**0,008***	**0,020***	**0,002***	**0,011***
Orthostatic hypotension	0,137	p>0,05	p>0,05	>0,05	p>0,05	p>0,05	p>0,05
EMG	0,806	0,737	0,524	0,705	0,607	0,417	0,579
30/15 ratio	0,849	>0,05	0,985	0,876	0,766	0,112	0,608
Heart rate response to deep breathing	>0,05	>0,05	0,645	>0,05	p>0,05	p>0,05	p>0,05
RETINOPATHY							
Diabetic	**0,049***	0,420	0,313	0,418	0,315	0,230	0,315
hypertensive	0,768	0,842	0,387	0,639	0,626	0,251	0,450
NEPHROPATHY							
GFR	0,083	0,610	0,933	0,521	0,704	0,872	0,425
albuminuria	**0,046***	0,883	0,931	0,711	0,718	0,904	0,871

GFR: Glomerular Filtration Rate

ference between females and males in terms of ABI prevalence. In our study, no significant relationship was found between PAD and gender, similar to the study conducted by Bozkurt et al.

In a study by Monteiro et al. (15), a significant relationship was found between low ABI and the number of drugs used. In our study, there was a significant relationship between PAD and clopidogrel use in ABI-1G1, ABI-2G1, ABI-2G3, and Doppler USG groups. However, there was no significant relationship between clopidogrel use and PAD in other groups.

In a study by Escobedo et al. (16), a strong relationship was found between HbA1c levels and the risk of PAD in patients with DM. In a study conducted in China, no significant relationship was found between PAD and HbA1c levels (17). In our study, similar to the study in China, no significant relationship was found between PAD and HbA1c levels.

Monteiro et al. (15), found no significant difference between patients with and without PAD regarding GFR. In our study, a significant relationship was found between PAD and GFR in the Doppler USG group (p: 0.046). As GFR decreases, the rate of PAD increases significantly in diabetic patients. Escobedo et al. (16), showed a strong relationship between albuminuria and PAD in patients with DM. In our study, a significant relationship was found between PAD and albuminuria in the Doppler USG group (p: 0.046).

In a study conducted in United Kingdom with 3834 type 2 DM patients, a significant relationship was found between decreased sensation of vibration and PAD (18). Similarly, in our study, a significant correlation was found between decreased sensation of vibration and PAD (p <0.001).

In the studies of Fowkes et al. (11) and Langlois et al. (19), a significant relationship was found between high uric acid levels and low ABI. In our study, a significant relationship was found between PAD and uric acid levels in ABI-1G1, ABI-2G1, ABI-2G2 and Doppler USG groups (p <0.001 in all four groups).

Escobedo et al (16), showed a strong relationship between the duration of DM and PAD. In our study, a significant relationship was found between PAD and duration of DM in the Doppler USG group (p: 0.026). As duration of DM increases, the rate of PAD increases significantly. Escobedo et al. (16), revealed a significant relationship between low ABI values and obesity in men, but not in women. In our study, in ABI-1G2 and ABI-2G2 groups, a significant relationship was found between PAD and BMI (p:0.014 and p:0.011, respectively).

Uzun et al. (20), reported no relationship between the presence of HT and PAD. Similarly, there was no significant relationship between HT and PAD in our study. In a previous study, the prevalence of PAD was 16% in type 2 diabetic patients with hyperhomocysteinemia, whereas the prevalence of PAD in type 2 diabetic patients with normal homocysteine levels was only 3% (21).

In our study, a significant relationship was found between PAD and homocysteine levels in all groups. As the homocysteine level of diabetic patients' increases, the rate of PAD increases significantly. Balletshofer et al. (22), found that people with high insulin resistance had significantly more endothelial dysfunction. In our study, a significant relationship was found between PAD and HOMA-IR in all groups. As the insulin resistance of diabetic patients increases, the rate of PAD increases significantly.

As a result, in our study, as in other studies, the diagnosis of PAD made by ABI method was found to be highly concordant based on Doppler USG results. The superior side of our study compared to the other studies is that the ABI is calculated by 2 methods and the calculated ABI values

are divided into three groups according to cut off values (≤0.9; ≤0.9->1.30; ≤0.9->1.40). In order to make a definite diagnosis of PAD in risky patients, the evaluation of ABI with the second calculation method (dividing the lower of the SBPs taken from both ankle levels by the higher SBPs measured from both arms) is important in order to prevent future complications.

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no conflict of interest. No financial support was received.

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Research Article / Araştırma Makalesi

Interobserver reliability in the ultrasonography evaluation with Graf method of developmental dysplasia of the hip: the importance of education for ultrasonography classification

Gelişimsel kalça displazisinin Graf yöntemiyle ultrasonografi değerlendirmesinde gözlemciler arası güvenirlilik: ultrasonografi sınıflamasında eğitimin önemi

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ABSTRACT

Aim: Developmental dysplasia of the hip is a hip deformity that can be diagnosed early by numerous ultrasonography measurements. The purpose of this study was to evaluate the interobserver reliability of ultrasonography measurements using the Graf ultrasonography (USG) method.

Material and Method: Ultrasonography measurements that were used by Graf ultrasonography (USG) method were obtained at presentation for 62 randomized and consecutive patients. Bilateral hip USG measurements were made for each patient. Each USG outcome was evaluated on multiple occasions by an orthopedic surgery specialist, a pediatric specialist, and a 25-year experienced radiologist.

The statistical measurements were made by SPSS 20.0 (Windows, IL, USA). Intraclass Correlation Coefficient was determined. 95% Confidence Interval and F Test with True Value 0 was detected. P values were accepted as statistically significantly less than 0,05.

Results: At the evaluation before education for Graf USG classification, the results were established. In the interobserver reliabilities, Intraclass classification correlations (ICC) values were 0,939 for USG classification evaluation with Graf method for right hips. ICC values were 0,907 for USG classification evaluation with Graf method for left hips. At the evaluation after education for Graf USG classification, the results were established. ICC values were 0,975 for USG classification evaluation with Graf Method for right hips. ICC values were 0,970 for USG classification evaluation with Graf Method for left hips. All p values were significant. While before education for Graf USG classification ICC was 0,838 in right hips, 0,765 in left hips; after education, it was 0,928 in right hips, 0,915 in left hips.

Conclusion: Diagnosis of DDH deformity with USG is a complex and difficult condition that needs a serious education period. Graf USG method was found to have high interobserver reliability. And also, we detected that education for Graf USG classification increased intra-observer reliability, especially among pediatrists.

Keywords: Developmental dysplasia of the hip, ultrasonography, Graf, inter-observer reliability, intra-observer reliability

ÖZ

Amaç: Birkaç ultrasonografi (USG) ölçümüyle gelişimsel kalça displazisi (GKD) deformitesi erken dönemde teşhis edilebilmektedir. Bu çalışmanın amacı, Graf USG yöntemini kullanarak gözlemciler arası güvenirliliği değerlendirmekti.

Gereç ve Yöntem: Altmış iki rastgele ve ardışık olarak sağlanan hasta için Graf USG yöntemi ölçümlerde kullanıldı. Her hasta için her iki kalça US ölçümü yapıldı. Her US sonucu, multidisipliner yaklaşımla, ortopedik cerrahi uzmanı, çocuk hastalıkları uzmanı ve 25 yıl tecrübeli radyoloji uzmanıyla değerlendirildi. İstatistiksel ölçümler SPSS 20.0 (Windows, IL, ABD) ile yapıldı. Sınıf İçi Korelasyon Katsayısı belirlendi. % 95 Güven Aralığı ve Gerçek Değer 0 ile F Testi tespit edildi. 0,05'ten daha düşük p değerleri istatistiksel olarak anlamlı kabul edildi.

Bulgular: Graf USG eğitiminden önceki değerlendirmede bulgular belirlendi. Gözlemciler arası güvenirlilikte, Sınıf içi sınıflama korelasyonları (ICC) değerleri, sağ kalçalar için Graf yöntemiyle değerlendirmede USG sınıflamasında 0,939 idi. ICC değerleri, sol kalçalar için Graf yöntemiyle değerlendirmede USG sınıflamasında 0,907 idi.

Graf USG sınıflaması eğitiminden sonraki değerlendirmede, bulgular belirlendi. Graf yöntemiyle USG sınıflama değerlendirmesinde sağ kalçalar için ICC değerleri 0,975 idi. Graf yöntemiyle USG sınıflama değerlendirmesinde sol kalçalar için ICC değerleri 0,970 idi. Bütün p değerleri anlamlıydı.

Graf USG yöntemi için eğitimden önce ICC değerleri sağ kalçalarda 0.838, sol kalçalarda 0.765 iken, eğitim sonrası sağ kalçalarda 0.928, sol kalçalarda 0.915 idi.

Sonuç: USG ile GKD deformitesinin tanısı ciddi bir eğitim dönemi gerektiren karmaşık ve zor bir durumdur. Graf USG yöntemi yüksek gözlemciler arası güvenirliliğe sahip bulundu. Ayrıca Graf USG sınıflamasına yönelik eğitimin, özellikle çocuk doktorları arasında gözlemci içi güvenilirliği arttırdığını tespit ettik.

Anahtar Kelimeler: Gelişimsel kalça displazisi, ultrasonografi, Graf, gözlemciler arası güvenirlilik, gözlemci içi güvenirlilik

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INTRODUCTION

Developmental dysplasia of the hip (DDH) deformity is a possible disability condition of the hip (1). And it can result in gait dysfunction and severe functional limitations. Although surgical treatment for developmental hip dysplasia tends to decrease by the Graf ultrasonography (USG) methods, still more surgical treatment is an option for DDH management (2). Although early radiographs cannot be applied because of the radiation risks, hip ultrasound is a qualified diagnosis method. Developmental dysplasia of the hip measurements utilizing USG evaluation with Graf method and Graf DDH classification views allow one to characterize the hip deformity (3).

The purpose of this study was to determine interobserver reliability with varying levels of observer experience on Graf USG classification. And it aims to detect the importance of education on Graf USG classification personally.

METHODS

USG evaluation according to Graf measurements on 62 patients who presented to the clinic with complaints of DDH were examined. The group was chosen randomly. USG views were evaluated at the routine clinical trials. USG evaluation and classification according to Graf method were made by a 15-year experienced orthopedic surgery specialist, a 25-year experienced radiologist and an 11-year experienced pediatric specialist. Each observer obtained Graf USG education firstly by individual reading. This reading session was based on Graf's Hip USG evaluation of literature. And these articles were discussed with the senior author prior to making measurements. Measurements were made by each observer using the computer tools of our hospital. Each observer made measurements and classification independently and blindly. All measurements were made on three separate occasions with the order of images randomized.

These databases were collected from measurements made by an orthopedic surgery specialist who had been orientated on pediatric orthopedia, a pediatric specialist and a pediatric radiologist. These measurements were made in separate settings and were analyzed to determine interobserver reliability. A statistician at our institution's statistical consulting center determined interobserver reliability between three measurements. And then the results were compared to the same measurements made on USG.

Statistics

The statistical measurements were made by SPSS 20.0 (Windows, IL, USA). Intraclass Correlation Coefficient was determined. 95% Confidence Interval and F Test with True Value 0 were detected. P values were accepted as significantly less than 0,05.

Ethical Declaration

In this study, national and international ethical rules are observed. Ethical approval was obtained for this study from the Committee of Ethics Committee of Kafkas University, School of Medicine (Date 26/03/2019, 05/ 80576354-050-99/174 number and decision no).

RESULTS

At the evaluation before USG education, the results were established. In the interobserver reliabilities, Intraclass Classification Correlations coefficient (ICC) values were 0,939 for Graf USG classification evaluation for right hips. ICC values were 0,907 for USG classification evaluation with Graf Method for left hips. **Table 1** and **2** show these values along with the difference in interobserver reliability between three specialists before USG education.

At the evaluation after USG education, the results were established. ICC values were 0,975 for USG classification evaluation with Graf Method for right hips. ICC values were 0,970 for USG classification evaluation with Graf Method for left hips. **Table 3** and **4** show these values along with the difference in interobserver reliability between three specialists after USG education.

The interobserver reliabilities for all measurements on USG were statistically equal to zero according to p-values (**Table 1, 2, 3** and **4**). The difference between sessions as before and after education are statistically significant. The measurements had consistent high interobserver reliability on USG with Graf method. These values are listed in all tables, and all measures were significant as statistically (p=0.000). Intra Class Correlations were 0,838 in right hips, 0,765 in left hips before education; and 0,928 in right hips, 0,915 in left hips after education on Graf USG classification. All p values were equal to zero (p=0.000).

DISCUSSION

Hip ultrasonography for DDH diagnosis was first introduced in the 1980s by Graf. The Graf USG classification of hip development has improved early detection and accuracy for DDH (4). In 1992, Matos et al (5) stated that ultrasound of the infantile hip using the Graf USG method was an important tool for the assessment of developmental dysplasia of the hip and it could be used interpretation training and frequent use. Spaans et al (6) reported a correlated relationship between USG and radiographic imaging outcomes, in patients for treatment and follow-up of DDH.

In the criteria of Graf USG classification, there are four important points: i) angles (alpha and beta), ii) labrum position, iii) position of femoral head, iv) the ossification of the femoral head. The most important point is to make a differential diagnosis among type 2b and type 2c (7). Because patients with Type 2c needs treatment. In a study, 4222 hips were evaluated for DDH by Fan et al. (7). They emphasized that femoral head coverage can be used as a reference indicator for DDH classification. And they established femoral

Table 1. Right hip Graf USG evaluations before education

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	Lower Bound	Upper Bound	
Single Measures	,838[a]	,335	,941	57,905	61	122	,000
Average Measures	,939[c]	,602	,979	57,905	61	122	,000

Two-way mixed effects model where people's effects are random and measure effects are fixed.
a. The estimator is the same, whether the interaction effect is present or not.
b. Type A intraclass correlation coefficients using an absolute agreement definition.
c. This estimate is computed assuming the interaction effect is absent because it is not estimable otherwise.

Table 2. Left hip Graf USG evaluations before education

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Single Measures	,765[a]	,349	,898	,765[a]	,349	,898	,765[a]
Average Measures	,907[c]	,617	,964	,907[c]	,617	,964	,907[c]

Two-way mixed effects model where people's effects are random and measure effects are fixed.
a. The estimator is the same, whether the interaction effect is present or not.
b. Type A intraclass correlation coefficients using an absolute agreement definition.
c. This estimate is computed assuming the interaction effect is absent because it is not estimable otherwise.

Table 3. Right hip Graf USG evaluations after education

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Single Measures	,928[a]	,885	,956	,928[a]	,885	,956	,928[a]
Average Measures	,975[c]	,958	,985	,975[c]	,958	,985	,975[c]

Two-way mixed effects model where people's effects are random and measure effects are fixed.
a. The estimator is the same, whether the interaction effect is present or not.
b. Type A intraclass correlation coefficients using an absolute agreement definition.

Table 4. Left hip Graf USG evaluations after education

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Single Measures	,915[a]	,859	,949	,915[a]	,859	,949	,915[a]
Average Measures	,970[c]	,948	,982	,970[c]	,948	,982	,970[c]

Two-way mixed effects model where people's effects are random and measure effects are fixed.
a. The estimator is the same, whether the interaction effect is present or not.
b. Type A intraclass correlation coefficients using an absolute agreement definition.
c. This estimate is computed assuming the interaction effect is absent because it is not estimable otherwise.

head coverage (FHC) at different positions corresponds to different reference values, and they stated Neutral and Flexion Positions (FHC-D) can be used as a quantitative indicator for assessment of hip stability (8). Choudry et al. (9) founded the positive predictive value (PPV) of clinical screening was found as 4.0% and the PPV of sonography was found as 16.1%. They evaluated previously published 10 or 15-year studies, and they found a deterioration in the PPV in those with potential instability of the hip. These findings were evaluated as a paradox Roposch et al. (10) have found that Graf classification showed moderate reliability. And they emphasized using self-study was not quite as effective as by a structured program. In another study about the USG evaluation for DDH, Kolb pointed to the examiner should pay attention to avoid transducer inclinations in the frontal plane and a combination of posterior and cranial inclination (11). Bilgili et al. (12) studied the reliability of computer-assisted and manual measurement methods for assessment of Graf type 1 and type 2 hip sonograms. They have established the alpha angle measurements had a high concordance, but low concordance for beta angle measurements. The two measurement methods were reliable and consistent with each other.

In this study, the interobserver reliability of the Graf USG classification among three-branch specialists before and after education was evaluated. The evaluation consisted of two steps. The first step was self-study and the second step two was learning the method by using standard teaching material developed by Graf USG professional personal trainer. Training studies were assumed at the same by three observers as performed self-study (before USG education) and teaching sessions (after USG education). Interobserver reliability was 0.602 (95% CI=0.335-0.979) for right hips, and 0.617 (95% CI=0.349-0.964) for left hips. After education, interobserver reliability was 0.958 (95% CI=0.885-0.985) for right hips, and 0.948 (95% CI=0.859-0.982) for left hips. All p values were equal to zero (p=0,000). Intra class correlation was 0,838 in right hips, 0,765 in left hips before education for Graf USG classification, and 0,928 in right hips, 0,915 in left hips after education for Graf USG classification.

In this study, our intention was to assess the interobserver reliabilities on the differing capability of three observers on Graf USG classification. Our hypothesis was that the USG education for the Graf classification method was better in improving interobserver reliability as statistically significant. Education increased the ability to make a differential diagnosis between Type 2b and 2c. And this effect reflected in conservative treatment decisions. Also, the variability in the three evaluations reflects each individual's experience and training level.

The most important point that must be emphasized is the misclassification may effect treatment incorrectly. An education for Graf classification can increase interobserver reliability, and it can provide a safer differential diagnosis for Type 2b and 2c for early diagnosis and treatment.

Limitation of this study is that the USG measurements were made in separate settings, which could cause bias among observers. Also, the study group could be larger.

CONCLUSION

We believe that the USG evaluation chosen to characterize developmental dysplasia of the hip deformity should be easy to teach to observers and have high reliability. This is the only way that results from different specialists can be meaningfully compared. These measurements must also be simple to perform uniform and adequate treatment.

We found that the Graf USG method is the most reliable measurement for early diagnosis and treatment on DDH management. Better education for Graf USG classification provides more accurate USG evaluation for early treatment.

DECLARATION OF INTEREST STATEMENT

The authors declared that this study has received no financial support.

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Research Article / Araştırma Makalesi

Konsantre büyüme faktörünün random patern cilt flep yaşayabilirliği üzerine etkisi: deneysel çalışma

Effect of concentrated growth factor on random pattern skin flap viability: experimental study

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ÖZ

Giriş: Plastik ve rekonstrüktif cerrahide, random patern cilt flepleri çeşitli nedenlerle oluşan doku defektlerinin onarımında sıklıkla kullanılmaktadır. Bu tür fleplerde en sık karşılaşılan sorun yetersiz kan akımına bağlı flep distalinde görülen iskemik nekrozdur. Flep kayıplarını ortadan kaldırmak ve azaltmak için çok sayıda teknik tanımlanmıştır. Bu çalışmadaki amacımız tam kandan elde edilen ve büyüme faktörleri açısından zengin olan konsantre büyüme faktörünün (CGF) daha önce çalışılmamış olan random patern cilt flep yaşayabilirliği üzerine etkisini incelemektir.

Gereç ve Yöntem: Çalışmada 50 adet Spraque–Dawley cinsi sıçan kullanıldı. Sıçanların 40 tanesi deney için, 10 tanesi CGF elde etmek için kullanıldı. Sıçanların sırt bölgelerinden kaldırılan 3x9 cm'lik distal bazlı McFarlene fleplerinin altına çalışma grubunda (n:20) CGF matriks yerleştirildi. Kontrol grubunda (n:20) herhangi bir tedavi uygulanmadı. İşlem sonrası 7. günde değerlendirme için fotografik, sintigrafik ve histopatolojik inceleme yapıldı.

Bulgular: Alan hesaplamaları sonucunda kontrol grubunda flep yaşayabilirlik oranlarının ortanca değerleri %53 (47-58) olarak bulundu. CGF uygulanan grupta ise flep yaşayabilirlik ortanca oranı %58 (55-64) olarak bulundu. CGF grubunda elde edilen yüksek flep yaşayabilirlik oranları istatistiksel olarak anlamlı bulundu (p<0,001). Sintigrafik perfüzyon alan hesaplamaları sonucunda kontrol grubunda flep yaşayabilirlik oranlarının ortanca değerleri %54 (51-60) iken bu değer CGF uygulanan grupta %61 (59-64)'di. Elde edilen artış istatistiksel olarak anlamlı bulundu (p=0,001). Histopatolojik değerlendirme sonucunda çalışma grubunda elde edilen vaskülarite skorlarının ortanca değerlerinin (10,0 (8,25-11,75)) kontrol grubuna (7,5 (6,25-9,00)) göre fazla olduğu görüldü ve bu fark istatistiksel olarak anlamlı bulundu (p=0,003). İnflamasyon skorları açısından gruplar arasında anlamlı farklılık saptanmadı (p=0,246).

Sonuç: Bu çalışmanın sonuçları lokal olarak flep altına uygulanan konsantre büyüme faktörünün flep yaşayabilirliğini artırdığını ve bu etkinin anjiyogenez ile ilişkili olabileceği gösterilmiştir, ancak bu konuda ileri çalışmalara ihtiyaç vardır. Flep cerrahisinde, iskemi öngörülen durumlarda büyüme faktörlerinden zengin CGF'in uygulanmasının flep yaşayabilirliği üzerine olumlu etkileri olabilir.

Anahtar Kelimeler: Konsantre büyüme faktörü, CGF, cilt flebi, flep yaşayabilirliği

ABSTRACT

Introduction: Random pattern skin flaps are used frequently for the reconstruction of random pattern skin defects from various causes in plastic and reconstructive surgery. Most frequent complication with these flaps is the necrosis of distal parts due to inadequate blood supply. Various types of techniques have been defined to avoid and reduce flap loss. The aim of this study is, for the first time, to evaluate the effect of concentrated growth factor (CGF), which is derived from whole blood and is rich in growth factors, on the survival of random pattern skin flaps.

Material and Method: Fifty Sprague-Dawley rats were used for the study. Forty of them were used for the experiment group and ten for the preparation of CGF. Distal based 3x9 cm McFarlene flaps were elevated from the back of the rats and in CGF group (n:20), CGF matrix was placed before closure. No treatment was given to the control group (n:20). On postoperative day 7, the flaps were evaluated by photographic, scintigraphic, and histopathological methods.

Results: The median rate of surviving flap areas in control group was 53% (47-58). In CGF group the median rate of flap viability was %58 (55-64). The higher flap viability rates obtained from CGF applied group was found to be statistically significantly higher from control group (p<0,001). For the scintigraphic perfusion area evaluations, the median rate of flap perfusion was 54% (51-60) for the control group and 61% (59-64) for the CGF group. The higher flap perfusion rates obtained from CGF applied group was found to be statistically significantly higher from control group (p=0,001). The median rate of vascularity scores in CGF treated group was 10,0 (8,25-11,75) and 7,5 (6,25-9,00) in control group. The difference was found to be statistically significantly higher in the study group (p=0,003). Inflammation scores were not found to be statistically significantly different (p=0,246).

Conclusions: This study shows that locally applied CGF under skin flap increases flap viability which might be associated with the increase in angiogenesis. This study for the first time shows that in flap surgery, applying CGF which is rich in growth factors, might have favorable effects on the flaps that are prone to ischemia but further studies are needed.

Keywords: Concentrated growth factor, CGF, skin flap, flap viability

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GİRİŞ

Plastik ve rekonstrüktif cerrahide, random patern cilt flepleri travma, doğumsal malformasyonlar, kanser cerrahisi sonrası veya diğer nedenlerle oluşan doku defektlerinin onarımında sıklıkla kullanılmaktadır. Bu tür fleplerde en sık karşılaşılan sorun yetersiz kan akımına bağlı flep distalinde görülen iskemik nekrozdur (1). Kısmi veya tam kat flep kayıpları vasküler yapıların zarar görmesi sonucu gözlenmektedir ve kan desteği fleplerin yaşamasında en önemli faktördür. Flep kayıplarını ortadan kaldırmak ve azaltmak için çok sayıda teknik tanımlanmıştır. İki basamaklı bir işlem olan cerrahi geciktirme (delay fenomeni), iskemik ön koşullandırma, vasküler implantasyon ile flep prefabrikasyonu kullanılabilmekle beraber pratik uygulamada daha çok sempatolitik, vazodilatör, kalsiyum kanal blokörü, antikoagülan, serbest radikal temizleyici antioksidan ilaçlar ve büyüme faktörleri (bFGF, PDGF, TGF ve VEGF) kan akımının artırılması ve neovaskülarizasyonun sağlanması için kullanılmıştır (2-8). Kullanılan diğer bir alternatif de tam kandan elde edilen trombositten zengin ürünlerdir (1, 8-10).

Trombositlerde bulunan alfa granülleri yüksek miktarda büyüme faktörü içerir. Bu büyüme faktörlerinden fibroblastlar ve düz kas hücreleri için güçlü bir mitojen olan trombosit kaynaklı büyüme faktörü (PDGF) mezenkimal hücre yaşamı ve migrasyonunda rol alır ve dönüştürücü büyüme faktörü β (TGF- β) ile birlikte, doku vaskülarizasyonunu, fibroblast proliferasyonunu, kollajen formasyonunu destekler ve aynı zamanda granülasyon doku üretimini stimüle eder, hücre epitelizasyonunu artırır (1,10,11). TGF- β aktive olduğunda doku onarım basamaklarının büyük kısmını etkilemektedir. Fibroblastları prokollajen üretimleri konusunda aktive edebilir. Vasküler endotelial büyüme faktörü (VEGF) anjiogenezi, endotelial hücre proliferasyonunu stimüle eder ve vasküler geçirgenliği artırır. Epidermal büyüme faktörü (EGF), fibroblastlar için kemotaktiktir, hücre proliferasyonunu ve diferansiasyonunu destekler. Fibroblast büyüme faktörü (FGF) granülasyon doku formasyonunda, reepitelizasyonda ve doku yeniden şekillendirilmesinde rol alır (1). İnsülin benzeri büyüme faktörü 1 (IGF-1), hücre apopitozunu geciktirir ve IGF-1 reseptörü endotelial cevapta önemli rol alır (12). Granüller içinde ayrıca trombosit kaynaklı anjiogenez faktörü (PDAF), trombosit faktör 4 (PF-4), keratinosit büyüme faktörü (KGF) ve interlökin 1 (IL-1) bulunmaktadır (8, 11). Bu kadar farklı ve çeşitli büyüme faktörlerini içeren trombositler, doku rejenerasyonu ve iyileşmesinde kullanılmış ve cilt fleplerinin yaşayabilirliği üzerine olumlu etkileri gösterilmiştir (8-11, 13, 14).

Konsantre büyüme faktörü (CGF), Sacco ve ark. (15) tarafından 2006 yılında tanımlanmıştır ve venöz kanın farklı hızlarda santrifüjü sonrasında elde edilmektedir. Büyüme faktörleri açısından trombositten zengin plazma (PRP) gibi diğer trombosit ürünlerine göre daha yoğun ve zengindir. Teorik olarak CGF'de bulunan büyüme faktörleri normal doku iyileşmesine yakın hızlarda yavaş salınmaktadır (16) ve yapılan çalışmalarda CGF in yağ, kemik, kıkırdak, mukoza, eklem, maksiller sinüs ve alveolar sırt bölgesinde olumlu etkileri gösterilmiştir (15,17-19).

Bu çalışmadaki amacımız tam kandan elde edilen CGF'in daha önce çalışılmamış olan random patern cilt flep yaşayabilirliği üzerine etkisini incelemektir.

GEREÇ VE YÖNTEM

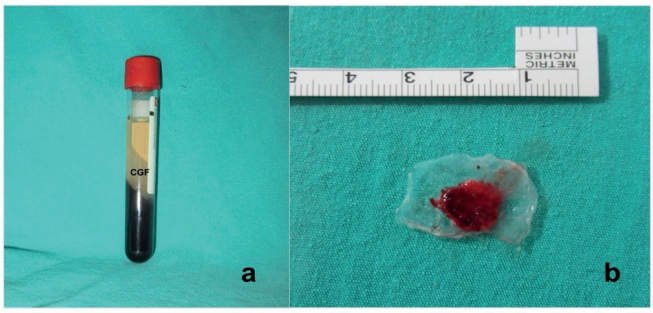
Çalışmada 50 adet, ağırlıkları 240-270 g arasında değişen dişi Spraque–Dawley cinsi sıçan kullanıldı. Sıçanların 40 tanesi deney için, 10 tanesi CGF elde etmek için kullanıldı. Deney için kullanılan sıçanlar rastgele iki gruba ayrıldı (n:20). Sıçanlar 12 saatlik ışık-karanlık döngüsünde, kontrollü hava akımının olduğu, sıcaklığın 22-24 ^{0}C de, nemin kontrol altında tutulduğu standart odalarda takip edildi. Hayvanlar standart sıçan yemi ve musluk suyu ile ad libitum beslendi. Flep cerrahisi sonrası hayvanlar ayrı kafeslerde takip edildi.

Konsantre Büyüme Faktörü Hazırlanması

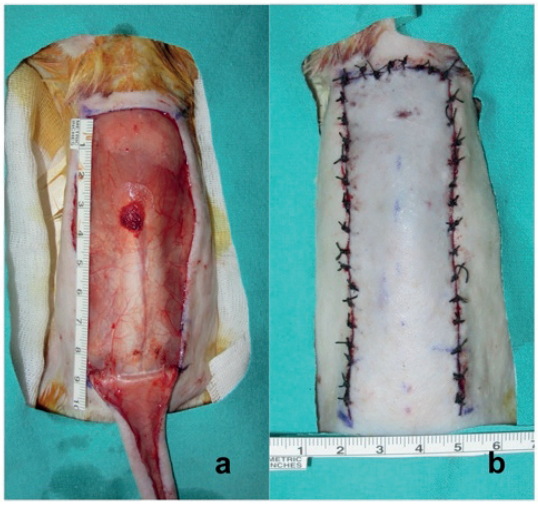
CGF elde edilmesi için 10 adet sıçanın kanları kullanıldı. İşlem standardizasyonunun sağlanması için CGF tek bir araştırmacı (M.A.) tarafından hazırlandı. Steril antikoagülansız 10 mL Vacuette tüplerine alınan venöz kanlar CGF elde etmek için özel üretilmiş olan cihaza yerleştirildi (Medifuge, Silfradentsrl, Italy). Cihaz içindeki kan tüpleri 30 saniye hızlanma, 2 dakika 2700 rpm (600 g), 4 dakika 2400 rpm (400 g), 4 dakika 2700 rpm (600 g), 3 dakika 3000 rpm ve 36 saniye yavaşlama içeren bir programla çalışıldı. İşlem sonunda 3 farklı kan fraksiyonu tanımlandı; 1) en üstte sıvı yapıda trombositten fakir plazma (PPP), 2) ortada daha katı kıvamda CGF, 3) en altta ise kırmızı kan hücrelerinden oluşan tabaka (12). Alınan orta CGF tabakası özel bir maşa yardımı ile sıkıştırılarak ince bir CGF matriks elde edildi (**Şekil 1**).

Cerrahi Teknik

Sıçanlara intramusküler ketamin hidroklorid (Ketalar, Pfizer,) (87,5 mg/kg) ve ksilazin hidroklorid (Rompun, Bayer) (12,5 mg/kg) anestezisi verilip steril şartlar sağlandıktan sonra sırt bölgelerinden 3x9 cm'lik cilt, cilt altı doku ve pannikulus karnosusu içeren distal bazlı McFarlene flepleri kaldırıldı. Çalışma grubunda flepler yerlerine adapte edil-



Şekil 1. Santrifüj sonrası orta CGF tabakası (a) alınıp özel bir maşa yardımı ile sıkıştırılarak ince bir CGF matriks elde edildi (b)



Şekil 2. CGF tabaka flep altına yerleştirildikten (a) sonra flep yerine adapte edildi (b)

meden önce flep yatağının orta 1/3 kısmına denk gelecek şekilde heterolog CGF matriks yerleştirildi. Kontrol grubuna herhangi bir uygulama yapılmadı. 5 dakika bekleme süresi sonrasında flepler yerlerine 4/0 ipek sütürler (Doğsan, Türkiye) ile adapte edildi (**Şekil 2**).

Flep cerrahisi sonrası 7. günde flep yaşayabilirlik oranlarının değerlendirilmesi için standart fotoğraflar çekildi ve sintigrafik olarak değerlendirmeler yapıldı. Sonrasında flepler, histopatolojik inceleme için formaldehit solüsyonu içerisine konuldu.

Flep Yaşam Ölçümleri

Yaşayan flep oranları 1 metre mesafeden standart çekilmiş olan fotoğraflar üzerinden analiz programı (Digimizer, Belçika) ile çalışmacılardan birisi (M.A.) tarafından çalışma ve kontrol gruplarına kör olarak yapıldı. Yaşayan flep oranları toplam flep yüzeyine orantılanarak sonuçlar yüzde olarak hesaplandı.

Radyonüklid Sintigrafik Ölçümler

İşlem sonrası 7. günde deney sonlandırılmadan ve sıçanlar sakrifiye edilmeden önce cilt flepleri sütürler alınarak donör bölgeden kaldırıldı. Flep altına ve çevresine kurşun plakalar yerleştirildi. Kurşun tabakadaki olası radyoaktif madde ile kirlenmeyi önlemek için her çekimde kurşun tabakalar yeni su geçirmez örtüler ile değiştirildi. Kuyruk venine yerleştirilen 24 G intraketten 0,1 ml serum fizyolojik içinde 1mCi (37MBq) Teknesyum perteknetat ($Tc99m\text{-}PO^4$) enjekte edildi. 5 dakika beklendikten sonra kan havuzu fazında 256x256 matriks ile 5 dakika süresince Gama kamera ile (Siemens eCAM, Hoffman Estates, IL, USA) pinhole kolimatör kullanılarak elde edilen görüntülerden kanlanan flep alanlarının ölçümü yapıldı. Ayrıca flepler distal kesimlerinden kesilerek hayvanlardan ayrıldı ve geri plan (Background) aktivitesinin olumsuz etkileri ortadan kaldırılarak sadece flepler üzerinden sintigrafik olarak sayımlar alındı. Elde edilen görüntülerden yapılan ölçümde flebin görüntüsel olarak en boy oranına sadık kalınarak sintigrafik görüntülerde flep tabanı üzerinden hesaplanan genişlik hesabına sadık kalınarak çizilen flep görüntüsü üzerinden yaşayan flep alanı hesaplandı. Hesaplama toplam flep alanının yüzdesi olarak yapıldı.

Histopatolojik Değerlendirme

Sıçanlar sakrifiye edildikten sonra flepler formaldehit içinde patoloji bölümüne gönderildi. Nekrotik alan ve yaşayan kısım arasında bulunan geçiş hattından 2 cm'lik spesimenler alınıp %4'lük formaldehit içinde fikse edildi ve parafin bloklara gömüldü. 3 µm kalınlığında kesitler alınıp sonrasında parçalar konvansiyonel hematoksilen-eosin (HE) ve Masson Trichrome boyaları ile boyandı ve ışık mikroskopisi altında histolojik inceleme yapıldı. Histopatolojik inceleme sırasında damarlanma sayısı ve inflamasyon skorlarına bakıldı. Vaskülarizasyon için sağlıklı geçiş bölgesindeki 10 büyütme alanı içinde papiller dermiste yer alan damarlar sayıldı ve ortalaması alındı. İnflamasyon yoğunluğu kriteri için nötrofil ve lenfosit yoğunluğu değerlendirildi. Skorlama sistemi 0 ile 3 arasında yapıldı (0:Yok, 1:Hafif, 2:Orta, 3:Yoğun). Bütün analizler aynı patolog (N.Y.) tarafından çalışma ve kontrol gruplarına kör olarak yapıldı.

İstatistiksel Analiz

Hastalardan toplanan bilgiler IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., ABD) programına girilerek veri kümesi oluşturuldu ve istatistiksel analizler yapıldı. Değişkenlere ait tanımlayıcı istatistikler (Frekans, Yüzdeler, Ortalama ± Standart Sapma, Ortanca (1. – 3. çeyrek değerleri)) tablolar ile belirtildi. Değişkenlerin parametrik test varsayımlarını karşılayıp karşılamadığını tespit etmek amacıyla Kolmogorov-Smirnov ve Shapiro-Wilk testleri ile normal dağılıma uygunluk tespit edildi. Normal dağılıma uymadığı tespit edilen nicel değişkenler Mann-Whitney U testi ile karşılaştırıldı. Kategorik değişkenlerin karşılaştırılmasında Pearson Ki-kare testi kullanıldı. $P<0,05$ değeri istatistiksel olarak anlamlı kabul edildi.

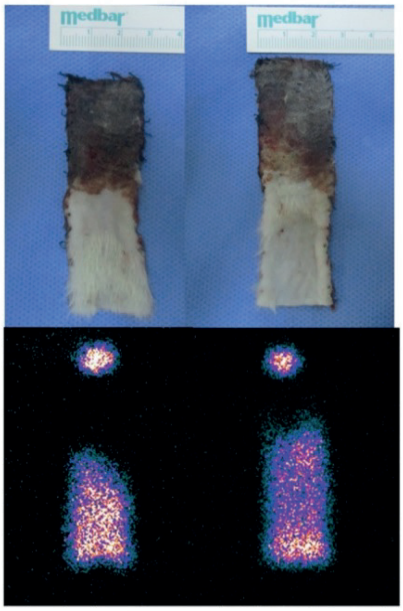
Etik Durum

Bu deneysel çalışma için S.B. Ankara Eğitim ve Araştırma Hastanesi hayvan deney çalışmaları etik kurulundan izin alındı (Tarih: 17/10/2019 ve Protokol No: 56/595). Bu deneysel, prospektif ve kör çalışmada kullanılan hayvanların hakları "Guide for the Care and Use of Laboratory Animals" (https://www.nap.edu/catalog/5140/guide-for-the-care-and-use-of-laboratory-animals) prensipleri doğrultusunda korunmuştur.

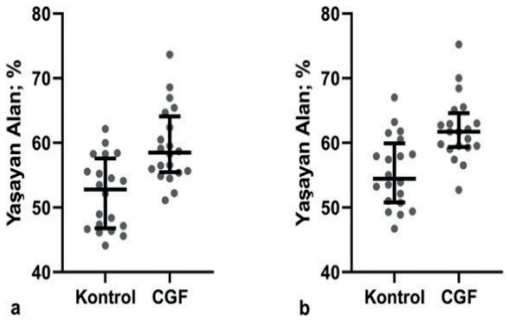
SONUÇLAR

Fotoğraf Alan Hesaplaması Sonuçları

Alan hesaplamaları sonucunda kontrol grubunda flep viabilite oranlarının ortanca değerleri %53 (47-58) olarak bulundu. CGF uygulanan grupta ise flep viabilite oranı %58



Şekil 3. Kontrol (a, c) ve CGF (b, d) uygulanan fleplerin fotografik (a, b) ve sintigrafik (c, d) görüntüsü

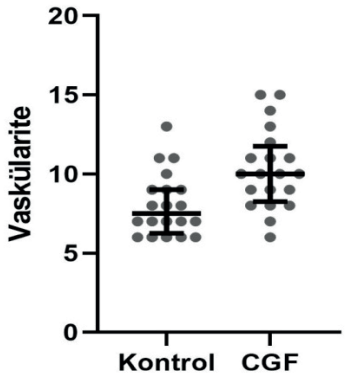


Şekil 4. Yaşayan flep alanlarının fotografik (a) ve sintigrafik (b) sonuçları

(55-64) olarak bulundu. CGF grubunda elde edilen yüksek flep viabilite oranları istatistiksel olarak anlamlı bulundu (p<0,001) (**Şekil 3 ve 4**).

Sintigrafi Alan Hesaplaması Sonuçları

Sintigrafik alan hesaplamaları sonucunda kontrol grubunda flep viabilite oranlarının ortanca değerleri %54 (51-60) olarak bulundu. CGF uygulanan grupta ise flep viabilite oranı %61 (59-64) olarak bulundu. Fotoğraf alan hesaplamalarına benzer biçimde, CGF grubunda elde edilen flep viabilite oranlarındaki artış istatistiksel olarak anlamlı bulundu (p=0,001) (**Şekil 3 ve 4**).



Şekil 5. Kontrol ve CGF gruplarında vaskülarite değerleri

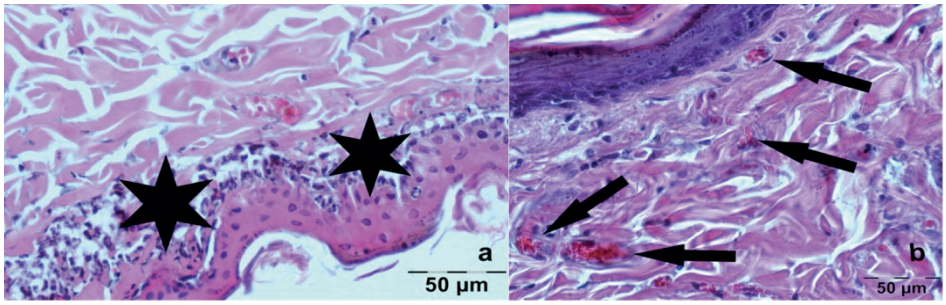
Histopatolojik Değerlendirme Sonuçları

Histolojik değerlendirme sonucunda CGF grubunda elde edilen vaskülarite sayıları ortanca değerlerinin (10,0 (8,25-11,75)) kontrol grubuna (7,5 (6,25-9,00)) göre fazla olduğu görüldü ve bu fark istatistiksel olarak anlamlı bulundu (p=0,003) (**Şekil 5**). İnflamasyon skorları değerlendirildiğinde CGF grubunda hafif skorları daha sık iken; kontrol grubunda orta ve yoğun skorları daha sık görülmekteydi, ancak istatistiksel olarak anlamlı farklılık saptanmadı (p=0,246) (**Şekil 6**) (**Tablo**).

Tablo. İnflamasyon ve vaskülarite değerlerinin analizi.

		KONTROL	CGF	p
		N (%)	N (%)	
İNFLAMASYON	Hafif	5(%25)	10(%50)	0,246*
	Orta	11(%55)	8(%40)	
	Yoğun	4(%20)	2(%10)	
VASKÜLARİZASYON	x̄±SD	8,05±1,99	10,3±2,51	
	Md (Q1-Q3)	7,5 (6,25-9)	10,0 (8,25-11,75)	0,003**

x̄±SD: Ortalama ± Standart Sapma Değeri) *: Pearson Ki Kare Testi
Md (Q1-Q3): Ortanca (1. ve 3. Çeyrek
**: Mann-Whitney U Test



Şekil 6. H&E ile boyanmış flep kesitleri. Kontrol (a) ve CGF (b) grubu. (* inflamasyonu ve → vaskülariteyi göstermektedir.)

TARTIŞMA

Random patern cilt flepleri, rekonstrüktif cerrahide farklı defektlerin onarımlarında artan oranlarda kullanılmaktadır. Bu fleplerin herhangi bir spesifik arteriovenöz dolaşım sistemleri yoktur ve esas olarak cilt pedikülünden ve subdermal kapiller ağ perfüzyonu ile beslenirler. Cilt fleplerinin iskemik nekrozunun patogenezi hala net değildir ve ortak fikir, temel nedeninin; proinflamatuar mediatörlerin hücresel aktivasyonu, yetersiz kanlanma ve tromboz olduğu yönündedir (1). Cerrahi sonrası yaşayan flep sınırlarının tahmin edilmesi zordur ve flep distalindeki dolaşım sorunu hala çözümü net ortaya konulabilmiş değildir. Bu tip fleplerin yaşayabilirliğinin artırılması için etkinliği gösterilmiş ürünlerden bir tanesi de kandan elde edilen konsantre trombosit ürünleridir (2).

Trombositler yara iyileşmesini başlatan ve düzenleyen çeşitli proteinler, sitokinler ve diğer biyoaktif faktörleri içermektedir. Yüksek konsantrasyonlarda büyüme faktörü elde etmenin kolay ve ucuz yöntemi trombositlerin kullanılmasıdır. Bu büyüme faktörleri mezenkimal hücreleri ve epitelyal hücreleri migrasyon ve bölünme açısından uyarır, kollajen ve matriks sentezini artırır, hücre diferansiyasyonu ve anjiyogenezde önemli roller alır.

Otolog platelet konsantrelerinin ilk jenerasyonu 1970 yılında tanımlanmış olan trombositten zengin plazma (PRP)'dır (17). PRP'nin hücre proliferasyonunu, anjiogenez, kollajen ve matriks biyosentezini stimüle ettiği rapor edilmiştir ve yara iyileşmesi, yağ ve kemik greftlerinin iyileşmesinin arttırılmasında çokça kullanılmaktadır (20). Flep yaşayabilirlik üzerine yapılan çalışmalarda, PRP enjeksiyonlarının kapiller proliferasyonu indükleyip, "choke" damarlarını genişlettiği ve inflamatuar hücre infiltrasyonunu azaltarak iskemi reperfüzyon (I/R) hasarına uğramış fleplerde, flep yaşayabilirliğini artırdığı gösterilmiştir (10,11,13,21). Bununla beraber PRP'nin hazırlanmasında artifisyel trombin ve antikoagülan aditifleri kullanıldığı için biyo-güvenilirlik ve dayanıklılıkları konusunda büyük tartışmalar ve çekinceler mevcuttur (22).

Trombosit ürünlerinin en son ortaya çıkan jenerasyonu olan CGF, 2006 yılında Sacco tarafından geliştirilmiştir. CGF'in çok büyük rejeneratif kapasitesi ve çok yönlülüğü vardır (17). Farklı santrifüj hızları büyüme faktörlerinden zengin ve yoğun fibrin matriks elde edilmesine olanak sağlar. PRP'ye göre daha yoğundur ve daha yüksek miktarlarda büyüme faktörü içermektedir ve trombin olmadan aktive olabilmektedir (16,17). Aynı zamanda vasküler dayanıklılık, neovaskülarizasyon ve anjiogenezde rol alan CD34+ kök hücre içermektedir (15,23). CGF'in yumuşak doku iyileşmesinde PRP'ye göre daha etkili olduğu raporlanmıştır (24). Sonuçta önceki çalışmalarda CGF'in inflamasyona aracılık ettiği, hücre proliferasyonunu arttırdığı ve doku onarımını indüklediği belirtilmiştir. Klinik ve deneysel çalışmalarda CGF'in kemik, kıkırdak ve periferik sinir doku üzerine proliferatif etkileri ve greftlerin yaşayabilirliği üzerine olumlu etkileri gösterilmiştir (17,18).

Yaptığımız çalışmada heterolog hazırlanan CGF'in, random patern cilt fleplerinin yaşayabilirliği üzerine olumlu etkileri görüldü. CGF uygulanan fleplerde yaşayan flep oranları kontrol gruplarına göre daha fazla bulundu. Benzer şekilde CGF uygulanan fleplerin vaskülarizasyonlarının da kontrol grubuna göre daha fazla olduğu görüldü.

Her ikisi de trombosit ürünü olan PRP ve CGF'in karşılaştırıldığı çalışmada; PRP preparatları ile kıyaslandığında CGF'in daha fazla trombosit ve trombosit kaynaklı büyüme faktörü içerdiği; TGF-β1, PDGF-BB, VEGF düzeylerinin, CGF'de PRP'ye göre anlamlı yüksek olduğu gösterilmiştir (23, 25). PRP ile yapılan çalışmalarda %84'e varan flep yaşayabilirlikleri gösterilmiştir (8). CGF'nin içeriği nedeni ile teorik olarak anjiogenez ve beraberinde yara iyileşmesini, doku rejenerasyonunu PRP'den daha fazla artırması beklenir. Ancak uygulama farklılıkları vardır. Fleplere PRP uygulanan çalışmalarda, PRP'nin enjeksiyon yöntemi ile uygulanmış olması da dikkate alınması gereken bir faktördür çünkü bu yöntemin flep yaşayabilirliğini arttırdığını gösteren çalışmalar mevcuttur (26). Dolayısıyla, nihai sonuç, sadece PRP etkinliği olarak değerlendirilmemelidir. Diğer taraftan bu çalışmada CGF'in fleplere ek bir işlem yapılmadan uygulanmış olması, etkinin sadece CGF'ye bağlı olduğu düşündürmektedir. CGF ile yapılan flep çalışması olmamakla birlikte, kemik, yağ, kıkırdak, mukoza üzerine etkilerini inceleyen çalışmalarda, CGF uygulanan gruplarda daha yüksek iyileşme görülmüştür (17-19,23,27). Bu çalışmada ise CGF'in flep yaşayabilirliği üzerinde de anlamlı artış sağladığı görüldü.

Histolojik olarak elde edilen sonuçlar değerlendirildiğinde; CGF uygulanan fleplerde damar sayısının kontrol grubuna göre daha fazla olduğu görüldü. İnflamasyon skorları düşük olmakla beraber istatistiksel olarak anlamlı bulunmadı. CGF'in kıkırdak, kemik ve yağ üzerine etkisini inceleyen çalışmalarda da benzer şekilde inflamasyon skorları daha az, vaskülarizasyon skorlarının daha yüksek olduğu gösterilmiştir (18,23,27). Trombosit ürünleri ile yapılan diğer çalışmalarda da benzer şekilde vaskülarizasyon sayılarında artış ve inflamasyon skorlarında azalma görülmüştür (1,9-11,13,14). Trombosit ürünleri ile yapılan çalışmalarda, vaskülarizasyon sayılarında görülen artış esas olarak trombositlerin içinde bulunan VEGF'ye bağlanmıştır (10). VEGF ile yapılan çalışmalarda da VEGF'nin flep yaşayabilirliğini artırdığı gösterilmiştir (5). Bizim çalışmamızda da flep yaşayabilirliği üzerine elde edilen yüksek değerlerin en önemli nedeninin trombositlerin içinde bulunan VEGF olduğu düşünülebilir.

Flep kayıplarının önemli nedenlerinden bir tanesi de revaskülarizasyon sonrası görülen iskemi reperfüzyon (I/R) hasarıdır (2). Trombositler I/R hasarının giderilmesinde anahtar komponentlerden birisidir. Bu etkinin anti-inflamatuar etki ve NF-kB aktivitesini azaltmasından kaynaklandığı düşünülmüştür. NF-kB aktivasyonu inflamasyona neden olmaktadır. Yapılan çalışmada trombosit konsantrelerinin Ccl2, TNF, Il1b ve Il6 gibi inflamatuar sitokinlerin ekspresyonunu etkili biçimde suprese ettiği gösterilmiştir. Trombosit konsantrelerinin cilt flebi, miyokard, böbrek ve

over dokusunda I/R hasarını azalttığı gösterilmiştir (21). Yaptığımız çalışmada benzer şekilde inflamasyon skorları kontrol grubuna göre istatistiksel olarak anlamlı olmamakla birlikte daha düşük bulunmuştur.

CGF hazırlanmasında farklı santrifüj hızları kullanılmaktadır. Son zamanlarda düşük santrifüj hız ve zamanları, lökosit sayıları ve beraberinde salınan büyüme faktörlerinin optimizasyonu için önerilmektedir. Düşük hız konseptinin büyüme faktörü salınımında artışa neden olduğu ve sonrasında fibroblast migrasyonu, proliferasyonu ve kollajen mRNA seviyelerini artırarak doku rejenerasyonunu etkilediği gösterilmiştir (16).

Pediküllü cilt flepleri, artmış iskemi gradiyenti ve sonrasında distal marjinlerinde rejyonel doku nekrozu gösterirler. Bu süreç nörotransmitterler tarafından yönetilmektedir ve bu süreç yeterli kanlanan proksimal kısım ile yetersiz kanlanan distal iskemik alan arasındaki geçiş bölgesinde yer aldığı ileri sürülmektedir (7). Çalışmamızda, CGF matriksini özellikle bu bölgeye uygulama amacımız geçiş bölgesinde yer alan nekroz hattını flepin distaline ilerletebilmek ve flep yaşayabilirliğini artırmaktır.

Otolog CGF biyo-uyumlu ve güvenlidir. Bundan dolayı klinik kullanım sırasında antikor oluşumu veya donörden kaynaklı enfeksiyon riski bulunmamaktadır. Kullanılan denek türü küçük olmasından ve CGF elde etmek için yeterli kan alınamayacağından dolayı çalışmada heterolog ürün kullanılmıştır. Trombosit ürünlerinin flep viabilitesi üzerine etkisini inceleyen çalışmalarda sıçanlarda bu konuda herhangi bir sorun ile karşılaşılmamıştır (1,8,11). İnsanlarda yapılan çalışmalarda ise yeterli kan miktarından dolayı otolog ürünlerin tercih edilebilirliği avantaj sağlayabilir. Sıçanların ve insanların metabolizmaları farklılık gösterdiği için büyüme faktörlerinden zengin olan bu kan ürünü ile ilgili ileri çalışmalara ihtiyaç vardır.

SONUÇ

Bu çalışmanın sonuçları lokal olarak flep altına uygulanan heterolog hazırlanmış konsantre büyüme faktörünün flep yaşayabilirliğini artırdığını ve bu etkinin anjiyogenez ile ilişkili olabileceği gösterilmiştir, ancak bu konuda ileri çalışmalara ihtiyaç vardır. Konsantre büyüme faktörü flep yaşayabilirliğini artırmaktadır ve bu etkisinin olası nedenleri arasında damar sayısının artırılması ve inflamasyonun baskılanmasını düşünebiliriz. Flep cerrahisinde, iskemi öngörülen durumlarda büyüme faktörlerinden zengin CGF'in uygulanmasının flep yaşayabilirliği üzerine olumlu etkileri olabilir.

MADDİ DESTEK VE ÇIKAR İLİŞKİSİ

Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkara dayalı ilişkisi yoktur.

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Research Article / Araştırma Makalesi

Radyoterapi çalışanlarının maruz kaldığı radyasyon miktarının dozimetrik değerlendirilmesi

Dosimetric evaluation of the ramount of radiation exposure on radiotherapy workers

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ÖZ

Amaç: Radyoterapi en sık kullanılan kanser tedavi yöntemlerinden birisidir ve tedavi etkisinin sağlanabilmesi için yüksek doz iyonize radyasyon kullanılmaktadır. Bu radyasyonlu ortamda çalışan radyoterapi personelleri için radyasyona maruz kalmak en büyük mesleki sorundur ve ciddi korunma önlemleri alınması gerekmektedir. Bu doğrultuda radyasyon onkolojisi kliniğimizde çalışan tüm personelimizin son 3 yıllık kişisel dozimetre değerleri incelenmiş ve radyasyon maruziyet oranları değerlendirilmiştir.

Gereç ve Yöntem: 2015-2018 yılları arasında tüm personelin kişisel dozimetreleri TLD ölçümleri verileri kaydedilmiş, ölçüm periyotlarındaki maruz kaldıkları doz miktarları, meslek gruplarına göre ölçüm periyotlarındaki maruz kalınan ortalama dozlar, yıllık toplam doz maruziyeti ve gruplar arasındaki farklılıklar değerlendirilmiştir.

Bulgular: Toplam 18 periyottaki ölçümlerde, her bir personelin periyot başına ortalama maruz kaldığı doz miktarı 0,21 mSv'dir. Meslek gruplarına göre değerlendirildiğinde; kliniğimiz doktorlarında 0,20 mSv, medikal fizik uzmanlarında 0,20 mSv, teknikerlerimizde ise 0,21 mSv'dir. Gruplar arasında anlamlı istatistiksel fark saptanmamıştır. Periyotların ayrı ayrı değerlendirilmesinde genel olarak meslek gruplarının maruz kaldıkları dozlar birbirine benzerken sadece 2016 1. Periyot ve 2017 5. periyotta doktorlar ile diğer gruplarda, 2018 3. periyot vücut dozları ve 4. periyotta medikal fizik uzmanları ile diğer iki grup arasında anlamlı farkın olduğu görülmektedir.

Sonuç: Bir radyasyon onkolojisi merkezinde personel eğitimleri, dozimetrik ölçümler ve denetimler son derece önemlidir. Yaklaşık 30 yıldır radyoterapi tecrübemizin olduğu kliniğimizde yapılan tüm ölçüm değerlerimizin uluslararası standartlara uygun olarak saptanmıştır. Bu doğrultuda radyasyonla çalışan tüm merkezlerin mevcut değerlendirmeleri titizlikle yapmaları önerilmektedir.

Anahtar Kelimeler: Radyoterapi, radyasyon çalışanları, kişisel dozimetre

ABSTRACT

Aim: Radiotherapy is one of the most commonly used cancer treatment methods and high dose ionizing radiation is used to provide therapeutic effect. Radiation exposure is the biggest occupational problem for radiotherapy personnel working in this radiation environment and serious protection measures must be taken. In this regard, the last 3 years of personal dosimeter values of all the staff working in our radiation oncology clinic were examined and radiation exposure rates were evaluated.

Material and Method: Between 2015 and 2018, the data of TLD measurements of personal dosimeters of all personnel were recorded, the amount of doses exposed in the measurement periods, the average doses exposed in the measurement periods according to occupational groups, the annual total dose exposure and the differences between the groups were evaluated.

Results: For measurements in a total of 18 periods, the average amount of dose each employee is 0.21 mSv per period. When evaluated according to profession groups they were; 0,20 mSv in physicians, 0,20 mSv in medical physicists and 0,21 mSv in technicians. There was no statistically significant difference between the groups. While evaluating the periods individually, the doses exposed by the occupational groups are similar, but there is a significant difference between the physicians and other workers in the 1st period of 2016 and the 5th period of 2017 and between the medical physicists and other workers in the 3rd period body doses of 2018 and the fourth period of 2018.

Conclusion: Staff training, dosimetric measurements, and inspections are extremely important in a radiation oncology center. All of our measurement values performed in our clinic, where we have radiotherapy experience for nearly 30 years, were determined in accordance with international standards. In this direction, it is recommended that all centers working with radiation should make current evaluations meticulously.

Keywords: Radiotherapy, radiation workers, personal dosimeter

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GİRİŞ

Radyoterapi, iyonize radyasyon kullanılarak kanserli hücrelerin öldürüldüğü ya da çoğalmasının engellendiği, kanser tedavisinde en sık kullanılan tedavi yöntemlerinden birisidir (1). Normal hücrelere göre çok daha hızlı bölünüp çoğalabilen tümör hücreleri üzerine oldukça etkilidir. Bu etkinin sağlanabilmesi için tedavide yüksek doz iyonize radyasyon kullanılmaktadır (1,2). Modern cihazlar ve planlama sistemleri sayesinde uygulanan radyasyon miktarları hasta üzerinde dozimetrik olarak ayrıntılı bir biçimde gösterilebilmektedir. Fakat bütün mesai saatini bu radyasyonlu ortamda geçiren radyoterapi personelleri için radyasyona maruz kalmak en büyük mesleki sorundur ve ciddi korunma önlemleri alınması gerekmektedir.

Bu önlemlerin en önemli basamağı yeni cihaz kurumu sırasında olmalıdır. Herhangi bir kuruma yeni kurulacak ya da yenilenecek cihazlar, hasta tedavisine başlanmadan önce Türkiye Atom Enerjisi Kurumu (TAEK) tarafından yönetmeliklere uygun şekilde detaylı bir şekilde denetlenmekte ve gerekli tüm tedbirler alındığı takdirde cihaza çalışma onayı verilmektedir (3). Personel bazında değerlendirme ise periyodik eğitimler ve kişisel dozimetrik kontroller ile yapılmaktadır. Tüm radyasyon personeli, Sağlık Bakanlığı Radyasyon Güvenliği Yönetmeliği gereğince çalışma esnasında kişisel dozimetre taşımak zorundadır (4). Bu dozimetreler belirli periyotlarda TAEK'e gönderilerek ölçümleri yapılır. Yönetmeliğe göre radyasyon personelinin aylık maksimum maruz kalabileceği radyasyon miktarı 0,2 mSv olarak belirtilmiştir. Bu değer normal toplum için 0,01 mSv'dir ve bu dozların üzerinde olması durumunda kayıt tutulması zorunludur. Yine aynı yönetmelikte radyasyon görevlileri için etkin doz ardışık beş yılın ortalaması 20 mSv'i, herhangi bir yılda ise 50 mSv'i geçemez şeklinde madde bulunmaktadır. Bu değerler toplum üyesi kişiler için sırası ile 1 mSv ve 5 mSv'dir (4). Kabul edilebilir bu maksimum değerler, uluslararası standartlarla uyumludur (5,6). Bu değerler üzerindeki maruziyet, radyasyon çalışanlarında oluşabilecek biyolojik etkilerde risk artışı olarak karşımıza çıkabilir.

Bu veriler doğrultusunda çalışmamızda, cihaz parkı ve deneyimli personeli ile Türkiye'nin en köklü ve önde gelen onkoloji merkezlerinden biri olmanın yanı sıra Avrupa'nın da sayılı tedavi merkezleri arasında yer alan radyasyon onkolojisi kliniğimizde çalışan personellerimizin son 3 yıllık kişisel dozimetre değerleri incelenmiş ve radyasyon maruziyet oranları değerlendirilmiştir.

GEREÇ VE YÖNTEM

Kliniğimiz 1989 yılında hizmete açılmış ve şu anda 3 adet modern LİNAC, 1 adet Tomoterapi, 1 adet cyber-knife, 1 adet HDR brakiterapi ve 1 adet intraoperatif radyoterapi cihazları ve 93 personeli ile yıllık yaklaşık 3000 hasta tedavi eden bir radyasyon onkolojisi merkezidir. Personelimizin tamamına işe başlangıçta ve çalışma süresince yılda iki kez radyasyon etkileri ve radyasyondan korunma yöntemleri ile ilgili eğitim verilmektedir. Tüm personelin klinik içerisinde kişiye özel Termoluminesans Dozimetre (TLD) kullanması zorunludur ve bu dozimetreler senede 6 kez ikişer aylık periyotlar şeklinde TAEK'e gönderilerek ölçümleri yapılmaktadır. Her ölçümde personelin cilt ve vücut olmak üzere iki ölçüm değeri belirlenmektedir. Veriler kayıt altına alınarak her personelin ayrı ayrı ölçüm değerleri yönetmeliğe uygunluk yönünden incelenmektedir.

Çalışmamızda kliniğimizde çalışan 93 personelin 2016-2018 yıllarında TLD ölçümlerinden elde edilen verileri kaydedilerek değerlendirilmiştir. 3 yılda toplam 18 periyot şeklinde yapılan bu ölçümlerde tüm personelin ölçüm periyotlarındaki maruz kaldıkları doz miktarları, meslek gruplarına göre ölçüm periyotlarındaki maruz kalınan ortalama dozlar, yıllık toplam doz maruziyeti ve gruplar arasındaki farklılıklar çalışmada değerlendirilmiştir.

Ortalama değerler, her ölçüm periyodu için SPSS (Statistical Package for the Social Sciences) versiyon 17 kullanılarak Descriptive analiz yapılarak saptanmıştır. Meslek grupları arasındaki fark karşılaştırması ise One-Way ANOVA yöntemi ile yapılmıştır. Ayrıca yıllara göre ortalama dozlar ve tüm çalışma periyodundaki 3 yıllık ortalama dozlar personelin tamamında ve gruplarda değerlendirilmiştir. $p<0,05$ değeri istatistiksel olarak anlamlı kabul edilmiştir.

Etik Durum

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BULGULAR

Çalışmamızın yapıldığı 2016-2018 yıllarında toplam 18 periyottaki ölçümlerde, her bir personelin periyot başına ortalama maruz kaldığı doz miktarı 0,21 mSv'dir. Meslek gruplarına göre ortalama periyot dozları değerlendirildiğinde; kliniğimiz doktorlarında 0,20 mSv, medikal fizik uzmanlarında 0,20 mSv, teknikerlerimizde ise 0,21 mSv'dir. Gruplar arasında anlamlı istatistiksel fark saptanmamıştır.

Yıllık değerlendirmede 2016 yılında ortalama maruz kalınan bir priyotluk doz miktarı 0,20 mSv'dir. Meslek gruplarına göre 2016 yılındaki ortalama periyot dozları; doktorlarda 0,21 mSv, medikal fizik uzmanlarında 0,20 mSv, teknikerlerimizde ise 0,21 mSv'dir. Bu değerler 2017 yılında tüm personelde 0,19 mSv, doktorlarda 0,19 mSv, medikal fizik uzmanlarında 0,19 mSv, teknikerlerde 0,21 mSv; 2018 yılında ise tüm personelde 0,21 mSv, doktorlarda 0,20 mSv, medikal fizik uzmanlarında 0,21 mSv, teknikerlerde 0,22 mSv'dir. Hiçbir personelin kişisel dozimetre verileri yıllık 20 mSv üzerinde bulunmamıştır.

Her ölçüm periyodunda tüm personelin ve farklı meslek grupların ortalama değerleri de ayrıca incelenmiştir. Bu değerler yıllara göre **Tablo 1**'de verilmiştir.

Ortalama ölçüm değerlerinin meslek gruplarına göre farlılıklarının araştırılmasında sadece 2016 1. periyotta (vücut p=0,000, cilt p=0,000), 2017 5. periyotta (vücut p=0,021, cilt p=0,037), 2018 3. periyot vücut dozlarında (p=0,02) ve 4. periyotta (vücut p=0,000, cilt p=0,000) istatistiksel an-

Tablo 1. Yıllara ve meslek gruplarına göre ortalama dozimetre ölçüm değerleri

		N	Mean (2016)	Mean (2017)	Mean (2018)
1. periyot vücut	Doktor	33	,1870	,1952	,1915
	Teknisyen	48	,2100	,2019	,1973
	Med. Fiz.	12	,2092	,1825	,1883
	Total	93	,2017	,1970	,1941
1. periyot cilt	Doktor	33	,1836	,1900	,1924
	Teknisyen	48	,2092	,2000	,1925
	Med. Fiz.	12	,2083	,1800	,1850
	Total	93	,2000	,1939	,1915
2. periyot vücut	Doktor	33	,1942	,1985	,1942
	Teknisyen	48	,1996	,2127	,1963
	Med. Fiz.	12	,1975	,1992	,1892
	Total	93	,1974	,2059	,1946
2. periyot cilt	Doktor	33	,1915	,1955	,1927
	Teknisyen	48	,1990	,2100	,1925
	Med. Fiz.	12	,1942	,1975	,1883
	Total	93	,1957	,2032	,1920
3. periyot vücut	Doktor	33	,2209	,2112	,1897
	Teknisyen	48	,2169	,2346	,2119
	Med. Fiz.	12	,1967	,2125	,1817
	Total	93	,2157	,2234	,2001
3. periyot cilt	Doktor	33	,2218	,2088	,1903
	Teknisyen	48	,2123	,2323	,2067
	Med. Fiz.	12	,1992	,2133	,1800
	Total	93	,2140	,2215	,1974
4. periyot vücut	Doktor	33	,2088	,2194	,2473
	Teknisyen	48	,2217	,2331	,2856
	Med. Fiz.	12	,2058	,2108	,2917
	Total	93	,2151	,2254	,2728
4. periyot cilt	Doktor	33	,2039	,2136	,2479
	Teknisyen	48	,2177	,2283	,2850
	Med. Fiz.	12	,2025	,2092	,2858
	Total	93	,2109	,2206	,2719
5. periyot vücut	Doktor	33	,2261	,1833	,1970
	Teknisyen	48	,2146	,2033	,2052
	Med. Fiz.	12	,2100	,1725	,2083
	Total	93	,2181	,1923	,2027
5. periyot cilt	Doktor	33	,2197	,1809	,2000
	Teknisyen	48	,2117	,1996	,2033
	Med. Fiz.	12	,2050	,1708	,2058
	Total	93	,2137	,1892	,2025
6. periyot vücut	Doktor	33	,2145	,1712	,1882
	Teknisyen	48	,1829	,1852	,1977
	Med. Fiz.	12	,2258	,1800	,1892
	Total	93	,1997	,1796	,1932
6. periyot cilt	Doktor	33	,2106	,1679	,1918
	Teknisyen	48	,1798	,1823	,1992
	Med. Fiz.	12	,2225	,1767	,1917
	Total	93	,1962	,1765	,1956

Tablo 2. Meslek grupları ortalama değerlerinin karşılaştırılması

	2016		2017		2018	
	F	Sig.	F	Sig.	F	Sig.
1. periyot vücut	10,443	,000	2,088	,130	1,156	,319
1. periyot cilt	12,458	,000	2,871	,062	,607	,547
2. periyot vücut	,228	,797	2,740	,070	,495	,611
2. periyot cilt	,443	,644	2,694	,073	,184	,832
3. periyot vücut	,373	,690	1,812	,169	4,095	,020
3. periyot cilt	,354	,703	1,630	,202	2,752	,069
4. periyot vücut	1,375	,258	2,784	,067	11,709	,000
4. periyot cilt	1,488	,231	2,450	,092	9,487	,000
5. periyot vücut	,260	,772	4,046	,021	2,559	,083
5. periyot cilt	,220	,803	3,430	,037	,452	,638
6. periyot vücut	2,544	,084	1,601	,207	1,758	,178
6. periyot cilt	2,969	,056	1,586	,210	1,022	,364

lamlı fark saptanmıştır. One-Way ANOVA değerlendirmesinde; 2016 1. Periyot ve 2017 5. periyotta doktorlar ile diğer gruplarda, 2018 3. periyot vücut dozları ve 4. periyotta medikal fizik uzmanları ile diğer iki grup arasında anlamlı farkın olduğu görülmektedir. Yıllara göre grup karşılaştırma verileri **Tablo 2**'de verilmiştir.

TARTIŞMA

Çalışmamız, Türkiye'de bir radyasyon onkolojisi merkezi çalışanlarının kişisel dozimetre verilerinin incelendiği ilk çalışmadır. Radyasyonun insan vücudu üzerine olan zararlı etkileri hem deneysel çalışmalar, hem de klinik araştırmalar ile gösterilmektedir (7-9). Bu etkiler düşük dozlarda halsizlik, yorgunluk, hemogram ve biyokimyasal değerlerde değişme şeklinde görülebilirken doz miktarında artışla birlikte kilo kaybı, gen mutasyonları, kromozom kırıkları, anti-kanser immünitesinde değişikliklikler ve kanser oluşumları, kısalmış yaşam süresi olarak karşımıza çıkabilir (7-9). Bunun yanında düşük doz radyasyonun, normal hücresel fonksiyonlar için gerekli olduğunu savunan yayınlar da bulunmaktadır (10). Bu nedenle ülkemizde ve tüm dünyada radyasyon çalışanları için maruz kalınabilecek maksimum dozlar belirlenmiş ve gerekli ölçümler ile denetimleri yapılmaktadır (3-6). Ülkemizin en köklü radyasyon onkolojisi merkezlerinden birisi olan kliniğimiz çalışanlarında hem tek ölçüm verilerinde hem de ortalama ölçüm değerlerinde bu doz limitlerinin aşılmadığı saptanmıştır.

Uluslararası yayınlar incelendiğinde yaklaşık 300 çalışanı olan iki büyük merkezde radyasyon çalışanlarının maruz kaldıkları dozlar incelenmiş ve yıllık ortalama 30 mSv'in altında olduğu gösterilmiştir (11,12). Doz maruziyeti sağlık merkezleri dışında da değerlendirilmektedir. Ritz ve arkadaşları (13)'nın nükleer santral çalışanları üzerinde yaptıkları çalışmada, çalışanların %87,3'ünün 0-20 mSv, %10,8'inin 21-100 mSv ve %1,9'unun ise 100 mSv üzerinde doz aldıklarını saptamışlardır. Çalışmamızda da yıllık ortalama maruz kalınan doz 0,21 mSv olarak saptanmış ve mevcut literatür verileri ile uyumluluk göstermektedir.

Meslek gruplarına göre farklılıkların incelenmesi sonucunda da genel olarak anlamlı farklılıklar bulunamamıştır. 36 inceleme periyodunun sadece dördünde personeller arası istatistiksel fark elde edilmiştir. Bu farkların da işgücü yoğunluğu, cihaz ölçüm periyotları ve radyasyon izin dönemlerinin farklılıklarından kaynaklandığı düşünülmektedir.

Radyasyon çalışanlarının maruz kaldıkları bu dozlar ülkemiz ve uluslararası belirlenen limitler ile uyumludur. Fakat nükleer kazalar sonrası yapılan bazı çalışmalarda (14-16) ve Lee ve ark. (9)'nın radyasyon çalışanlarında topluma göre daha yüksek oranda tiroid kanseri saptadıkları çalışmalarında 200 mSv altındaki düşük doz iyonize radyasyon maruziyetinin insan sağlığı üzerine olan etkileri hala belirsizliğini korumaktadır. Bu nedenle tüm radyasyon çalışanları üzerinde ayrıntılı klinik çalışmalara ihtiyaç bulunmaktadır.

Kliniğimiz, ülkemizde 30 yılın üzerinde aktif hizmet veren nadir radyasyon onkolojisi kliniklerinden birisidir. Personelimiz tamamına görev başlangıcında bilgilendirme eğitimleri ve görev sırasında hizmet içi eğitimleri verilmektedir. Tüm personelin kişisel dozimetre taşıdıkları düzenli olarak değerlendirilmekte ve ölçüm verileri titizlikle incelenerek kayıt edilmektedir. Cihaz ölçümleri düzenli periyotlarda ayrıntılı bir şekilde yapılmaktadır. Personellerin radyasyon izni ve mesai süreleri takipleri yapılmaktadır. Bu eğitimler, ölçümler ve denetimler sonucunda yapılan tüm ölçüm değerlerimizin uluslararası standartlara uygun olduğu düşünülmekte ve radyasyonla çalışan tüm merkezlerin mevcut değerlendirmeleri titizlikle yapmaları önerilmektedir.

Çalışmamız, ülkemizde radyasyon çalışanlarının değerlendirildiği ilk çalışma olduğundan hem ulusal hem de uluslararası literatüre katkı sağlayacağı düşünülmektedir. Fakat sadece radyasyon onkolojisi değil bütün radyasyon çalışanlarının değerlendirildiği, daha uzun ölçüm periyotlarını

kapsayan ve çalışanların klinik gözlem verilerinin de dahil edildiği çalışmalar ile mevcut çalışmamız desteklenebilir.

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Research Article / Araştırma Makalesi

The value of late phase imaging with FDG-PET/CT in liver metastases of colorectal carcinoma

Kolorektal kanserli hastalarda metastatik karaciğer lezyonlarının geç faz FDG-PET/BT görüntülemesinin değerlendirilmesi

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ABSTRACT

Aim: Our aim was to investigate the role of late-phase imaging with FDG-PET/CT in colorectal carcinoma patients with liver metastases.

Material and Method: Dual-phase FDG-PET/CT scan was retrospectively evaluated in colorectal carcinoma patients with liver metastases. Late phase imaging was acquired 92-253 minutes (mean 158.53±35.7 minutes) after the FDG injection. Sixty-eight metastatic lesions were determined in 37 patients. Mean lesion SUVmax and lesion-to non-tumorous liver tissue ratio was calculated and results of routine FDG-PET imaging were compared with late-phase imaging.

Results: Metastatic lesion sizes were 9 to 230 mm (mean 3.71±3.7 cm). SUVmax values of the metastasis and non-tumorous liver SUVmax for routine and late-phase imaging were as follows respectively; 7.19±3.8, 10.3±5.4; 2.98±0.7, 2.41±0.6. In the late phase imaging metastatic liver lesions SUVmax values were increased ($p< 0.01$) and non-tumorous liver SUVmax values were decreased ($p< 0.01$). Compared to routine imaging, in late phase, lesion to non-tumorous liver tissue was increased ($p< 0.001$). Lesion retention index was increased by 45.74±31.8% and the non-tumorous liver index was decreased by 18.63±10.4%.

Conclusion: The results of this study indicate that normal liver FDG uptake decreases in time and late-phase imaging improves the tumor to normal tissue ratio and enables differentiation of metastatic liver lesions from normal liver.

Keywords: Dual-Phase, FDG-PET, liver metastases, colorectal carcinoma

ÖZ

Amaç: Amacımız, karaciğer metastazı olan kolorektal karsinom hastalarında FDG-PET/BT ile geç faz görüntülemenin rolünü araştırmaktır.

Gereç ve Yöntem: Karaciğer metastazı olan kolorektal karsinomlu hastalarda çift fazlı FDG-PET/BT taraması retrospektif olarak incelendi. FDG enjeksiyonundan 92-253 dakika (ortalama 158,53±35,7 dakika) sonra geç faz görüntüleme alındı. Otuz yedi hastada 68 metastatik lezyon saptandı. Ortalama lezyon SUVmax ve lezyon/tümor olmayan karaciğer doku oranı hesaplandı ve rutin FDG-PET görüntüleme sonuçları geç faz görüntüleme sonuçları ile karşılaştırıldı.

Bulgular: Metastatik lezyon boyutları 9-230 mm bulundu (ortalama 3,71±3,7 cm). Rutin ve geç evre görüntüleme için metastaz ve tümörsüz karaciğer SUVmax değerleri SUVmax olarak sırasıyla; 7,19±3,8, 10,3±5,4 ve 2.98±0.7, 2,41±0,6 olarak hesaplandı. Geç evre görüntülemede metastatik karaciğer lezyonlarında, SUVmax değerleri artmış ($p<0.01$) ve tümör içermeyen karaciğer dokusunda SUVmax değerleri azalmıştır ($p<0.01$). Rutin görüntülemeye kıyasla geç faz görüntülerinde lezyon/ tümörsüz karaciğer dokusu oranı artmıştır ($p=0.001$). Karaciğer metastatik lezyon retansiyon indeksi %45,74±31,8 oranında artmış ve tümör içermeyen karaciğerde ise retansiyon indeksi %18,63±10,4 oranında azalmıştır.

Sonuç: Bu çalışmanın sonucu olarak, normal karaciğer dokusunda FDG tutulumunun zamanla azaldığını, geç faz görüntüleme ile de lezyon/tümörsüz karaciğer dokusu oranının belirginleştirdiği ve metastatik karaciğer lezyonlarının normal karaciğerden daha kolay ayırt edilmesini sağladığını göstermektedir.

Anahtar Kelimeler: Geç faz, FDG-PET, karaciğer metastazı, kolorektal karsinom

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INTRODUCTION

In colorectal cancers, the liver is one of the most common regions of metastases in hematogenous spread of disease (1). On the course of cancer, depending on the primary tumor type liver metastasis can be found in 25% of all cases. The detection of liver metastasis not only results in the spread of the disease but also effects the treatment approaches from local treatment surgical approaches to systemic chemotherapies. However, surgical removal of liver metastases can provide cure especially in colorectal cancers and increase the 5-year survival rate by 58-61% (2).

For the detection of liver lesions, PET (positron emission tomography) is a functional imaging method based on the metabolic information of increased glucose utilization in cancer cells (3). A meta-analysis comparing imaging modalities found that FDG (Fluorine-18 labeled fluoro-2-deoxy-glucose)-PET has sensitivity of 93.8% and specificity of 98.7% per patient basis in detecting liver metastasis of colorectal carcinomas (4).

FDG accumulation is usually expressed in Standard Uptake Value (SUV) which is calculated by dividing the ratio of tissue activity (mCi/mL) to the patient's body weight (5). However, though SUV over 2.5 or over in many literatures is usually accepted as malignant (6) there is no specific limit for SUV values and especially in tissues with high physiological uptake and there is no definite limit in the malignant benign distinction for SUV values. Apart from the SUV, the retention index can be used for quantification. The retention index of the lesion is calculated by the difference of SUV between the images. Retention index=SUV2-SUV1/SUV1x100% (7).

Besides, by calculating the ratio of acquired counts from the region of interest of the lesion to the non-tumorous surrounding tissue (L/B) can be used for quantification. In general, unlike other tissues, liver tissue FDG uptake is physiologically heterogeneous. Nevertheless, the physiologically intense and heterogeneous FDG uptake observed in liver leads to difficulty in the evaluation of liver lesions. The FDG-PET sensitivity is lower than many other malignant tumors due to the variable levels of glucose-6-phosphatase depending on the type of lesion for liver lesions. The value of late-phase imaging in various cancers such as lung, pancreas, head and neck, breast and cervical cancers has been emphasized in differentiating benign lesions from malignant lesions (8–12).

Although the FDG-PET imaging procedure is recommended to be performed after 45 minutes, at the 60th minute on average, various differences are observed in the literature in this regard. A study performed late imaging study to differentiate the malignancy from inflammatory lesions by Zhuang et al. (13) showed that in malignant tissues FDG uptake continues to accumulate over time.

In a study with advanced or recurrent cervical cancer, they found that late phase FDG-PET images caused changes in the treatment of 31% of patients (14). A study with head and neck cancer compared the ratio of the tumor SUV value to the contralateral the tissue SUV value in acquisitions between 47-112 min and 77-142 min, and they found that the ratio was increased by 23±29% and this rate has increased even more in late-phase images taken after 30 minutes (15).

Regarding the discussed literature above in performing late phase imaging, we hypothesized that the sensitivity may be higher in late-phase acquisition which may facilitate the separation of metastatic liver lesions from the liver and provide an accurate interpretation. We have evaluated the diagnostic efficiency of late-phase FDG-PET/CT in patients with a liver metastasis in addition to the routinely acquired images.

MATERIAL AND METHOD

All the patients underwent a 6 to 8-hour fasting and diabetic patients using insulin were not included in this study to minimize the level of glucose competing with FDG in the blood to avoid increased liver uptake. All patients were advised to be hydrated and not to exercise on the day and the day before the PET/CT scan. There were 37 patients in total, (14 female, 23 male) and all the 68 lesions were suspected of liver metastases. The patients' ages were between 23 to 80 years and mean patient age was 55.51±11.7 years. All the patients were injected FDG dose once and both of the acquisitions were acquired by the same FDG dose. Intravenous FDG of 0.2 mCi/kg was given through an antecubital catheter and patients were kept immobile and silent in a dimly lighted room for around 20 minutes prior to and after injection. FDG-PET/CT images were acquired at a 4 sliced multidetector helical CT scanner and a bismuth germanate crystal equipped Discovery ST PET/CT (GE Healthcare, Milwaukee, WI, USA). Emission data were acquired starting from calvarium base till mid-thigh. The transmission time required for each bed position was 3 minutes. Mean PET/CT acquisition time was 22 minutes and mean number of required bed positions was 5 to 7 beds. CT images were utilized to obtain attenuation maps for the attenuation correction of PET images. Furthermore, CT images were fused to PET images automatically to determine the exact anatomical location. The CT transmission scan was acquired with 140 kVp and 110 mA and 3.5 mm slice thickness.

Both the routine and the late phase F18-FDG PET/CT images were evaluated both visually and semi-quantitatively by two nuclear medicine physicians.

For each lesion, the maximum standard uptake value (SUVmax) was calculated automatically. Both in the routine and the late phase image evaluation, CT images were utilized to obtain the anatomical position of the lesions. At the late phase images, CT images acquired during the routine FDG-PET/ CT were utilized for attenuation correction and these images were fused to late-phase images. Non-tumorous liver tissue SUVmax value was calculated by drawing multiple regions of interest (ROI) and mean SUVmax was calculated by getting the average value of

those ROIs. The ratio of the routine FDG-PET/CT image to the late phase FDG-PET/CT was calculated for each lesion and also for the non-tumorous liver tissue. It has been demonstrated that other SUV, the retention index can be utilized for quantification. Lyshchik et al. (7) have calculated the retention index by subtracting the routine FDG-PET/CT SUVmax value from the late phase FDG-PET/CT image SUVmax value, divided by the routine FDG-PET/CT SUVmax value. According to this formula retention index was calculated for each lesion.

Statistical Analysis

The data were analyzed with Statistical Package for Social Sciences for Windows software (SPSS version 23.0, SPSS Inc., Chicago, Illinois, USA). The Mann–Whitney U tests were used for statistical evaluation of the difference between the two imaging sessions both in liver metastases and in non-tumorous liver tissue. To evaluate the relation between the acquisition times and the metastatic lesion and the non-tumorous liver tissue the Pearson correlation test was used. A value of $p < 0.05$ was accepted as statistically significant.

Ethical Declaration

Retrospectively, all the patients with colorectal carcinoma who were referred to FDG-PET/CT due to staging, re-staging, response to therapy with a liver lesion were included in this study. The procedures were followed according to the regulations established by the Clinical Research and Ethics Committee (Ankara Training and Research Hospital, Date 22/03/2011, number 0120, decision 2541) and to the Helsinki Declaration of the World Medical Association. All the patients were given signed informed consent.

RESULTS

The routine FDG-PET/CT images were acquired between 46-91 minutes after the FDG injection. The mean acquisition time for the routine FDG-PET/CT images were 63.88±12.3 minutes. The late phase FDG-PET/CT images were acquired between 92-253 minutes (mean: 158.53±35.7) after the routine FDG-PET/CT injection for 1 or 2-bed position, covering the liver area. The range of the metastatic lesion size varied between 0.9 to 23 cm (mean lesion size: 3.71±3.7). Three lesions were smaller than or equal to 1 cm. The calculated mean SUVmax of the non-tumorous liver tissue in routine FDG-PET/CT images was 2.98±0.7, however, in the late-phase the calculated mean SUVmax of the non-tumorous liver tissue was decreased to 2.42±0.6. The difference in the non-tumorous liver tissue between the routine F18-FDG-PET/CT images and the late phase FDG-PET/CT images were statistically significant ($p<0.01$) (**Table 1**).

A patient example was demonstrated in **Figure 1.**

The visual evaluation revealed that all the lesions demonstrated increased FDG uptake. The calculated SUVmax in the liver metastases in routine FDG-PET/CT images was 7.19±3.8, the calculated mean SUVmax of the late phase was increased to 10.30±5.4. The difference in the liver

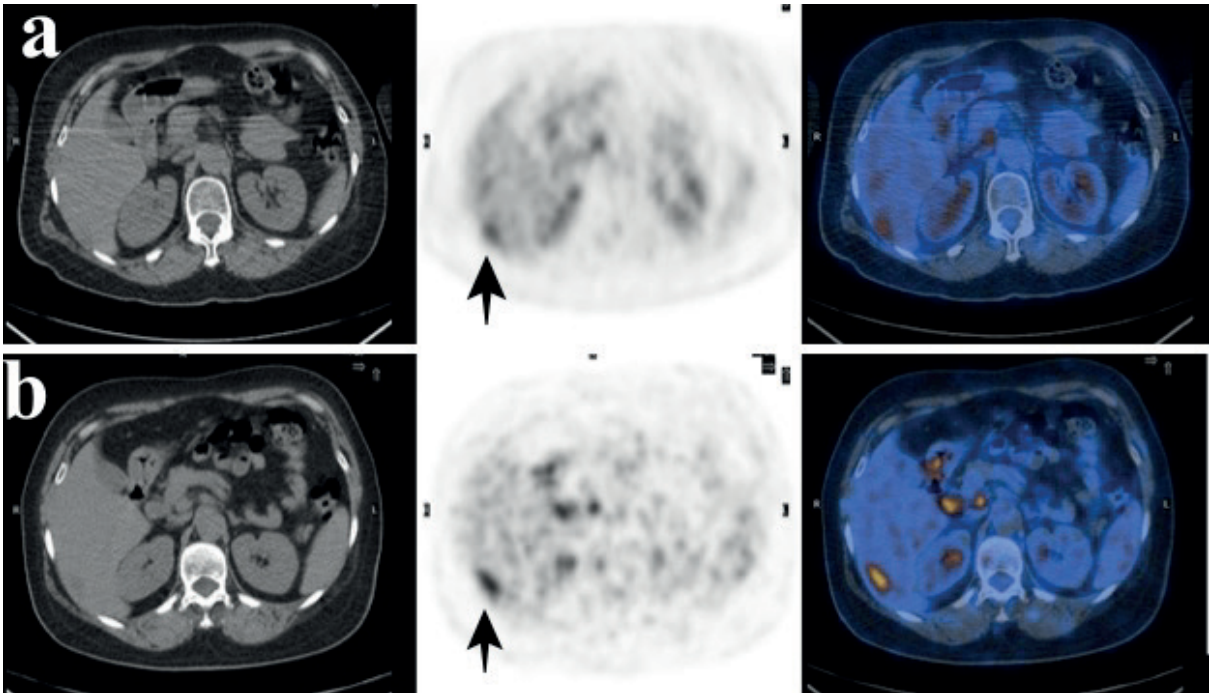

Figure 1. 53 year-old female colorectal carcinoma was referred to PET/CT for the evaluation of a liver mass. The routine FDG-PET images were presented in row a). CT, FDG-PET and fused FDG-PET/CT images respectively presented a lesion with SUVmax: 4.37 and non-tumorous liver SUVmax:3.2. The late phase FDG-PET images in row b) CT, FDG-PET and fused FDG-PET/CT images respectively, the mass shown with black arrow has shown a much higher SUVmax: 7.04 compared to routine FDG-PET images and non-tumorous liver has shown a decreased SUVmax:2.9. The calculated retention index was 97%.

Table 1. Liver metastatic lesions and Non-tumorous liver SUVmax values

	Routine Imaging SUVmax	Late Phase Imaging SUVmax	p values
Liver metastases	7.19±3.8	10.30±5.4	< 0.01
Non-tumorous Liver	2.98±0.7	2.41±0.6	< 0.01

metastases between the routine FDG-PET/CT images and the late phase FDG-PET/CT images was statistically significant (p<0.01) (**Table 1**).

The routine FDG-PET/CT mean lesion L/B ratio was 2.48±1.2 and the late phase FDG-PET/CT was 4.38±2.1 and the difference between L/B ratios was statistically significant (p<0.001) (**Table 2**).

Table 2. Lesion-to non-tumorous liver tissue SUVmax values

	Routine Imaging	Late Phase Imaging	p values
Lesion-to non-tumorous liver tissue (L/B)	2.48±1.2	4.38±2.1	< 0.001

There was moderate positive correlation between the time of acquisition and L/B ratio (p<0.05, r:0.56). And there was moderate positive correlation between the time of acquisition and the SUVmax decrease of the non-tumorous tissue (p< 0.001, r: 0.472). Nevertheless, there was no correlation between the time of acquisition and SUVmax of the metastatic lesion (p>0.05, r<0.2), or the time of acquisition and the size of the metastatic lesions and (p>0.05, r<0.2), or between the time of acquisition and the patient age (p>0.05, r<0.2).

The mean RI was calculated for lesions was 45.74±31.8% (range 1.10-193.75) and the decrease of mean RI of non-tumorous liver was 18.63± 10.4% (range 4.43-40.83) (**Table 3**).

Table 3. Liver metastases and lesion-to non-tumorous liver tissue SUVmax values

	Retention Index
Non-tumorous liver tissue	-18.63± 10.4%
Liver metastases	45.74±31.8%
p value	< 0.001

Furthermore, in 3 patients with colorectal carcinoma, referred due to elevated CEA levels, in total 4 new liver metastatic foci were identified which were not apparent in the routine FDG-PET/CT images. One of those patients are presented in **Figure 2** and **Figure 3**.

193.75%.

DISCUSSION

Liver is considered as the most common region of metastasis in many cancers including colorectal cancers (2). Liver metastasis affects the course of the primary disease, life span, mode of treatment, and operability. It has been shown that the life span is prolonged after surgery in the presence of liver metastasis before the disease becomes widespread (16).

The heterogeneous FDG uptake is thought to be due to the hexokinase enzyme that converts FDG into FDG-6P

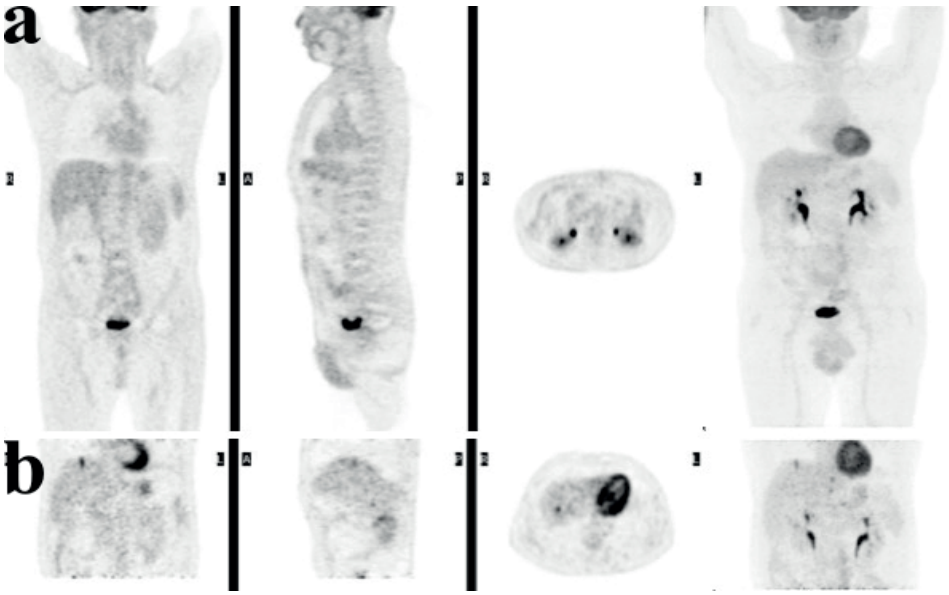

Figure 2. 50 year-old male rectum carcinoma was referred due the increase in CEA levels. The routine FDG-PET images were presented in row a) Coronal, sagittal, transaxial and maximum intensity projection images respectively did not presented any lesion and non-tumorous liver SUVmax:3.26. The late phase FDG-PET images in row b) Coronal, sagittal, transaxial and maximum intensity projection images images respectively, has shown a focal FDG uptake with an SUVmax: 7.99 and non-tumorous liver SUVmax was decreased to 2.95. The calculated retention index was from the same region was

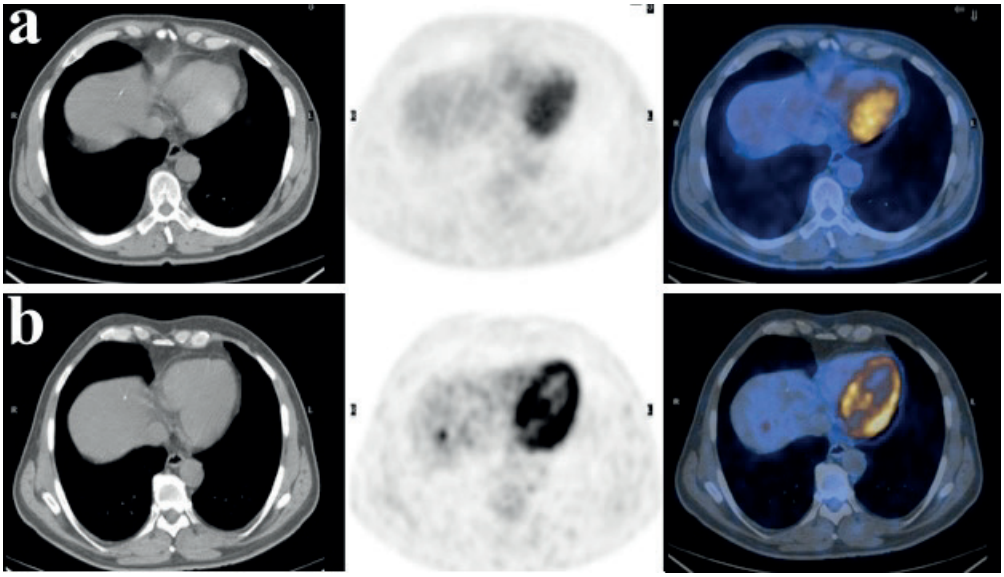

Figure 3. a). Respectively, CT, FDG-PET and fused FDG-PET/CT images acquired at routine FDG-PET/CT of the patient presented in **Figure 2. b)** CT, FDG-PET and fused FDG-PET/CT images respectively, at late phase FDG-PET/CT.

as well as the glucokinase enzyme specific to the liver. Although this situation causes more FDG uptake in the first phase of the FDG uptake, the FDG uptake in the liver decreases over time as in the other tissues. The glucose-6-phosphatase enzyme (G6Pase), which converts FDG-6P to FDG, is quite high in normal liver tissue, it is almost zero in metastatic liver tumors and variable in primary liver tumors (17).

A study with pancreatic cancer patients found that FDG PET has a sensitivity of 68% in liver lesions, whereas, in metastatic liver lesions larger than 1 cm, the specificity of FDG-PET was found to be 97% and in metastatic lesions less than or equal to 1 cm, the FDG-PET sensitivity was 43% (18). In a study with liver metastases of colorectal tumors smaller than 1 cm, the false negativity of FDG-PET was reported as 5% (19). Through the literature, it has been suggested that specificity may increase even in lesions smaller than 1 cm as a result of decrease in FDG accumulation in time and decrease in metastatic liver lesions (20,21). In our study, the lesions sizes range from 14 to 230 mm and the average size of the lesions was 3.71±3.7 centimeters and we had 3 lesions equal to 1 cm or under 1 cm. All those 3 lesions were clearly visible.

In colorectal cancer studies for staging of liver, the sensitivity of FDG-PET was around 90-97%, while the specificity was 88-100% (4,15,22). In these studies, lesion imaging was routinely performed between 45 and 70 minutes. A study in patients with suspected liver metastases reported that 11.1% of the liver lesions were present only at late PET images (23). In our study, in all 37 patients and 68 lesions in late images liver lesions were detected. Compared to late-phase imaging, the routine FDG-PET images localized 64 of the 68 lesions and the sensitivity of late-phase imaging to detect liver lesions on lesion basis was calculated as 94.11%. On patient basis, in 3 of 37 patients with metastatic liver lesions, routine images could not detect all the liver lesions that late-phase imaging has detected and the sensitivity to detect liver metastatic lesions was calculated as 91.89%.

In a study consisting of primary liver tumors and metastatic liver lesions, reported that late phase images are useful in distinguishing liver lesions, more prominent in metastatic tumors (24). A study evaluate liver metastasis in patients with pancreatic carcinoma found that rather than the images acquired at 1 hour or 3 hours, the images acquired at 2 hours were thought to be more useful to rule out the presence of liver metastasis (25). Regarding this, we also evaluating the metastatic lesion by the time of acquisition. In our study, the mean time difference was 94.65±38.2 minutes in all patients (range between 14-178 minutes) and we grouped the patients according to the time differences of acquisitions, we had 3 groups. The first group had their late phase imaging within an hour, consisting of 34 patients; the second group had their late phase imaging between an hour and 2 hours, consisted of 14 patients; the third group had their late phase imaging after 2 hours, consisted of 20 patients. However, we did not find any differences between groups in terms of retention index of the metastatic lesions.

A dual time point study evaluating liver metastases in colorectal carcinoma, confirmed the metastatic lesions by histopathology and found that a delayed scan is more favorable (26). One of the main limitations of our study is, we did not confirm the lesions by histopathology, however other than the patients with known liver metastasis, all the other patients liver masses were confirmed by conventional imaging methods. We had 4 additional lesions in 3 patients' that were identified in late-phase imaging which were not localized in the early study. All of the 4 lesions were larger than 1 cm, the lowest SUVmax of those lesions in routine images was 2.72 and the lowest late-phase imaging SUVmax was 4.88. However, due to the heterogenous

liver background, it was not possible to identify the lesions in the routine FDG-PET imaging.

Delbeke D et al. (22) reported that in a group of patients liver masses including liver metastases, cholangiocellular carcinoma, primary liver tumors, and liver abscesses, FDG-PET showed high sensitivity in metastatic liver tumors. They also found that lesion/ non-tumorous liver ratio was higher than 2 and SUV values were greater than 3.5 in all malignant tumors and lesion/ non-tumorous liver ratio was less than 2 and the SUVmax values were less than 3.5 in benign lesions. In our study the mean lesion/ non-tumorous liver ratio was higher than 2 (2.48±1.2) in the routine FDG-PET/CT imaging and much higher in late-phase imaging (4.38±2.1) (**Table 2**) and in all lesions SUVmax was higher than 2, however, in 31 lesions the mean lesion/ non-tumorous liver ratio was less than 2 which suggested that the threshold of 2 for the lesion/non-tumorous liver was not as reliable as SUVmax in the quantitative evaluation of metastatic liver lesions for our study.

In the study of Condrad et al. (27) in thoracic malignancies with the same method, they accepted a 5% increase in the images taken 30 minutes after the first acquisition and emphasized that late-phase is important for malignant benign distinction. In our study, mean retention index in liver lesions calculated in this study was found 45.73±31.8% (**Table 3**). Our results support that quantification values such as the retention index used alongside the SUV are useful when evaluated together with visual evaluation.

Concordant with the literature (28), in our study, we found that the presence of heterogeneous non-tumorous liver, decreases by time and late-phase imaging may help us to find new metastatic foci in liver. The non-tumorous liver tissue SUVmax decrease is correlated by time of acquisition. The late acquired images increase the reliability of detection of metastatic liver lesions by the separation of the lesion from the non-tumorous liver tissue, thanks to the utilization of FDG, decreasing over time from the liver parenchyma.

CONCLUSION

Our results support that the FDG-PET sensitivity of detecting metastatic liver lesions in colorectal carcinoma patients may be increased by acquiring late-phase images. Besides late-phase images can facilitate the identification of suspicious metastatic lesions from the non-tumorous liver tissue and facilitate accurate interpretation.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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Sağlık Bilimleri ve Tıp Dergisi

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Research Article / Araştırma Makalesi

Dahiliye polikliniğine başvuran tip 2 diyabet hastalarında başvuru anındaki klinik ve laboratuvar verilerinin retrospektif incelenmesi

Retrospective analysis of clinical and laboratory data at the time of admission in type 2 diabetic patients admitted to the internal medicine outpatient clinic

Ömer Akyürek
Özel Farabi Hastanesi, İç Hastalıkları Bölümü, Konya, Türkiye

ÖZ

Amaç: Tip 2 diabetes mellitus insülin eksikliği ya da insülin etkisindeki defektler nedeniyle gelişen sürekli tıbbi bakım gerektiren, kronik bir metabolizma hastalığıdır. Çalışmada, tip 2 diabetes mellituslu hastaların klinik ve laboratuvar bulgularının değerlendirilmesi ve bulguların literatür eşliğinde tartışılması amaçlanmıştır.

Gereç ve Yöntem: Bu çalışma hastanemiz genel dâhiliye polikliniğine, 3 yılda 'tip 2 diabetes mellitus'' tanı kodu ile kaydedilen, 18 yaş üstü toplam 186 hastanın değerlendirildiği tanımlayıcı kesitsel bir araştırmadır.

Bulgular: Çalışmamıza 186 hasta dâhil edildi (Yaş ortalaması 59,7±11,5 yıl). Olguların hastalık süresi 1-27 yıl aralığında olup ortancası 9 yıldı ve %37,1'inde (n:69) hastalık süresi 10 yıldan fazla idi. Hastaların HbA1c ortalaması %7,9±1,7, ortalama HDL düzeyi 46,2±10,9 mg/dL, ortanca LDL düzeyi 117 mg/dL idi. Tip 2 diabetes mellitus hastalarının %55,9'inde (n:104) HbA1c düzeyi %7 ve üzeri idi, LDL kolesterol düzeyi 100 ve üzeri olan hasta oranı %58 (n:108) idi.

Sonuç: Diyabetli hastaların risk faktörlerini azaltmaya yönelik yeni sağlık politikalarının planlanması, diyabete bağlı gelişebilecek komplikasyonların erken saptanması ve tedavi maliyetlerinin azaltılması için muhakkak daha ayrıntılı incelenip multidisipliner bir yaklaşımla tedavi edilmesi gerekmektedir.

Anahtar Sözcükler: Tip 2 diabetes mellitus, glisemik kontrol, lipid kontrol

ABSTRACT

Aim: Type 2 diabetes mellitus is a chronic metabolic disease requiring continuous medical care due to insulin deficiency or insulin-effect defects. The aim of this study is to evaluate the clinical and laboratory findings of type 2 diabetes mellitus patients and discuss the findings in the light of literature.

Material and Method: This study is a descriptive cross-sectional study with 186 patients over the age of 18 who were enrolled in three years in the general internal medicine outpatient clinic of our hospital with the diagnosis code 'type 2 diabetes mellitus.

Results: 186 patients were included in the study (mean age 59.7±11.5 years). The disease duration of the patients was 1-27 years, the median was 9 years and 37.1% (n: 69) of them had a disease duration of over 10 years. The mean HbA1c of the patients was 7.9±1.7%, the mean HDL level was 46.2 ± 10.9 mg/dL, and the median LDL level was 117 mg/dL. In 55.9% (n: 104) of type 2 diabetes mellitus patients, HbA1c level was 7% and above, and the percentage of the patients with LDL cholesterol level of 100 and above was 58% (n: 108).

Result: New health care policies to mitigate the risk factors of diabetic patients should be planned and such factors should be examined in depth for the early detection of diabetes-related complications and reduction in treatment costs and treated with a multidisciplinary approach.

Keywords: Type 2 diabetes mellitus, glycemic control, lipid control

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GİRİŞ

Diyabet; insülin eksikliği ya da insülin etkisindeki defektler nedeniyle organizmanın karbonhidrat, yağ ve proteinlerden yeterince yararlanamadığı, sürekli tıbbi bakım gerektiren, kronik bir metabolizma hastalığıdır. İnsülin direnci zemininde gelişen Tip 2 diabetes mellitus (DM) vakalarının %90-95'ini oluştururken, Tip 1 diyabet ise vakalarının % 5-10'unu oluşturur ve genellikle mutlak insülin yetmezliğine sebep olan β hücre harabiyeti vardır (1).

Tahminen 2011'de 366 milyon insanın DM hastası olduğu düşünülmektedir, 2030'a kadar bunun 552 milyona yükselmesi beklenmektedir (2). Türkiye'de 2002 ve 2010 yıllarında epidemiyolojiye yönelik yapılan iki çalışmadan Türkiye Diyabet Epidemiyoloji Çalışması (TURDEP-I)'e göre 2002 yılında T2DM prevalansı %7,2 bulunmuştur. Bu hastaların %67,7'si bilinen DM tanısı olan hastalar olup, % 32,3'ü ise yeni tanı Dm vakasıdır (3). TURDEP-II çalışmasında 2010 yılında Türk erişkin toplumunda, yani 20 yaş ve üstü nüfusta Dm sıklığının %13,7'ye (yaklaşık 6,5 milyon kişi) ulaştığı görülmüştür. Bunların sadece %54'ünün bilinen diyabet tanısı olup geriye kalan yaklaşık %46 diyabetlinin (yaklaşık 3 milyon kişi) hastalığının farkında olmadığı saptanmıştır. DM tanısı olan hastaların da %64,5'inin kan şekeri kontrolünün yetersiz olduğu görülmüştür (4). Bu sonuçlara göre diyabetin 12 yıllık artış hızı %90 gibi ciddi bir rakam olmuştur (4). 2018 yılında yayınlanan PURE (The Prospective Urban Rural Epidemiology)-Türkiye çalışmasına göre 2015 yılı için Türkiye'de Dm prevalansı %21'e yükselmiştir (5).

Diyabet her geçen gün hem ülkemiz hem de tüm dünya için giderek ciddiyeti artan ve üzerinde multidisipliner çalışma gerektiren bir hastalıktır. Bu veriler diyabet hastalığının ciddiyetini ortaya koymaktadır. Tüm bu nedenlerden dolayı Dünya Sağlık Örgütü DM'yi günümüzün en önemli halk sağlığı sorunlarından biri olarak kabul etmiştir (6). Ülkemizde diyabetli hastalarda diyabet regülasyonunun ne durumda olduğu hakkında çalışmalar sınırlıdır. Bu nedenle hastanemizde tedavi hedeflerine ne düzeyde ulaşıldığı, Dm hastalarında klinik ve laboratuvar verilerinin hangi düzeyde olduğunu araştırmak için bu çalışma planlandı.

GEREÇ VE YÖNTEM

Bu çalışmaya hastanemiz genel dahiliye polikliniğine, 3 yılda 'tip 2 DM'' tanı kodu ile kaydedilen, 18 yaş üstü toplam 186 tip 2 DM hastası alındı. Diyabet dışı nefrotik sendrom, akut böbrek yetmezliği olanlar, malignitesi olan hastalar ve bilinen romatizmal ve spesifik enfektif hastalığı olanlar çalışma dışı bırakıldı. Hastaların elektronik dosyalarından, yaş, cinsiyet, diyabet süresi, açlık kan şekeri, HbA1c düzeyi, lipid profili (trigliserit, total kolesterol, HDL, LDL), albümin, ürik asit, kreatinin ve komorbiditesi (hipertansiyon, kronik böbrek hastalığı varlığı koroner arter hastalığı) incelenerek kaydedildi

İstatistik

İstatistiksel değerlendirme Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc. Chicago, IL) programı kullanılarak yapıldı. Verilerin normal dağılımı Kolmogorov-Smirnov testi ile değerlendirildi. Sayısal değişkenlerden normal dağılım sergileyenler ortalama±standart sapma olarak, normal dağılım sergilemeyenler ortanca (min - max) olarak gösterildi. Kategorik değişkenler sayı ve yüzde olarak belirtildi. Sayısal değişkenlerin cinsiyet grupları, glisemik kontrol olup olmaması, lipid kontrol olup olmaması, Student t testi (normal dağılım sergileyen sayısal değişkenlerde) veya Mann Whitney U testi (normal dağılım sergilemeyen sayısal değişkenlerde) kullanıldı. Kategorik verilerin kıyaslanmasında Ki-Kare ve Fisher'in Kesin Ki-kare testi kullanıldı. İstatistiksel analizlerde $p< 0.05$ değeri anlamlı olarak kabul edildi.

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Çalışmada Helsinki Bildirgesi İlkeleri'ne bağlı kalınmış ve hasta mahremiyetine dikkat edilmiştir. Ayrıca çalışma için ve verilerin kullanımı için kurum onayı alınmıştır (Tarih/Sayı: 2020/201).

BULGULAR

Araştırma popülasyonu 186 tip 2 DM hastasından oluştu. Hastaların ortalama yaşı 59,7±11,5 yıl olup %68,8 (n:128) 65 yaş altı idi, erkeklerin oranı %35,5 (n:66) ve kadınların oranı %64,5 (n:120) idi. Tip 2 DM hastalarının hastalık süresi 1-27 yıl aralığında olup ortancası 9 yıldı ve %37,1'inde (n:69) hastalık süresi 10 yıldan fazla idi. Hastaların HbA1c yüzdesi 5,3-15,2 aralığında olup ortalaması %7,9±1,7, ortalama HDL düzeyi 46,2±10,9 mg/dL, ortanca LDL düzeyi 117 mg/dL idi. T2DM hastalarının %55,9'inde (n:104) HbA1c düzeyi %7 ve üzeri idi, LDL kolesterol düzeyi 100 ve üzeri olan hasta oranı %58 (n:108) idi. Kadın tip 2 DM hastalarında hastalık süresi 10 yıl üzeri olanların oranı erkek hastalara kıyasla yüksek (%44,1 karşı %41; p=0,050) saptandı. Kadınlarda erkeklere kıyasla ortanca kreatinin düzeyi (0,7 mg/dL karşı 0,9 mg/dL; p<0,001), ortalama ürik asit düzeyi (4,9±1,8 karşı 5,7±1,5; p<0,001) düşük saptandı. Yine kadın hastalarda ortanca trigliserit düzeyi (161 karşı 144; p<0,001), ortalama total kolesterol düzeyi (198,1±49,2 karşı 176,9±36,4; p<0,001), ortalama HDL düzeyi (49,5±11,6 karşı 43,8±10,9; p<0,001) ve ortanca LDL düzeyi (120 karşı 112; p<0,001) yüksek saptandı. Kadınlarda erkeklere kıyasla glisemik kontrolü olan hastaların oranı anlamlı farklılık göstermedi (%45 karşı %42,4 p=0,196), lipid kontrolü olan hastaların oranı (%37,5 karşı %46,9; p<0,001) düşük saptandı (**Tablo 1**).Tüm popülasyonda hastaların %43'ünde (n:80) hipertansiyon, %18,8'ünde (n:35) koroner arter hastalığı, %3,2'sinde (n:6) kronik böbrek yetmezliği belirlendi. Kadınlarda erkeklere kıyasla HT oranı (%45,8 karşı %39,4; p<0,001) yüksek saptandı, koroner arter hastalığı oranı (%18,3 karşı %27,2 p<0,001) erkeklerde yüksekti.

TARTIŞMA

Diyabet prevalansı, tüm dünyada obezitenin ve metabolik sendromun artış göstermesiyle birlikte ciddi derecede artış göstermeye başladı. Dünyada birçok ülkede yapılan farklı

Tablo 1. Demografik ve laboratuvar bulguların dağılımı

	DM N=186	Erkek N=66	Kadın N=120	p
Cinsiyet, n(%)				
Erkek	66(35,5)	66(100,0)	-	-
Kadın	120(64,5)	-	120(100,0)	
Yaş (yıl)	59,7±11,5	61,8±9,4	59,1±10,1	0,164
<65 yıl, n(%)	68,8 (n:128)	46(69,7)	82(68,3)	0,556
≥65 yıl, n(%)	31,2 (n:58)	20(30,3)	38(31,7)	
DM süresi (yıl)	9(1-27)	9(1-27)	9(1-27)	0,080
>10 yıl, n(%)	69(37,1)	27(41)	53(44,1)	0,050*
AKŞ (mg/dL)	148(44-504)	147(44-504)	149(52-489)	0,814
HbA1c (%)	7,9±1,7	8,0±1,8	7,8±1,9	0,834
<%7, n(%)	82(44,1)	28(42,4)	54(45)	0,196
≥%7, n(%)	104(55,9)	38(63,6)	66(55)	
Kreatinin (mg/dL)	0,8(0,3-6,2)	0,9(0,5-6,2)	0,7(0,3-5,7)	<0,001*
Albumin (g/L)	4,4±1,5	4,4±0,5	4,4±1,6	0,846
Ürik asit (mg/dL)	5,1±1,7	5,7±1,5	4,9±1,8	<0,001*
Trigliserit (mg/dL)	155(30-1644)	144(52-1644)	161(30-1604)	<0,001*
Total Kol. (mg/dL)	190,9±48,8	176,9±36,4	198,1±49,2	<0,001*
HDL (mg/dL)	46,2±10,9	43,8±10,9	49,1±12,6	<0,001*
LDL (mg/dL)	117(18-311)	112(340-277)	120(18-311)	<0,001*
<100, n(%)	76(42)	31(46,9)	45(37,5)	<0,001*
≥100, n(%)	108(58)	35(53,1)	75(62,5)	
İlaç Kullanımı, n(%)				0,114
İlaç Yok	6(3,2)	2(3)	4(3,3)	
Sadece İnsülin	32(17,2)	10(15,2)	22(18,3)	
Sadece OAD	76(40,9)	26(39,4)	50(41,7)	
OAD+ İnsülin	72(38,7)	28(42,4)	44(36,7)	

Sayısal değişkenler ortalama±standart sapma veya ortanca (min-max) olarak gösterildi.
Kategorik değişkenler sayı(%) olarak gösterildi. * p<0,05 istatistiksel anlamlılık göstermektedir.
Kısaltmalar: DM: Diabetes Mellitus, AKŞ: Açlık Kan Şekeri, HbA1c: Hemoglobin A1C, Kol: Kolesterol.

çalışmalarda diyabet prevalansının daha önceki çalışmalara kıyasla katlanarak arttığı gözlenmektedir. Shaw ve ark. (7)'nın yaptıkları bir meta–analizde diyabetli hasta sayısı 2009 yılında 285 milyon (%6,4) iken 2030 yılındaki tahmini değerin 438 milyon (%7,7) olacağı yönündedir. Bu çalışmanın sonuçlarına göre 2009 yılından 2030 yılına geçişte diyabet prevalansının gelişmekte olan ülkelerde %69, gelişmiş ülkelerde ise %20 oranında artacağı yönündedir. Ülkemizde 2002 yılında yapılmış TURDEP – I çalışmasının sonucunda diyabet prevalansı %7,2 olarak bulunmuştur (3). Aynı ekip tarafından 2010 yılında bu çalışma TURDEP – II çalışması olarak tekrarlanmış ve bu çalışmanın sonucunda TURDEP – I çalışmasına kıyasla diyabet prevalansı %90 artarak %13,7'ye yükselmiştir (5).

Hızla artan diyabet insidansı, diyabete bağlı mikrovasküler ve makrovasküler komplikasyon düzeyinde de ciddi artışa neden olmaktadır. International Diabetes Management Parctise Study (IDMPS) üçüncü dönem verileri incelendiğinde T2DM hastaların en az %41,5'inde diyabete bağlı geç komplikasyon gelişmiştir (8). Bizim araştırmamızda T2DM hastaların %43'inde (n:80) hipertansiyon, %18,8'ünde (n:35) KAH, %3,2'sinde (n:6) KBH belirlendi. Bundan dolayı son dönemlerde diyabetin önlenmesi veya etkin tedavi edilebilmesi için çok fazla sayıda klinik çalışma yapılmaktadır. Tüm dünyada diyabetin durumu ve eşlik eden metabolik ve vasküler komplikasyonların son durumu hakkında gerçek yaşam verileri ile analizler sunulmaktadır. Bizim ülkemizde T2DM hastalarında diyabet tedavisi, tedavi rejimleri ve eşlik eden metabolik problemler hakkında sınırlı sayıda çalışma mevcuttur. Bizim çalışmamız tek merkezden elde edilen 186 hasta ile yapıldı. Hastaların ortalama yaşı 59,7±11,5 yıl olup T2DM hastalarının hastalık süresi ortancası 9 yıldı ve %37,1'inde (n:69) hastalık süresi 10 yıldan fazla idi. Ortanca açlık kan şekeri düzeyi 148 mg/dl, HbA1c ortalaması %7,9±1,7 olarak saptandı.

Türkiye'de 2000 yılından önce yapılan çalışmalarda ortalama HbA1c düzeyleri %9,5, 2010 yılında TURDEP – II çalışmasında %8 olarak saptanmıştır(4). Sönmez ve ark.(9)'nın yaptıkları çalışmada ortalama diyabet süresi çalışmamıza benzer şekilde ortalama 10,84±7,53 olarak saptanmış ve

ortalama HbA1c düzeyi 7.73±1,74 tespit etmişlerdir(9). Bizim çalışmamızda da hastaların %55,9 'sinde HbA1c düzeyi %7'nin üstünde saptanmıştır. TURDEP – II çalışmasında bu oran bizim çalışmamızdan farklı olarak hastaların yaklaşık %51,2'sinde %7 ve üzerinde bulunmuştur(4). Sonmez ve ark.(9)'nın çalışmasında ise vakaların %59,9'unda HbA1c düzeyi %7'nin üzerinde saptanmıştır. Ülkemizde değişik zamanlarda yapılan çalışmalarda farklı sonuçlara ulaşılmıştır (10). Dünya verilerini inceleyecek olursak; Si ve ark. (11) Avusturalya'da genel popülasyonda HbA1c düzeyi ≥%7 olan diyabetli hasta popülasyonu oranı %43 oranında, Yeni Zelanda'da hastaların %73'ünde HbA1c düzeyinin %8'in altında olduğunu, Amerika Birleşik Devletlerinde 18–75 yaş arası popülasyonda hastaların yalnızca %30'unda HbA1c düzeyi <%7 olduğunu saptanmışlardır (11). Halen tüm dünyada diyabet hastalarında HbA1c düzeyleri beklenilen düzeyde değildir.

Bizim çalışmamızda Sonmez ve ark.(9)'nın çalışmasına oranla LDL (108 mg/dl - 113,92±36,17 mg/dl) ve trigliserit düzeyi (155 mg/dl - 181,75±128,47 mg/dl) tüm popülasyonda daha düşük saptanmıştır. Bizim çalışmamızda HDL düzeyi 46,9 ± 12,6 mg/dl, Sönmez ve ark.'nın çalışmasında ise 46,58±12,93 olarak tespit edilmiş ve benzer olduğu görülmüştür. Çalışmamızda LDL düzeyi hedef aralıkta (<100 mg/dl) olan hasta düzeyi %42 olarak bulunmuştur. Avusturalya'da LDL düzeyinin hastaların %82'sinde normal aralığın üstünde, Yeni Zelanda'da LDL düzeyi normal aralıkta olan hasta sayısı %31–43 arasında saptanmış ve İngiltere'de hastaların %73'ünde kolesterol düzeyleri normal aralıkta saptanmıştır (11). IDPMS beşinci dönem Türkiye verilerinde dislipidemisi olan T2DM hasta oranı %55,8 saptanmıştır (12).

Bizim çalışmamızda vakaların %45,5'inde hipertansiyon tanısı olduğu saptandı. Sonmez ve arkadaşlarının (9) çalışmasında vakaların %59,3'ünün antihipertansif tedavi aldığı saptanmıştır. IDPMS beşinci dönem Türkiye verilerinde T2DM hastalarının %62,7'sinde hipertansiyon saptanmıştır (12). Diyabete özgü komplikasyonlar yeni tanı konulan T2DM hastalarının %20-50'sinde mevcut olabilir. Komplikasyonlar ve diyabetle ilişkili olan obezite, HT, dislipidemi, kardiyovasküler hastalık durumları diyabet tanısı konulunca değerlendirilmeli ve tedavisi planlanmalıdır.

Diyabet sağlık harcamalarının yaklaşık %12'sini oluşturmaktadır (6). Ayrıca bu harcamaların önemli bir kısmını ise diyabet komplikasyonları sebep olmaktadır (6). Diyabetin ekonomik yükünde direkt hastalık nedeniyle yapılan harcamalar (ilaçlar, hastalık takibi ve komplikasyon tedavisi) dışında diyabette indirekt ekonomik kayıplarda yaşanmaktadır (13). Türkiye'de 2008 yılında yaklaşık 4,5 milyar tl olan diyabetin toplam maliyeti 2012 yılında yaklaşık 10 milyar TL olmuştur (14). Ayrıca toplam sağlık harcamalarında diyabetin oranı 2008 yılında %16,4 iken 2012 yılında bu oran %22,6' ya kadar yükselmiştir (14). Diyabet ile ilgili harcamalarda doğrudan hastalığın maliyeti %26 iken diyabetin neden olduğu komplikasyonların maliyeti ise %74 olarak hesaplanmıştır (14). DSÖ'nün 2016 verilerine göre tüm dünyada diyabet harcamalarının artmaya devam edeceği bununla beraber az gelişmiş ve gelişmekte olan ülkelerdeki diyabet harcamalarının gelişmekte olan ülkelere oranla daha yüksek paya sahip olacağı öngörülmektedir (15).

Diabetes mellitus toplum sağlığını ciddi bir şekilde etkilemekte, tedavi maliyetleri ve işgücü kayıplarından dolayı ciddi bir yük oluşturmakta ve olumsuz sonuçlara yol açmaktadır (16). Bu nedenle, diyabete bağlı gelişebilecek komplikasyonların erken saptanması, koruyucu önlemlerin alınarak diyabetli hastalarda yaşam kalitesinin artırılması ve tedavi maliyetlerinin azaltılması için muhakkak daha ayrıntılı incelenip multidisipliner bir yaklaşımla tedavi edilmesi ve bu konuda risk faktörlerini azaltmaya yönelik yeni sağlık politikalarının planlanması gerekmektedir. Bu açıdan tip 2 diyabetin tedavisinde izlenecek en uygun yaklaşıma katkı sağlamaya yönelik klinik çalışmalara ihtiyaç vardır.

MADDİ DESTEK VE ÇIKAR İLİŞKİSİ

Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkara dayalı ilişkisi yoktur

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Research Article / Araştırma Makalesi

Rheum ribes L.'nin etanol ekstraktlarının malign melanoma hücreleri üzerine anti-kanser etkinliği

The anti-cancer effect of Rheum ribes L.'s ethanol extracts on malign melanoma cells

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ÖZ

Amaç: Işkın bitkisi (*Rheum ribes L.*) Çin, Hindistan, İran ve Türkiye'de yabani olarak yetişmekte olup birçok tıbbi amaç için kullanılmaktadır. Bu çalışmanın amacı *Rheum ribes L.*'nin kök, gövde ve kabuklarının etanol ekstraktlarının malign melanoma hücrelerine karşı anti-kanser etkinliğini araştırmaktır.

Gereç ve Yöntem: Bitkinin oda sıcaklığında kurutulan kök, gövde ve kabuk kısımları mikser ile toz haline getirilip %50 etanol ile 24 saat inkübe edildi. Organik faz rotary evaporatörde uçurulup, su fazı liyofilizatörde ayrıldıktan sonra elde edilen ekstraktlarda; öncelikle total fenol ve total flavonoid düzeyi ile total antioksidan kapasite (TAS) fotometrik yöntemlerle ölçüldü. Daha sonra kültüre edilen malign melanoma hücreleri (B16F10) bu ekstraktların farklı konsantrasyonları ile 24 saat inkübe edildi. İnkübasyondan sonra sitotoksisite düzeyi; kolorimetrik MTT [3-(4,5-diMetilTiyazol-2-il)-2,5-difenil Tetrazolyum bromür] metodu ile, apoptozis düzeyi ise; Akridin Oranj-Etidyum Bromit (AO/EB) ikili boyama yöntemi ile tespit edildi.

Bulgular: Hem sitotoksisite hem de apoptozis sonuçları incelediğinde aynı konsantrasyonlarda en yüksek sitotoksik ve apoptotik etkinliğin kök ekstraktları ile sağlandığı görüldü ($p<0,001$).

Sonuç: *Rheum ribes L.'nin* malign melanoma hücreleri üzerine antikanser etkinliğinin doza bağımlı olarak arttığı ve bitkinin gövde ve kabuklarına kıyasla kök ekstraktının anti-kanser ilaç olma potansiyelinin daha yüksek olduğu kanaatine varılmıştır.

Anahtar Kelimeler: Işkın; anti-kanser; malign melanoma

ABSTRACT

Objective: Iskin plant (*Rheum ribes L.)* grows in China, India, Iran and Turkey in the wild and it is used for many medicinal purposes. The aim of this study is to investigate the anti-cancer efficacy of the ethanol extracts of the roots, stems and barks of *Rheum ribes L.* on the malignant melanoma cells. **Material and Method:** Root, stem and bark of the plant dried at room temperature were powdered with a mixer and incubated with 50% ethanol for 24 hours. The organic phase was evaporated in a rotary evaporator and the water phase is separated in the lyophilizer. Total phenol, total flavonoid level and total antioxidant capacity (TAS) were measured by photometric methods. Then, the cultured malignant melanoma cells (B16F10) were incubated for 24 hours with different concentrations of these extracts. Cytotoxicity level was determined by colorimetric MTT [3- (4,5-diMethyl Thiazol-2-yl) -2,5-diphenyl Tetrazolium bromide] and the apoptosis level by Acridine Orange-Ethidium Bromide (AO/EB) double staining method.

Results: When examining the results of both cytotoxicity and apoptosis, it was observed that the highest cytotoxic and apoptotic activity was achieved with root extracts at the same concentrations ($p <0.001$).

Conclusion: It was concluded that the anti-cancer efficacy of *Rheum ribes L.* on malignant melanoma cells increased in a dose-dependent manner and the root extract had a higher anti-cancer drug potential compared to the stem and shells of the plant.

Keywords: Rheum ribes L, anti-cancer, malignant melanoma

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GİRİŞ

Rheum ribes L. Çin, Hindistan, İran ve Türkiye'de yabani olarak yetişmektedir (1). Türkiye'de ise Doğu Anadolu ve özellikle Van ili civarında yetişmektedir (2). *Rheum ribes L.* Türkiye'de yabani olarak yetişen tek rheum türüdür (3). Bitkinin aerial kısımlarının daha önce yapılmış içerik çalışmalarında krizofanol, fiskiyon ve emodol antrakinonları ile kuersetin, 5-dezoksikuersetin, kuersetin 3-0-ramnozit, kuersetin 3-0- galaktozit, kuersetin 3-0-rutinozit flavonoidleri içerdiği tespit edilmiştir (2). Ayrıca C vitamini açısından zengin olduğunu ortaya koyan çalışmalar da mevcuttur (4).

Rheum ribes L. halk arasında birçok klinik faydalarının yanında az bilinen anti-kanser özelliği için de kullanılmaktadır. Halk arasında birçok hastalığın tedavisinde kullanılmış olması bu bitki üzerine bilimsel çalışmaları cazip hale getirmiştir. *Rheum ribes L.*'in antioksidan, anti-bakteriyel, anti-trikomonas, anti-viral, anti-fungal, anti-diyabetik, anti-dayareik, anti-hiperlipidemik özellikleri üzerine çok sayıda çalışma yapılmıştır (5-14). Ayrıca Alzheimer hastalığının hafif ve orta şiddetteki vakalarında kullanılan asetil kolin esteraz inhibitörleri gibi etki gösterdiğini ortaya koyan çalışmalar da mevcuttur (15). Bildiğimiz kadarıyla, anti-kanser (10, 16-20) özelliği üzerine de çalışmalar yapılmıştır. Bununla birlikte malign melanoma hücre hattı üzerine yapılmış sadece bir çalışmaya rastladık (10). Bu çalışmada da *Rheum ribes L.*, fraksiyonlarına ayrılmayıp bir bütün halinde değerlendirilmiştir.

Malign melanoma, cilt kanserinden ölümlerin %75'ini teşkil etmektedir. Agresif lokal büyüme ve metastaz malign melanomanın yaygın özellikleri olup nodal yayılımı olan hastalarda beş yıllık sağkalım oranı yaklaşık %36'dır ve uzak metastazı olanlarda bu oran %5'e kadar düşmektedir (21). Sağkalım oranının düşüklüğü, etkin bir tedavinin halen mevcut olmayışı nedeniyle malign melanoma için etkin tedavi arayışları halen sürmektedir.

Bu çalışmada, *Rheum ribes L.*'nin kök, gövde ve kabuklarının ayrı ayrı ekstraktlarının antioksidan özelliklerini ve farklı konsantrasyonlarının malign melanoma (B16F10) hücre hattı üzerindeki sitotoksik ve apoptotik etkinliğini araştırmayı amaçladık.

GEREÇ VE YÖNTEM

Bitki Malzemeleri: Bu çalışmada *Rheum ribes L.*, taze olarak Van ilinden toplanmıştır. Bitki, üzerindeki toz kalıntıları su ile temizlenerek kökü, gövdesi ve gövdesinin yeşil kabukları olmak üzere üç kısma ayrılıp ince dilimler haline getirildikten sonra 1 hafta boyunca gölgede kurutuldu. Şahit numuneler Harran Üniversitesi herbaryumunda 5994 numarası ile kaydedildi. Bitkinin kuru halde olan kök, gövde ve kabukları çırpıcı ile kaba toz haline getirildi.

Ekstraktların Hazırlanması: Toz haline getirilen bitki materyalleri %50 etanolde 24 saat kapalı amber beherde manyetik karıştırıcı ile karıştırıldıktan sonra rotary evaporatörde organik fazı ayrılarak, sonrasında liyofilizatörde su fazı uçurularak ekstrakte edildi.

Etken maddenin hazırlanması: Elde edilen bitki ekstraktları, farklı konsantrasyonlarda hazırlanmak için önce %0,1 DMSO (Dimetil Sülfoksit) ile çözülüp stok solüsyonu hazırlandıktan sonra PBS (Phosphate Buffered Saline) ile seyreltildi.

Toplam Fenolik İçerik Ölçümü: *Rheum ribes L.*'nin toplam fenolik içeriğini belirlemek için Folin-Ciocalteu yöntemi (22) kullanıldı. Filtrelenmiş 50 µL numune ve 250 µL 0,2 N Folin-Ciocalteu reaktifi vortex ile karıştırıldı ve oda sıcaklığında 5 dakika tutuldu. Daha sonra 200 µL 0,7 mol/L Na_2CO_3 ile karıştırıldı. Oda sıcaklığında 2 saat inkübasyondan sonra, reaksiyon karışımının absorbansı, bir spektrofotometre (Varioskan Flash Multimode Reader, Thermo Scientific, ABD) kullanılarak bir köre karşı 760 nm'de ölçüldü. Kalibrasyon eğrisini çizmek için standart olarak gallik asit (0-300 mg/L) kullanılmıştır. Hesaplamalarda üç ölçümün ortalaması kullanılmıştır ve toplam fenolik içerik, µg/mL gallik asit eşdeğerleri cinsinden ifade edilmiştir.

Toplam Flavonoid İçerik Ölçümü: *Rheum ribes L.* örneklerinin toplam flavonoid içeriği, Zhishen vd. tarafından geliştirilen kolorimetrik test yöntemine göre belirlenmiştir (23). Filtrelenmiş 50 µL numune 250 µL distile su ve 15 µL %5 $NaNO_2$ çözeltisi ile karıştırıldı. 6 dakika sonra 30 µL %10 $AlCl_3$ çözeltisi, daha sonra 100 µL 1 mol/L NaOH ilave edildi ve çözelti, 5 dakika daha oda sıcaklığında inkübe edildi. Reaksiyon karışımı iyice karıştırıldı ve kırmızı renkli flavonoid-alüminyum kompleksinin yoğunluğu, bir spektrofotometre (Varioskan Flash Multimode Reader, Thermo Scientific, ABD) kullanılarak 510 nm'de ölçüldü. 5 ila 50 mg/L'lik bir konsantrasyon aralığında standart bir kuersetin eğrisi çizildi. Toplam flavonoid içeriği, µg/mL kuersetin eşdeğeri olarak ifade edildi.

Toplam Antioksidan Kapasite Ölçümü: Erel vd. (24) geliştirdiği yönteme göre yapıldı. Prensip, ekstrakttaki antioksidanların koyu mavi yeşil renkli ABTS radikalini, renksiz ABTS formuna indirgemesi esasına dayanır. Standart olarak 1 mM troloks kullanılıp, ekstraktlar için sonuçlar mM troloks eşdeğeri olarak ifade edildi.

Hücre hattının Hazırlanması: B16F10 hücre hattını çoğaltmak için D'MEM'e (Dulbecco's Modified Eagle Medium) FBS %10 (Fetal Bovine Serum) ve P/S %1 (penisilin 100 U/mL ve streptomisin 100 U/mL) eklenen vasat kullanıldı. Hücreler %5 CO_2, %95 nem ve 37˚C'de sıcaklık ortamında inkübatörde tutuldu. Hücreler konflüe olduktan sonra tripsinizasyonla hasat edilip etken madde uygulanacak 96'lık ve 6'lık platelere ekildi. 96'lık platelerde her kuyucuğa $10x10^3$ hücre, 6'lık platelerde her kuyucuğa $10x10^5$ hücre ekilip 24 saat inkübe edildi. Hücre canlılığı tripan mavisi ile kontrol edildi ve canlılığın %95'in üzerinde olduğu görülerek deneylere devam edildi.

Sitotoksisite Analizi: Farklı konsantrasyonlardaki ekstraktlar 96'lık platelerde ekili olan B16F10 hücre hattı üzerinde üçerli tekrar olacak şekilde ve 24 saat süreyle uygulandı. Negatif kontrol olarak, tedavi yapılan kuyucuklardaki en yüksek düzeye eşit konsantrasyonda (%0,1) DMSO

eklendi. Ekstraktlarla tedaviden sonra her kuyucuğa 5 µg/mL konsantrasyondaki MTT'den [3-(4,5-diMetilTiyazol-2-il)-2,5-difenil Tetrazolyum bromür] 20 µL eklenerek 4 saat süreyle 37˚C'de karanlıkta inkübe edildikten sonra kuyucuklardaki içerik döküldü. Daha sonra her kuyucuğa 150 µL DMSO eklenerek oda sıcaklığında ve çalkalayıcıda 20 dk çalkalanmaya bırakıldı. Ardından spektrofotometreyle 470 nm'de (Varioskan LUX Multimode Microplate Reader- Thermo Fisher Scientific) absorbans ölçümü yapıldı.

Apoptozis Tayini: 200 µg/ml ve altı konsantrasyonlardaki ekstraktlar 6'lık platelerde ekili olan B16F10 hücre hattı üzerinde 24 saat süreyle uygulandıktan sonra apoptotik hücre oranlarının mikroskobik tespiti için Akridin Oranj–Etidyum Bromit ikili boyama yöntemi kullanıldı. Hücre incelemesi floresan mikroskop (Leica DM 1000, Solms, Germany) altında gerçekleştirildi. Her konsantrasyon için test üç tekrarlı yapıldı.

İstatistiksel Analiz

Tüm analizler üç tekrarlı olarak gerçekleştirilmiştir. Veriler ortalama ± SD olarak ifade edildi. Grafiksel değerlendirmeler ve IC_{50} değerlerinin hesaplanması için Microsoft Excel 2010 (Roselle, IL, ABD) programı kullanılmıştır. Çoklu grupların karşılaştırılmasında tek yönlü ANOVA, post hoc analizinde Tukey testi kullanılmıştır.

Etik Durum

Çalışmamız herhangi bir insan veya hayvan üzerinde veya onların biyolojik materyalleri kullanılarak yapılmış olmadığı; sadece satın alınan dünyaca kabul görmüş kuruluşların standardize ettiği hücre serileri kapsamındaki hücre serisi üzerinde yapılmış in vitro laboratuvar çalışması olduğundan herhangi bir etik kurul iznine gerek duyulmamıştır.

BULGULAR

Total Fenolik ve Flavonoid Aktivite: *Rheum ribes L.*'nin farklı kısımlarının %50 etanolik ektraktları, kateşin ve gallik asit'e kıyasla önemli ölçüde fenolik ve flavonoid aktivite göstermiştir. Fenolik aktivite düzeyi 200 µg/mL konsantrasyonda gövde, kabuk ve kök için sırasıyla 53,74±3,56, 57,16±3,97 ve 69,49±4,17 µg GAE (Gallik Asit Eşdeğeri)/mL idi. Flavonoid aktivite düzeyi de 200 µg/mL konsantrasyonda gövde, kabuk ve kök için sırasıyla 76,55±5,72, 83,80±4,29 ve 99,11±6,85 µg KE (Kuersetin Eşdeğeri)/mL idi. *Rheum ribes L.'nin* gövde, kabuk ve kök ekstraktlarının total fenolik ve flavonoid aktivite ölçüm sonuçları **Şekil 1** ve **Şekil 2**'de görülmektedir.

Total Antioksidan Kapasite: Total antioksidan kapasite 200 µg/mL konsantrasyonda gövde, kabuk ve kök için sırasıyla 1,10±0,06, 1,29±0,02 ve 1,48±0,07 mmol troloks/L idi (**Şekil 3**).

Hücre Canlılık Oranı: MTT ile yapılan canlılık testinde *Rheum ribes L.'nin* fraksiyonları arasında en yüksek oranda fenolik ve flavonoid içeriğe sahip olan kök ekstraktının düşük dozlarda (15 µg/mL) hücre canlılığında hafif bir artışa, yüksek dozlarda ciddi düzeyde canlılık azalışına neden olduğu; bir tepe noktasından sonra da bu etkinlikte hafif bir azalmaya doğru bir gidiş olduğu saptandı. Gövde, kabuk ve kök ekstraktlarının 500 µg/mL konsantrasyonda hücre canlılığını sırasıyla %85,01±7,20, %65,01±7,53 ve %8,08±5,88 düzeyine kadar düşürdükleri saptandı ($p<0,001$) **(Şekil 4) (Tablo)**. Kök ekstraktı için IC_{50} değeri 61 µg/mL olarak saptandı. Çalışması yapılan konsantrasyonlarda kontrole kıyasla sitotoksisite etkinlik farkı kök için 62 µg/mL'den itibaren ($p<0,001$) istatistiki olarak belirginlik gözlenirken, kabuk için 125 µg/mL'den ($p=0,002$) itibaren belirginlik saptanmıştır. Gövdede ise kontrole kıyasla farklılık saptanmamıştır **(Şekil 4)**.

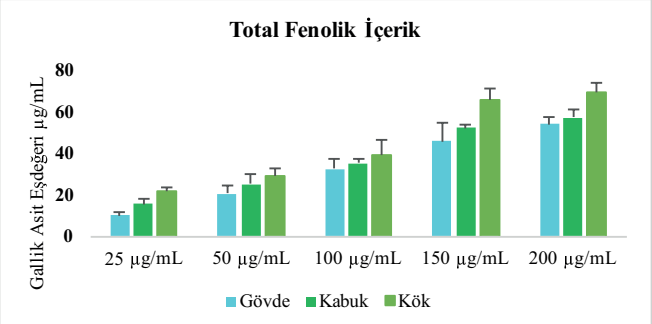
AO/EB yöntemiyle yapılan incelemede *Rheum ribes L.*'nin gövde, kabuk ve kök ekstraktlarının 200 µg/mL konsantrasyonda B16F10 malign melanoma hücre hattında hücre canlılığını sırasıyla %69,16±3,50, %57,14±4,14 ve %40,24±3,57 düzeyine kadar düşürdüğü saptandı ($p<0,001$) **(Şekil 5) (Tablo 1)**. Kök ekstraktı için IC_{50} değerinin 188 µg/mL olduğu saptanmıştır. Çalışması yapılan konsantrasyonlarda kontrole kıyasla apoptozis etkinlik farkı kök için 50 µg/mL'den itibaren ($p=0,001$) istatistiki olarak belirginlik gözlenirken, kabuk ve gövde için 100 µg/mL'den ($p<0,001$) itibaren belirginlik saptanmıştır **(Şekil 5)**.

TARTIŞMA

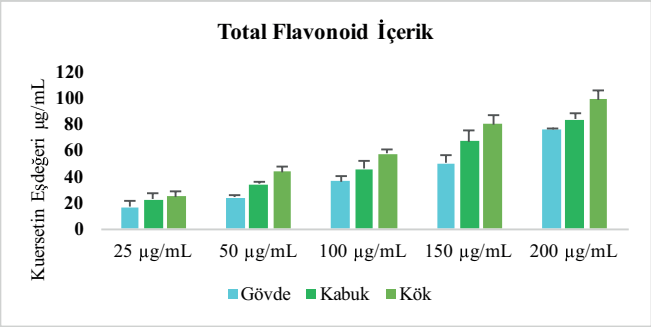
Bu çalışmada *Rheum ribes L.'nin* çeşitli kısım ekstraktlarının farklı düzeyde polifenolik içeriğe sahip olduğu ve antikanser etkinliklerinin de farklı olduğu saptandı.

Önceki çalışmalarda *Rheum ribes L.'nin* kök ve gövdesinin fenolik ve flavonoid aktivitesinin ölçümü yapılmış ve kök ekstraktında bu içeriğin daha yüksek düzeyde olduğu, bununla orantılı olarak antioksidan kapasitenin de kök ekstraktında daha yüksek olduğu saptanmıştır (6,12). Polifenol içerikle orantılı olarak antioksidan aktivitenin arttığı bilinmektedir (25-27). Bununla uyumlu olarak biz de çalışmamızda bu bulguları teyit ettik (**Şekil 1-3**).

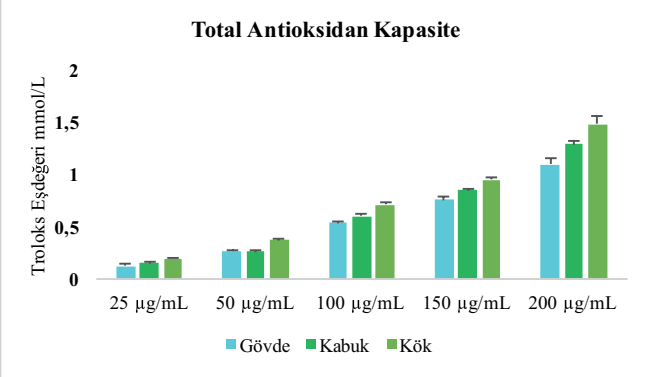
Kanser hücrelerinin bazal redoks seviyeleri normal hücrelerden farklıdır. Kanser hücrelerinde daha yüksek seviyelerde serbest metal iyonları ve daha yüksek endojen ROS



Şekil 1. *Rheum ribes L.*'nin farklı kısım ekstraktlarının Total Fenolik İçerik düzeyleri



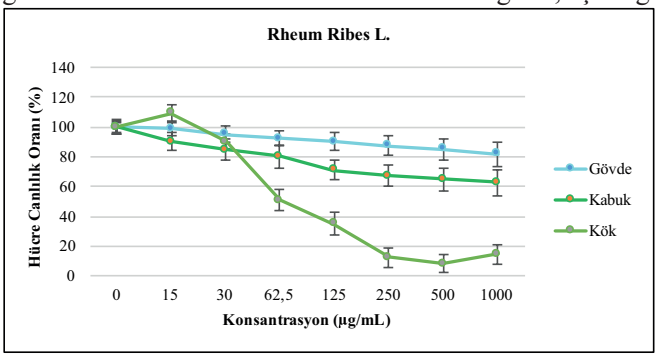
Şekil 2. *Rheum ribes L.*'nin farklı kısım ekstraktlarının Total Flavonoid İçerik düzeyleri



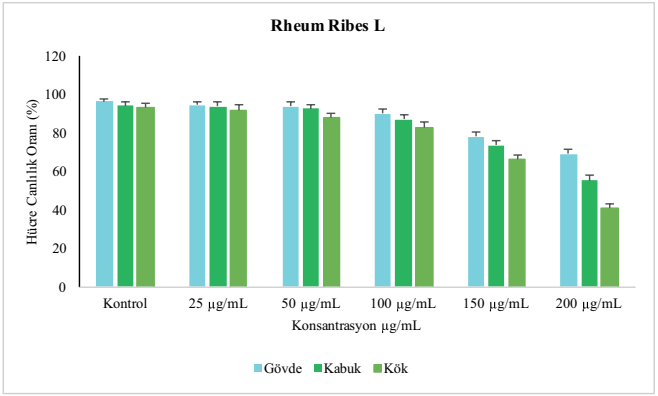
Şekil 3. *Rheum ribes L.*'nin farklı kısım ekstraktlarının Total Antioksidan Kapasite düzeyleri

üretimleri, onları oksidan sitotoksisiteye neden olan fitokimyasallara daha duyarlı hale getirir (28). Bu bağlamda birçok medikal ilaç ROS üretebilme potansiyeli ile orantılı olarak antikanser etkinlik göstermektedir (29).

Rheum ribes L., antioksidan etkinliğinin yanısıra antikanser özellik de göstermiştir (**Şekil 4,5**). Bu bağlamda antioksidan aktivitesi iyi tanımlanmış fitokimyasalların yüksek dozlarda ve demir, bakır gibi metal iyonlarının varlığı gibi belirli koşullar altında pro-oksidasyon aktivite de gösterebildikleri ortaya konmuştur (30,31). Bu açıdan bakıldığında *Rheum ribes L.'nin* antikanser özelliğinin, içerdiği



Şekil 4. *Rheum ribes L.*'nin farklı kısım ekstraktlarının B16F10 hücre hattı üzerinde 24 saat uygulandıktan sonra sitotoksik etkinliğinin MTT yöntemi ile değerlendirilmesi. Kontrole kıyasla sitotoksisite etkinlik farkı kök için 62 µg/mL'den itibaren (p<0,001) istatistiki olarak belirginlik gözlenirken, kabuk için 125 µg/mL'den (p=0,002) itibaren belirginlik saptanmıştır. Gövdede ise kontrole kıyasla farklılık saptanmamıştır.



Şekil 5. *Rheum ribes L.*'nin farklı kısım ekstraktlarının B16F10 hücre hattı üzerinde 24 saat uygulandıktan sonra apoptotik etkinliğinin Akridin Oranj – Etidyum bromit boyaları ile floresan mikroskopta değerlendirilmesi. Kontrole kıyasla apoptozis etkinlik farkı kök için 50 µg/mL'den itibaren (p=0,001) istatistiki olarak belirginlik gözlenirken, kabuk ve gövde için 100 µg/mL'den (p<0,001) itibaren belirginlik saptanmıştır.

antioksidan polifenollerin yüksek konsantrasyonda pro-oksidan aktivite göstermesine bağlı gibi görünmektedir.

Rheum ribes L.'nin anti-kanser (10,16-20) etkinliği üzerine bazı çalışmalar yapılmıştır. Esmaeilbeig vd.'nin (16) yaptığı çalışmada *Rheum ribes L.*'nin K-562 (Kronik miyelojen lösemi) hücre hattı üzerinde antikanser etkinliği (IC_{50}: 115 µg/mL) saptanmıştır. Ayrıca Sardari vd. *Rheum ribes L.* ekstraktını malign melanoma A375 hücre hattı üzerinde denemiş ve IC_{50}: 21,3 µg/mL olarak bulmuşlardır (10). Abudayyak vd. *Rheum Ribes L*'nin hepatosellüler kanser hücre hattı üzerinde IC_{50} değerini 14.29-31.94 mg/mL olarak saptamışlardır. Keser vd. ise insan meme kanseri (MCF-7), kolon kanseri (HCT-116), over kanseri (A2780) ve prostat kanseri (PC-3) hücre hatları üzerinde sitotoksik etkinliğini saptamışlardır. Bu çalışmalar *Rheum ribes L.*'nin antikanser etkinliği açısından bizim bulgularımızı teyit etmektedir. Bununla birlikte Sardari vd. (10)'nin yaptığı çalışmada *Rheum ribes L.*, fraksiyonlarına ayrılmayıp bir bütün halinde değerlendirilmiştir. Bizim çalışmamızda buna ek olarak *Rheum ribes L.*'nin kök ekstraktlarının gövde ve kabuk ekstraktlarına kıyasla malign melanoma hücreleri üzerinde istatistiksel olarak daha yüksek sitotoksik ve apoptotik etkinlik gösterdiği saptanmıştır (p<0,001) (**Tablo**).

Tablo. Rheum ribes L.'nin farklı kısım ekstraktlarının B16F10 hücre hattı üzerine MTT yöntemiyle sitotoksisite ve AO-EB yöntemiyle apoptozis oranlarının karşılaştırılması.

	MTT (% Canlılık Oranı) 250 µg/mL	AO-EB (%Canlılık Oranı) 200 µg/mL
Gövde	87,41±6,5	69,16±3,50
Kabuk	67,54±4,5 Ω	57,14±4,14 Ψ
Kök	12,43±4,3 *	40,24±3,57 *

Veriler kontrole kıyasla ortalama ± SS olarak ifade edilmiş ve gruplar arası fark ANOVA testiyle değerlendirilmiştir.
*: p<0,001 kabuk ve gövdeye kıyasla, Ω: p<0,01 gövdeye kıyasla, Ψ: p< 0,001 gövdeye kıyasla.

Bundan sonraki hedefimiz *Rheum ribes L*. kök ekstraktının içerik analizini yapmak, anti-kanser etki mekanizmasını ve kanser hücre hatlarına kıyasla normal insan hücre hatlarına ne denli etki ettiğini aydınlatmaya çalışmak olacaktır.

SONUÇ

Elde edilen sonuçlar, önceki çalışmalarla uyumlu olarak *Rheum ribes L.'*nin antikanser etkinliğinin varlığını ortaya koymaktadır. Bu sonuçlar doğrultusunda *Rheum ribes L*.'nin anti-kanser ilaç üretiminde kullanılabileceği ümit edilebilir. Bununla birlikte bitkinin kök ekstraktının, gövde ve kabuk kısmına kıyasla daha etkili olduğu görünmektedir. Bundan sonra, *Rheum ribes L*. ile ilgili yapılacak anti-kanser etkinlik çalışmalarında bitkinin kök kısmına odaklanılmasının daha uygun olacağı kanaatine varılmıştır.

Çıkar Çatışması: Yazarlar, aralarında çıkar çatışması olmadığını beyan ederler.

Maddi Destek: Hiçbir kuruluş tarafından maddi destek alınmamıştır.

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Research Article / Araştırma Makalesi

Prevalence and risk factors of hemodynamic instability during endoscopic transsphenoidal pituitary surgery: a retrospective analysis

Endoskopik transsfenoidal hipofiz cerrahisi sırasında hemodinamik instabilite prevalansı ve risk faktörleri: retrospektif bir analiz

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ABSTRACT

Background: Intraoperative hemodynamic instability in patients undergoing endoscopic transsphenoidal pituitary surgery (ETSS) for pituitary adenoma may lead to significant complications. We aimed to investigate the prevalence of hemodynamic instability and its associated risk factors in these patients.

Material and Method: This retrospective study included patients who underwent ETSS at Ankara Numune Training and Research Hospital between 14 January 2010 and 20 March 2014. Intraoperatively occurring episodes of bradycardia, hypotension, and hypertension were recorded. Distribution of hemodynamic instability was determined based on age groups, ASA class, tumor type, and anesthesia method.

Results: A total of 323 patients met the study criteria. Mean age of the patients was 46.88 ± 13.91 years and 54.5% were female. Intraoperative bradycardia was detected in 137 patients (42.41%), hypotension in 57 patients (17.65%), and hypertension in five patients (1.55%). Hemodynamic instability occurred in all of the patients over the age of 51. Patients classified as ASA III were more likely to have hemodynamic instability than patients with ASA I and ASA II ($P<0.05$). All 18 patients who were transferred to intensive care unit were in the age group of 61 years and over. The rate of hemodynamic instability was higher in patients with non-functioning tumor compared to that in patients with functioning tumors.

Conclusion: The rate of hemodynamic instability during ETSS is higher in the elderly, ASA III class and non-functioning tumors. These patients need a carefully planned anesthetic management.

Keywords: Endoscopic transsphenoidal pituitary surgery, pituitary adenoma, anesthesia, hemodynamic instability

ÖZ

Amaç: Hipofiz adenomu için endoskopik transsfenoidal hipofiz cerrahisi geçiren hastalarda intraoperatif dönemde ortaya çıkan hemodinamik instabilite önemli komplikasyonlara yol açabilir. Bizim amacımız bu hastalarda hemodinamik instabilitenin prevalansını ve onunla ilişkili risk faktörlerini araştırmaktır.

Gereç ve Yöntem: Endoskopik transsfenoidal hipofiz cerrahisi geçiren hastalarda intraoperatif dönemde ortaya çıkan bradikardi, hipotansiyon ve hipertansiyon kaydedildi. Yaş grupları, ASA sınıfı, tümör tipi ve anestezi metoduna göre hemodinamik instabilite dağılımı belirlendi.

Bulgular: Toplam 323 hasta çalışma kriterlerini karşıladı. Ortalama yaş 46,88±13,91 ve %54,5'i kadın cinsiyette idi. Intraoperatif dönemde 137 hastada bradikardi (%42.41), 57 hastada hipotansiyon (%17,65) ve 5 hastada hipertansiyon (%1,55) tespit edildi, 51 yaşın üzerindeki hastaların tamamında hemodinamik instabilite görüldü. ASA III olarak sınıflandırılan hastalarda ASA I ve ASA II'ye göre daha fazla oranda hemodinamik instabilite görüldü ($p<0.05$). Postoperatif dönemde yoğun bakıma transport edilen 18 hastanın tamamı 61 yaş ve üzeri hasta grubunda idi. Non-functioning tümör olan hastalarda functioning tümöre sahip olanlara göre hemodinamik instabilite oranı daha yüksekti.

Sonuç: ETSS sırasında hemodinamik instabilite oranı yaşlılarda, ASA III sınıfında ve çalışmayan tümörlerde daha yüksektir. Bu hastalar dikkatle planlanmış bir anestezi yönetimine ihtiyaç duyar.

Anahtar Kelimeler: Endoskopik transsfenoidal hipofiz cerrahisi, hipofiz adenomu, anestezi, hemodinamik instabilite

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INTRODUCTION

Maintaining the heart rate and blood pressure within normal limits is very critical to provide an adequate tissue perfusion. Abnormal excursions of heart rate and blood pressure lead to the clinical condition called as hemodynamic instability. If perioperatively emerging hemodynamic instability is severe and long-lasting, it may cause significant complications such as myocardial ischemia, cerebrovascular events, and acute kidney injury (1-4). A number of factors trigger hemodynamic instability during ETSS performed for pituitary adenomas. Bradycardia and arterial blood pressure changes may occur via trigemino-cardiac reflex during transsphenoidal surgery. Trigemino-cardiac reflex results from stimulation of trigeminal nerve receptors in the nose, and trigeminal ganglion and its roots (5,6). This reflex was reported in 10% of patients undergoing transsphenoidal surgery for pituitary adenomas (7).

Patients scheduled for ETSS may have comorbidities such as diabetes mellitus, hypertension, coronary artery disease, cardiomyopathy, and hyperthyroidism, which contribute to perioperative hemodynamic instability. If a patient has a functioning pituitary adenoma, several symptoms exist due to hormone excess. For example, the prevalence of clinical hypertension is 50% in patients with acromegaly, and it resolves markedly after ETSS (8,9). Eighty percent of patients with Cushing's disease have hypertension. Thyrotropic adenoma may cause pituitary hyperthyroidism. Failure to normalize thyroid functions preoperative may lead to perioperative hemodynamic instability (10).

Advanced age may pose an important risk for perioperative hemodynamic instability. Elderly patients who underwent ETSS for nonfunctioning pituitary adenoma were reported to be more likely to have elevated blood pressure than that in their younger counterparts (11).

As performed in nasal region, ETSS elicits intense pain. Unless adequately deep anesthesia is established, severe pain leads to blood pressure elevation. Since it is very important to ensure and maintain an appropriate field of surgical vision, any blood pressure elevation that can increase bleeding should not be allowed. Nevertheless, a very deep anesthetic due to concern about the occurrence of pain and awareness causes bradycardia and hypotension. While the rate of tachycardia was high in patients who received sevoflurane through inhalation anesthesia, bradycardia was more common in patients who were administered propofol by total intravenous anesthesia (12). Another cause of hypotension in the perioperative period is hypovolemia (1).

There are limited number of large-scale clinical studies regarding hemodynamic alterations emerged during ETSS. This study aimed to investigate the prevalence of hemodynamic instability and its associated risk factors in patients who underwent ETSS for pituitary adenoma in a time-period of more than four years in a tertiary care hospital.

MATERIAL AND METHOD

The data of patients who underwent ETSS in our hospital were obtained from medical records. Their age, gender, ASA (American Society of Anesthesiologists class score), and accompanying diseases were recorded. Pituitary tumors were classified as functioning and nonfunctioning. Duration of the surgery, anesthesia methods, and muscle relaxants were recorded. Intraoperatively, bradycardia (heart rate<50 beat/min), hypotension (an episode of a mean arterial pressure of <50 mmHg) or hypertension (systolic pressure>160 mmHg) were identified (13,14). It was determined whether patients were transferred into the intensive care unit in the postoperative period.

All patients electively underwent a standard ETSS. The patients were in the supine position, and pituitary tumor was removed with a surgical microscope. Patients were fasted for at least 6 hours preoperatively per standard anesthesia protocol in our hospital. Premedication was performed with intramuscular midazolam. Intraoperative monitoring was performed through electrocardiogram, heart rate, pulse oximetry, end-tidal concentration of carbon dioxide, and continuous monitoring of arterial blood pressure with indwelling radial artery catheter. Anesthesia was induced with propofol (0.5-3 mg/kg) and fentanyl (1-2 μg/kg) followed by muscle relaxation with vecuronium (0.15 mg/kg) or rocuronium (0.6 mg/kg). After the trachea was intubated, mechanical ventilation was initiated with air and oxygen mixture (FiO2 of 0.3-0.5). Maintenance anesthesia was established with sevoflurane (1%-3%) and remifentanil (0.1 to 0.2 μg/kg/min) or with total intravenous anesthesia (TIVA) protocol (propofol 4-12 mg/kg/h and remifentanil 0.1-2 μg/kg/min). The choice of muscle relaxants and the agents used for maintaining anesthesia was in anesthesiologist's discretion. The patients who had clinical deterioration such as requiring continuous monitoring and respiratory or cardiovascular support, or neurologic deficits were transferred to the intensive care unit.

Statistical Analysis

Data analysis was made through SPSS v. 19.0 for Windows (SPSS, Inc., Chicago, IL, USA). statistical package software. The Kolmogorov-Smirnov test was used to determine the distribution of variables. Categorical comparisons were made using Pearson Chi-Square, Fisher Chi-Square, or Yates Chi-Square tests. Intergroup parametric variables were compared with Student's t-test. Non-parametric variables were compared with the U-test. An overall 5% Type-I error level was used to infer statistical significant difference.

Ethical Declaration

This study was planned as a retrospective cross-sectional study. It was approved by the local ethics committee (Approval Number is 20796219/ E-14-219) and conducted in accordance with the ethical principles described by the Declaration of Helsinki.

RESULTS

A total of 323 patients met the study criteria and included to the statistical analyses. **Table 1** shows patient characteristics. Mean age of the patients was 46.88 ± 13.91 years and 54.5% were female. Over half of patients (52.9%) were ASA class II, and 26.9% had systemic comorbidity. The rate of patients with non-functioning tumors was 53.3%. Intraoperative data are presented in **Table 2**.

Table 1. Patient characteristics

		n	%
Age			
	21-30	35	10.8
	31-40	89	27.6
	41-50	83	25.7
	51-60	70	21.7
	61 and over	46	14.2
Gender			
	Male	147	45.5
	Female	176	54.5
ASA			
	ASA I	89	27.6
	ASA II	171	52.9
	ASA III	63	19.5
	ASA IV	-	-
Comorbid disease			
	Cardiovascular	34	10.5
	Respiratory	43	13.3
	Endocrine	68	21.1
	Comorbid diseases related to multiple systems	87	26.9
Pituitary tumor			
	Functioning	151	46.7
	Non-functioning	172	53.3

Table 2. Distribution of patients by surgery time, anesthesia method, and muscle relaxant agent

		n	%
Surgery time			
	0-2 Saat	52	16.1
	2-3 Saat	197	61.0
	3-4 Saat	74	22.9
Anesthesia methods			
	Sevoflurane	127	39.3
	TIVA	196	60.7
Muscle relaxant agents			
	Vecuronium	78	24.1
	Rocuronium	245	75.9

The mean duration of anesthesia and surgery was 114.52 ± 23.03 min and 97.00 ± 23:49 min, respectively. Maintenance anesthesia was established with TIVA in 60.7% of patients and with sevoflurane in 39.3%. Rocuronium was administered as neuromuscular blocking agents in 75.9% of patients and vecuronium in 24.1% of patients. Functioning tumor was more likely to be detected ≥61-year-old age group than that in other age groups (**Table 3**).

Table 3. Distribution of tumortypes by age groups

	Functioning		Non-functioning		P value
Age group	**n**	**%**	**n**	**%**	
21-30 years	22	14.6	13	7.6	<0.001
31-40 years	48	31.8	41	23.8	
41-50 years	51	33.8	32	18.6	
51-60 years	19	12.6	51	29.7	
>60 years	11	7.3	35	20.3	

Statistically significant difference existed in the distribution of functioning and non-functioning tumors among different age groups ($p<0.001$). While 33% of patients with functioning tumors were in 41-51 years' age group, 29.7% of those with non-functioning tumors were in 52-62 years' age group. Comparison of tumor types between sexes showed a statistically significant difference ($p<0.05$). While 58.5% of women had functioning tumor, 67.3% of male patients were detected to have non-functioning tumor.

Intraoperative bradycardia was detected in 137 patients (42.41%), hypotension in 57 patients (17.65%), and hypertension in five patients (1.55%). The distribution of hemodynamic instability by age groups, ASA class, tumor type, and anesthetic method was shown in Table 4. Hemodynamic instability occurred in all of the patients over the age of 51. Bradycardia was detected in 60% and hypotension in 40% of patients who were in 51-60 years' age group. In patients who were in ≥61-years' age group, 63.1% had hypotension and 36.9% had bradycardia. Patients classified as ASA III were more likely to have hemodynamic instability than patients with ASA I and ASA II ($p<0.05$). In patients with functioning tumor, 39.7% had bradycardia, 7.2% had hypotension, and 3.3% had hypertension. On the other hand, 44.7% of patients with non-functioning tumor had bradycardia and 26.7% had hypotension. Patients with functioning and non-functioning tumors significantly differed in terms of hemodynamic instability ($p<0.05$). Bradycardia was observed in 51.1%, hypotension in 22.8%, and hypertension in 3.9% of the patients who were administered sevoflurane. On the other hand, bradycardia and hypotension occurred in respectively 36.7% and 14.2% of the patients who received TIVA. The rates of hemodynamic instability were significantly different between patients who received sevoflurane and TIVA ($p<0.05$), (**Table 4**).

Table 4. Distribution of hemodynamic instability by age groups, ASA class, tumor type, and anesthesia method

	Bradycardia		Hypotension		Hypertension	
	n	%	n	%	n	%
Age						
21-30years (n=35)	8	22.8	0	0	5	14.2
31-40years (n=89)	43	48.3	0	0	0	0
41-50 years (n=83)	27	32.5	0	0	0	0
51-60years (n=70)	42	60.0	28	40.0	0	0
>60 years (n=46)	17	36.9	29	63.1	0	0
ASA						
ASA I (n=89)	25	28.0	-	-	5	5.6
ASA II (n=171)	78	45.6	28	16.3	-	-
ASA III (n=63)	34	53.9	29	46.0	-	-
ASA IV (n=0)	-	-	-	-	-	-
Tumor type						
Functioning (n=151)	60	39.7	11	7.2	5	3.3
Non-functioning (n=172)	77	44.7	46	26.7	--	--
Anesthesia method						
Sevoflurane (n=127)	65	51.1	29	22.8	3.9	100,0
TIVA (n=196)	72	36.7	28	14.2	--	--

Eighteen patients were transferred to intensive care unit in the postoperative period. All these patients were in the age group of 61 years and over. No need for intensive care was determined in patients below the age of 61. Eleven patients (17.4%) in ASA III class and seven patients (7.8%) in ASA II class were transferred to the intensive care unit. Neither tumor type nor anesthetic method altered the need for intensive care. All patients were extubated in the operating room after the completion of surgery. None of the patients who were transported to the intensive care unit were intubated. Sedation was not required for patients in the postoperative period. None of the patients had clinical situations associated with hemodynamic instability such as myocardial infarction, cerebrovascular accident or acute renal failure.

DISCUSSION

The rate of bradycardia, hypotension, and hypertension during ETSS was 42.41%, 17.65%, and 1.55%, respectively among patients in our study. Hemodynamic instability developed more common in elderly than in young patients, and postoperative intensive care was only required in older patients. This study demonstrated the importance of anesthesia management for ETSS in elderly patients. Comorbid diseases and invasiveness of surgical procedure is associated with mortality in patients with advanced age (15). The potency of anesthetic drugs increases in the elderly. Minimum alveolar concentration value of volatile anesthetics decreases with increasing age and elicits hemodynamic effects at higher doses (16). Administration of propofol is more likely to cause hypotension in the elderly than in younger patients (17). Older people are more susceptible to opioids than young people (18). In our study, we thought that the higher intraoperative occurrence of hypotension and bradycardia in the elderly patients resulted from the fact that the elderly are more sensitive to anesthetics. Intraoperative hypotension, even for a short time, is associated with poor perioperative outcomes (19). Therefore, doses of anesthetics should be carefully adjusted in elderly patients. Elderly population was associated with increased risk of postoperative residual neuromuscular blockade due to administration of either vecuronium or rocuronium. Therefore, postoperative muscle weakness, airway obstruction, and hypoxemia are more common in the elderly than young patients (20).

Reich et al. showed that hypotension that occurred after induction of anesthesia was associated with ASA III-IV class, having the age over 50, and administration of higher doses of propofol and fentanyl. The authors reported the incidence of hypotension as 7.7% and 12.6% of patients with ASA I-II class and ASA III-IV class, respectively (21). Consistently, we also detected higher prevalance of hemodynamic instability in patients with ASA III class than that in patients with ASA I-II class.

Hypertension and ischemic heart disease are more common than normal population in patients who have pituitary tumor leading to Cushing's disease secondary to ACTH hypersecretion. In fact, these conditions constitute the major reason for perioperative mortality. Activation of renin-angiotensin-aldosterone system cause increased blood volume and hypertension in 80% of patients with Cushing's disease (22,23). We found a higher incidence of intraoperative hypertension in patients with functioning tumors. Non-functioning tumors, i.e. tumors that do not secrete hormones, often lead to panhypopituitarism due to mass effect. Thyroid hormone and glucocorticoid replacement might be necessary in these patients preoperatively. They also tend to intraoperative hypotension due to increased sensitivity to general anesthetic agents (22-25). In our study, intraoperative hypotension and bradycardia were more common in patients with non-functioning tumors.

Due to the long duration of neurological surgery, complications such as meningitis, extracranial infection and pulmonary embolism may occur. Korinek et al reported that the risk of meningitis was a 70% increase if the duration of neurological surgery was longer than 4 hours (26). In our study, no operation lasted longer than 4 hours.

The major limitation of our study is that retrospective design does not allow us to investigate the mechanisms that mainly trigger hemodynamic instability. In addition, data on prevalence of trigeminocardiac reflex, volume status, and doses of anesthetic agents were not collected. Further prospective studies should be warranted in this manner.

In conclusion, we found a higher prevalence of intraoperative hemodynamic instability in elderly patients who underwent ETSS. While older patients required intensive care postoperatively, no young patient needed intensive care. Also, to have non-functioning tumor and to be in ASA III class increased the risk of intraoperative hemodynamic instability. Specific anesthesia protocols should be developed to reduce the risk of hemodynamic instability in the patients scheduled for ETSS.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Noninvasive assesment in differentiating benign and malign pancreatic lesions with endosonographic elastography score and strain ratio

Benign ve malign pankreas lezyonlarının ayırıcı tanısının endosonografik elastografi skoru ve sertlik oranları ile noninvaziv değerlendirilmesi

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ABSTRACT

Background: We aimed to evaluate the diagnostic capability of endoscopic ultrasound elastography (EUS-EG) score and strain ratio (SR) for differentiating benign pancreatic lesions from the malign lesions

Material and Method: We retrospectively evaluated well collected data of patients who undergone EUS-EG in a single center during the period of January 2016-June 2019. Patients who had pancreatic disorders were further evaluated for the study. The final diagnosis of solid pancreatic lesions (SPL) was made by histopathologic examination. Control group consisted of patients with chronic pancreatitis (CP) who diagnosed according to Rosemont criteria. Elastography was evaluated by a qualitative (elastography scores) and a quantitative method SR.

Results: A total of 66 patients (42 (63.6%)female/24(63.6%)male) with mean age of 58.88±15.32 (19- 80) were included in the study. Thirty-eight patients had SLP, remain 28 patients were CP. In SPL group, 32 (84.2%) had adenocarcinomas and 6 (15.8%) had neuroendocrine tumors. Among 28 patients with benign pancreatic lesions, 23 (82.1%) had CP while five (17.9%) had autoimmune pancreatitis. Median SR values were significantly higher in patients with SPL than those with CP (44.0 (10.0-110.0) vs 7.0 (2.6-14.6), p<0.001). Elasticity scores were also significantly different between patients with SLP and CP (p<0.001). Elasticity scores were significantly different between adenocarcinomas and CP (p<0.001). A 14 cut-off value of SR had 97% sensitive and 100% specificity for SPL and receiver-operating characteristic curves showed an area under the curve of 0.99.6. Likelihood Ratio test revealed that SR appears as the best parameter in discrimination of lesion type either as benign or malignant (X2 = 54.031, p<0.001).

Conclusion: Our study suggested that EUS-elastography and SR scores are highly effective in differentiating malign-benign pancreatitis lesions

Keywords: Chronic pancreatitis, endoscopic ultrasound, elastography, solid pancreatic lesions, strain ratio

ÖZ

Amaç: Endosonografik elastografi skoru (EUS-EG) ve sertlik oranının (strain ratio (SR)) benign ve malign pankreatik lezyonların ayırıcı tanısındaki etkinliğini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Ocak 2016-Haziran 2019 döneminde tek merkezde EUS-EG uygulanan hastaların verileri retrospektif olarak değerlendirildi. Çalışmada kronik pankreatit tanısı kesin olan hastaların endosonografik bulguları ile solid pankreatik lezyonların endosonografik bulguları karşılaştırıldı. Solid pankreas lezyonlarının (SPL) kesin tanısı histopatolojik inceleme ile konuldu. Kontrol grubunda biası önlemek için Rosement A kriterlerini karşılayan kronik pankreatitli (CP) hastalar değerlendirmeye alındı. Rosement B-C değerlendirmeye alınmadı. Elastografi kalitatif (elastografi skorları) ve kantitatif SR yöntemi ile değerlendirildi.

Bulgular: Ortalama yaş 58,8±15,3 (19-80) olan toplam 66 hasta (42 (%63,6) kadın / 24 (%63,6) erkek) çalışmaya dahil edildi. Otuz sekiz hastada SLP; 28 hastada CP vardı. SPL grubunda 32'sinde (%84,2) adenokarsinom, 6'sında (%15,8) nöroendokrin tümör vardı. Benign pankreatik lezyonu olan 28 hastanın 23'ünde (%82,1) CP, 5'inde (%17,9) otoimmün pankreatit vardı. SPL'li hastalarda medyan SR değerleri CP'li hastalardan anlamlı olarak daha yüksekti (44,0 (10,0-110.0) ve 7,0 (2,6-14,6), p<0.001). Sertlik skorları da SLP ve CP'li hastalar arasında anlamlı olarak farklıydı (p<0.001). Elastikiyet skorları adenokarsinom ve CP arasında anlamlı olarak farklıydı (p<0.001). Sertlik skoru için cut-off değeri 14 olarak belirlendi, SPL ve alıcı işletim karakteristik eğrileri için %97 duyarlı ve %100 özgüllüğe sahipti ve 0.99.6 eğrisinin altında bir alan gösterdi. Likelihood Ratio test, benign ve malign lezyonların ayırt edilmesinde en iyi parametrenin SR olduğunu göstermiştir (X^2=54,031, p<0.001).

Sonuç: Çalışmamız, EUS-EG ve SR skorlarının malign ve benign pankreatit lezyonlarını ayırmada oldukça etkin bir yöntem olduğunu göstermiştir.

Anahtar Kelimeler: Endosonografik-elastografi, kronik pankreatit, sertlik oranı, solid pankreas lezyonları

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INTRODUCTION

Endoscopic ultrasound (EUS) is currently thought of as the reference method in detecting to solid pancreatic lesions; this is particularly true for lesions measuring less than 2 cm in size (1). Although EUS has high specificity, its sensitivity is somehow less, ranging between 87% to 100% among studies. Moreover, EUS has a limited ability to differentiate benign lesions from malignant ones. EUS guided fine needle aspiration is used to provide a pathologic diagnosis for these lesions. However, the sensitivity of EUS-FNA also suffers from moderate sensitivity (78-94.3%) (2). Moreover, it is an invasive method with attendant complications and cannot be performed due to difficult locations of the target lesions. In addition, sampling error is always a problem with EUS-FNA. These shortcomings and limitations prompted the efforts to devise a noninvasive, real-time method with relatively high sensitivity and specificity to differentiate benign pancreatic lesions from malignant lesions.

Elastography is a novel technique that is based on the principle that benign and malignant lesions have distinct tissue properties in terms of hardness and stiffness (3). While malignant lesions are more heterogenous and hard, benign lesions tend to be softer and more homogenous. Elastography utilizes this innate feature of the lesions as applying pressure on the target tissue and representing tissue strain response as color-coded areas. However, this method is qualitative and is subject to considerable intra and interobserver variability. To overcome this subjectivity, a quantitative method, namely, strain ratio (SR), was developed. This method, by the help of dedicated software, compares the elasticity of a region of interest to the surrounding healthy tissue. A more objective technique so-called hue histogram further reduces subjectivity with computer-assisted calculation of the strain patterns (4). Nowadays, quantitative and qualitative elastography can be used with EUS. Thus, elastography offers an opportunity of noninvasively and accurately distinguishing benign from malignant pancreatic lesions in addition to its role as a reliable guide to FNA. In a meta-analysis that involved studies evaluating EUS-elastography in differentiating benign and malignant pancreatic lesions, the authors found the sensitivity and specificity of EUS elastography as 95% and 69%, respectively (2). It appeared that owing to its high sensitivity, EUS-elastography would be used to exclude malignancy and thus avoid unnecessary biopsies and consequent complications. On the other hand, the specificity of the EUS elastography was much lower than that of EUS-FNA.

Several studies to date assessed the diagnostic accuracy of EUS elastography techniques in differentiating benign pancreatic lesions from malignant lesions (1,5-7). However, to the best of our knowledge, the optimal cut-off values to distinguish malign and benign pancreas lesions have not been determined yet. Thus, we aimed to evaluate the diagnostic capability of EUS elastography along with strain ratio for differentiating benign pancreatic lesions from the malignant lesions and determined the optimal cut-off value to distinguish malign and benign pancreatic lesions.

MATERIAL AND METHOD

Patients and Design

This was a prospective chart review study in which patients who underwent endosonographic elastography (EUS-EG) at Kırıkkale University Faculty of Medicine Hospital between January 2016 and June 2019 were performed. The primary objective of the study was the assessment of the ability of EUS-EG in differentiating malign and benign pancreatic lesions. Of all included patients (n=636), 186 patients had CP, cystic and solid pancreatic lesions. The inclusion criteria were as follows: (1) having a solid pancreatic lesion that is diagnosed histopathologically based on the biopsy material obtained by endosonographic fine needle aspiration or surgery, (2) having chronic pancreatitis and fulfilling Rosemont criteria of "consistent with chronic pancreatitis" assessed by EUS. Exclusion criteria involved (1) having cystic pancreatic lesions and fulfilling Rosemont diagnostic criteria for "suggestive for CP" and "indeterminate for CP". Flow-chart of patient selection is depicted in **Figure 1**.

Ethical Declaration

Kırıkkale University Faculty of Medicine Ethics Committee approved the study protocol (Date: 07.08.2019, Ethics No: 2019.08.03).

Final Diagnoses of Chronic Pancreatitis and Malignant Pancreatic Masses

The final diagnosis of solid pancreatic lesions was determined via pathologic evaluation of the biopsy specimens that were obtained through EUS-FNA or surgery. Pancreatic lesion biopsies were examined by the same pathologist. Clinical history and medical records of the patients, along with pancreatic imaging findings (computed tomography,

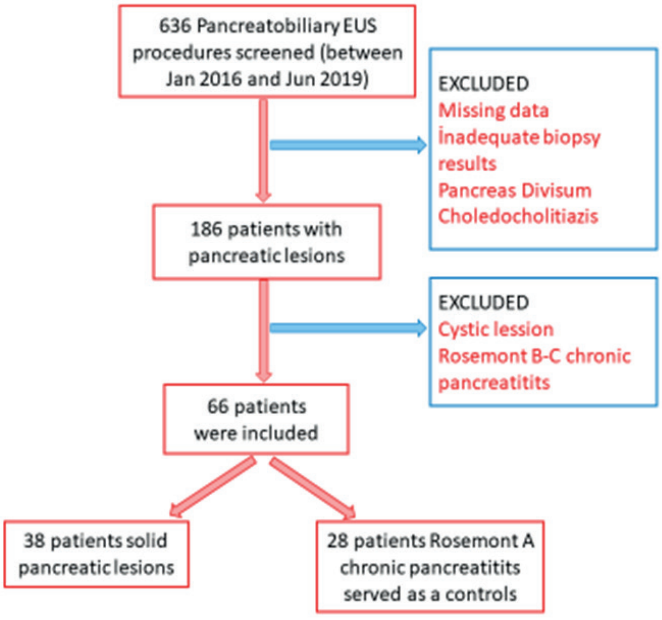

Figure 1. Flow Charts

endosonography and ultrasound) were used to diagnose chronic pancreatitis. Among the CP patients who underwent EUS, each patient who met the Rosemont diagnostic criteria for "consistent with chronic pancreatitis" were included in the study as the control group. Patients meeting the "suggestive for CP" or "indeterminate for CP" was not included to ascertain that we only recruited the patients with definite chronic pancreatitis. Rosemont chronic pancreatitis criteria are shown in **Table1** (8).

Table 1. Rosemont criteria for chronic pancreatitis

Major criteria A	1) Hyperechoic foci with shadowing (echogenic structure ≥2 mm length and width that shadow) 2) Lobularity with honeycombing (well circumscribed, ≥5 mm structures with enchaining rim and relatively echo pure center, contiguous ≥3 lobules) 3) Main pancreatic duct calculi (echogenic structure(s) within main pancreatic duct with shadowing)
Major criterion B	Honeycomb pattern of lobularity
Minor criteria	1) Lobularity without honeycombing (noncontiguous lobules) 2) Hyperechoic foci without shadowing 3) Cysts 4) Strands 5) Irregular pancreatic duct contour 6) Dilated side branches 7) Main pancreatic duct dilatation 8) Hyperechoic duct wall

Rosemont criteria denoting ''consistent with CP'' is defined as (1) 1 major A criteria and ≥3 minor criteria, (2) 1 major A and major B criteria, or (3) 2 major A criteria.

Endoscopic Ultrasound and Evaluation of Elasticity

Endoscopic Ultrasound

All EUS procedures were performed by the same endoscopist who had sufficient EUS-EG experience. EUS examination was performed by means of Pentax EG3830UT linear echo-endoscope (HOYA Corporation, PENTAX Lifecare Division, Showanomori Technology Center, Tokyo, Japan) connected to a Hitachi EUB-7000 HV ultrasound unit (Hitachi Medical Systems, Tokyo, Japan), which contain an elastography module. After identification of a solid pancreatic lesion and/or Rosemont diagnostic criteria for "consistent with chronic pancreatitis", EUS-EG was performed. The lesion was visualized in B-mode ultrasound, and then elastography mode was utilized with color-coded duplex features. B mode image at 7.5 MHz and an overlay mode image with elastography color scale are demonstrated simultaneously at the console.

Elastography Score and Strain Ratio

We evaluated elastic features of the pancreatic lesions by means of a qualitative and a quantitative scoring system. In the qualitative method, we adopted the "elasticity score" reported by Giovanni and colleague(9). In this scoring system, Elastography score 1 (ES_1) represented normal tissue and used when homogeneous green area was seen, ES_2 denoted inflammation or fibrosis and used when a heterogeneous green area was predominant, ES_3 denoted indeterminate for malignancy and used when a heterogeneous blue dominant area was seen, ES_4 represented malignant lesion and used when a homogeneous blue area was seen, and ES_5 represented necrosis in an advanced malignant lesions and used when a mainly dark (blue) tissue with areas of heterogeneous soft tissue (green, red) was seen.

The strain ratio method was used to evaluate the elasticity of the tissues quantitatively. Perception depth and the entire targeted area were set according to the lesion location for strain ratio value. Since elastography strain values are demonstrated corresponding to the adjacent tissue, which operates as an inner reference norm, we accepted a ratio of pattern to neighboring tissue of 1:1 in this study. The strain ratio was measured when a steady image of at least 5 seconds course was attained for quantitative measurement and final pattern description. Two distinct areas to the mass lesion and/or chronic pancreatic tissue (B) and normal adjacent tissue (A) were selected for quantitative elastographic measurement. To prevent selection bias of areas A and B, each measurement of elasticity was repeated three times in all patients. The mean value of three measurements was accepted as the final strain ratio value. We used the receiver-operating characteristic (ROC) curve to evaluate the best possible cut-off value of strain ratio to differentiate chronic pancreatitis lesions from neoplastic lesions.

Statistical Analysis

Chi-square test, Mann Whitney U test, and Kruskal Wallis test were used to making comparisons of non-parametric variables between the groups. In the comparisons of the paired groups, the Chi-square test and Mann Whitney U test were used. To evaluate differences between the groups involving parametric data, the Independent Samples t-test or One-Way Analysis of Variance (ANOVA) test were used, and in the post-hoc comparisons, the Tukey Multiple Comparisons test was used. ROC-Curve test was used to determine the sensitivity and specificity of the study parameters, which could predict the diagnosis. Likelihood-Ratio test was used to the variables for the prediction of the "best" diagnostic variable. A p-value <0.05 was deemed significant.

RESULTS

Patient Characteristics

A total of 66 patients, of whom 28 (42.4%) had chronic pancreatitis, and 38 (57.3%) had a SPLs, were included in the study. The majority of patients were male (63.6%). There were significantly more female patients in the SPLs group. The mean age of the patients with a SPL was significantly higher than that of patients with a CP (64.7±11.1 vs. 46.1±13.7, $p<0.001$). Of all malignant SPLs, 32 (48.5%) were adenocarcinomas, and 6 (9.1%) were neuroendocrine

Table 2. Mean age, gender distribution, pancreatic mass localization, elasticity scores and strain rations in patients with malignant mass and chronic pancreatitis

Variable		Total	Chronic pancreatitis	Malignant mass	p
Age (year)		58.8±15.3	46.1±13.7	64.7±11.1	<0.001*
Gender	Male	42 (63.6%)	22 (33.3%)	20 (30.3%)	0.030***
	Female	24 (36.4%)	6 (9.1%)	18 (27.3%)	
Localization	Head	20 (30.3%)	1 (1.5%)	19 (28.8%)	<0.001***
	Body	9 (13.6%)	1 (1.5%)	8 (12.1%)	
	Tail	4 (6.1%)	0 (0.0%)	4 (6.1%)	
	Uncinat	7 (10.6%)	0 (0.0%)	7 (10.6%)	
	Diffuse	26 (39.4%)	26 (39.4%)	0 (0.0%)	
Diagnotic methods	EUS-FNA	39 (59.1%)	5 (7.6%)	34 (51.5%)	<0.001***
	Surgery	4 (6.1%)	0 (0.0%)	4 (6.1%)	
	EUS+Imaging+Laboratory	23 (34.8%)	23 (34.8%)	0 (0.0%)	
Final Diagnosis	Adenocarcinoma	32 (48.5%)	0 (0.0%)	32 (48.5%)	<0.001***
	Neuroendocrine tumors	6 (9.1%)	0 (0.0%)	6 (9.1%)	
	Chronic pancreatitis	28 (42.4%)	28 (42.4%)	0 (0.0%)	
Elasticity Score	ES2	10 (15.2%)	9 (13.6%)	1 (1.5%)	<0.001***
	ES3	35 (53.0%)	18 (27.3%)	17 (25.8%)	
	ES4	21 (31.8%)	1 (1.5%)	20 (30.3%)	
Strain Ratio		21.8 (2.6-110.0)	7.0 (2.6-14.6)	44.0 (10.0-110.0)	<0.001**

(*) Independent Samples t test (**) Mann Whitney U test (***) Chi-square test

tumors. Among 28 patients with CPs, 23 (34.8%) patients had been following with chronic pancreatitis. Five patients (7.6%) who presented with pancreatic mass were diagnosed with autoimmune pancreatitis via EUS-FNA. The underlying causes of chronic pancreatitis were biliary pancreatitis in 8 patients, and alcoholic pancreatitis in 15 patients. In the malignant mass group, the most common location of the pancreatic mass was the head of the pancreas (50%). Mean age, gender distribution and characteristics of the pancreatic mass are depicted in **Table 2**.

Elastography Score and Strain Ratio

Median strain ratio values were significantly higher in malignant pancreatic mass compared with chronic pancreatitis (benign pancreatic mass) (44.0 (10.0-110.0) vs 7.0 (2.6-14.6), p<0.001) (**Table 2 and Figure 2**).

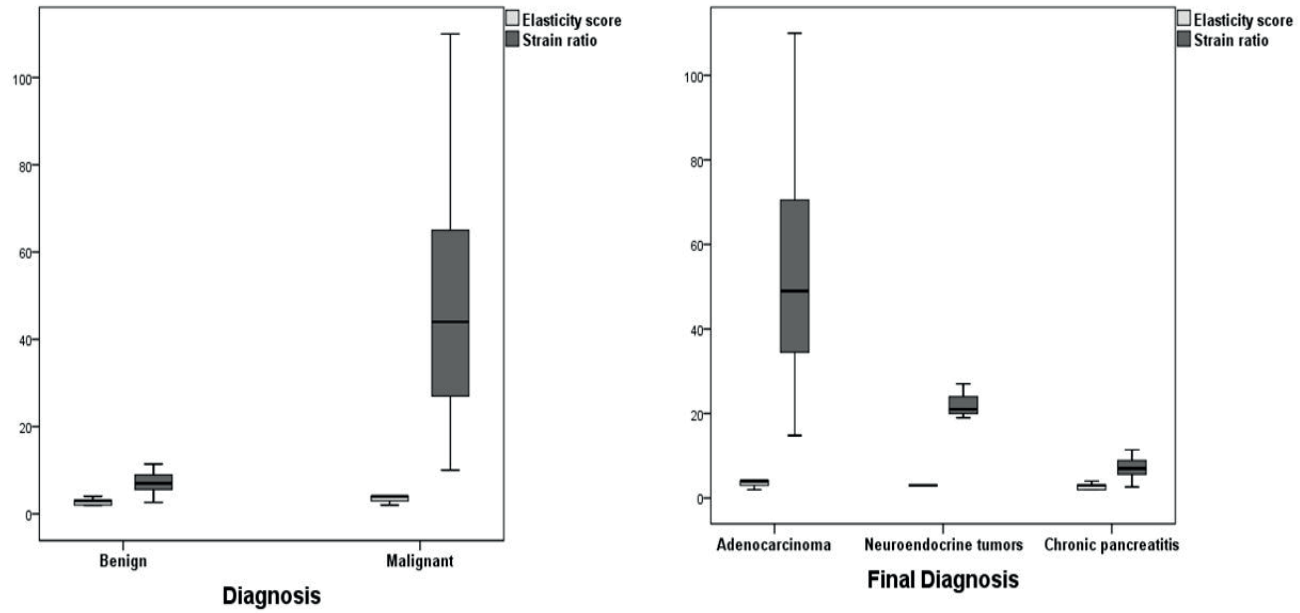

Figure 2. Comparison of strain ratio and elastography between chronic pancreatitis and solid pancreatic lesions. Comparison of strain ratio and elastography between chronic pancreatitis, adenocarcinomas and neuroendocrine tumors

Table 3. Mean age, gender distribution, elasticity scores and strain ratio in patients with adenocarcinoma, neuroendocrine tumor and chronic pancreatitis

Variable		Adenocarcinoma	Neuroendocrine tumors	Chronic pancreatitis	p
Age (year)		65.47±10.77 a	61.17±13.15 a,b	46.14±13.74 b	<0.001*
Gender	Male	18 (27.3%) a	2 (3.0%) a	22 (33.3%) a	0.054***
	Female	14 (21.2%)	4 (6.1%)	6 (9.1%)	
Localization	Head	16 (24.2%) a	3 (4.5%) a	1 (1.5%) b	<0.001***
	Body	5 (7.6%)	3 (4.5%)	1 (1.5%)	
	Tail	4 (6.1%)	0 (0.0%)	0 (0.0%)	
	Uncinat	7 (10.6%)	0 (0.0%)	0 (0.0%)	
	Diffuse	0 (0.0%)	0 (0.0%)	26 (39.4%)	
Diagnostic methods	EUS-FNA	28 (42.4%) a	6 (9.1%) a	5 (7.6%) b	<0.001***
	Surgery	4 (6.1%)	0 (0.0%)	0 (0.0%)	
	EUS+Imaging+Laboratory	0 (0.0%)	0 (0.0%)	23 (34.8%)	
Elasticity_Score	ES2	1 (1.5%) a	0 (0.0%) a,b	9 (13.6%) b	<0.001***
	ES3	12 (18.2%)	5 (7.6%)	18 (27.3%)	
	ES4	19 (28.8%)	1 (1.5%)	1 (1.5%)	
Strain Ratio		49.00(14.79-110.00) a	21.00 (10.00-89.00) b	7.00 (2.60-14.60) c	<0.001**

(*) One-Way Analysis of Variance (ANOVA) test (**) Kruskal Wallis test (***) Chi-square test

Elasticity scores were also significantly different between benign and malignant lesions (p<0.001). Out of 38 SPLs, only one is classified as ES2 (inflammation or fibrosis), and out of 28 chronic pancreatitis lesions, only one labeled as ES4 (malignant lesion). On the other hand, a considerable percentage of patients in each group was diagnosed as ES3 (indeterminate for malignancy) in both groups based on qualitative elastography (**Table 2**).

When malignant lesions are further characterized, median strain ratio values were 49.00(14.79-110.00) for adenocarcinomas and 21.00 (10.00-89.00) for neuroendocrine tumors (**Table 3**). Both values were significantly different from each other and from the chronic pancreatitis strain ratio values (p<0.001). Elasticity scores were significantly different between adenocarcinomas and chronic pancreatitis (p<0.001). However, there was no significant difference between neuroendocrine tumors and chronic pancreatitis. It should be emphasized once more that many cases were classified as ES3 bot in chronic pancreatitis and adenocarcinoma groups (**Table 3**).

The ROC-Curve test demonstrated that if the cut-off value for SR level were >14, it would be 97% sensitive and 100% specific in distinguishing the CP from the malignant tumor (area=0.996, p<0.001). Likelihood Ratio test revealed that strain ratio appears as the best parameter in discrimination of tumor type either as benign or malignant (X^2=54.031, p<0.001) (**Table 4 and Figure 3**).

Table 4. Receiver operating characteristic (ROC) analysis and likelihood test comparing differentiation of strain ratio and eastography scores in various diagnostic pairs.

		ROC-Curve				Likelihood Ratio	
Groups (I/J)	Variable	Area	p	Cut-off value		X^2	p
Bening/ Malignant	Elasticity Score	0.809	<0.001	>3.5	Sensitivity 53% Specificity 97%	0.400	0.527
	Strain Ratio	0.996	<0.001	>14	Sensitivity 97% Specificity 100%	54.031	<0.001
Adenocarcinoma/ Neuroendocrine tumor	Elasticity Score	0.701	0.123	-	-	0.628	0.428
	Strain Ratio	0.826	0.012	>29.00	Sensitivity 84% Specificity 83%	2.675	0.102
Adenocarcinoma/ Chronic pancreatitis	Elasticity Score	0.829	<0.001	>3.50	Sensitivity 60% Specificity 97%	0.000	0.985
	Strain Ratio	1.000	<0.001	>14.69	Sensitivity 100% Specificity 100%	55.683	<0.001
Neuroendocrine tumor/ Chronic pancreatitis	Elasticity Score	0.699	0.130		-	0.000	0.998
	Strain Ratio	0.976	<0.001	>16.80	Sensitivity 83% Specificity 100%	19.163	<0.001

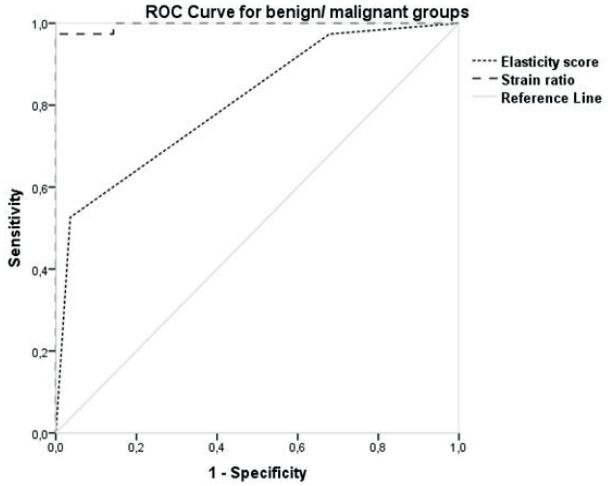

Figure 3. Receiver operating characteristic curve analysis of the strain ratio for the detection of benign pancreatic lesions and malign pancreatic lesions

On the other hand, receiver operating characteristics (ROC) Curve analysis demonstrated that when the ES cut-off value was taken >3.5, it was 60% sensitive and 97% specific in distinguishing the adenocarcinoma from chronic pancreatitis (area = 0.829, p <0.001). Furthermore, if SR level was >14.69, it could be 100% sensitive and 100% specific in distinguishing the adenocarcinoma from chronic pancreatitis (area=1.000, p<0.001, cut-off value = 14.69). Likelihood Ratio test revealed that SR value was determined to be the best parameter in making the decision for discrimination of adenocarcinoma from chronic pancreatitis (X^2=55.683, p<0.001) (**Table 2 and Figure 4**).

DISCUSSION

The salient finding of the present study was that strain ration, which is calculated using EUS-elastography, was a sensitive and specific method to differentiate malign pancreatic lesions from the benign ones with considerably high sensitivity and specificity. On the other hand, elasticity scores were not as robust as strain ratio in making this distinction. Many masses, both benign and malignant, were labeled as indeterminate with this method.

Distinguishing mass-forming chronic pancreatitis from pancreatic malignancy might be challenging owing to common features both have in imaging characteristics and clinical presentation. In many instances, only a combination of a number of imaging modalities can provide the differential diagnosis (10). Because of this difficulty, several studies tried to differentiate chronic pancreatitis related masses from malignant pancreatic masses by means of EUS elastography modalities. However, there was still enough data with this regard in a Turkish population.

Several studies have demonstrated that EUS has a higher sensitivity for the detection of small pancreatic masses compared with other imaging modalities such as computed tomography, magnetic resonance imaging, and positron emission tomography (11,12). EUS, along with EUS-guided FNA, is currently accepted as the gold standard method for the diagnosis of pancreatic masses. On the other hand, it might be problematic to differentiate malignant masses from the inflammatory masses seen in chronic pancreatitis by means of EUS alone (13). EUS-guided FNA of pancreatic masses has a 97% accuracy rate in the detection of malignant lesions (14). However, since sampling error is always an issue, it cannot be used to exclude malignancy when FNA results show benign changes. Moreover, as it is more invasive, although considered relatively safe by many authors, FNA is associated with some complications such as pancreatitis (15). Thus, it is an actual clinical need to be able to decide whether a pancreatic lesion is benign or malignant with sufficient accuracy without the need for FNA. Elastography is being considered by many to offer such an opportunity in the evaluation of pancreatic masses.

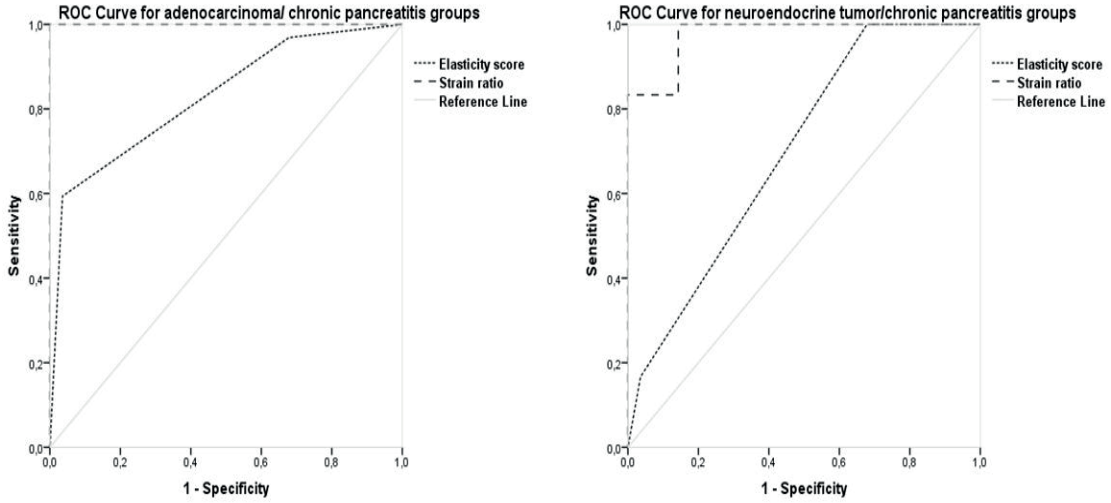

Figure 4. Receiver operating characteristic curve analysis of the strain ratio for the detection of (a)adenocarcinoma and chronic pancreatitis, (b) neuroendocrine tumors and chronic pancreatitis

The diagnostic value of EUS FNA may be increased by performing targeted biopsy with elastography. Thus, false negative results can be avoided.

Several meta-analyses have evaluated the value of different elastography techniques in differentiating benign from malignant pancreatic masses (16-18). The latest of these reported by Zhang and colleagues analyzed data of 1687 patients. The results showed that both qualitative and quantitative modalities of EUS elastography had high accuracy rates in the diagnosis of malignant pancreatic masses. The pooled analysis revealed that the sensitivity for the diagnosis of malignant pancreatic masses were 0.98 for qualitative EUS elastography, and 0.95 for quantitative EUS elastography, respectively. On the other hand, the specificity of both methods was around 60% (17). Thus, as in the previous meta-analyses, the authors concluded that EUS elastography cannot replace EUS-FNA but might be used in addition to it to avoid unnecessary biopsies in benign lesions owing to its considerably high sensitivity to detect malignant pancreatic masses.

The original method first introduced with elastography was based on the evaluation of color-codes reflecting different strain levels in the region of interest. While blue predominant areas represent harder tissues, hence with a more probability for malignancy, the green predominant areas mean that the imaged tissue is softer and more likely to be benign. Since this method is more subjective and operator dependent, new elastography modalities such as hue histogram and elasticity score were devised to render the methods more objective and reproducible. Although it seems counterintuitive, the sensitivity rates have been found to be similar for qualitative and quantitative elastography methods (16,17). In our study, the strain ratio method was more sensitive and specific compared with elastography scores. When a strain ratio value of 14 was taken as cutoff, the sensitivity and the specificity of the method was 97% and 100% respectively in differentiating benign lesion from the malignant ones. SR was less efficient in the differentiation of neuroendocrine tumors from adenocarcinoma. With a cutoff point of 29, the sensitivity and the specificity were 84% and 83%, respectively.

In a recent study, Kim et al. (7) evaluated the capability of the EUS elastographic strain ratio in differentiating malign pancreatic masses from focal pancreatic masses related to chronic pancreatitis. The authors found that the median SR for pancreatic cancer was 18 (13.1-26.6) whereas, the value was 15.1 (9.5-18.7) for mass-forming chronic pancreatitis. With an optimal cutoff value of 6.0 for strain ratio, the sensitivity and specificity for the diagnosis of pancreatic cancer were 97.8% and 86.7%, respectively. An older meta-analysis specifically included the studies that evaluated the accuracy of EUS elastography for distinguishing pancreatic adenocarcinoma from chronic pancreatitis associated inflammatory masses (18). The authors revealed that the pooled sensitivity and specificity were 0.99 (0.97-1.00), 0.76 (0.67-0.83), respectively, with qualitative EUS elastography. Hue histogram method had a sensitivity of 0.92 (0.89-0.95), and specificity 0.68 (0.57-0.78) to distinguish inflammatory lesions from malignant masses in the pancreas.

Some limitations of the current study deserve mention. First, our study was retrospective in nature. Second, our sample size was relatively small to provide a clear-cut cutoff value to measure the sensitivity and specificity of the differential ability of the compared diagnostic methods. Third, patients with diagnosis of CP who haven't inflammatory masses did not undergo FNA. These group was diagnosed with appropriate history, medical records, and a combination of imaging modalities.

In conclusion, our study showed that EUS elastography measurements when performed in experienced centers have strong diagnostic value with high sensitivity and specificity in differentiating benign and malignant pancreas lesions. Strain scores appeared to have a high accuracy rate with this regard. Larger studies to give more clear-cut cutoff values for differentiation based on elastography score are needed in the pancreas lesions.

DECLARATION OF INTEREST STATEMENT

All authors meet the ICMJE authorship criteria. The manuscript has not been published, accepted or under simultaneous review for publication elsewhere. The all authors declare that there is no conflict of interest.

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Research Article / Araştırma Makalesi

The effects of dialysis modalities on sexual hormone levels in male patients

Erkek hastalarda diyaliz modalitelerinin cinsel hormon düzeyleri üzerine etkisi

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ABSTRACT

Introduction: Low testosterone level is association with low quality of life and cardiovascular risk factors. The dialysis modality effects on testosterone levels remain unclear. To investigate the haemodialysis (HD) and peritoneal dialysis (PD) effects on male sexual hormones.

Material and Method: Serum total testosterone (TT), free testosterone (FT), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and sex hormone-binding globulin (SHBG) were investigated. Serum TT below 3 ng/ml was considered a low TT. Sociodemographic data and an Index of Independence in Activities of Daily Living were recorded.

Results: This study included adult male HD (n=71) and PD (n=24) patients. Age and dialysis duration were similar between groups. Serum TT and FT levels were significantly higher in the PD group (p=0.01 and p=0.05, respectively). There were no differences between the HD and PD groups with regard to SHBG, FSH, LH or PRL levels (p=0.353, p=0.858, p=0.410 and p=0.410, respectively). The number of patients who were capable of performing Index of Independence in Activities of Daily Living was higher in the PD group (p=0.033) and with normal TT levels (p=0.027). Binary regression analysis showed more favourable effects in the PD group on testosterone levels (OR=4.659; 1.477–14.704 95% CI Exp B).

Conclusion: PD has favourable effects on testosterone levels compared to HD. Mental and physical well-being resulting from PD and its technique affect TT levels.

Keywords: Chronic renal failure, haemodialysis, peritoneal dialysis, testosterone, index of independence in daily living activities

ÖZ

Amaç: Düşük testosteron düzeyi, kardiyovasküler risk faktörleri ve düşük yaşam kalitesi ile ilişkilidir. Diyalizin testosteron düzeyi üzerine etkisi halen net değildir. Amaç hemodiyaliz (HD) ve periton diyalizi (PD) tekniklerinin erkek cinsel hormonları üzerine etkisinin araştırmaktır.

Gereç ve Yöntem: Serum total testosteron (TT), serbest testosteron (ST), follikül stimulan hormon (FSH), luteinizan hormon (LH), prolaktin (PRL) and seks hormone bağlayıcı globulin (SHBG) incelendi. Serum TT, 3 ng/ml'nin altında ise düşük TT düzeyi kabul edildi. Sosyodemografik veriler ve günlük yaşam aktivitesinde bağımsız olabilme indeksi kaydedildi.

Bulgular: Çalışmaya erişkin erkek HD (n=71) ve PD (n=24) hastaları dahil edildi. Her iki grubun yaş ve diyaliz süreleri benzerdi. Serum TT ve ST düzeyleri anlamlı düzeyde PD grubunda yüksek idi (p=0.01 and p=0.05, respectively). PD ve HD grupları arasında SHBG, FSH, LH veya PRL düzeyleri açısından farklılık yoktu (p=0.353, p=0.858, p=0.410 and p=0.410, sırasıyla). Günlük yaşam aktivitesinde bağımsız olabilme indeksi aktivitelerini gerçekleştiren hasta sayısı PD grubu (p=0.033) ve normal TT düzeyli grupta (p=0.027) daha fazlaydı. Binary regresyon analizinde, PD grubunda testosteron düzeyine etkinin daha olumlu olduğu gösterilmiştir (OR=4.659; 1.477–14.704 95% CI Exp B).

Sonuç: Periton diyalizinin HD ile karşılaştırıldığında testosteron düzeyleri üzerine olumlu etkisi vardır. PD tekniğinin kendisi ve sağladığı fiziksel ve zihinsel iyilik hali testosteron düzeylerini etkilemektedir.

Anahtar Kelimeler: Kronik böbrek yetmezliği, hemodiyaliz, periton diyalizi, testosteron, günlük yaşam aktivitesinde bağımsız olabilme indeksi

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INTRODUCTION

Chronic kidney disease (CKD) embodies several factors that affect an individual's quality of life, such as anaemia, bone mineral disorder, malnutrition, inflammation and hormonal imbalances. The incidence of these factors and their effects on quality of life may vary. An androgen deficiency resulting from disturbances in the hypothalamic-pituitary-gonadal axis together with the influence of a uremic environment is one of the major problems in dialysis patients (1). In addition, Handelsman reported that a uremic environment may cause primary hypogonadism with decreases in both testicular blood flow and testosterone production (2). Changes in hormone production and metabolism lead to a loss of libido, infertility, physical and mental problems as well as in increase in cardiovascular risk factors. Serum testosterone levels reaches normal range after the kidney transplantation. A recent study has reported a normalization of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels during the early period following a renal transplantation, while a slower normalization was noted in Sertoli cell functions compared to that in Leydig cells (3). Another recent study demonstrated that a low testosterone level at time of transplantation is a risk factor for graft loss and mortality (4). All CKD patients who have not had the chance of pre-emptive transplantation must receive dialysis treatment. In our study, we aimed to investigate the differences between haemodialysis (HD) and peritoneal dialysis (PD) techniques in terms of hypogonadism.

MATERIAL AND METHOD

Ethical Declaration and Patients

This study was conducted with adult male patients, 71 of whom were receiving HD four hours a day, three days a week at the HD centre of our hospital, and 24 patients were under follow-up care at our PD unit. All participants gave written informed consent, and the study protocol was reviewed and approved by the **local ethics committee** (approval date and number: 15.05.2017 and 38/11). This study was carried out in compliance with the principles of the Declaration of Helsinki. With regard to the patients, those with a serum total testosterone (TT) level <3.0 ng/ml were considered to have low testosterone levels or hypogonadism (normal range for TT: 3–10.5 ng/ml). Sex hormone-binding globulin (SHBG), TT, free testosterone (FT), FSH, LH and prolactin (PRL) serum levels were tested in serum samples in line with the Society of Endocrinology and Metabolism guidelines (5). Blood samples were collected in the morning after fasting for 10–12 hours. The samples were centrifuged for 10 minutes at 3000 revolutions per minute. Serum samples were stored at -20 °C. FSH (mlU/ml), LH (mlU/ml), PRL (ng/ml) and TT (ng/ml) assays were run on a Unicel Dxl 800 (Beckman Coulter Inc., California, USA) device by means of a chemiluminescent immunoassay, while FT (pg/ml) and SHBG (nmol/L) were measured on a LB 2111 Gamma Counter (Berthold Technologies GmbH and Co., Bad Wildbad, Germany) via a radioimmunoassay. Other biochemical tests (urea, creatinine, sodium, potassium, calcium, phosphorus, albumin, total protein, uric acid, low density lipoprotein (LDL], high density lipoprotein (HDL], ferritin, intact parathormone, total cholesterol, triglyceride, C-reactive protein (CRP), glycosylated haemoglobin (HbA1c) and haemoglobin were performed during routine controls and the data were retrieved from the patients' files. Employment status, marital status, education level and comorbidities were retrieved from files or recorded via face-to-face interviews.

Katz's Index of Independence in Activities of Daily Living (Index of ADL) was used to determine activity scores (6). This is a survey where feeding, transfer, continence, toileting, bathing and dressing are assessed on a scale from 0 to 6 and each domain is scored as 1 or 0.

Statistical Analysis

The Statistical Package for Social Sciences for Windows version 25.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. Descriptive analysis results are expressed as mean ± standard deviation, while variables with non-normal distributions are expressed as a median and interquartile range. To determine whether all variables were distributed normally, the Kolmogorov-Smirnov test was used. The *t*-test was used for variables with continuous normal distributions and the Mann-Whitney test was used for variables with non-normal distributions. Pearson's and Spearman's tests were employed to assess correlations. Statistical evaluations of categorical variables were performed with chi-square tests, while Pearson's or Fisher's exact tests were used for the assessment of the findings. In the present study, Index of Independence in Activities of Daily Living with a survey result of six full points were regarded as active or "1", and others (< 6 for a total score) were recorded as not active or "0". In this way, the index was adapted as a categorical variable for statistical evaluation. Binary logistic regression tests were used to determine the factors with an independent influence on testosterone levels. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The present study included 71 male patients undergoing HD and 24 male patients undergoing PD. While there were no differences between the HD and PD groups in terms of SHBG, LH or PRL (p=0.353, p=0.858, p=0.410 and p=0.410, respectively), TT and FT levels were significantly higher in the PD group (p=0.01 and p=0.05, respectively). Serum total cholesterol and LDL levels were also significantly higher in the PD group (p=0.031 and p=0.016, respectively). Serum intact parathormone levels, body mass index (BMI) and haemoglobin values, which have been associated with testosterone levels, were not significantly different between the HD and PD groups (p=0.543, p=0.577 and p=0.429, respectively). Education level, employment status and the Index of ADL scores were significantly different in the patients receiving PD (p=0.004, p=0.024 and p=0.033, respectively). Among the concomitant diseases (diabetes mellitus [DM], coronary artery disease, hypertension and cancer), only DM

Table 1. Demographics and clinical data of all participants

Variables		Haemodialysis paients	Peritoneal dialysis patients	P
Count (n)		71	24	
Age (years)		53.59±15.17	47.67±17.12	0.113
Smoke (n)			0.774	
	Smoking (n. %)	24 (% 36.4)	8 (% 34.8)	
	No smoking (n,%)	18 (% 27.3)	8 (% 34.3)	
	Smoking cessation (n,%)	24 (% 36.4)	7(% 30.4)	
Education			0.004	
	University (n,%)	0 (% 0)	4 (% 17.4)	
	High school (n,%)	6 (% 9.0)	3 (% 13.0)	
	Middle school (n,%)	17 (% 25.4)	2 (% 8.7)	
	Elementary school (n,%)	35 (% 52.2)	13 (% 56.5)	
	Illiterate (n)	9 (% 13.3)	1 (% 4.3)	
Marital status			0.481	
	Married (n,%)	49 (% 73.1)	17 (% 73.9)	
	Single (n,%)	10 (% 14.9)	5 (% 21.7)	
	Divorced /widow (n,%)	8 (% 11.9)	1 (% 4.3)	
Employment status			0.024	
	Employee (n,%)	9 (% 13.4)	6 (% 26.1)	
	Unemployed (n,%)	36 (% 53.7)	8 (% 34.8)	
	Retired (n,%)	22 (% 32.8)	9 (% 39.1)	
Active (n,%)		49 (% 70)	22 (% 91.7)	0.033
Diabetes (n,%)		28 (% 40.6)	3 (% 12.3)	0.012
Cancer (n,%)		5 (% 6.8)	1 (% 4.2)	0.537
Hypertension (n,%)		45 (% 65.2))	16 (% 66.7)	0.898
Coronary artery disease (n,%)		22 (% 31.9)	6 (% 25)	0.527
BMI (kg/m2)		23.99±4.24	24.57±4.74	0.577
Dialysis duration (months)		52 (33-147)	48 (5-156)	0.156
Haemoglobin (g/dl)		10.91±1.80	11.23±1.44	0.429
HbA1C (%)		7.20 (5.30-8.40)	5.40 (4.90-6.20)	0.002
CRP (mg/L)		17.51±23.42	8.36±9.06	0.021
Albumin (g/L)		3.76±0.49	3.76±0.43	0.994
Uric acid mg/dl)		6.56±1.35	6.78±0.97	0.404
Calcium (mg/dl)		9.02 (6.30-9.20)	9.17 (8.08-10.69)	0.143
Phosphorus (mg/dl)		5.38±1.31	5.01±1.15	0.224
HDL (mg/dl)		38.36±9.24	39.75±9.95	0.536
LDL (mg/dl)		105.06±35.07	125.83±38.011	0.016
Total cholesterol (mg/dl)		156.59±42.99	179.42±46.49	0.031
Triglyceride (mg/dl)		179.32±108.13	196.67±90.75	0.483
Ferritin (mcg/L)		496.22±372.17	251.74±184.27	0.003
iPTH (pg/ml)		366.30 (8.60-2536)	368.40 (160.00-1019.00)	0.543
25(OH) vitamin D (ng/ml)		15.08 (5.47-74.39)	10.56 (4.29-37.16)	0.160
Total testosterone (ng/ml)		2.36 (0.41-6.22)	3.67 (0.28-20.50)	0.010
Free testosterone (pg/ml)		19.44±16.63	28.59±22.60	0.050
SHBG (nmol/L)		21.66±14.51	25.45±19.21	0.353
FSH (mlU/ml)		7.08 (1.26-70.31)	7.63 (1.70-26.03)	0.858
LH (mlU/ml)		7.68 (3.06-85.77)	9.18 (2.63-25.28)	0.410
PRL (ng/ml)		13.70 (3.88-467.00)	16.77 (6.11-73.62)	0.410

BMI: Body mass index, CRP: C-reactive protein, iPTH: Intact parathormone, SHBG: Sex hormone-binding globulin, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, PRL: Prolactin, Activity: Active=1 (Index of independence in activities of daily living index score=6), No active=0 (Index of independence in activities of daily living index score <6), $p<0.05$

had a significantly higher incidence in the HD group compared to the PD group (40.6% versus 12.3%, respectively, p=0.012). Age and dialysis vintage were similar between patients receiving HD and PD (p >0.05 for both). Serum CRP and HbA1c values were significantly higher in the HD group than in the PD group (p=0.021 and p=0.002, respectively). Serum albumin levels were not different between the groups (p=0.994). Demographics and clinical data of all participants are presented in **Table 1**.

Table 2. Demographics and laboratory data of dialysis patients with serum testosterone deficiency (TT<3 ng/ml)

Variables	Hemodialysis paients	Peritoneal dialysis patients	P
Age (years)	55.55±15.51	38.51±11.41	0.016
Count (n)	31 (% 43.6)	6 (% 25)	
Dialysis duration (months)	55 (33-147)	50 (13-151)	0.844
BMI (kg/m^2)	24.92±4.76	25.78±5.30	0.696
HbA1C (%)	6.55 (4.20-8.80)	5.20 (4.20-5.70)	0.224
CRP (mg/L)	19.45±23.77	7.31±5.14	0.018
Albumin (g/L)	3.82±0.59	3.76±0.24	0.804
Uric acid mg/dl)	6.70±1.35	6.60±0.94	0.872
Calcium (mg/dl)	8.55±0.79	8.94±0.63	0.264
Phosphorus (mg/dl)	5.46±1.32	5.06±1.52	0.518
iPTH (pg/ml)	342.33±268.76	543.77±154.99	0.087
LDL (mg/dl)	116.89±39.68	121.83±21.72	0.771
Total cholesterol (mg/dl)	170.54±47.23	173.33±24.99	0.890
Triglyceride (mg/dl)	179.18±101.58	246.50±117.44	0.161
Haemoglobin (g/dl)	11.12±1.79	11.51±2.16	0.634
Free testosterone (pg/ml)	11.30 (2.62-65.77)	9.39 (4.39-41.38)	0.471
SHBG (nmol/L)	16.82±13.16	24.15±14.88	0.255
FSH (mIU/ml)	6.91 (1.26-70.31)	3.77 (1.70-12.66)	0.126
LH (mIU/ml)	7.91 (3.06-85.77)	7.14 (2.63-13.94)	0.340
PRL (ng/ml)	55.88±90.66	21.62±18.86	0.069
Cancer (n)	2	0	0.690
Hypertension (n, %)	19 (% 61)	3 (% 50)	0.351
Diabetes (n, %)	12	0	0.055
Coronary artery disease (n, %)	9 (% 29)	1 (% 16.6)	0.644
Active (n, %)	20 (% 64.5)	5 (% 83.3)	0.640

BMI: Body mass index, CRP: C-reactive protein, iPTH: Intact parathormone, SHBG: Sex hormone-binding globulin, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, PRL: Prolactin, Activity: Active=1 (Index of independence in activities of daily living index score=6), No active=0 (Index of independence in activities of daily living index score <6), p<0.05

Table 3. Evaluation of dialysis patients with and without testosterone deficiency

Variables	TT<3 ng/ml	TT ≥ 3 ng/ml	p
Age (years)	52.63±16.09	51.85±16.67	0.835
Count (n, %)	37 (% 39)	58 (% 61)	
Dialysis duration (months)	55 (13-151)	49 (5-156)	0.460
BMI (kg/m^2)	24.88±4.85	23.79±3.83	0.301
HbA1C (%)	6.06±1.42	6.18±1.07	0.786
CRP (mg/L)	17.13±21.88	11.68±19.79	0.289
Albumin (g/L)	3.81±0.53	3.77±0.39	0.740
Uric acid mg/dl)	6.67±1.26	6.81±1.04	0.610
Calcium (mg/dl)	8.63±0.78	8.88±0.77	0.188
Phosphorus (mg/dl)	5.35±1.35	4.91±1.81	0.165
iPTH (pg/ml)	381.02±260.00	475.82±295.75	0.158
LDL (mg/dl)	116.26±37.45	111.21±40.58	0.596
Total cholesterol (mg/dl)	169.23±43.46	164.03±48.42	0.646
Triglyceride (mg/dl)	188.37±105.51	187.12±83.04	0.957
Haemoglobin (g/dl)	11.21±1.81	11.34±1.53	0.746
Free testosterone (pg/ml)	19.63±16.77	25.62±21.66	0.195
SHBG (nmol/L)	18.04±14.21	28.06±17.11	0.009
FSH (mIU/ml)	6.61 (1.26-70.31)	7.52 (1.69-25.69)	0.823
LH (mIU/ml)	7.95 (2.63-85.77)	8.03 (4.12-25.28)	0.764
PRL (ng/ml)	17.03 (6.11-467.00)	14.04 (3.88-73.62)	0.084
Cancer (n, %)	2 (% 33.3)	4 (% 66.7)	0.473
Hypertension (n, %)	22 (% 59.4)	23 (% 39.6)	0.676
Diabetes (n, %)	12 (% 38.7)	19 (% 61.3)	0.431
Coronary artery disease (n, %)	10 (% 35.7)	18 (% 64.3)	0.633
Active (n, %)	17 (% 43.1)	33 (% 56.9)	0.027

BMI: Body mass index, CRP: C-reactive protein, iPTH: Intact parathormone, SHBG: Sex hormone-binding globulin, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, PRL: prolactin, Activity: Active=1 (Index of independence in activities of daily living index score=6), No active=0, (Index of independence in activities of daily living index score <6), p<0.05

Patients with serum TT levels <3.0 ng/ml were considered to have low testosterone levels or hypogonadism (normal range for TT: 3–10.5 ng/ml). There were 31 of 71 patients in the HD group (43.6%) and 6 of 24 patients in the PD group (25%) with serum TT levels<3 ng/ml. The patients receiving HD treatments with serum TT levels<3 ng/ml were older than those in the PD group (p = 0.016). Serum CRP levels were significantly higher in the HD group (p=0.018). No significant differences were noted in dialysis vintage, BMI, haemoglobin or rate of comorbidities between the PD and HD groups with low testosterone levels (p > 0.05 for all). There was also no significant difference in the Index of ADL for both groups with low TT levels (p=0.640). Furthermore, there were no differences between the HD and PD groups in terms of SHBG, FSH, LH and PRL results (p=0.255, p=0.126, p=0.340 and p=0.069, respectively). Data from patients with low TT levels undergoing HD and PD are shown in **Table 2**.

We evaluated all participants (71 HD patients and 24 PD patients) according to whether their serum testosterone levels were low (TT <3 ng/ml) or normal (TT ≥3 ng/ml). From this data, we determined the relationship between TT and both SHBG (p=0.009) and Index of ADL. The latter scores were statistically significant in favour of TT ≥3 ng/ml (p=0.027, r=0.267). No significant difference was noted in other parameters (p >0.05 for all; **Table 3**).

We evaluated the associations of TT levels (TT below 3 ng/ml = 1 and TT ≥ 3 ng/ml = 2) with renal replacement therapy (PD=1 and HD=2) and DM (patients with DM=1 and patients without DM=2) by chi-square tests. These results

showed a statistically significant correlation was not observed for DM (p=0.431), while renal replacement therapy had both relationship (p=0.004) and correlation (r=0.341).

The effects of DM and renal replacement therapy were also assessed with binary regressions. These results showed that PD had an odd ratio=4.659 (1.477–14.704 95% CI Exp B) and p=0.009. For DM, the p=0.992.

DISCUSSION

Hypogonadism in males refers to low TT and/or FT in the serum together with associated signs and symptoms. The Society of Endocrinology and Metabolism guidelines recommend routine screening for hypogonadism in healthy males of advanced age (7). This is based on the insidious onset of the clinical picture where tests and screenings may facilitate the diagnosis (8). The incidence has been reported from 25% to 66% in patients with CKD (9). However, the highest incidence may be observed in CKD patients undergoing dialysis.

Comorbidities render hypogonadism more severe. DM has been reported to be associated with lower testosterone levels in earlier stages. In the present study, only the incidence of DM was higher in the HD group among the comorbidities (**Tables 1** and **2**). When evaluated, for all patients receiving PD and HD with serum TT levels normal or below 3 ng/ml, the number of patients with DM was not significantly different (**Table 3**). Regarding TT levels, a binary regression analysis and chi square test revealed no influence of DM on TT levels in our study. Shi and colleagues, in their 5-year study, have shown that annualized TT changes are associated with obesity, being unmarried and smoking at baseline, but not with DM, hypertension or cardiovascular disease in the general population (10).

The number of male patients 65 years of age or older was 53 of 71 (74.6%) in the HD group and 19 of 24 (79.2%) in the PD group (p=0.665). Advanced age is an important factor in terms of low testosterone levels both in the general population and in patients with CKD. Although age was not different across the PD and HD groups in this study, serum TT and FT were lower in the HD group compared to the PD group (**Table 1**).

There was no statistically significant difference in terms of dialysis duration between the PD and HD groups in the present study (48 (5–156) months, and 52 (33-147) months, respectively, p=0.156). Moreover, the dialysis durations were similar between patients with low and normal TT levels (55 (13–151) months and 49 (5–156) months, respectively, p=0.460). Cigarran's study reported that dialysis durations were significantly different between patients with low and normal TT levels, while the differences in testosterone levels between the two dialysis techniques cannot be justified by the time on renal replacement therapy (11). This is consistent with the results of the present study.

The adequacy of dialysis was assessed in the HD group and on a weekly basis in the PD group. A normal value for adequacy of dialysis was accepted as Kt/V ≥1.4 for the HD group and Kt/V >1.7/week for the PD group. There was no inadequacy of dialysis in either the PD or HD groups. A study evaluating the effect of HD adequacy on testosterone levels by Kim and colleagues demonstrated no significant difference in terms of testosterone levels between the groups with and without HD adequacy (12). As in the study of Kim et al, this study was demonstrated that dialysis adequacy was not associated with serum testosterone levels. Although urea and creatinine values of PD patients scratch the plateau, TT and FT levels are better, which indicates that the situation cannot be explained only by the uremic environment. This shows that we should investigate the causes other than uremia.

About 50–60% of testosterone is bound to SHBG, while 40–50% is albumin-bound and 1–2% is in free form. In our study, no difference was noted in serum albumin and SHBG values in the HD and PD groups (p=0.994 and p=0.353, respectively). In CKD, it appears that SHBG levels are unaffected by a decline in glomerular filtration rate (GFR) (13).

One of the two studies investigating the superiority between HD and PD reported that testosterone levels were higher in patients undergoing PD (11). The second study demonstrated the benefit of a nightly nocturnal home HD to conventional HD (14). Our study is important in that it confirms the findings of these studies (11,14).

Patients who choose PD are often those who are able to perform personal activities and prefer not spending four hours on HD three times a week. Furthermore, male patients receiving PD treatments are usually married and able to obtain full support from their family. A study that followed men aged 35 years and older for 5 years in Australia reported that being unmarried predicted a decrease in testosterone, whereas men who were married were more likely to have an increase in testosterone (10). When adequately informed, improved treatment satisfaction and compliance to treatment have been observed in patients receiving PD compared to those receiving HD (15,16).

Heiman highlights that the rate of sexual dysfunction is 10–52% in the general population and he reported that adequate sexual functioning also appears to be associated with personal well-being and relationship stability, although this may be more accurate for men than women (17). Additionally, Azevedo stated that sexual dysfunction is strongly associated with an impaired quality of life (18).

Clinical outcomes of low TT and FT levels are muscle wasting, frailty, loss of physical performance and sexual dysfunction (19). There is no opinion suggesting the opposite association in the literature. A study conducted in Singapore reported favourable effects of exercise on testosterone and sexual function in obese individuals (20). The PD group in our study exhibited superiority compared with the HD group in terms of the Index of ADL scores (p=0.033; **Table 1**).

As stated in both a previous study (11) and in our study, higher testosterone levels in patients receiving PD compared to HD has a positive effect on physical performance on testosterone levels. Additionally, in their study evaluating quality of life parameters among patients receiving PD or HD treatments, Russo and colleagues reported better psychophysical well-being in the PD group. They also reported patients receiving HD treatments are associated with a greater tendency of having depression (21).

We believe that taking an active part in the treatment of patients receiving PD by applying the treatment themselves may contribute to improving testosterone levels. Russo et al. suggested that PD appears to have clear advantages in terms of quality of life thanks to the possibility of performing treatments independently at the patient's home (21).

The limitations of this study are as follows: 1) We had a low number of patients, especially those on PD. 2) There was a lack of testosterone assays performed with dialysate fluids. A study involving male patients on HD and healthy participants who received transdermal testosterone replacement reported that the amount of testosterone removed via dialysis was very low (22). No such study has been conducted for PD.

Considering the associations between testosterone and mortality, quality of life and graft loss, further studies with multi-centre designs involving a greater number of patients would be useful.

CONCLUSION

The effect of dialysis techniques on TT has not been fully clarified. However, the benefits of PD versus HD have been confirmed with the present study. We believe that PD, and the technique itself, offer enhanced mental and physical well-being with positive benefits on TT levels.

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Research Article / Araştırma Makalesi

Investigation of the neutrophil/lymphocyte and monocyte to high-density lipoprotein cholesterol ratios in differentiated thyroid cancers

Diferansiye tiroid kanserlerinde nötrofil/lenfosit oranı ve monosit/ yüksek dansiteli lipoprotein kolesterol oranının araştırılması

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ABSTRACT

Introduction: Thyroid cancers are the most common malignant tumors of endocrine origin. They are classified depending on their histopathological and clinical behaviors. Papillary and follicular cancers are classified as differentiated thyroid carcinomas (DTCs). The neutrophil/lymphocyte ratio (NLR) and the monocyte to high-density lipoprotein cholesterol ratio (MHR) have recently been shown to be powerful markers of oxidative stress and systemic inflammation, and the MHR has been revealed as a potent marker of mortality in coronary heart disease associated with coronary atherosclerosis. The aim of this study was to evaluate these markers in patients diagnosed with DTC.

Material and Method: One hundred twenty-five patients newly diagnosed with DTC and a 75-member control group consisting of entirely healthy individuals were included in the study. The patient and control groups were evaluated by investigation of cholesterol and hematological parameters following 12-h fasting.

Results: Significant differences were determined between the patient and control groups in terms of mean NLR (3.2±2.8 vs 2.4±1.3, respectively, p=0.013) and MHR (0.102±0.079 vs, 0.038±0.052 respectively, p<0.001) values. In the correlation analysis, positive correlation was determined between the NLR and white cell count (r=0.530, p<0.001), neutrophil count (r=0.293, p<0.001) and C reactive protein (CRP) (r=0.371, p=0.005), while negative correlation was determined between the NLR and lymphocyte count (r=-0.271, p=0.001).

Conclusion: The study data show that DTCs increase systemic inflammation.

Keywords: Differentiated thyroid carcinoma, neutrophil/lymphocyte ratio, monocyte to high-density lipoprotein cholesterol ratio

ÖZ

Amaç: Tiroid kanserleri endokrin kökenli en yaygın malign tümörlerdir. Tiroid kanserleri histopatolojik ve klinik davranışlara göre sınıflandırılır. Papiller ve foliküler kanserler diferansiye tiroid kanserleri (DTK) olarak sınıflandırılırlar. Son zamanlarda nötrofil/lenfosit oranı (NLO) ve monosit/yüksek dansiteli lipoprotein kolesterol oranı (MHO) oksidatif stres ve sistemik inflamasyonun güçlü göstergeleri olduğu ve MHO' nun koroner ateroskleroz ile ilişkili koroner kalp hastalığında mortalitenin güçlü göstergeleri olduğu ortaya konuldu. Bu çalışmamızda DTK tanısı konulmuş olan hastalarımızda bu belirteçleri değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmamıza DTK tanısı yeni konulan 125 hasta ve tamamen sağlıklı kişilerden oluşan 75 kişi kontrol grubu olarak dahil edildi. Hasta ve kontrol grubunun 12 saatlik açlık sonrası kolesterol parametreleri ve hematolojik parametreleri çalışılarak değerlendirmeye alındı.

Bulgular: Hasta ve sağlıklı grubun sırası ile NLO ortalaması (3,2±2,8 ve 2,4±1,3 p=0,013) ve MHO (0,102±0,079 ve 0,038±0,052, p<0,001) şeklinde tespit edildi ve aralarında istatistiki açıdan anlamlı bir fark olduğu tespit edildi. Yapılan korelasyon analizinde ise NLO ile sırası ile beyaz küre (r=0,530, p<0,001), nötrofil (r=0,293, p<0,001) ve C reaktif protein (CRP) (r=0,371, p=0,005) arasında pozitif korelasyon tespit edilirken, NLO ile lenfosit arasında (r=-0,271, p=0,001) negatif korelasyon tespit edildi.

Sonuç: Bu çalışma ile elde edilen verilere göre diferansiye tiroid kanserlerinin sistemik inflamasyonu artırdığı gözlenmektedir.

Anahtar kelimler: Diferansiye tiroid kanserleri, nötrofil/lenfosit oranı, monosit/yüksek dansiteli lipoprotein kolesterol oranı

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INTRODUCTION

Papillary and follicular cancers arising from thyroid follicular cells are classified as differentiated thyroid carcinomas (DTCs), and the prognosis is generally good. DTCs comprise 90% of thyroid cancers (1), and their incidence is growing worldwide (2,3). Thyroid cancer is currently the third fastest- growing cancer diagnosis in the USA, and the incidence has doubled in the last 30 years (4).

Numerous studies have shown that chronic inflammation plays an important role in the development of various cancers (5,6). One such association is between thyroid malignancies and autoimmune thyroiditis and high inflammation marker levels. Patients with autoimmune thyroiditis are at a significant risk of developing well-differentiated carcinomas (7,8).

The neutrophil-to-lymphocyte ratio (NLR) has recently been identified as a powerful indicator of oxidative stress and systemic inflammation (9). Studies have also showed elevated NLR in many types of cancer, such as colorectal, gastric, and biliary tract cancers (10,11,12). Monocyte count and high-density lipoprotein (HDL)-cholesterol ratio are important hallmarks of atherosclerosis, in the development of which inflammation again plays an important role (13). A high monocyte count and low HDL cholesterol levels may be related to a pro-inflammatory state in atherosclerosis. However, no previous research has investigated the association between MHR and cancer development, which is strongly associated with chronic inflammation, as described above.

The aim of the present study was to evaluate the levels of these simple, inexpensive, and readily available inflammation markers (NLR and MHR) in patients with DTC.

MATERIAL AND METHOD

One hundred twenty-five DTC patients (47 papillary, 46 micropapillary and 32 follicular) and 75 healthy controls were recruited into the study. Erzurum Regional Training and Research Hospital records were screened from December 2014 to December 2018, and individuals diagnosed of DTC based on histopathological examination were enrolled. Cases with hematological disease, ongoing infection, and chronic inflammatory disease were excluded from the study. All the controls were selected from the same hospital's internal medicine outpatient department. All the controls and patients were over 18 years old. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 **Helsinki Declaration** and its later amendments or comparable ethical standards. All lipid and hematological parameters in the patient and control groups were evaluated after 12-h fasting.

Statistical Analysis

All statistical analyses in this study were performed on SPSS version 17.0 (SPSS, Chicago, IL, USA) for Windows software. The Mann–Whitney U Test or the Independent Sample-t Test were used following analysis of variance. Unless otherwise stated, the results were expressed as mean ± standard deviation, and p values <0.05 were considered statistically significant.

Ethical Declaration

The study was approved by the Health Sciences University, Region Training and Research Hospital Local Ethical Committee, Erzurum, Turkey (2018/05-32).

RESULTS

Sociodemographic characteristics, complete blood count, and biochemical parameters for both groups are summarized in Table 1. No significant difference was determined between the patient and control groups in terms of mean age or mean body mass index (p=0.106 and p=0.589, respectively). The 125 DTC cases consisted of 47 papillary, 46 micropapillary, and 32 follicular carcinomas. Liver and kidney tests exhibited no significant difference between the patients and controls. TSH values were higher in the controls than in the cancer cases, while free-T3 and free-T4 values were significantly higher in the cancer cases than in the controls. Mean MHR and NLR values both differed significantly between the patient and control groups (p<0.000 and p=0.013, respectively). Lymphocyte,and monocyte counts also differed significantly between two groups **(Table 1).**

Table 1. The demographic, clinical and biochemical features of the patients with and without differentiated thyroid cancer

Characteristics	DTC Patients* (n=125) Mean±SD	Controls (n=75) Mean±SD	p
Age (years)	45.9±14.7	48.2±7.7	0.106
BMI (kg/m^2)	28.0±4.7	27.2±3.3	0.589
Hemoglobin (gr/dl)	14.0±1.6	14.0±2.0	0.854
White blood cell (x10.e3/uL)	8.4±2.8	8.2±2.7	0.552
Neutrophil (x10.e3/uL)	5.6±2.5	6.7±6.8	0.001
Lymphocytes (x10.e3/uL)	2.0±0.7	3.0±1.4	<0.001
Monocyte (x10.e3/uL)	0.5±0.5	6.3±1.6	<0.001
Monocyte/HDL-cholesterol ratio	0,102±0,079	0.038±0.052	<0.001
Neutrophil-to-lymphocyte ratio	3.2±2.8	2.4±1.3	0.013
C-reactive protein (mg/dl)	8.2±26.0	2.7±1.9	<0.001
Total cholesterol (mg/dl)	199±46	193±44	0.485
LDL-cholesterol (mg/dl)	132±77	121±36	0.419
HDL-cholesterol (mg/dl)	48±14	50±13	0.275
Triglycerides (mg/dl)	158±71	151±113	0.018
TSH (mIU/L)	1. 3±1.3	1.5±1.3	0.305
Free T_3 (pg/ml)	3.2±0.77	2.7±0.66	<0.001
Free T_4 (ng/dl)	1.20±0.30	1.1±0.27	<0.001
Creatinine (mg/dl)	0.90±1.67	0.85±0.92	0.761
Alanine aminotransferase (U/L)	21±11	23±14	0.629
Aspartate aminotransferase (U/L)	22±6	24±9	0.342

The DTC cases consisted of 47 papillary, 46 micropapillary, and 32 follicular carcinomas.
BMI: Body Mass Index, TSH: Thyroid Stimulating Hormone, T_3: Triiodothyronine, T_4: Thyroxine,

The NLR exhibited positive correlation with the inflammatory markers C-reactive protein (CRP) (r=0.371, p=0.005), white blood cell count (r=0.530, p<0.001), and neutrophil count (r=0.293, p<0.001), and negative correlation with lymphocyte count (r=- 0.271, p=0.001). **(Table 2).**

Table 2. Correlation findings between NLR and other parameters

Characteristics	Correlation Coefficient	p
White blood cell	0.530	<0.001
Neutrophil	0.293	<0.001
C-reactive protein	0.371	0.005
Lymphocytes	-0.271	0.001

The MHR exhibited positive correlation with CRP (r=0.346, p=0.017), monocyte count (r=0.494, p<0.001) and neutrophil count (r=0.268, p=0.001).

DISCUSSION

The findings of this study showed that DTC increased systemic inflammation. NLR and MHR are indices that can be easily calculated with routine laboratory tests and that show the systemic inflammatory response. MHR has also recently emerged as a cardiovascular prognostic marker.

Oxidative stress and inflammation are closely linked pathophysiological processes. Oxidative stress is known to play a key role in several chronic diseases (14,15). Chronic inflammation has been associated with various diseases, such as diabetes mellitus (16), cardiovascular diseases (17), pulmonary diseases (18) and different types of cancer (19).

A relationship has also long been reported between thyroid carcinoma and inflammatory processes (20,21). In the physiological process of hormonogenesis, thyroid cells are exposed to high levels of reactive oxygen species (ROS) capable of involvement in the pathogenesis of thyroid cancer or of exhibiting cytotoxic effects (22). Oxidative stress has therefore been described as capable of impacting on thyroid malignancy.

Studies concerning the use of the NLR in various types of thyroid cancer have reported that the ratio cannot replace biopsy in diseases of the thyroid but that it may be a useful clinical marker.

In their study of histologically diagnosed cases of lymphocytic thyroiditis (LT), multinodular goiter (MNG), papillary thyroid carcinoma with lymphocytic thyroiditis (PTC-LT), and papillary thyroid carcinoma, Koçer et al. observed significantly higher NLR values in PTC and LT-PTC cases than in the other two groups, and suggested that this might be a useful guide in differentiating between benign and malignant thyroid diseases (23).

Studies examining the relationships between the clinicopathological characteristics of papillary thyroid carcinomas and the NLR have emphasized that perioperative high NLR values may be associated with tumor size and extrathyroidal extension (24), and also with extra-thyroidal invasion, presence of bilateral location and multifocal tumor, and lymph node positivity (25). Lee et al. reported that a high NLR was associated with an incomplete treatment response in DTCs (26).

We also observed a significant difference in NLR values between cases of DTC and the control group Our scan of the literature revealed no studies investigating the value of the MHR in cases of DTC. Similarly to the NLR, we also observed a significant difference in MHR values between the two groups.

The relationship between thyroid functions and the cardiovascular system is well known. The cardiovascular effects of abnormal thyroid functions, and particularly on atherosclerotic processes, such as adverse impacts on cardiac contractility, systolic and diastolic hypertension, a poor LDL cholesterol profile, and a tendency to cardiac arrhythmias, have been described in the literature (27,28). MHR has recently been described as a novel marker in adverse cardiovascular outcomes. In the present study, we observed significantly higher MHR values in cases of DTC compared to the control group. Research and clarification with more detailed studies of MHR in DTC patients are needed.

Also, we detected in our study, TSH values were higher in the controls than in the cancer cases, while free-T3 and free-T4 values were significantly higher in the cancer cases than in the controls. TSH, the major growth factor for thyroid cells, is a thyroid function regulator (29) and independent predictor for the diagnosis of thyroid malignancy in patients with nodular thyroid disease. In addition, preoperative serum TSH concentrations are higher in patients with more aggressive tumors (30) and advanced tumor stages (31), and this suggests a potential role for TSH in the progression of different thyroid cancers (30).

We attribute the lower mean TSH level in the DTC group in this study than that in the control group, although the difference was not statistically significant, and the significantly higher FT3 and FT4 values to our patients having had diseases requiring L-thyroxine therapy, such as Hashimoto thyroiditis and multinodular goiter, in the period prior to diagnosis of DTC and to their receiving L-thyroxine therapy at the time of diagnosis.

The limitation of this study is that our DTC group patients were not divided into subgroups based on the type of thyroid disease before diagnosis, if present, and on L-thyroxine use. Another limitation is that statistical evaluations have not been made according to these subgroups.

In conclusion, we think that this may be significant as an inflammatory index showing an increased inflammatory response in DTCs.

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no conflict of interest. No financial support was received.

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Review/Derleme

Distraksiyon ostegenezisi ve güncel maksillofasyal bölge uygulamaları: sistematik derleme

Distraction osteogenesis and current maxillofacial region applications: systematic review

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ÖZ

Distraksiyon osteogenezisi kallusu gererek yeni kemik oluşturma tekniğidir. Bu teknik ilk olarak Rus bilim adamı Ortopedist İlizarov tarafından uzun kemiklerde geliştirilmiştir. Distraksiyon osteogenezisi; osteotomi periyodu, latent periyod, distraksiyon periyodu ve konsolidasyon periyodu olmak üzere 4 safhadan oluşmaktadır. Kolay, etkili ve komplikasyonu az olan bir uygulamadır. Distraksiyon osteogenezis, maksillofasiyal cerrahide yaygın olarak kullanılabilmesi için modifikasyonlara ve geliştirmelere ihtiyaç duyulan bir tekniktir. Distraksiyon osteogenezisi tekniğinin geliştirilmesi için materyal ve teknik olarak birçok araştırma yapılarak önemli gelişmeler kaydedilmiştir. Ancak bu araştırmalar henüz tam bir kesinlik kazanmamakla beraber olumlu sonuçlar vermektedir.

Anahtar Kelimeler: Distraksiyon osteogenezisi maksillofasiyal bölge, kallus, yeni kemik oluşturma

ABSTRACT

Distraction osteogenesis is a new bone-forming technique by stretching the callus. This technique was first developed in the long bones by the Russian orthopedist Ilizarov. Distraction osteogenesis consists of 4 stages; osteotomy period, latent period, distraction period and consolidation period. It is easy, effective and less complicated. Distraction osteogenesis is a developing technique that requires modifications and improvements to be widely used in maxillofacial surgery. A lot of research has been made in material and technique for the development of distraction osteogenesis technique and many improvements have been made. However, although these studies have not yet been completely accurate, they give positive results.

Keywords: Distraction osteogenesis, maxillofacial region, new bone-forming

GİRİŞ

Maksillofasiyal bölgeyi ilgilendiren deformasyonlar geleneksel olarak çeşitli rekonstrüktif yöntemler ve ortognatik cerrahi işlemler ile düzeltilebilmektedir. Her yöntemin kendine göre avantaj ve dezavantajları vardır. Konvansiyonel cerrahi yöntemlerinin en önemli dezavantajlarından bir tanesi, kemikte yapılan değişikliklerin kısa sürede gerçekleştirilmesi nedeni ile yumuşak dokuların bu hızlı değişime ayak uyduramamasıdır. Büyük kemik hareketleri sırasında kemiğin etrafındaki yumuşak dokuların, oluşan yeni pozisyona adapte olamaması; dejeneratif değişikliklere, fonksiyon ve estetik problemlere yol açabilmektedir. Bu tip problemlerin ortadan kaldırılması amacıyla zaman içinde çeşitli yeni tekniklere başvurulmuştur. Bu alternatif metotların bir tanesi de distraksiyon osteogenezisi (DO)'dir. Bu teknik, yeni kemik dokusu elde etme ve kemik hacmini artırmak amacıyla uygulanması ile beraber, bu esnada komşu yumuşak dokularda da, kemik gelişimine paralel olarak bir takım hacimsel değişikliklere neden olmaktadır (1).

Distraksiyon osteogenezisi kemiklerde osteotomi ya da kortikotomi sonrası kemik segmentlerine distraksiyon aygıtı yerleştirilerek yavaş çekme kuvveti uygulanması ile

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beraber, kallusun indüklenmesi sonucunda, segmentlerin birbirlerine bakan yüzeylerinde yeni kemik ve komşu bölgelerde yumuşak doku formasyonunun meydana geldiği ve şekillendiği biyolojik bir olaydır. Bu yöntem uzun süreli, progresif ve kan desteğini bozmayan, kademeli uzatma esasına dayanır. Söz konusu olan iki temel hücresel süreçten birisi kallus formasyonu diğeri de distraksiyon ile yeni kemiğin meydana getirilmesidir (2).

Distraksiyon osteogenezisinde çekme kuvvetinin oluşturduğu gerilim distraksiyon vektörüne paralel olacak şekilde yeni kemik formasyonunu stimüle eder. Sert dokuya uygulanan bu kuvvetler, periost, diş eti, deri, kas, kartilaj, kan damarları ve periferal sinirler gibi çevre yumuşak dokularda da gerilim oluşturmakta ve distraksiyon histogenezisi adı verilen adaptif değişiklikler de meydana gelmektedir. Bu adaptif değişiklikler ise geleneksel cerrahi tekniklerle yapılamayacak kadar büyük iskeletsel hareketlere izin vermekte ve relaps riskini minimale indirmektedir (3).

TARİHÇE

Kemik segmentlerinin mekanik manipülasyonu ile ilgili prensipler yaklaşık 2500 yıl öncesine dayanmakla birlikte DO yöntemi kemiklerin uzatılması amacıyla 20. yüzyılın başlarından itibaren kullanılmaya başlanmıştır. İlk defa 1905 yılında Codivilla (4) deformite nedeniyle kısa kalmış olan femura oblik yönde yapılan osteotomiyi takiben, topuğa yerleştirdiği pinler ile eksternal kuvvet uygulayarak tekrarlayan bu yoğun aksiyal çekme kuvvetleri ile ekstremitelerin uzatılmasını göstermiştir.

Abbott (5) 1927 yılında bilateral aygıt ile modern DO'ne benzer şekilde tibiada uzama sağlamıştır. Ancak uygulamanın lokal ödem, deri nekrozu, pin yolu enfeksiyonu ve düzensiz ossifikasyon gibi yüksek komplikasyon riskleri nedeniyle klinik olarak bu görüş pek kabul görmemiştir.

Rus Ortopedist Gavril Abramovich Ilizarov (6) yaptığı çalışmalar sonucunda 1952 yılında DO'nin biyolojik temellerini ve başarılı yeni kemik formasyonu için fizyolojik ve mekanik faktörleri tanımlayarak modern uygulamalara geçişi sağlamış, canlı dokular üzerinde dereceli traksiyonun oluşturduğu stresin doku rejenerasyonunu stimüle ettiğini ve belli dokularda büyümeyi aktive ettiğini açıklamıştır.

Kraniomaksillofasiyal bölgede ilk DO uygulaması 1973 yılında Snyder ve ark. (7) tarafından İlizarov ilkeleri esas alınarak yapılan, köpek mandibulasının, transkütanöz endosseöz pinler ile tutturularak, ekstraoral bir aygıt vasıtasıyla gerçekleştirilmiştir. 1977 yılında Micheli ve Miotti (8) yine köpekte geliştirdikleri diş destekli intraoral distraksiyon aygıtı ile mandibulaya distraksiyon uygulamışlar ve başarılı olmuşlardır. Karp ve ark. (8) yaptıkları çalışmalarla, köpeklere uygulanan mandibular DO'ni takiben meydana gelen ossifikasyon sürecini radyolojik, histolojik ve vital boyama metodları ile detaylı bir şekilde incelemişlerdir. Yapılan başarılı deneysel çalışmaların ışığında ilk kez 1992 yılında McCarthy ve ark. (9) konjenital mandibuler anomalili çocuk hastaları DO yöntemiyle tedavi etmişlerdir. Hemifasiyal mikrosomi ve Nager's sendromlu hastalarda kademeli distraksiyon ile ekstraoral bir fiksasyon düzeneği kullanarak mandibular uzatma işlemini klinik olarak ilk kez başarıyla uygulamışlardır. Bu uygulamanın ardından kullanılan apareylerde uygun değişiklikler yapılarak kraniomaksillofasiyal bölgeye adapte edilmiştir(10). Bu gelişmeler kraniomaksillofasiyal bölgedeki defekt, anomalilerin orijinal kendi kemiğiyle düzeltilmekte ve elde edilen yeni kemik klasik yöntemlere göre önemli üstünlükler sağlamaktadır.

DİSTRAKSİYON OSTEOGENEZİSİNİN BİYOLOJİK TEMELLERİ

Distraksiyon teknikleri gerilim kuvvetlerinin uygulandığı yere göre kallotazis ve fiziyal distraksiyon olmak üzere iki sınıfa ayrılmaktadır:

I- Kallotazis

Kırık kallusunun distraksiyonudur. Osteotomi veya kırık nedeniyle devamlılığı bozulmuş kemik segmentleri arasında yer alan tamir kallusunun kademeli gerilimidir.

Kallotazis klinik olarak birbirini takip eden 5 periyodu kapsamaktadır:

Osteotomi Periyodu: Osteotomi kemiğin cerrahi olarak iki parçaya ayrılmasıdır.

Latent Periyot: Kemiğin cerrahi olarak iki parçaya ayrılması ile distraksiyon kuvvetlerinin uygulanmaya başlaması arasındaki dönemdir. İki parçaya ayrılmış olan kemik segmentleri arasında tamir kallusunun oluşması için gerekli olan süreyi ifade etmektedir.

Distraksiyon Periyodu: Kemik segmentlerine kademeli çekme kuvvetlerinin uygulandığı ve kemik segmentleri arasındaki bölgede yeni kemik veya distraksiyon rejeneratının oluştuğu dönemdir. Bu dönem süresince iki büyük parametre kritik öneme sahiptir: Distraksiyon ritmi ve distraksiyon oranı. Distraksiyon oranı kemik segmentlerinin toplam günlük hareket miktarını ifade ederken, distraksiyon ritmi ise distraksiyon oranının günlük kaç kerede uygulandığını ifade eder.

Konsolidasyon Periyodu: Bu periyot istenilen miktarda uzatma sağlanıp distraksiyon kuvveti kesildikten sonra başlar, distraksiyon aygıtı çıkarılıncaya kadar devam eder. Bu süre yeni oluşmuş kemik dokusunun mineralizasyonuna ve kortikalizasyonuna izin verir.

Remodeling Periyodu: Distraksiyon aygıtının çıkarılmasından sonraki dönemdir. Bu süre genellikle distraksiyonun tamamlanmasından 1 yıl sonraya kadar devam etmesine rağmen, yeni oluşan kemiğin remodelingi distraksiyonun tamamlanması ile başlar ve konsolidasyon periyodu boyunca da devam eder (11).

II- Fiziyal Distraksiyon

Kemik büyüme plaklarının distraksiyonudur. Bu teknik temel olarak büyüme plaklarına uygulanan distraksiyon oranına göre ikiye ayrılır:

A. Distraksion Epifiziyolizis: Büyüme bölgelerine günde 1-1,5 mm'lik bir distraksiyon aralığında yapılan hızlı bir fiziyal distraksiyon tekniğidir. Hızlı ve artan derecedeki gerilim büyüme plaklarında kırılmaya neden olur. Daha sonra epifizin metafizden kademeli olarak ayrılması, büyüme plağı kartilajının yerinin trabeküler kemikle dolmasına neden olmaktadır.

B. Kondrodiatazis: Günlük 0,5 mm'den daha az olacak şekilde çok yavaş bir ayrılma ile meydana gelir. Bu, büyüme plağının kırılma olmaksızın gerilmesine izin verir. Yavaş bir şekilde gerilen büyüme plağındaki gerilimsel stres kıkırdak hücrelerinin biyosentetik aktivitesini arttırır ve sonuçta hızlanmış bir osteogenezis oluşmasına neden olur(12).

DİSTRAKSİYON OSTEOGENEZİSİ HİSTOLOJİSİ

DO evrelerinin histolojisi farklılık göstermektedir:

1- Latent dönem: DO kortikotomiyi takip eden bir latent dönemle başlar. Kortikotomi sonrası latent periodun kırık iyileşmesinin başlangıç safhasından hiçbir farkı yoktur. Kortikotomi bölgesindeki boşluk fibrin kılıfla çevrelenen iltihabi hücre infiltrasyonu ve hematomla dolar. DO başlamadan mezenkimal hücrelerinin immatür vasküler sinüzoidler ve kollajen köprüler oluşturmak üzere organize oldukları görülmektedir.(13)

2- Distraksiyon başlangıcı: Fibrovasküler köprünün kendini distraksiyon yönünde organize ettiği görülmektedir. Kollajen ağı tendon gibi daha yoğun ancak daha az vasküler bir hal almaktadır. Bu dönemde aradaki yapının gerilim toleransını aşmayan hız ve ritmin uygulanması çok önemlidir.

3- Distraksiyonun 1. haftası: Birinci hafta sonunda 6-7 mm olan distraksiyon aralığındaki fibröz, avasküler doku, fibröz interzon olarak adlandırılan ve kollajen lifleri arasında iğ şeklinde fibroblastlar içeren yapı halini almaktadır. Osteoid ve osteoblast halen mevcut değildir.

4- Distraksiyonun 2. haftası: Fibröz interzonunun her iki tarafında, vasküler sinüzoidlere komşu kümeler halinde osteoblastik hücreler ortaya çıkar. Kollajen demetleri osteoid benzeri bir matriks ile kaynaşır. İkinci haftanın sonuna doğru osteoid hücreleri mineralize olarak primer mineralizasyon öncüsü adı verilen kemik spiküllerini oluşturur. Bu osteojenik proses periost, korteks, medüller kanal olmak üzere kesilen tüm yapıları içermektedir.

5- Distraksiyonun 3. haftası: Osteojenik yapılanma, kemik spiküllerinin uzaması, mikrokolon formasyonu ve fibröz interzon ossifikasyonu göze çarpar. Uzama, oluşan kemik spiküllerinin etrafında devam etmektedir.

6- Distraksiyonun son haftası: Fibröz interzon tam olarak ossifiye olur. Aralık tam köprüleşir ve bir tane kalın mikro kolon oluşur.(14)

7- 6 haftalık konsolidasyon ve yük verme sonrası: Konsolidasyon döneminde vasküler sinüzoidler köprüleşir ve fibröz interzonun mineralize mikrokolon haline dönüşümüyle aralık kapanır ve canlı kemik dokusu oluşur. Osteojenik ara bölge korteks ve medulla olarak remodele olur. Kemik kolonları lameller ve laküner özellikler gösterir. Kemik kolonlar arasındaki fibrovasküler doku normal ilik dokusuna dönüşür.(15,16)

Başarılı Distraksiyonun temel biyolojik parametreleri

1. Osteojenik dokuların ve periosteal /endosteal kan desteğinin maksimum korunduğu düşük enerjili osteotomi

2. Kırık kallusunun oluşmasına izin verecek kadar yeterli latent periyot.

3. Kemik segmentlerinin stabil fakat rijit olmayan fiksasyonu.

4. Tam olarak hesaplanmış distraksiyon yönü.

5. Optimal distraksiyon oranı ve ritmi.

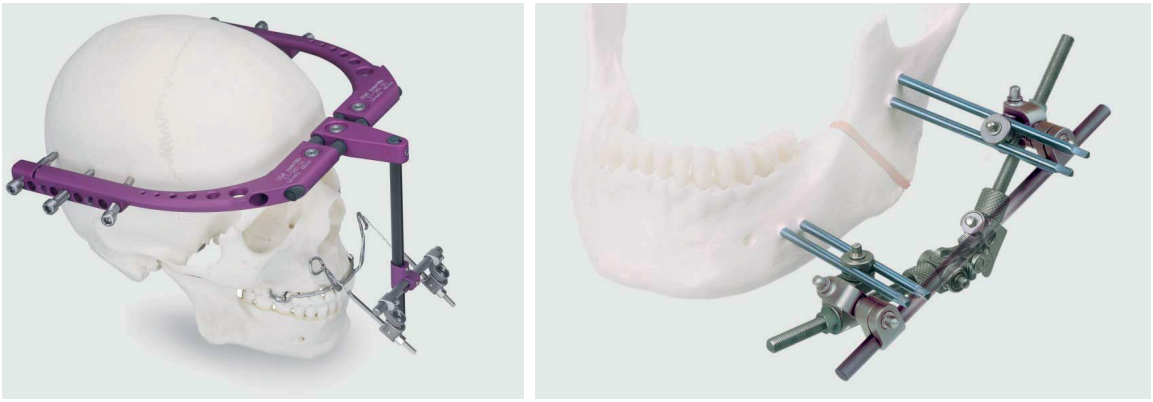
6. Kontrolsüz fonksiyonel yüklemeden önce yeni oluşan kemiğin remodelingi ve konsolidasyonu için yeterli zaman.

7. Yeni oluşmuş kemiğin mekanik yüklemesi ve kan desteği arasındaki oransal ilişki (17).

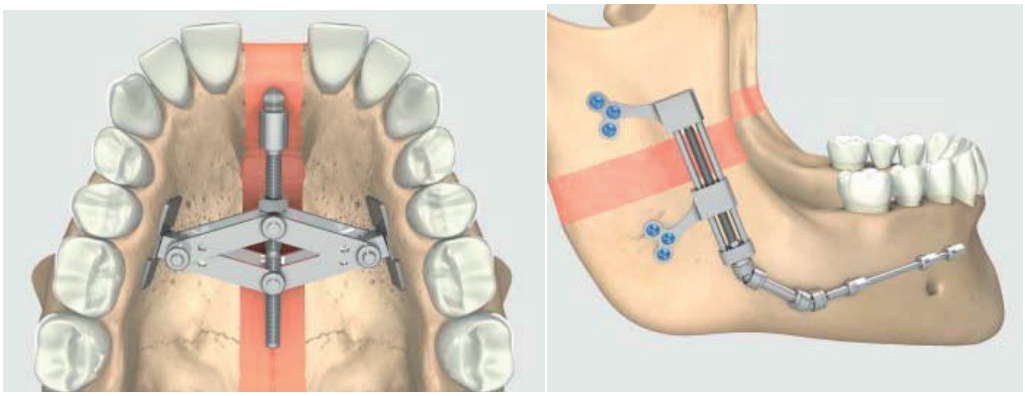
DİSTRAKSİYON OSTEOGENEZİSİ AYGITLARI

Kraniyofasiyal osteodistraksiyon tekniklerinde uygulanan distraksiyon aygıtlarının değişik çeşitleri bulunmaktadır. Distraktörler, kullanılan alan ve olguya göre, ağız dışı (ekstra-oral=eksternal) (Şekil 1) ve ağız içi (intra-oral=internal) (Şekil 2) olarak tasarlanmıştır (18).

Eksternal cihazlarla sadece kemik ile bağlantı sağlanırken; internal cihazlarla diş ile bağlantı, kemik ile bağlantı, hem kemik hem de diş ile bağlantı (hibrit) sağlanmaktadır. Her iki tip cihazında tek yönlü, çift yönlü ya da çok yönlü etki gösteren türleri bulunmakta ve tedavide defektin lokalizasyonu ve genişliğine göre bu türlerden biri tercih edilmektedir (19).



Şekil 1. Eksternal orta yüz ve mandibula distraksiyon aygıtları (https://www.klsmartin.com/fileadmin/user_upload/Homepage/Mediathek/90-173-02_Distraction_ product_overview.pdf adresinden alınmıştır)



Şekil 2. İnternal maksilla ve mandibula distraktör aygıtları (https://www.klsmartin.com/fileadmin/user_upload/Homepage/Mediathek/90-173-02_Distraction_ product_overview.pdf adresinden alınmıştır)

DİSTRAKSİYON OSTEOGENEZİSİNİN AVANTAJLARI VE DEZAVANTAJLARI

DO'nin geleneksel ortognatik cerrahi tekniklere göre birçok avantajı vardır. Bu avantajlar;

1. DO ile kemik greftine ihtiyaç duyulmaksızın 20 mm veya daha fazla mandibuler ilerletme yapılabilir. Greft ihtiyacının ortadan kalkması yanında donör sahanın enfeksiyon riski, morbidite ve skar gibi riskler yaşanmaz (20).
2. DO bebeklerde ve çocuklarda uygulanabilir. Bu yaş grubundaki hastalar yetersiz kemik dokusu ve gelişen diş köklerine zarar verme riski yüzünden geleneksel osteotomiler için uygun değildir.

3. TME'de sagittal split osteotomisine göre daha az distorsiyon ve yüklenme görülür.

4. DO, mandibulanın ilerletilmesi, genişletilmesi ve yüksekliğinin arttırılması olmak üzere üç boyutta gerçekleştirilebilir.

5. DO'nde relaps görülme oranı daha azdır. Yumuşak dokudaki adaptif değişiklikler, akut ortopedik düzeltmelerle meydana gelebilen potansiyel relapsı önleyen geniş iskeletsel hareketlere izin verir (21).

6. DO'nde inferior alveoler sinire zarar verme ihtimali daha azdır.

7. Operasyon zamanı daha kısadır.

8. Özellikle intraoral aygıtların geliştirilmesiyle hastanın operasyonu kabullenmesi daha kolay olmaktadır (22-24).

DO'nin başlıca dezavantajları ise apareylerin çıkarılması için ikinci bir operasyon gerekmesi ve ekstraoral apareylerin pinlerinin yerleştirilmesine bağlı skar dokusu gelişebilmesidir. Bununla birlikte toplam tedavi süresinin uzun olması da önemli bir dezavantajdır (25).

Distraksiyon Osteogenezisinde Gelişmeler

DO sisteminin gelişmesi ve hastalara daha hızlı daha konforlu bir iyileşme sağlayabilmek için çeşitli araştırmalar yapılmış ve yeni teknikler geliştirilmeye çalışılmıştır. Bunlardan bazılarına aşağıda değinilmiştir:

1-Distraksiyon bölgesine kompresyon uygulanması: Çeşitli araştırmacılar konsolidasyon periyodunu kısaltmak için ve daha kaliteli kemik elde etmek için distraksiyon bölgesine kompresyon uygulamışlardır. Bu amaçla Kim ve ark. (26) modifiye distraksiyon osteogenezis ve konvansiyonel distraksiyon osteogeneziste kemik iyileşmesini karşılaştırdıkları ve konsodilasyon periyodu boyunca basınç uygulayan modifiye distraksiyon osteogenezis protokolünü anlamak için bilgisayar stimülasyonu kullandıkları çalışmalarında, konsolidasyon periyodunda kompresyon uygulamasının

hızlı ve yoğun bir kemik rejenerasyonu sağladığını bulmuşlardır.

2-Elektrik sitimülasyonunun distraksiyon osteogenezise etkisi: Araştırmacılar kemik kalitesini artırmak için distraksiyon periyodunun çeşitli aşamalarında distraksiyon bölgesine, doğru akım uygulamışlardır. Doğru akım, stimulasyonu klinik olarak değişik ortopedik problemlerin tedavisinde kullanılmaktadır. Bunun, yeni kemik oluşumunda kayda değer bir artış ve mekanik açıdan daha kuvvetli bir kemik oluşturduğu bulunmuştur.Elektrik akımının distraksiyon osteogenezis üzerine olan etkisi ve farklı distraksiyon evrelerindeki en iyi uygulama periyodunu belirlemek amacıyla El-Hakim ve ark. (27) tarafından yapılmış bir çalışmada distraksiyon bölgesine, distraksiyon ve konsolidasyon periyodunda uygulanan doğru akımın mandibular distraksiyonla sinerji oluşturduğu gösterilmiştir.

3.İrradyasyon ve hiperbarik oksijen tedavisinin distraksiyon osteogenezise etkisi: İrradyasyon ve hiperbarik oksijen tedavisi kemiği etkileyen tedavilerdir. Bu tedavileri alan hastalarda kemik formasyonu değişimleri ile ilgili yapılmış bir araştırmada Muhonen ve ark. (28) mandibular distraksiyon sırasındaki kemik formasyonunu değerlendirmek için floride (18F−) emisyon tomografisi kullanmışlardır. Çalışma göstermiştir ki operasyondan önce verilen irradyasyon kemik formasyonunu bozmaktadır. Hiperbarik oksijen tedavisi osteogenezisi uyarmamaktadır fakat osteojenik aktiviteyi uzatarak kemik formasyonunu artırabilmektedir.

4. Kalsiyum sülfatın distraksiyon osteogenezise etkisi: Distraksiyonu geliştirmek için distraksiyon bölgesine çeşitli maddeler uygulanmıştır, bunlardan birisi de kalsiyum sülfattır. Rezorbe olabilen kalsiyum sülfat uygulamasının yeni distrakte olmuş kemikte osteogenezisi ve konsolidasyonu hızlandırdığı Al Ruhaimi ve ark. (29)'nın yaptığı bir çalışmada gösterilmiştir. Çalışmada kalsiyum sülfat uygulamasının yeni distrakte kemikteki osteogenezis ve kalsifikasyon oranını arttırdığı gösterilmiştir.

5. Kök hücrelerin distraksiyon osteogeneziste kullanılması: Popülerlik kazanan kök hücreler distraksiyon osteogenezisde de kullanılmıştır. Birçok araştırmacı DO de kök hücre uygulamasının başarılı sonuçları olduğunu bildirmiştir (30).

Otolog kemik iliği mezenşimal kök hücre transplantasyonu, distraksiyon bölgesindeki kemik rejenerasyonunu hızlandıran ve konsolidasyonu geliştiren bir metottur (31). Maksiller ve mandibular DO'de mezenşimal kök hücrelerinin uygulanması, ossifikasyon ve konsodilasyon periyodunu hızlandırarak kemik formasyonunu artırmaktadır (32).

Song ve ark. (33), mezenşimal kök hücre olan dental pulpa kaynaklı kök hücrelerinin yeni kemik formasyonuna olumlu etkilerinin yanı sıra, dental pulpa kaynaklı kök hücreleri kullandıkları grupta kontrol grubuna oranla kemik mineral yoğunluğu ve içeriğinin daha iyi olduğunu gözlemlemişlerdir.

6.Lazerin distraksiyon osteogenezise etkisi:Lazerin kontrollü enerji salınımı ile yara iyileşmesini hızlandırma özelliğinden yararlanmak amacıyla,araştırmacılar, DO'de düşük enerji seviyeli lazer tedavisi (DESLT)'ni kullanmışlardır.Bu çalışmalardan birinde, Medeiros ve ark. (34), distraksiyon sırasında uygulanan DESLT'nin kemik rejenerasyonunu arttırdığını ve konsolidasyon periyodunu kısalttığını, böylelikle apareylerin erken çıkarılmasına olanak sağladığını ve apareylerin uzun süreli kullanımına bağlı oluşan sorunları da ortadan kaldırdığını göstermişlerdir.

7. Lokal olarak uygulanmış nevre growth faktörün distraksiyon osteogenezise etkisi: Çeşitli hormonların distraksiyon osteogenezis üzerine etkili olabileceği saptanmış ve bu konuda çeşitli araştırmalar yapılmıştır. Bunlardan birisi de lokal olarak uygulanmış nevre growth faktördür. Lokal olarak uygulanan nerve growth faktörün, kallus maturasyonunu hızlandırarak konsolidasyon periyodunu kısaltması klinik olarak yararlı olabilir (30).

8.Radyasyonun distraksiyon osteogenezise etkisi: Radyasyonun vücudun tüm dokularına olduğu gibi kemik üzerine de etkileri vardır. González-García ve ark.(35) tarafından radyoterapi sonrası distraksiyon uygulanmış 6 hastanın dahil edildiği çalışmada, 5 hastada DO'in başarılı olduğu ve radyoterapinin DO'i direk olarak etkilemediği rapor edilmiştir.

9.Bisfosfonatların distraksiyon osteogenezise etkileri: Bir bifosfonat türü olan zoledronik asidin kemik üzerine etkileri bilinmektedir. Pampu ve ark. (36), DO'de zoledronik asidin kemik mineral yoğunluğuna ve içeriğine olan etkilerini araştırdıkları bir çalışmada, zoledronik asidin yeni kemik formasyonunda ve distraksiyon alanında pozitif etkisi olduğunu ortaya çıkarmışlardır.

10. Piezoelektrik cerrahinin distraksiyon osteogenezisde kullanılması: Piezoelektrik osteotomların, bitişik yumuşak dokuların bütünlüğünü koruyarak kemik yapılarının hassas, güvenli ve temiz bir şekilde kesilmesini sağladığı düşünülmektedir. González-García ve ark. (37), alveoler distraksiyonda piezoelektrik osteotominin intra-operatif komplikasyon insidansını azaltırken piezoelektrik osteotominin post-operatif ve post-distraksiyon komplikasyon riskini artırdığını göstermişlerdir.

11. Trombosit konsantratlarının distraksiyon osteogenezise etkisi: Trombositten zengin plazma (TZP) ve trombositten zengin fibrin (TZF), ağız, diş ve çene cerrahisi alanında, kemik içi defekt rekonstrüksiyonu, sinüs lift prosedürleri, kist enükleasyonu sonrası oluşan kaviteler, alveoler yarık rekonstrüksiyonu ve dental implant çevresinde oluşan kemik kayıplarının tedavisi gibi çeşitli rejeneratif prosedürlerde kullanılmaktadır (38).

Fibrin matris içerisindeki büyüme faktörleri ve sitokinler sinerjik etkileşim ile sert ve yumuşak dokuların doğal yolla ve daha hızlı iyileşmesine olanak tanır, ayrıca vasküler bütünlüğün sağlanmasında rol oynar (39).

Xu ve ark.'nın (40) DOde TZP uygulayarak yaptıkları çalışmada, TZP'nin yara iyileştirmesini hızlandırmasının yanı sıra, kemik rejenerasyonuna da olumlu etkileri olduğunu bildirilmişlerdir.

12. Cerrahi rehberlerin distraksiyon osteogenezis de kullanılması: Üç boyutlu (3B) yazılım nesnelerin dijitalleştirilmiş modellerden doğru şekilde yeniden oluşturulmasına olanak sağlar. 3B yazılım tekniği, cerrahi riskleri etkin bir şekilde azaltma ve tedavi doğruluğunu artırma potansiyeline sahiptir.

Mao ve ark.(41)'nın Pier Robin sendromlu hastalarda cerrahi rehber kullanarak yaptıkları distraksiyon osteogenezisde cerrahi rehber kullanımı ile operasyon süresinin oldukça kısaldığı, hastanede kalış süresinin azaldığı ve postoperatif komplikasyonların minimuma indiği görülmüştür. Chen ve ark. (42), Foley ve ark. (43) cerrahi rehber kullanımının,tedavi planlamasını, doğru osteotomiyi, kemik segmentlerinin repozisyonunu ve osteotomi hatlarının konturunu kolaylaştırdığını ve distraksiyon aygıtının yerleştirilmesinde etkinliğini arttırdığını bildirmişlerdir.

SONUÇ

Konvansiyonel distraksiyon osteogenezis yıllardır başarıyla uygulanmış ve başarılı sonuçlar alınmıştır. Bu araştırmalarda distraksiyonun gelişmesi yönünde olumlu gelişmeler olmuştur, ancak bulunan yeni yöntem ve materyaller üzerinde daha çok araştırma yapılması gerekmektedir.

MADDİ DESTEK VE ÇIKAR İLİŞKİSİ

Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkar dayalı ilişkisi yoktur.

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Review/Derleme

Kistik fibrozis hastalığında tıbbi beslenme tedavisi

Medical nutritional therapy in cystic fibrosis disease

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ÖZ

Kistik fibrozis (KF), çocuklarda ve yetişkinlerde görülen, multisistem tutulum gösteren genetik bir hastalıktır. Yenidoğanlarda en sık Avrupa kökeninde görülmekle bilrikte farklı etnik kökenlerde hastalığın görülme sıklığı 1/3500 ila 1/30000 arasında değişmektedir. KF hastalığında beklenen yaşam süresinin uzatılması ve sağ kalım oranının artırılması için tanının erken konulması, tedaviye erken başlanması gerekmektedir. KF'de sindirim yetersizliği dolayısıyla enerjinin etkili olarak kullanılmaması, enerji ihtiyacının artması, gastrointestinal problemler ve iştah azalmasına bağlı olarak yetersiz beslenme; malabsorbsiyon kaynaklı olarak da vitamin mineral yetersizlikleri yaygın olarak görülmektedir. Beslenme gereksinimlerinin artmasına karşın ihtiyacın karşılanamamasından dolayı KF'li bebek ve çocuklarda malnütrisyon görülmektir. Bu derlemede kistik fibrozis hastalığında tıbbi beslenme tedavisi ilkelerinin ve beslenme tedavisinin öneminin ortaya konması amaçlanmıştır.

Anahtar Kelimeler: Beslenme, diyet, emilim bozukluğu, kistik fibrozis, malnütrisyon

ABSTRACT

Cystic fibrosis (CF) is a genetic disorder with multisystem involvement in children and adults. In Newborns, the most frequent occurrence of European ancestry is the frequency of having a disease in different ethnic origins, ranging from 1/3500 to 1/30000. In order to extend the expected life span and increase the survival rate in patients with CF, it is necessary to make diagnosis early and to start treatment early. Inadequate use of energy due to digestive failure in CF, increased energy need, gastrointestinal problems and inadequate nutrition due to reduced appetite; Vitamin mineral deficiencies are also frequently seen as malabsorption origin. Malnutrition is seen in CF infants and children because of the increased need for nutrition and the inability to meet the needs. In this review, it is aimed to introduce the principles of medical nutrition therapy principles and nutritional therapy in cystic fibrosis.

Keywords: Cystic fibrosis, diet, malabsorbtion, malnutrition, nutrition

GİRİŞ

Kistik fibrozis hastalığı kistik fibrozis transmembran regülatör (KFTR) proteinini kodlayan gendeki mutasyonun neden olduğu, birden çok organ ve ekzokrin bezleri etkileyen, otozomal resesif geçişli, mortalitesi yüksek, kronik ve progresif bir hastalıktır (1). KFTR proteini, epitelin apikal membranlarında klor dolaşımını düzenler. Bu proteinin yapısal ve işlevsel bozukluğu, epitel hücre salgılarının bileşiminde değişiklikler ile sonuçlanır. Hastalık terde elektrolit yoğunluğunun artması, pankreatik yetersizlik, mukus akışkanlığı klirensinde azalma, akut pulmoner alevlenme atakları ve kronik akciğer infeksiyonu ile karakterizedir (2).

Kistik fibrozis insidansı; Avrupa ve Avrupa kökenli popülasyonda 1/3.500, Afrikalılar, Afrikan Amerikalılarda 1/15.000, Asyalı Amerikalılarda 1/30.000, taşıyıcı frekansı beyaz ırkta 1/22-28'dir (3). Kistik fibrozis tarama programlarından önce hastaların çoğuna kistik fibroz semptomları görüldükten sonra tanı konulmaktaydı. Son 10 yılda yenidoğan tarama programları genişletilmesi sayesinde KF vakalarının çoğunda semptomlar görülmeden önce tanı konulmaya başlandı. Amerika'da 2001 yılında yenidoğan tarama programı sırasında KF vakalarının %10'undan daha azı teşhis edilirken, 2011 yılında taramaların %60'ında teşhis konulmuştur (4). Ülkemizde 1 Ocak 2015 itibariyle KF Yenidoğan Tarama Programı uygulanmaya başlanmıştır (5).

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ABD Kistik Fibrozis Sağlığı Hasta Kayıt Defteri Yıllık Veri Raporu'na göre, KF için beklenen yaşam süresi; 1985 yılında 25 yıl ilken 2008 yılında 37 yıla yükselmiştir. Birleşik Krallık'ta 2000 yılından itibaren yenidoğan tarama testleriyle birlikte KF'li hastalarda beklenen yaşam süresinin 50 yıl olduğu bildirilmiştir (6). Tıbbi beslenme tedavisi KF'li hastalarda yaşam kalitesi ve yaşam süresinin artmasında büyük önem taşımaktadır. Bu derlemede KF'li hastalığına yönelik tıbbi beslenme tedavisi ilkelerinin ortaya konması amaçlanmıştır.

KİSTİK FİBROZİS PATOGENEZİ

Kistik fibrozis geni, 'kistik fibroz transmembran regülatör (KFTR)' adlı proteinin sentezini kodlamaktadır. KFTR epitalyel hücrelerde eksprese olur ve iyon transportunu düzenler. KFTR ile ilgili gen mutasyonları epitel hücrelerde iyon transportu işlevinin bozulmasına neden olur. Solunum yolu epitelindeki transport defekti tuzun ve suyun hücre dışına salgılanamamasına ve bunun sonucu olarak salgıların yeterli derecede su içerememesine neden olur. Benzer olaylar pankreas ve safra kanallarında da meydana gelir, bu sekresyonlar da kururlar ve bulundukları kanalları tıkarlar (7). Hastalardaki koyu kıvamlı salgılar pankreas kanallarında tıkanma yaparken aynı zamanda, içinde taşıdığı proteolitik enzimler ile pankreas dokusuna zarar vermeye başlamakta, gittikçe fibrozis ve atrofi gelişen pankreasta yağlanma oluşmaktadır. Önce ekzokrin fonksiyonlarında sonra endokrin fonksiyonlarında bozulmanın sonucunda adacık hücreleri, zarar görür ve insülin eksikliği ortaya çıkmaktadır (8). Ter bezlerinde ise tuzun geri emilimi bozulmuştur. Bu nedenle bu yoldan da fazla miktarda tuz kaybı olmaktadır (7).

KİSTİK FİBROZİS HASTALIĞINDA KLİNİK BULGULAR

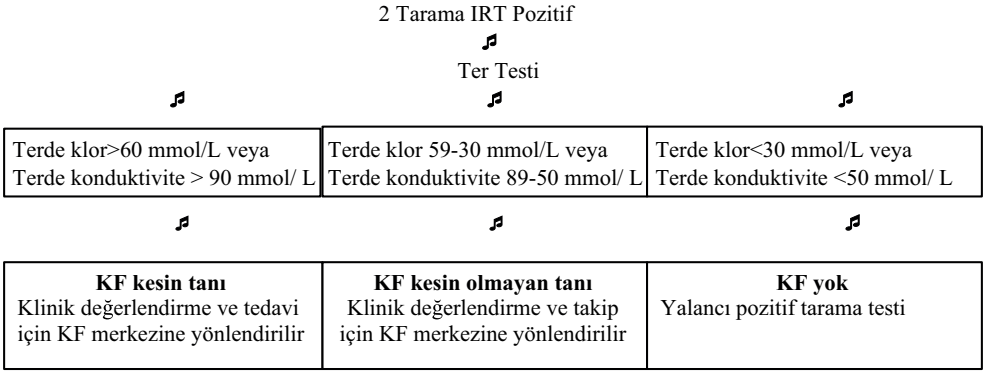
Kistik fibrozis mutasyonlarının heterojen oluşu ve çevre farklılıkları nedeni ile akciğer, gastrointestinal sistem ve diğer organlar değişken biçimde tutulurlar. KF'de en sık rastlanan klinik bulgular alt solunum yolunda reaktif havayolu hastalığı, tekrarlayan bronşiyolit bronşektazi, çomak parmak, tekrarlayan pnömoni, atelektazi, pnömotoraks, hemoptizi, solunum yetersizliği; pankreasta ekzokrin yetersizlik, yağda eriyen vitamin eksikliği, tekrarlayan pankreatit, insüline bağımlı diabetes mellitus; mide-bağırsak yolunda mekonyum ileusu, distan intestinal obstrüksiyon, rektal prolapsus, invajinasyon, kronik ishal; karaciğer- safra yollarında; uzamış yeni doğan sarılığı, neonatal kolestaz, biliyer siroz, portal hipertansiyon, hepatosplenomegali, kolesistit ve kolelityazis, üst solunum yolunda nazal polipozis ve pansinüzittir. KF'li hastaların değişik yaş gruplarında en sık başvuru nedenleri **Tablo 1**'de özetlenmiştir (9).

Tablo 1. Semptom ve bulguların yaşa göre sınıflaması

Sütçocukluğu	Çocukluk	Ergen/Yetişkin
Mekonyum ileusu	Büyüme geriliği	Püberte gecikmesi
Obstriktif sarılık	Steatore	Obstrüktif azospermi
Büyüme geriliği	Hipolektrolitemi ve yorgunluk	Kronik bronşit
Hipoproteinemi ve anemiyle	Bronşektazi	Kronik abdominal ağrı
Birlikte ödem	Nazal polip	Pankreatit
Tekrarlayan bronşiyolit	Rektal prolapsus	Siroz
Rektal prolapsus	Tuz tadı alınan cilt	Hemoptizi
Tuz tadı alınan cilt	Pnömotoraks	

KİSTİK FİBROZİS HASTALIĞINDA TANI

Son 10 yılda KF hastalarında en önemli gelişme birçok ülkede yenidoğan tarama programınnn uygulamaya konmasıdır. KF yenidoğan tarama testi çok pahalı olmayan ve geniş gruplara uygulanabilen topuk kanında immunreaktif tripsinojen (IRT) analizine dayanır (10). Immünoreaktif tripsinojen (IRT) pankreas tarafından yapılan bir enzim prekürsörüdür. IRF düzeyleri pankreas yetersizliği olmayan hastalar dahil tüm KF hastalarında yüksektir. Topuk kanından alınan örneklerde immun reaktif tripsinojen (IRT) ölçümü yapılmakta, IRT değeri belirlenen düzeyin üzerinde bulunan bebekler 2. kez topuk kanından IRT ölçümü için çağrılmaktadır. İki IRT değeri de belirlenen eşik değerin üzerinde çıkan bebekler ter testi yapan merkezlere yönlendirilmektedir. Ülkemizde terde klor ölçümü çok az merkezde yapılmakta genellikle terde konduktivite ölçülmektedir. Konduktivite ölçümünde terde klor yanında sodyum, potasyum gibi diğer iyonlarında ölçümü yapıldığından ter testi için pozitiflik değerleri değişmektedir. Yenidoğan tarama testi sonrası hastaları yönlendirme şeması **Şekil**'de gösterilmiştir (5).



Şekil. Yenidoğan tarama testi sonrası hastaları yönlendirme şeması

TIBBİ BESLENME TEDAVİSİ

Kistik fibrozis hastalığının beslenme yönetimini; beslenme durumunun değerlendirilmesi, artan enerji gereksiniminin karşılanması, pankreatik enzim replasman tedavisi (PERT) ve vitamin ve mineral desteği oluşturmaktadır (11).

Beslenme Durumunun Değerlendirilmesi

Avrupa Klinik Beslenme ve Metabolizma Birliği (AKBMB/ESPEN) KF'li bebek, çocuk ve yetişkinler için beslenme bakım rehberine göre, KF'li hastaların beslenme durumunun değerlendirilmesinde büyüme/ağırlık izlemlerinin yapılması, nutrisyonel izlem ve diyet değerlendirilmesi yapılmalıdır. Büyüme izleminde bebeklerde yeterli beslenme sağlanana kadar her hafta kontrol önerilir. Yeterli beslenme sağlandıktan sonra yaşamın ilk yılında her ay, ilk yıldan sonra üç ayda bir kontrol önerilir. Nutrisyonel izlemde her yıl kan sayımı, demir seviyesi, plazma A, D, E, K vitamini düzeyleri, plazma/serum fosfolipid yağ asidi seviyeleri, karaciğer fonksiyonu ve elektrolit ölçümleri değerlendirilmelidir. Malnütrisyon riski için; Akciğer fonksiyonu 3 ayda bir değerlendirilmeli, pankreas fonksiyonu yıllık değerlendirilmelidir. Büyüme ve beslenme durumu incelenerek PERT ihtiyacı değerlendirilmelidir (12).

Pankreatik ekzokrin fonksiyonları değerlendirmek amacı ile 72 saatlik dışkıda yağ miktarının ölçülmesi, dışkıda fekal elastaz tayini ya da sekretin ve kolesistokinin stimülasyonu sonrası toplanan duodenal sıvıda pankreatik enzim miktarlarının ölçümü yöntemleri kullanılabilir (13). Diyet değerlendirmesinde diyet önerilerine uyma konusundaki sorular da dahil olmak üzere çocukların 3 ayda bir, yetişkinlerin 6 ayda bir diyet incelemesine tabi tutulması önerilmektedir (12).

Enerji Gereksinimi

Kistik fibrozisli çocuklar ve adölesanlar enerjinin gastrointestinal düzeyde sindirim yetersizliği yüzünden etkili olarak kullanılamaması ve artmış enerji ihtiyacından dolayı enerji gereksinimini karşılayamamaktadır (3). Enerji kaybının temel nedeni intestinal lümene yetersiz pankreas enzimi salınımından kaynaklanan sindirim yetersizliğidir. Sağlıklı insana kıyasla KF'li bireylerde pankreatik yetersizliklerden dolayı enerji ihtiyacı daha fazladır (7). Pulmoner inflamasyon, gastrointestinal problemler ve ilaçların yan etkileri de iştahı düşürebilir ve besin alımını engelleyebilir. KF'li bebek ve çocuklarda yetersiz besin alımı bodurlukla sonuçlanır (14). KF mutasyonuna, hastanın yaşına ve mevcut sağlık durumuna bağlı olarak, sağlıklı çocuklar ve yetişkinler için gereken enerjinin %120-150'si kadar olmalıdır. Enerjinin %40-45'i yağlardan, %40-45'i karbonhidratlardan, %20'si proteinlerden sağlanmalıdır (15).

Avrupa Kistik Fibröz Derneği Hasta Kayıt 2010 verilerine göre Avrupa'da KF'li bebek ve çocukların yarısı beslenme hedeflerini/gereksinimlerini karşılayamamaktadır (16). Amerika'da Kistik Fibrozlu BKI persentilleri 2001 yılında 41 iken, 2011 yılında 51.3'e çıkmıştır. Bununla birlikte ABD KF Vakfı Hasta Kayıt Raporuna göre çocukların yaklaşık 4'te 1'i cinsiyete göre 10. persentilin altında kalmaktadır. KF'li hastalardan enerji gereksiniminin sağlanması büyük önem taşımaktadır (17).

Pankreatik Enzim Replasman Tedavisi

Pankreatik enzim replasman tedavisi (PERT) yeterli beslenme durumunun sağlanması için hayati önem taşımaktadır. PERT, gastrik boşalma yoluyla verilen; protein ve yağların sindirimi için gereken pankreatik enzimlerin, özellikle proteazın ve lipazın oral yoldan verilmesini içerir. Bu şekilde tedavi intestinal yoldan protein ve yağ kayıplarını azaltır. Yağ emilimi enzim kullanımı ile %80-90 düzeyine çıkabilir (18).

Pankreatik enzim replasman tedavisinin yeterliliğinin belirlenmesi için; bebeklere 3 ayda bir, çocuklara ve adölesanlara 6 ayda büyüme ve beslenme durumunun izlenmesi önerilir (19).

Kistik Fibrozis Vakfi konsensüs rehberine göre, 0-12 aylık bebeklerde 120 ml formüla veya anne sütü ve 1 gram diyet yağı için 2000-4000 ünite lipaz, 1-4 yaş arası çocuklarda 1 gram diyet yağı için 2000-4000 ünite lipaz, 4 yaşından büyük çocuk ve yetişkinlerde 1000 – 2500 ünite lipaz /kg/gün alınması önerilmektedir (20).

Vitamin ve Mineral Desteği

Kistik fibroziste sık görülen artmış terleme, bağırsak malabsorbsiyonu ve kronik enflamasyonun bir sonucu olarak tuz, kalsiyum, demir, çinko ve selenyuma olan gereksinim artar. Kaybedilen sodyumun replasmanı için 0-6 aylık bebeklere 20-40 mg/kg/gün sodyum takviyesi yapılması ve çocuklarda tuzlu besinlerin ve sodyum klorid kapsüllerinin kullanılması önerilmektedir. Çinko yetersizliği olan hastalarda 2 yaşından küçük bebek ve çocuklara 1 mg/kg/gün; 2-18 yaş arasındaki çocuklara 15 mg/gün çinko takviyesi önerilmektedir. KF'de kalsiyum dengesi malabsorbsiyon, dışkı ile kalsiyum kaybının artması, diyet alımının yetersiz olması ve glukokortikoid tedavisi nedeniyle bozulabilir. Düşük kemik mineral yoğunluğu KF'de sıklıkla görülür. Kalsiyum yetersizliği kemik mineralizasyonun azalmasına ve sekonder osteoporoza yol açabilir. Kalsiyum takviyesi KF'li çocuk ve yetişkinlerde yararlıdır. Kalsiyum 0-6 aylık bebeklerde 210 mg/gün, 7-12 aylık bebeklerde 270 mg/gün, çocuklarda 500-800 mg/gün, adölesanlarda 1300 mg/gün ve yetişkinlerde 1000 mg/gün olarak yeterli miktarda alınmalıdır. Pankreas yetersizliğinden kaynaklı yağ emiliminin azalması, yağda eriyen vitaminlere olan gereksinimi artırır. KF'li hastalara yağda eriyen vitamin takviyeleri rutin olarak verilmelidir ve yıllık olarak takip edilmelidir. KF'li hastalarda yağda eriyen vitamin gereksinimleri **Tablo 2**'de verilmiştir. Vitamin takviyesi ile istenilen sonuç alınamayan hastalarda daha yüksek dozlar gerekebilir (21).

SONUÇ VE ÖNERİLER

Kistik fibrozis komplikasyonlarından dolayı enerji ve protein ihtiyacının arttığı malnütrisyon ile karakterize bir has-

Tablo 2. Yağda eriyen vitaminler için günlük önerilen dozlar

Yaş	Günlük Doz Önerisi			
	A (IU)	E (IU)	D (IU)	K (IU)
0-12 ay	1500	40-50	400	0,3-0,5
1-3 yıl	5000	80-150	400-800	0,3-0,5
4-8 yıl	5000-10.000	100-200	400-800	0,3-0,5
>8 yıl	10.000	200-400	400-800	0,3-0,5

talıktır. Optimal düzeyde büyüme gelişmenin sağlanması, malnütrisyonun önlenmesi, hayat kalitesinin yükseltilmesi ve sağ kalım oranının artırılması için tıbbi beslenme tedavisi büyük önem taşımaktadır. Yenidoğan tarama programlarının geliştirilmesi ile birlikte erken tanı konulması, hastalığın tıbbi tedavisine olanak sağlamakta ve beslenme tedavisine erken başlanarak organ hasarının önlenmesi hedeflenmektedir. Hastalığın beslenme tedavisinde artan enerji gereksiniminin karşılanması, yağların sindirimi için enzim replasman tedavisinin yapılması yağda eriyen vitaminlerin ve kaybedilen mineral takviyelerinin alınması, hastanın belirli aralıklarla hekim tarafından sağlık durumunun ve diyetisyen tarafından beslenme durumunun kontrol edilmesi tedavinin en önemli basamaklarını oluşturmaktadır.

MADDİ DESTEK VE ÇIKAR İLİŞKİSİ

Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkara dayalı ilişkisi yoktur.

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Sağlık Bilimleri ve Tıp Dergisi

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Case Report / Olgu Sunumu

Cervical aortic arch

Servikal aortik ark

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ABSTRACT

Cervical aortic arch is an unusual congenital anomaly. It is usually symptomatic in childhood, but sometimes this abnormality is diagnosed during radiological examination because of unrelated causes in asymptomatic adults. We aimed to report a case of cervical aortic arch and review of the literature which presented with cough and shortness of breath.

Keywords: Cervical, aortic arch, shortness of breath, dyspnea

ÖZ

Servikal aortik ark, nadir görülen bir konjenital anomalidir. Çocuklukta genellikle semptomatiktir, ancak asemptomatik erişkinlerde bazen bu anormallik farklı nedenlerden dolayı radyolojik görüntüleme sırasında teşhis edilir. Biz bu yazıda öksürük ve nefes darlığı ile başvuran servikal aortik ark olgusunu ve literatürün gözden geçirilmesini amaçladık.

Anahtar Kelimeler: Servikal, aortik ark, nefes darlığı, dispne

INTRODUCTION

Cervical aortic arch (CAA) is a unusual congenital anomaly in which the ascending aorta elongate in such a way that the aortic arch is situated higher to its usual position (1) . The position of the arch different from lightly superior to normal to very high in the neck, lying on either side of the trachea . Patients is usually asymptomatic. But, it may cause cough and dyspnea, especially during exercise (2). We here report a cases of CAA, which is a rare case, presented with cough and shortness of breath.

CASE REPORT

A 48-year-old female was admitted to emergency department suffering from cough and shortness of breath during both exercise. She was on salbutamol inhalation as well as budesonide/formoterol inhalation for 5 years and the symptoms did not ameliorate. Her blood pressure was 120/80 mmHg and all peripheral pulses were palpable. The breath and pulse rate were within normal limits. She had no significant family history. There was no history of change in voice, trauma, dysphagia, hemoptysis, or arm claudication. On inspection, the neck swelling appeared pulsatile. There was a pulsatile left supraclavicular and suprasternal mass. The palpation revealed an elongated soft neck swelling on the left side. There was no skin discoloration or erythema over the swelling. Routine laboratory investigations were normal. Chest radiograph did not reveal any information except a homogeneous shadow with sharp margins in the left apex and continuous with superior mediastinum (**Figure 1**).

Computed tomography (CT) revealed an elongated ascending aorta with cervical aortic arch and the tracheal compression (**Figure 2-3**). The arch was at the level of C-7 vertebra. The descending aorta was seen to cross the midline to descend on the left side. No vessel was seen arising from the aortic arch. The brachiocephalic trunks were absent and all major vessels were arising separately. No associated cardiac anomaly was evident on CT scan. The patient was referred to cardiovascular surgery with a diagnosis of CAA.

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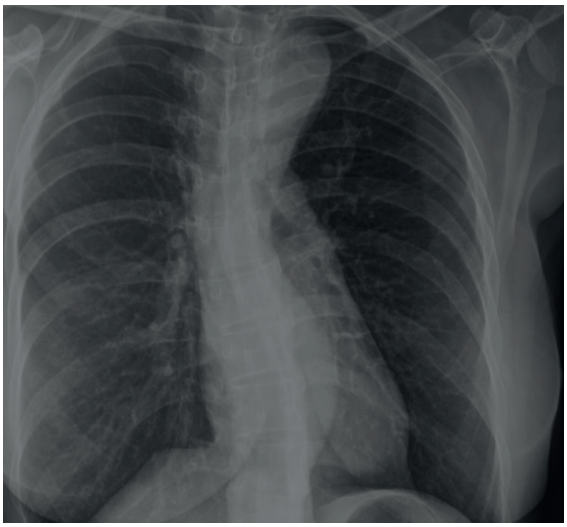

Figure 1. Chest radiograph shows homogeneous shadow of the aortic knob

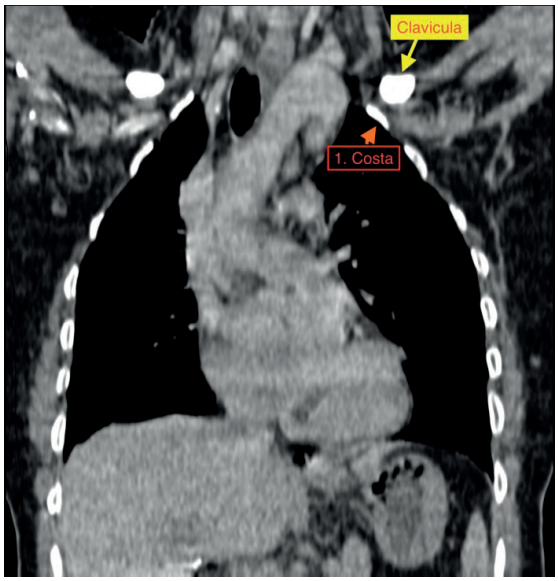

Figure 2. Computed tomography of the thorax shows cervical aortic arch at the level of C-7 vertebra

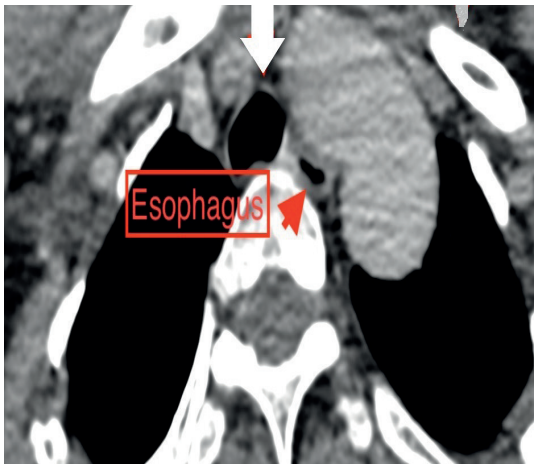

Figure 3. Computed tomography of the thorax, compressed tracheal lumen and cervical aortic arch is shown (white arrow: trachea)

DISCUSSION

CAA is a rare congenital anomaly resulting from cephalic displacement of the aortic arch. Haughton identified five different forms on the basis of the aortic configuration, brachiocephalic branching and embryogenesis sequence. Type A has separate external and internal carotid artery branches from the aortic arch, type B has dual common carotid arteries, type C is a left cervical arch with right sided descending aorta and bicarotid trunk, type D has normal brachiocephalic branching, redundant transverse aorta and left-sided descending aorta, type E is a right cervical aortic arch with a right descending aorta and an aberrant left subclavian artery (3).

Most patients with this malformation are asymptomatic, but patients may present with esophageal and tracheal vascular compression indicative of dysphagia, dyspnea or frequent pulmonary infection. Dysphagia and respiratory distress are associated with the right cervical arc, while aneurysmal formations are more commonly associated with the left cervical arc. Physical examination reveals a large pulsatile mass in the supraclavicular area. CT, angiography and magnetic resonance angiography are useful for specific diagnosis (4). The case of our patient demonstrates the importance of suspicion of a vascular ring in patients with frequent respiratory symptoms. This congenital vascular abnormality and others are diagnosed optically by computed tomography or magnetic resonance imaging and angiography.

A variety of cervical arched associations include aneurysms, aortic coarctation, aortic pseudo-coarctation, congenital cardiac anomalies, Di George's syndrome and Turner's syndrome. Complications usually result from the compression of the brachial plexus resulting in aneurysmal dilatation, coarctation, dissection or cervical radiculopathy. These conditions require surgical management (5).

Investigations suggest that a cervical aortic arch is associated with deletions in the chromosome 22q11 and therefore may be included in the spectrum of defects known as catch 22. This syndrome, described by Wilson and colleagues in 1993, is characterized by cardiac defects, facial dysmorphic, thymic hypoplasia, cleft palate, hypocalcaemia and a deletion in chromosome 22 (2).

Treatment includes excision and thoracic re-localization and surgical correction. Other options include an endovascular stent to exclude aneurysms and dissections (6).

In conclusion, CAA might be considered in differential diagnosis of patients who admitted to emergency department suffering from cough and shortness of breath.

ETHICAL DECLARATION

Patient approval was obtained.

MATERIAL SUPPORT AND RELATIONSHIP

There is no person / organization to support the work financially and the authors have no conflict of interest.

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JOURNAL OF HEALTH SCIENCES AND MEDICINE

Sağlık Bilimleri ve Tıp Dergisi

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Case Report / Olgu Sunumu

A rare cause of severe earache: a tick in the external auditory canal

Şiddetli kulak ağrısının nadir bir nedeni: dış kulak yolunda kene

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ABSTRACT

Although tick is rarely seen in the external auditory canal, it may cause pain and tinnitus in the ear. In addition, it is also important in terms of causing Crimean-Congo hemorrhagic fever disease. In this report, we presented the treatment of a 60-year-old patient who was admitted to the emergency service with pain in the left ear and who had a tick in the external auditory canal together with literature data. Removing the tick from the external ear canal with the alligator forceps in a careful manner is important in terms of preventing many diseases that might occur due to the tick.

Keywords: Tick; external auditory canal; foreign body

ÖZ

Dış kulak yolunda kene nadir görülmesine rağmen kulakta ağrı,tinnitus gibi durumlara yol açabilir. Ayrıca Kırım Kongo kanamalı ateşi gibi hastalıkları oluşturması açısından önemlidir. Bu raporda biz 60 yaşında sol kulakta ağrı şikayetiyle acil servise başvurmuş ve dış kulak yolunda kene bulunan hastanın tedavisini literatür eşliğinde sunduk. Kenenin dış kulak yolundan alligatör forsepsle dikkatli biçimde çıkarılması keneye bağlı oluşabilecek bir çok hastalığın oluşmasının engellenmesi açısından önemlidir.

Anahtar Kelimeler: Kene; dış kulak kanalı; yabancı cisim

INTRODUCTION

Ticks are blood-sucking ectoparasites that use mostly animals and rarely humans as their hosts and which are the vectors of many bacterial and viral diseases. Following the attachment to a place in the human body, they can transfer some viral and bacterial infectious agents through their saliva into the body. As well as these disease agents, they may also cause foreign body reactions, hypersensitivity and some neurological complications through neurotoxins they secrete (1).

In emergency departments (ED), it is possible to see foreign bodies such as cotton, toy parts, and fruit seeds as well as flies and insects in the external ear canals of the patients who apply with pain in their external ear canals. A tick in the external auditory canal is a rare cause of application to ED. The symptoms such as pain, tinnitus, imbalance and facial paralysis were reported in the external auditory canal because of tick bites in previous studies (1,2). Sometimes no symptoms are seen in patients (2). The tick may adhere to the external auditory canal or tympanic membrane with the help of its mouth, and the enzymes secreted through its saliva are the cause of local pain (3).

In this case report, we presented the management and the treatment of a 60-year-old patient who applied to the ED with severe earache together with literature data.

CASE

A 60-year-old female patient who was living in rural area applied to the ED with complaints of severe pain in the left ear, pain that spread to the left side of the head, and loss of sensation in the left side of the face. There was also a fullness sensation in the left ear. The complaints of the patient had started one day before, and increased gradually.

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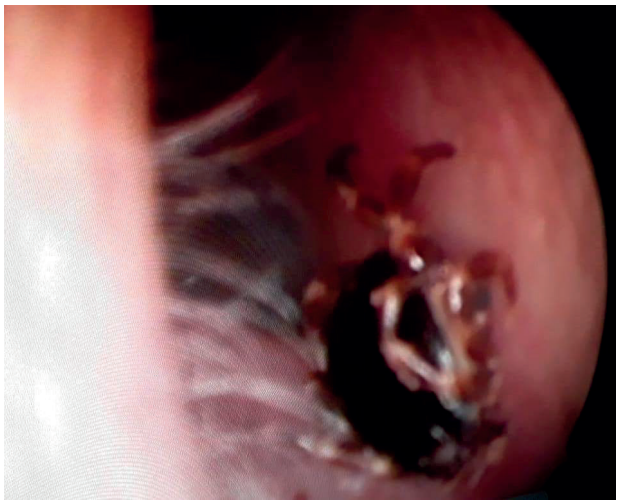

Figure. Tick in the external auditory canal

According to the anamnesis of the patient, she had no other chronic diseases. When she applied to the ED, she had body temperature as 36,7°, pulse was 82/min, and blood pressure was 130/80. The laboratory values of the patients were; white blood cell (WBC) amount was 10900µL, platelet count (PLT) was 240000 µL, hemoglobin (Hgb) level was 13.1 g/dL, alanine aminotransaminase (ALT) level was 21 unit/liter (U/L), aspartate aminotransaminase (AST) level was 13 U/L. In the autoscopic examination of the patient in the ED, a mobile tick was detected in the posterior wall of the left ear external auditory canal (**Figure**). The tympanic membrane was intact, and slight hyperemia was observed around the area where the tick was attached. No pathologies were detected in the other system examinations. Following local anesthesia in the left external ear canal, the tick was removed carefully with the help of the alligator forceps by ensuring the vision of the external ear canal with 0° endoscope. The tick that was removed was identified as *Hyalomma marginatum marginatum*, which is one of the hard tick types. After this process, the patient was followed-up in terms of Crimean-Congo hemorrhagic fever (CCHF). In the follow-ups, no increases were detected in the temperature, and no abnormal changes were observed in the laboratory findings. The earache and numbness of the ear regressed completely. No abnormal findings were detected in the examination of the ear in the 1st week.

DISCUSSION

Ticks are living organisms that have a life-cycle that goes on by absorbing blood. The hosts of the ticks consist of pets, humans and wild animals. Ticks can transmit some bacterial and viral agents through their mouths. As a result of this, life-threatening diseases like CCHF, Lyme disease, tularemia, babesiosis, and Rocky Mountain spotted-fever may be seen in humans (4). In Turkey, the first CCHF case was detected in 2002; and until December 8, 2015, a total of 9787 CCHF cases were identified (5).

In most patients who apply to ED, inorganic foreign bodies like cotton, metal pieces and pieces of toys are detected; and in a few of them, bees, flies, and ticks may also be detected in the ear in small quantities (6). The cases in which ticks are reported in the external auditory canal are observed especially in India, Sri Lanka, Nepal, South Africa, Chile, and Malaysia throughout the world (7). In the external auditory canal, the most frequently reported complications after ticks are earaches, bleeding, tinnitus, and facial paralysis (1,7,8). It is considered that pain and facial paralysis are associated with the neurotoxins transferred to the area through the saliva during the attachment of the tick to the skin (3).

It is important to remove the tick from the bite area as soon as possible to prevent the transfer of infectious agents to the human body. Although methods like petroleum jelly, gasoline, fingernail polish, or 70% isopropyl alcohol over the tick's mouthparts are suggested for removal of the tick, it has also been claimed that these methods are not effective (8). It has been argued that the most effective method is the complete removal of the tick by forceps (8). The removal of the tick from the external auditory canal or tympanic membrane might be challenging when compared with the other body regions since the ear region is relatively narrow. Since the removal of the tick with the help of autoscopy in the ED has the risk of possible complications, we removed the tick from the external auditory canal with the help of a 0° video endoscope and the alligator forceps. However, in some cases, it was observed that it is impossible to remove the tick completely in one piece because the body volume of it increases with the volume of the blood it sucks from humans (9).

Removing the tick from the area it affects is important to prevent the infection of disease factors as well as to avoid local granulomas and abscesses that might occur. Previous studies have shown that the risk of infection by the tick is high especially in the first 48 hours. In addition, the patient should be followed-up with clinical and serological tests in the period following the removal of the tick (10). In our case, the tick was removed within the first 24 hours, and no evidence of CCHF was found in the follow-ups.

As a result, careful removal of the tick from the external ear canal by an otolaryngologist is important to prevent possible diseases like CCHF. In this respect, alligator forceps are a safe and effective tool. Such patients should be followed-up with their clinical and laboratory findings after the tick is removed.

DECLARATION OF CONFLICTING INTERESTS

The author declared no conflicts of interest with respect to the authorship and/or publication of this article.

ETHICS

Permission was obtained from the patient to share information.

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Case Report / Olgu Sunumu

Sodium valproate use may result in hyponatremia

Sodyum valproat kullanımı hiponatremi ile sonuçlanabilir

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ABSTRACT

Sodium valproate is a drug used in neurological and psychiatric diseases and has side effects such as tremor, drowsiness, Reye-like syndrome, hepatic failure, thrombocytopenia, pancreatitis. Hyponatremia is another serious side effect of sodium valproate. Here, we report the case of a patient with hyponatremia associated with sodium valproate.

Keywords: Sodium valproate, hyponatremia, side effect

ÖZ

Sodyum valproat, nörolojik ve psikiyatrik hastalıklarda kullanılan ve titreme, halsizlik, Reye benzeri sendrom, karaciğer yetmezliği, trombositopeni, pankreatit gibi yan etkileri olan bir ilaçtır. Hiponatremi, sodyum valproatın bir başka ciddi yan etkisidir. Burada, sodyum valproat ile ilişkili hiponatremi gelişen bir hastayı ele aldık.

Anahtar Kelimeler: Sodyum valproat, hiponatremi, yan etki

INTRODUCTION

Sodium valproate (SV) is an FDA (United States Food and Drug Administration)-approved drug to treat seizures, to prevent migraine headaches, and bipolar disorder (BD). It is also used off-label (for unapproved uses) for other conditions, particularly for other psychiatric disorders. SV has psychiatric, neurological, dermatological, immunological, metabolic, gastroenterological and hematological side effects. Hyponatremia is another serious side effect that has also been previously associated with the use of carbamazepine, clozapine, and selective serotonin reuptake inhibitors (SSRIs) (1). Although cases of hyponatremia associated with SV have also been reported, recurrent use-associated hyponatremia cases are rare (1, 2). We discussed the treatment process and the training process related to compliance with treatment of a male patient who had side effects of hyponatremia due to SV several times in different hospitals and times.

CASE PRESENTATION

The patient is single and unemployed a man born in 1979 who has suffered from BD type 1 since the age of 24. He was admitted to the emergency department with complaints of headache, muscle weakness, irritability, and confusion. He was using quetiapine 300 mg/day per oral (PO), risperidone 3 mg/day PO, and biperiden 2 mg/day PO for nine months for BD in our outpatient clinic, was using desmopressin 40 µgr/day intranasal (IN) with a diagnosis of central diabetes insipidus (CDI) since childhood, and two weeks ago, it was learned that SV was started and the dose was increased to 1500 mg/day PO. The patient's initial baseline laboratory data at the time of admission were sodium 111 mmol/L and potassium 3.9 mmol/L. Drug-induced hyponatremia was considered due to antipsychotic and mood stabilizer use. He was hospitalized with the diagnosis of drug-induced hyponatremia plus BD according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) (3). Previously prescribed drugs used by the patient at the effective dose and time were chlorpromazine, olanzapine, risperidone, quetiapine, biperiden, diazepam, lorazepam, alprazolam, lithium, carbamazepine, mirtazapine, fluoxetine, desmopressin, and a few psychotropic combinations, etc. According to the story taken from his parents, after treatment for decreased need for sleep, feeling overly happy, increased sexual desire with various psychotropic drugs had failed, he finally received SV in

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2016 firstly and had a severe hyponatremia with the sodium level of 109 mmol/L and stopped using the SV. The patient had been examined in another city two weeks ago. The family stated that SV could have been rewritten there and that they were not aware of this situation. The serum SV level at this time was 82.9 mg/L. The patient's thyroid and liver function tests were within normal limits. The fasting blood glucose, protein level, and lipid profile were within normal limits. Chest X-ray, electrocardiogram, renal ultrasonography gave normal results. The patient and relatives stated that there was no change in dietary and fluid intake in recent days. The patient had no drug use other than quetiapine, risperidone, biperiden, desmopressin. He had no systemic disease such as hypertension or diabetes mellitus. A history of smoking, alcohol and substance abuse was not available. His family history was unremarkable apart from diabetes mellitus type 2 in his father. The hyponatremia was attributed to SV or unsuitable use of desmopressin as there were no obvious underlying disorders to cause the hyponatremia and firstly SV was stopped. Hyponatremia ceased 6 days after his SV intake was stopped. Quetiapine 300 mg/day PO, risperidone 3 mg/day PO, and biperiden 2 mg/day PO and desmopressin 40 µgr/day IN continued to be used. No additional treatment was applied for adverse effects. Lamotrigine 12.5 mg/day PO was added to treatment and it was titrated up to 100 mg/day PO. The patients and their relatives were informed about the effects and possible side effects of the treatment. No similar side effects were reported during the follow-up of the patient. Psychiatric complaints decreased partially. The patient and his relatives were warned about hyponatremia due to SV use and informed consent was obtained from them for their knowledge. Naranjo Adverse Drug Reaction Probability Scale (NADRPS) score of the patient was 7 (4).

DISCUSSION

This case report was evaluated as a case of hyponatremia due to SV. Because there was a temporal relationship between them, the side effect began with the addition of the drug and completely cured after discontinuation of the drug. Other causes of hyponatremia, such as volume depletion, hypothyroidism, adrenal insufficiency, and diuretic abuse, and vomiting were excluded. On the other hand, the patient has a history of SV-induced hyponatremia. Our patient had a history of CDI and desmopressin use. If desmopressin had not been used, serum sodium would have been elevated (5). However, the serum sodium level was decreased in our patient. It was thought that desmopressin high dose use might cause this condition, but SV was initially discontinued due to the history of SV-induced hyponatremia. The NADRPS score indicates a probable association between drug use and side effect (4). The mechanism by which SV could cause hyponatremia and syndrome of inappropriate secretion of antidiuretic hormone (SIADH) has not been fully elucidated. However, it was speculated that dopaminergic, serotonergic and noradrenergic systems may play a role in SIADH due to SV (6). SIADH due to drugs can be caused by stimulation of the release of ADH by the hypophysis. SV can make hypothalamic osmoreceptors less sensitive, can enhance action of ADH on the kidney, can act directly the kidney, can inhibit the vasopressinase activity, resulting in prolonged vasopressin half-life (1).

Hyponatremia due to SV could be a dose-related side effect. Some authors suggest that this side effect occurs in toxic doses (1). However, the serum SV level of our patient was within normal limits. On the other hand, the reported cases are known to be advanced age patients. Our patient was 39 years old. The number of cases in which the NADPRS score is 7, ie, cases of hyponatremia due to recurrent SV use, is not much. There were no cases of SV-induced hyponatremia accompanied by conditions related to sodium balance such as CDI and desmopressin. Differential diagnosis was difficult because of desmopressin and CDI. We attributed this drug-induced hyponatremia to SV because of the patient's side effect history and the lack of fluid intake change. When hyponatremia occurs, the patient's general medical condition should be reassessed and other organic conditions that may cause hyponatremia should be excluded. Drug dose can be reduced or the drug can be changed (7, 8). In our patient, there was no need for them, and when the drug was stopped supportive treatment was started, the hyponatremia disappeared. World Health Organisation (WHO) defines 'probable' as an event or laboratory test abnormality, with a reasonable time relationship to drug intake (9). WHO also says this relationship cannot be explained by disease or other drugs, response to withdrawal clinically reasonable, rechallenge (not necessary) (9). Factors influencing patients with psychiatric disorder compliance with medication include patient-related influences, physician-related variables, factors related to the patient's environment, treatment-related factors, and side effects. The influence of side effects has been demonstrated in patient's noncompliance with treatment. Sometimes, despite the side effects, some patients continue to be exposed to the drug. The level of functioning of the relatives of the patients, psychiatric or medical diseases which they have should be taken into consideration (10). For these reasons, we have warned the patient and relatives about this side effect.

As a result, this case report suggests that physicians and relatives should be aware that sodium valproate may induce hyponatremia with a low quality of life and low compliance. Further systemic research should be conducted with respect to sodium valproate-associated hyponatremia to provide a greater understanding of both its prevalence and etiology.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

FINANCIAL DISCLOSURE

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ETHICS

The patient's data was used to write this case report within the context of the institutional local ethics approval.

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JOURNAL OF HEALTH SCIENCE AND MEDICINE

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Ethical Guidelines: The protocol of clinical trials should be approved by the ethics committee. All work done on humans should include a statement that the work in the "Materials and Methods" section has been approved by the committee or that the work has been carried out in accordance with the **Helsinki Declaration of Principles** (www.wma.net/e/policy/b3.htm). All persons included in the study must be indicated in the text signed by the informed consent form. ***Journal of Health Sciences and Medicine*** shall be deemed to have been made in conformity with the Helsinki Declaration and that institutional ethics and legal permits shall be taken and shall not be held responsible for this matter. If the "animal" item is used in the study, the authors must indicate that they have protected animal rights and have been approved by the ethical committees of their institutions in line with the principles of the article in the Materials and Methods section of the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html).

Acknowledgment: If yes, it should be written after the sources.

Financial Support and Relationship Based on Self-interest: At the end of the article, if any, the persons and institutions that support the work financially, and if so, the interest relations of these institutions with the authors should be indicated. (In case of non-existence, it should be written as ***"There is no person / organization supporting the work financially and the authors have no relationship based on self-interest".***

Sources: Sources should be written according to the order of arrival on the article. All authors should be listed if the number of authors in the source is 6 or less, and the first 3 names if 7 or more and sould be added "et al". The format used for source writing should be as specified in Index Medicus (www.icmje.org). The resource list should include only works that have been published or accepted to be published or have received a DOI number. Magazine abbreviations should follow the style used in "Cumulated Index Medicus". It should be taken to limit the number of references to 40 in research and 60 in review articles, 20 in case presentations and 10 in editorials. References should be specified in parentheses immediately before the dot mark at the end of the sentence in the text. For example (4,5). The author (s) are responsible for the correctness of the sources. Importance should be given to the synthesis of domestic and foreign sources.

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Source Writing Examples

Excerpt from the journals;

Cesur S, Aslan T, Hoca NT, Çimen F, Tarhan G, Çifçi A. Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. Int J Mycobacteriol 2014; 3: 5-8.

Tos M. Cartilage tympanoplasty. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.

Excerpt from the books having only editor or writer;

Neinstein LS. The office visit, interview techniques, and recommendations to parents. In: Neinstein LS (ed). Adolescent Health Care. A practical guide. 3rd ed. Baltimore: Williams&Wilkins; 1996: 46-60.

Excerpt from the books having multiple writers or editörs;

Schulz JE, Parran T Jr: Principles of identification and intervention. In:Principles of Addicton Medicine, Graham AW. Shultz TK (eds). American Society of Addiction Medicine, 3rd ed. Baltimore: Williams&Wilkins; 1998:1-10.

If the editor is also a section author in the book;

Diener HC, Wilkinson M (editors). Drug-induced headache. In: Headache. First ed., New York: Springer-Verlag;1988:45-67.

Excerpt from Doctoral / Bachelor Thesis

Kılıç C. General Health Survey: A Study of Reliability and Validity. phD Thesis, Hacettepe University Faculty of Medicine, Department of Psychiatrics, Ankara; 1992.

Excerpt from a website;

The name of the site, URL address, author names, submission date should be given in detail.

Given DOI number;

Joos S, Musselmann B, Szecsenyi J. Integration of Complementary and Alternative Medicine into Family Practice in Germany: Result of National Survey. Evid Based Complement Alternat Med 2011 (doi: 10.1093/ecam/nep019).

Fort he other referance styles, visit "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References" page.

Scientific Responsibility Statement

Before an accepted article is published, each author must declare that he / she has participated in the search to share the responsibility of his / her content. This participation may include: **1.** Creation of concepts and designs of works, or collection of data **2.** analysis or expression **3.** Preparing the draft of the article or reviewing its scientific content **4.** Approving the final version of the article's print ready.

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6. Copyright Transfer Form

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JOURNAL OF HEALTH SCIENCE AND MEDICINE

YAYIN KURALLARI, YAYIN POLİTİKASI, GENEL İLKELER VE YAZIM KURALLARI

Tıp dergilerine gönderilecek makalelerin standart gereksinmeleri ile ilgili tüm bilgileri www.icmje.org internet adresinde bulabilirsiniz.

Amaç

Nisan 2018 itibariyle dergimiz ***"Journal of Health Sciences and Medicine"*** ilk sayısını çıkartarak yayın hayatına başlamış bulunmaktadır. ***"Journal of Health Sciences and Medicine"*** ulusal hakemli, açık erişimli ve periyodik olarak çıkan bir dergidir. Dergi yazım kurallarına göre düzenlenmiş makaleler ***DergiPark*** sistemi üzerinden kabul edilmektedir. Tüm sayılara web adresimizden ve Dergipark web sayfasından (http://dergipark.gov.tr/jhsm) ücretsiz olarak erişilebilmektedir**.** Amacımız uluslararası bir tabanda hastalıkların teşhis ve tedavisinde yenilikler içeren yüksek kalitede bilimsel makaleler yayımlamak ve bilime katkı sağlamaktır. Yılda dört kez çıkan bilimsel bir tıp dergisidir. Hakemli bir dergi olarak gelen yazılar biyomedikal makalelere ait Uluslararası Tıp Dergileri Editörleri Komitesi (www.icmje.org) tarafından tanımlanan standart gereksinimler ile ilgili ortak kurallara uygunluğu açısından değerlendirilmektedir. Dergimizde yayımlanmış makalelerin tamamına elektronik ortamdan ulaşabilir, web sitemizden okuyabilir, indirebilirsiniz. Amacımız siz meslektaşlarımızın göndermiş olduğu yayınların karar ve yayımlanma sürecini en kısa sürede sonuca ulaştırmaktır. Dergimizin kalitesini yükseltmek için her zaman önerilere ve yapıcı eleştirilere açık olduğumuzu ve bu konudaki bildirimlere gereken hassasiyeti göstereceğimizi belirtmek isterim.

Kapsam

Kapsam olarak tıbbın her dalı ile ilgili retrospektif/prospektif klinik ve laboratuvar çalışmaları, ilginç olgu sunumları, davet üzerine yazılan derlemeler, editöre mektuplar, orijinal görüntüler, kısa raporlar ve teknik yazıları yayımlayan bilimsel, ulusal hakemli bir dergidir. Derginin dili **İngilizce** ve **Türkçe**'dir. Makaleler hem **İngilizce** hem de **Türkçe** olarak kabul edilmektedir. Türkçe gönderilen makalelerde ayrıca İngilizce Başlık, Abstract, Keywords olmalı, İngilizce olarak gönderilen makalelerde de ayrıca Türkçe Başlık, Öz, Anahtar Kelimeler olmalıdır. Başka bir dergide yayımlanmış veya değerlendirilmek üzere gönderilmiş yazılar veya dergi kurallarına göre hazırlanmamış yazılar değerlendirme için kabul edilmez. Dergimizde yayımlanmış makalelerin tamamına elektronik ortamdan ulaşabilir, web sitemizden okuyabilir, indirebilirsiniz.

On-line Makale Gönderimi

Dergiyle tüm yazışmalar ve yazı gönderimleri http://dergipark.gov.tr/journal/2316/dashboard veya http://dergipark.gov.tr/jhsm üzerinden yapılmalıdır. Yazı gönderimi için detaylı bilgi bu internet adresinden edinilebilir. Gönderilen her yazı için özel bir numara verilecek ve yazının alındığı e-posta yolu ile teyit edilecektir. Makalelerin "full-text" pdf formuna bu sayfadan ulaşılabilir.

Derginin Yayın Sıklığı

Journal of Health Sciences and Medicine yılda dört kez olmak (Mart, Haziran, Eylül, Aralık) yayımlanan ulusal hakemli bir dergidir. Sağlık Bilimleri ve Tıp Dergisi duyurusu önceden yapılmak koşuluyla özel sayılar çıkarabilir.

Açık Erişim Politikası

Journal of Health Sciences and Medicine açık erişimi olan bir dergidir. Kullanıcılar yazıların tam metnine ulaşabilir, kaynak gösterilerek tüm makaleler bilimsel çalışmalarda kullanılabilir.

Aşağıdaki rehber dergiye gönderilen makalelerde aranan standartları göstermektedir. Bu uluslararası format, makale değerlendirme ve basım aşamalarının hızla yapılmasını sağlayacaktır.

Yazarlara Bilgi

Yazıların tüm bilimsel sorumluluğu yazar(lar)a aittir. Editör, yardımcı editör ve yayıncı dergide yayımlanan yazılar için herhangi bir sorumluluk kabul etmez.

Dergi Adının Kısaltması

İngilizce: J Health Sci Med / JHSM

Yazışma Adresi

Yazılar e-posta yoluyla sorumlu yazar tarafından, DergiPark'a kayıt olunduktan sonra http://dergipark.gov.tr/journal/2316/dashboard veya http://dergipark.gov.tr/jhsm linkine girip gönderilmelidir.

Makale Dili

Derginin dili **İngilizce** ve **Türkçe**'dir. Makaleler hem **İngilizce** hem de **Türkçe** olarak kabul edilmektedir. İngilizce makaleler gönderilmeden önce profesyonel bir dil uzmanı tarafından kontrol edilmelidir. Yazıdaki yazım ve gramer hataları içerik değişmeyecek şekilde İngilizce dil danışmanı tarafından düzeltilebilir. Türkçe yazılan yazılarda düzgün bir Türkçe kullanımı önemlidir. Bu amaçla, Türk Dil Kurumu Sözlük ve Yazım Kılavuzu yazım dilinde esas alınmalıdır.

Makalenin "Başka Bir Yerde Yayımlanmamıştır" İbaresi

Her yazar makalenin bir bölümünün veya tamamının başka bir yerde yayımlanmadığını ve aynı anda bir diğer dergide değerlendirilme sürecinde olmadığını, editöre sunum sayfasında belirtmelidirler. Kongrelerde sunulan sözlü veya poster bildirilerin, başlık sayfasında kongre adı, yer ve tarih verilerek belirtilmesi gereklidir. Dergide yayımlanan yazıların her türlü sorumluluğu (etik, bilimsel, yasal, vb.) yazarlara aittir.

Değerlendirme

Dergiye gönderilen yazılar format ve ***intihal*** açısından değerlendirilir. Formata uygun olmayan yazılar değerlendirilmeden sorumlu yazara geri gönderilir. Bu tarz bir zaman kaybının olmaması için yazım kuralları gözden geçirilmelidir. Basım için gönderilen tüm yazılar iki veya daha fazla yerli/yabancı hakem tarafından değerlendirilir. Makalelerin değerlendirilmesi, bilimsel önemi, orijinalliği göz önüne alınarak yapılır. Yayıma kabul edilen yazılar editörler kurulu tarafından içerik değiştirilmeden yazarlara haber verilerek yeniden düzenlenebilir. Makalenin dergiye gönderilmesi veya basıma kabul edilmesi sonrası isim sırası değiştirilemez, yazar ismi eklenip çıkartılamaz.

Basıma Kabul Edilmesi

Editör ve hakemlerin uygunluk vermesi sonrası makalenin gönderim tarihi esas alınarak basım sırasına alınır. Her yazı için bir doi numarası alınır.

Yayın Hakkı Devir Formu

Telif Hakkı Devir Formu, makalenin ana dilinde (makalenin dili İngilizce ise, İngilizce olmalıdır, makalenin dili Türkçe ise, Türkçe olmalıdır) doldurulmalı, http://dergipark.gov.tr/journal/2316/dashboard veya http://dergipark.gov.tr/jhsm adresi üzerinden online olarak gönderilmelidir. 1976 Copyright Act'e göre, yayımlanmak üzere kabul edilen yazıların her türlü yayın hakkı yayıncıya aittir.

Makale Genel Yazım Kuralları

Yazılar Microsoft Word programı ile çift satır aralıklı ve başlık yazıları (makale adı, öz, abstract, giriş, materyal metot, bulgular, tartışma, kaynaklar vs.) 12 punto olarak, makalenin diğer kısımları 11 punto olacak şekilde, her sayfanın iki yanında ve alt ve üst kısmında 2,5 cm boşluk bırakılarak yazılmalıdır. Yazı stili Times New Roman olmalıdır. "System International" (SI) unitler kullanılmalıdır. Şekil, tablo ve grafikler metin içinde refere edilmelidir. Kısaltmalar, kelimenin ilk geçtiği yerde parantez içinde verilmelidir. Türkçe makalelerde %50 bitişik yazılmalı, aynı şekilde İngilizcelerde de 50% bitişik olmalıdır. Türkçe'de ondalık sayılarda virgül kullanılmalı (55,78) İngilizce yazılarda nokta (55.78) kullanılmalıdır. Derleme 4000, orijinal çalışma 2500, olgu sunumu 1500, editöre mektup 500 kelimeyi geçmemelidir. Öz sayfasından itibaren sayfalar numaralandırılmalıdır.

Yazının Bölümleri

1. Sunum Sayfası

Journal of Health Sciences and Medicine"de yayımlanmak üzere değerlendirilmesi isteğinin belirtildiği, makalenin sorumlu yazarı tarafından dergi editörüne hitaben gönderdiği yazıdır. Bu kısımda makalenin bir bölümünün veya tamamının başka bir yerde yayımlanmadığını ve aynı anda bir diğer dergide değerlendirilme sürecinde olmadığını***, maddi destek ve çıkar ilişkisi*** durumu belirtmelidir.

2. Başlık Sayfası

Sayfa başında gönderilen makalenin kategorisi belirtilmedir (klinik analiz, orijinal çalışma, deneysel çalışma, olgu sunumu vs). Tüm yazarların ad ve soyadları yazıldıktan sonra üst simge ile 1'den itibaren numaralandırılıp, çalıştıkları kurum, klinik, şehir ve ülke yazar isimleri altına eklenmelidir. Bu sayfada ***"Sorumlu Yazar"*** belirtilmeli isim, açık adres, telefon ve e-posta bilgileri eklenmelidir (Dergimizin formatı gereği Adres bilgileri, kurumları makale dili Türkçe ise Türkçe olarak, İngilizce ise İngilizce olarak belirtilmelidir). Kongrelerde sunulan ***sözlü veya poster bildirilerin***, başlık sayfasında kongre adı, yer ve tarih verilerek belirtilmesi gereklidir.

3. Makale Dosyası

Yazar ve kurum isimleri bulunmamalıdır, bu bilgiler sadece başlık sayfasında olmalıdır).

Başlık: Kısa ve net bir başlık olmalıdır. Kısaltma içermemeli, Türkçe ve İngilizce olarak yazılmalıdır.

Öz: Türkçe ve İngilizce (Abstract) yazılmalıdır. Orijinal çalışmalarda Öz; ***Amaç, Gereç, Yöntem, Bulgular*** ve ***Sonuç*** bölümlerine ayrılmalı ve 400 kelimeyi geçmemelidir. Derleme, olgu sunumları ve benzerlerinde Öz; kısa ve tek paragraflık olmalı, derlemelerde 300, olgu sunumlarında 250 kelimeyi geçmemelidir.

Anahtar Kelimeler: Türkçe Öz ve İngilizce Abstract sonlarında bulunmalıdır. En az 3 en fazla 6 adet yazılmalıdır. Kelimeler birbirlerinden noktalı virgül ile ayrılmalıdır. İngilizce Anahtar Kelimeler (Keywords) "Medical Subject Headings (MESH)"e uygun (www.nlm.nih.gov/mesh/MBrowser.html) olarak verilmelidir.. Türkçe Anahtar Kelimeler "Türkiye Bilim Terimleri' ne uygun olarak verilmelidir (www.bilimterimleri.com). Bulunamaması durumunda bire bir Türkçe tercümesi verilmelidir.

Metin Bölümleri: ***Orijinal makaleler***; Giriş, Gereç ve Yöntem, Bulgular, Tartışma, Sonuç olarak düzenlenmelidir. ***Olgu sunumları***; Giriş, Olgu sunumu, Tartışma, Sonuç olarak düzenlenmelidir. Şekil, fotoğraf, tablo ve grafiklerin metin içinde geçtiği yerler ilgili cümlenin sonunda belirtilmeli metin içine yerleştirilmemelidir. Kullanılan kısaltmalar altındaki açıklamada belirtilmelidir. Daha önce basılmış şekil, resim, tablo ve grafik kullanılmış ise yazılı izin alınmalıdır ve bu izin açıklama olarak şekil, resim, tablo ve grafik açıklamasında belirtilmelidir. Tablolar metin sonuna eklenmelidir. Resimler/fotoğraf kalitesi en az 300 dpi olmalıdır.

Etik Kurallar: Klinik araştırmaların protokolü ***etik komitesi*** tarafından onaylanmış olmalıdır. İnsanlar üzerinde yapılan tüm çalışmalarda "Gereç ve Yöntem" bölümünde çalışmanın ilgili komite tarafından onaylandığı veya çalışmanın **Helsinki İlkeler Deklarasyonu'na** (www.wma.net/e/policy/b3.html) uyularak gerçekleştirildiğine dair bir cümle yer almalıdır. Çalışmaya dahil edilen tüm insanların "***Bilgilendirilmiş Onam Formu"***nu imzaladığı metin içinde belirtilmelidir. ***"Journal of Health Sciences and Medicine"***e gönderilen makalelerdeki çalışmaların Helsinki Deklarasyonu'na uygun olarak yapıldığını, kurumsal etik ve yasal izinlerin alındığını varsayacak ve bu konuda sorumluluk kabul etmeyecektir. Çalışmada "Hayvan" öğesi kullanılmış ise yazarlar, makalenin Gereç ve Yöntem bölümünde **Guide for the Care and Use of Laboratory Animals** (www.nap.edu/catalog/5140.html) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmek zorundadır.

Teşekkür Yazısı: Varsa kaynaklardan önce yazılmalıdır.

Maddi Destek ve Çıkar İlişkisi: Makale sonunda varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve varsa bu kuruluşların yazarlarla olan çıkar ilişkileri belirtilmelidir. Olmaması durumunda da "Çalışmayı maddi olarak destekleyen kişi/kuruluş yoktur ve yazarların herhangi bir çıkar dayalı ilişkisi yoktur" şeklinde yazılmalıdır.

Kaynaklar: Kaynaklar makalede geliş sırasına göre yazılmalıdır. Kaynaktaki yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 3 isim yazılıp **ve ark**. ("**et al**") eklenmelidir. Kaynak yazımı için kullanılan format Index Medicus'ta belirtilen şekilde olmalıdır (www.icmje.org). Kaynak listesinde yalnızca yayınlanmış ya da yayınlanması kabul edilmiş veya DOI numarası almış çalışmalar yer almalıdır. Dergi kısaltmaları "Cumulated Index Medicus" ta kullanılan stile uymalıdır. Kaynak sayısının araştırmalarda 40, derlemelerde 60, olgu sunumlarında 20, editöre mektupta 10 ile sınırlandırılmasına özen gösterilmelidir. Kaynaklar metinde cümle sonunda nokta işaretinden hemen önce parantez kullanılarak belirtilmelidir. Örneğin (4,5). Kaynakların doğruluğundan yazar(lar) sorumludur. Yerli ve yabancı kaynakların sentezine önem verilmelidir.

4. Şekil ve *Tablo Ba*şlıkları

Başlıklar kaynaklardan sonra yazılmalıdır. Her biri ayrı bir görüntü dosyası **(en az 300 dpi çözünürlükte, jpg)** olarak gönderilmelidir.

Makalenin basıma kabulünden sonra ***"Dizginin ilk düzeltme nüshası"*** sorumlu yazara e-posta yoluyla gönderilecektir. Bu metinde sadece yazım hataları düzeltilecek, ekleme çıkartma yapılmayacaktır. Sorumlu yazar düzeltmeleri 2 gün içinde bir dosya halinde e-posta ile yayın idare merkezine bildirecektir.

Kaynak Yazım Örnekleri

Dergilerden yapılan alıntı;

Cesur S, Aslan T, Hoca NT, Çimen F, Tarhan G, Çifci A. Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. Int J Mycobacteriol 2014; 3: 5-8.

Kitaptan yapılan alıntı;

Tos M. Cartilage tympanoplasty. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.

Tek yazar ve editörü olan kitaptan alıntı;

Neinstein LS. The office visit, interview techniques, and recommendations to parents. In: Neinstein LS (ed). Adolescent Health Care. A practical guide. 3rd ed. Baltimore: Williams&Wilkins; 1996: 46-60.

Çoklu yazar ve editörü olan kitaptan alıntı;

Schulz JE, Parran T Jr: Principles of identification and intervention. In:Principles of Addicton Medicine, Graham AW. Shultz TK (eds). American Society of Addiction Medicine, 3rd ed. Baltimore: Williams&Wilkins; 1998:1-10.

Eğer editör aynı zamanda kitap içinde bölüm yazarı ise;

Diener HC, Wilkinson M (editors). Drug-induced headache. In: Headache. First ed., New York: Springer-Verlag; 1988: 45-67.

Doktora/Lisans Tezinden alıntı;

Kılıç C. General Health Survey: A Study of Reliability and Validity. phD Thesis, Hacettepe University Faculty of Medicine, Department of Psychiatrics, Ankara; 1992.

Bir internet sitesinden alıntı;

Sitenin adı, URL adresi, yazar adları, ulaşım tarihi detaylı olarak verilmelidir.

DOI numarası vermek;

Joos S, Musselmann B, Szecsenyi J. Integration of complementary and alternative medicine into family practice in Germany: Result of National Survey. Evid Based Complement Alternat Med 2011 (doi: 10.1093/ecam/nep019).

Diğer referans stilleri için "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References" sayfasını ziyaret ediniz.

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