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Can Inflammatory Indices be Used to Predict Adverse Pregnancy Outcomes in Pregnant Women with Recurrent Urinary Tract Infection?

İnflamatuvar İndeksler Tekrarlayan İdrar Yolu Enfeksiyonu olan Gebelerde Olumsuz Gebelik Sonuçlarını Tahmin Etmek için Kullanılabilir mi?

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ABSTRACT

Aim: This study aimed to investigate the utility of inflammatory indices in predicting adverse maternal and neonatal outcomes in pregnant women with recurrent urinary tract infections.
Material and Methods: This retrospective study was conducted on pregnant women treated for symptomatic urinary tract infection (UTI) between 2017 and 2021. Pregnant women with two or more episodes of symptomatic UTI were included in the study group. Pregnant women with one UTI were included in the control group. The study group consisted of 91 (46.9%) patients and the control group consisted of 103 (53.1%) patients. The groups were compared in terms of clinical characteristics, adverse outcomes, and inflammatory indices.
Results: It was found that more adverse maternal and neonatal outcomes occurred in the study group compared to the control group (p=0.021, and p<0.001, respectively). The cut-off values for platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII) to predict adverse maternal outcomes were found 185.00 (p=0.015, area under the curve (AUC)=0.604, 95% confidence interval (CI)=0.558-0.782,), 4.34 (p=0.051, AUC=0.584, 95% CI=0.514-0.746), and 1210.48 (p=0.008, AUC=0.614, 95% CI=0.547-0.771), respectively. The cut-off values for PLR, NLR, and SII for predicting negative neonatal outcomes were found 192.98 (p=0.001, AUC=0.692, 95% CI=0.572-0.812), 4.67 (p=0.166, AUC=0.583, 95% CI=0.475-0.740), and 1339.47 (p=0.006, AUC=0.666, 95% CI=0.526-0.777), respectively.
Conclusion: Although the success of discrimination is weak, PLR and SII may be useful to predict adverse maternal and neonatal outcomes in pregnant women with recurrent UTI.
Keywords: Adverse pregnancy outcomes; inflammatory indices; recurrent urinary tract infections.

ÖZ

Amaç: Bu çalışmanın amacı tekrarlayan idrar yolu enfeksiyonu olan hamile kadınlarda inflamatuar indekslerin olumsuz maternal ve neonatal sonuçları tahmin etmedeki yararını araştırmaktır.
Gereç ve Yöntemler: Bu geriye dönük çalışma, 2017 ve 2021 yılları arasında semptomatik idrar yolu enfeksiyonu (İYE) nedeniyle tedavi edilen gebeler üzerinde yapılmıştır. İki veya daha fazla semptomatik İYE atağı olan gebeler çalışma grubuna dahil edilmiştir. Kontrol grubuna tek İYE geçiren gebeler dahil edilmiştir. Çalışma grubu 91 (%46,9) hastadan ve kontrol grubu ise 103 (%53,1) hastadan oluşmuştur. Gruplar klinik özellikler, olumsuz sonuçlar ve inflamatuar indeksler açısından karşılaştırıldı.
Bulgular: Kontrol grubu ile karşılaştırıldığında, çalışma grubunda daha fazla olumsuz maternal ve neonatal sonuçların meydana geldiği bulundu (sırasıyla p=0,021 ve p<0,001). Olumsuz maternal sonuçları öngörmek için trombosit-lenfosit oranı (TLR), nötrofil-lenfosit oranı (NLR) ve sistemik immün-inflamasyon indeksi (Sİİ) için kesim değerleri sırasıyla, 185,00 (p=0,015; eğri altında kalan alan (EAA)=0,604; %95 güven aralığı (GA)=0,558-0,782), 4,34 (p=0,051; EAA=0,584; %95 GA=0,514-0,746) ve 1210,48 (p=0,008; EAA=0,614; %95 GA=0,547-0,771) idi. Negatif neonatal sonuçları tahmin etmek için PLR, NLR ve Sİİ için kesim değerleri sırasıyla, 192,98 (p=0,001; EAA=0,692; %95 GA=0,572-0,812), 4,67 (p=0,166; EAA=0,583; %95 GA=0,475-0,740) ve 1339,47 (p=0,006; EAA=0,666; %95 GA=0,526-0,777) idi.
Sonuç: Ayırt etme başarısı zayıf olmakla birlikte, TLR ve Sİİ, tekrarlayan İYE'li gebe kadınlarda olumsuz maternal ve neonatal sonuçları tahmin etmek için faydalı olabilir.
Anahtar kelimeler: Olumsuz gebelik sonuçları; inflamatuar indeksler; tekrarlayan idrar yolu enfeksiyonları.

INTRODUCTION

Urinary tract infection (UTI) is the most common bacterial infection during pregnancy due to anatomical, hormonal, and immune changes (1,2). UTI is classified as asymptomatic and symptomatic UTI. Asymptomatic bacteriuria is defined as the presence of at least 105/ml bacterial colonies in the urine culture without any symptoms or signs. Symptomatic UTI is defined as the presence of bacteriuria accompanied by symptoms related to the localization of the infection in the urinary tract. Cystitis, which is a lower UTI, causes dysuria, frequency, urgency, and suprapubic pain and occurs in 1-2% of pregnant women. Pyelonephritis, an upper UTI, is characterized by symptoms, such as malaise, fever, nausea, vomiting, flank pain, and costovertebral angle tenderness, and its incidence is 0.5-1% (3).

Regardless of whether UTIs are symptomatic or asymptomatic, they have been associated with many adverse pregnancy outcomes, such as preterm birth, fetal growth restriction (FGR), low birth weight, and preeclampsia (4,5). Adverse pregnancy outcomes are more common in pyelonephritis that is characterized by renal parenchymal inflammation (6).

Recurrent urinary tract infection (RUTI) is the occurrence of two or more UTI episodes during pregnancy. Patients who have had UTI once are more likely to suffer from colonization of the urinary tract by the same or similar agents. UTI recurs in 4-5% of pregnancies (7,8). It has been demonstrated that continuous or postcoital antibiotic therapy in RUTI, which causes an increase in adverse pregnancy outcomes, reduces adverse outcomes (1,9,10).

Systemic inflammation is associated with changes in the number and function of blood cell components, and this underlies the inflammatory response. While clinical inflammation is characterized by symptoms, such as fever, pain, and redness, there is infiltration of the tissue by neutrophils, macrophages, and lymphocytes in subclinical inflammation (11). The level of inflammation can be evaluated by indices derived from full blood count (FBC) parameters. In recent years, the use of inflammatory indices obtained from FBC components to evaluate inflammation and predict adverse maternal and neonatal outcomes has become one of the research areas with increasing popularity. The mean platelet volume (MPV), red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are among the frequently used indices. The systemic immune-inflammation index (SII) is a new inflammatory index that has been developed in recent years and incorporates more FBC parameters, and a growing number of studies in the field of obstetrics have used SII (12-16).

The primary aim of this study is to calculate the cut-off values for inflammatory indices in predicting adverse maternal and neonatal outcomes in pregnant women with RUTI. The secondary aim is to analyze the outcomes of pregnancies treated for RUTI at our center.

MATERIAL AND METHODS

The study was conducted on pregnant women with recurrent, symptomatic UTI who were treated at Ankara Etlik Zübeyde Hanım Gynecology Training and Research Hospital between January 2017 and January 2021. The study was approved by the hospital's local ethics committee with a decision number 2021-03/6. Patient consent was renounced due to the retrospective nature of the study.

The diagnosis of UTI was based on the urine culture with a bacterial count $\geq 10^3$ colony-forming unit (CFU)/ml in pregnant women with symptoms, such as dysuria, frequency, urgency, suprapubic pain, fever, flank pain, and costovertebral angle tenderness. Cases of asymptomatic bacteriuria, defined as the presence of $\geq 10^5$ CFU/ml in the urine without any clinical symptoms, were not included in the study. The study group comprised singleton pregnant women with two or more episodes of symptomatic UTIs during pregnancy. The control group comprised pregnant women who had symptomatic UTI once at similar gestational weeks during pregnancy. Pregnant women with pregestational diabetes mellitus, chronic hypertension, collagen tissue disease, autoimmune disease, and smokers were excluded from the study. Multiple pregnancies were not included in the study. The data of the patients were obtained from the hospital's electronic archive and patient files.

The laboratory parameters included leukocyte, lymphocyte, neutrophil, and platelet counts, MPV, and RDW. While calculating inflammatory indices, absolute lymphocyte, neutrophil, and platelet counts in the FBC at the time of admission to the hospital and prior to the start of antibiotic therapy were used. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. The PLR was calculated by dividing the platelet count by the lymphocyte count. The following formula was used to calculate the SII: neutrophil x platelet/lymphocyte. In the study group, FBC values at the time of admission to the hospital in the last symptomatic UTI during pregnancy were used. In the control group, FBC values at the time of admission to the hospital due to UTI were used.

In the study, clinical data such as maternal age, gravida, parity, number of miscarriages, gestational week at diagnosis, gestational week at birth, mode of delivery, and adverse pregnancy outcomes, such as preterm birth, preeclampsia, FGR, small for gestational age (SGA), gestational diabetes mellitus (GDM), preterm premature rupture of membranes (PPROM), were evaluated. Birth weight, 1-minute, and 5-minute Apgar scores, and the need for neonatal intensive care were analyzed as newborn parameters. The diagnoses of preterm birth, FGR, SGA, preeclampsia, GDM, and PPROM were established according to the current guidelines (17-21).

An adverse maternal outcome was defined as the presence of any of the following conditions: preterm birth, FGR, SGA, preeclampsia, and PPROM. Adverse neonatal outcome was defined as the presence of any of the following findings: 1-minute Apgar score of <7, 5-minute Apgar score of <7, and the need for neonatal intensive care unit (NICU). The cut-off values for inflammatory indices were calculated for the prediction of composite adverse maternal and neonatal outcomes in the study group.

Statistical Analysis

Statistical analysis was performed using SPSS software version 26 (Armonk, NY: IBM Corp). Normality distribution was evaluated with the Kolmogorov-Smirnov test. Continuous data without normal distribution were expressed as median and interquartile range, and the

comparisons between the groups were made using the Mann-Whitney U test. Categorical data were expressed as number and percentage and analyzed using the chi-square and Fisher's exact tests. The performances of inflammatory indices in predicting adverse maternal and neonatal outcomes were evaluated using receiver operating characteristic (ROC) curve analysis. A p-value less than 0.05 was considered significant.

RESULTS

During the study period, 106 pregnant women were treated for RUTI. Seven patients were excluded from the study because of the presence of a concurrent disease during pregnancy (2 had familial Mediterranean fever, 1 had systemic lupus erythematosus, 3 had diabetes mellitus, and 1 had chronic hypertension). Four pregnant women with multiple pregnancies were excluded from the study. Pre-treatment laboratory parameters of four patients could not be accessed. The final study group included 91 patients. The control group comprised 103 patients who had had symptomatic UTI once during pregnancy and were matched for maternal age and gestational week. Demographic and clinical characteristics and laboratory parameters of the groups are presented in Table 1. The median gestational age at diagnosis was similar between the two groups (p=0.498). The median number of UTI episodes was 2 (range, 2-6) in the study group. 44 (48.4%) of the pregnant women in the study group and 66 (64.1%) of the pregnant women in the control group were nulliparous (p=0.027). Gravida and parity were significantly higher in the study group than in the control group (p=0.010, and p=0.008, respectively). Preterm birth and composite adverse maternal outcomes were more common in the study group than in the control group (p=0.001, and p=0.021, respectively). The need for admission to NICU and composite adverse neonatal outcomes were significantly more common in the study group (both p<0.001).

In the study, 196 urine cultures in the RUTI group and 103 urine cultures in the control group were analyzed. *E. coli* was the most commonly reproduced pathological agent in urine culture in both groups (n=141, 71.9% cases vs. n=73, 70.9% cases). *Klebsiella* spp., *S. aureus*, group B streptococci, and *Proteus* spp. were other common agents. The results of antimicrobial susceptibility tests were similar between the groups. In the entire population, the rate of drug resistance in *E. coli* isolates was 56.2% (n=168) for ampicillin, 23.1% (n=69) for ampicillin-sulbactam, 21.1% (n=63) for amoxicillin-clavulanic acid, and 25.1% (n=75) for trimethoprim/sulfamethoxazole. Mixed infection was detected in 5 (2.6%) cases in the RUTI group and 1 (0.97%) case in the control group.

FBC parameters and inflammatory indices in the groups are presented in Table 2. The platelet count was higher in the study group (p<0.001). SII and PLR were found to be significantly higher in the study group than in the control group (p=0.002, and p<0.001, respectively). The performances of inflammatory indices in predicting composite adverse maternal and neonatal outcomes were analyzed using the ROC curve (Table 3, Figure 1). The cut-off values in the ROC curves with the most ideal sensitivity and specificity balance that can be used to predict adverse maternal and neonatal outcomes were obtained using the Youden index.

Table 1. Comparison of demographic and clinical characteristics between pregnant women with and without recurrent UTI

	Recurrent UTI (n=91)	**Non-recurrent UTI (n=103)**	**p**
Age (years)	25 (7) [17-41]	25 (7) [17-45]	0.359
Gravida	2 (2) [1-11]	1 (1) [1-5]	**0.010**
Parity	1 (2) [0-6]	0 (1) [0-4]	**0.008**
Nulliparity, n (%)	44 (48.4%)	66 (64.1%)	**0.027**
Miscarriages	0 (0) [0-5]	0 (0) [0-2]	0.108
Gestational age at diagnosis (weeks)	28 (7) [20-37]	28 (8) [13-39]	0.498
Number of UTIs in pregnancy	2 (0) [2-6]	1 (0) [1-1]	**<0.001**
Hydronephrosis, n (%)	9 (9.9%)	2 (1.9%)	**0.017**
Preeclampsia, n (%)	10 (11.0%)	7 (6.8%)	0.303
Fetal growth restriction, n (%)	7 (7.7%)	5 (4.9%)	0.413
Small for gestational age, n (%)	11 (12.1%)	11 (10.7%)	0.758
Gestational diabetes mellitus, n (%)	4 (4.4%)	4 (3.9%)	1.000
Preterm premature rupture of membranes, n (%)	4 (4.4%)	3 (2.9%)	0.708
Preterm birth, n (%)	35 (38.5%)	17 (16.5%)	**0.001**
Composite adverse maternal outcomes, n (%)	42 (46.2%)	31 (30.1%)	**0.021**
Gestational age at delivery (week)	38 (3) [24-41]	38 (3) [26-41]	**0.004**
Route of delivery, n (%)			
Cesarean section	45 (49.5%)	46 (44.7%)	0.505
Vaginal delivery	46 (50.5%)	57 (55.3%)	
Birth weight (gram)	3000 (680) [565-4120]	3050 (515) [915-4100]	0.065
1st minute Apgar	9 (0) [2-9]	9 (0) [6-9]	**0.020**
5th minute Apgar	10 (1) [2-10]	10 (0) [7-10]	**0.019**
Neonatal intensive care unit admission, n (%)	21 (23.1%)	4 (3.9%)	**<0.001**
Composite adverse neonatal outcomes, n (%)	22 (24.2%)	5 (4.9%)	**<0.001**

UTI: urinary tract infection, descriptive statistics were presented as median (interquartile range) [min-max] for numerical variables, and n (%) for categorical variables

Table 2. Comparison of full blood count parameters and inflammatory indices in the groups

	Recurrent UTI (n=91)	Non-recurrent UTI (n=103)	p
Leukocytes (10^3/μL)	9.03 (4.5) [4.3-22.8]	8.2 (4.5) [3.9-21.6]	0.211
Neutrophils (10^3/μL)	7.4 (4) [3.6-21.7]	6.8 (4.1) [1.5-20.2]	0.224
Lymphocytes (10^3/μL)	1.5 (0.7) [0.3-2.9]	1.6 (0.6) [0.5-3.5]	0.489
Platelet (10^3/mm^3)	293 (92) [136-448]	251 (76) [104-504]	**<0.001**
Mean platelet volume (fL)	8.7 (1.6) [5.6-12.2]	8.8 (1.5) [5.2-11.5]	0.817
Red cell distribution width (%)	14.1 (1.5) [12.3-31.5]	14.5 (1.7) [12.4-19.9]	0.109
Neutrophil-to-lymphocyte ratio	4.5 (5) [1.7-25.3]	4.2 (3.4) [0.4-24.6]	0.158
Platelet-to-lymphocyte ratio	201.5 (91.7) [78.5-725.9]	166.9 (81.2) [82.4-494.1]	**<0.001**
Systemic immune-inflammation index (10^3/mm^3)	1402.3 (1611.5) [455.9-7615.3]	1111.4 (896.1) [181.9-6201.2]	**0.002**

UTI: urinary tract infection, descriptive statistics were presented as median (interquartile range) [min-max] for numerical variables, and n (%) for categorical variables

Table 3. ROC curve analysis for assessing the performance of NLR, PLR, and SII values in predicting composite adverse maternal and neonatal outcomes for RUTI

	AUC	95% CI	p	Cut-off	Sensitivity	Specificity
Maternal outcomes						
NLR	0.584	0.514 - 0.746	0.051	4.34	60.3	53.7
PLR	0.604	0.558 - 0.782	**0.015**	185.00	60.3	60.3
SII	0.614	0.547 - 0.771	**0.008**	1210.48	67.1	58.7
Neonatal outcomes						
NLR	0.583	0.475 - 0.740	0.166	4.67	59.3	58.3
PLR	0.692	0.572 - 0.812	**0.001**	192.98	74.1	60.1
SII	0.666	0.526 - 0.777	**0.006**	1339.47	70.4	60.1

ROC: receiver operating characteristic, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammatory index, RUTI: recurrent urinary tract infections, AUC: area under the curve, CI: confidence interval.

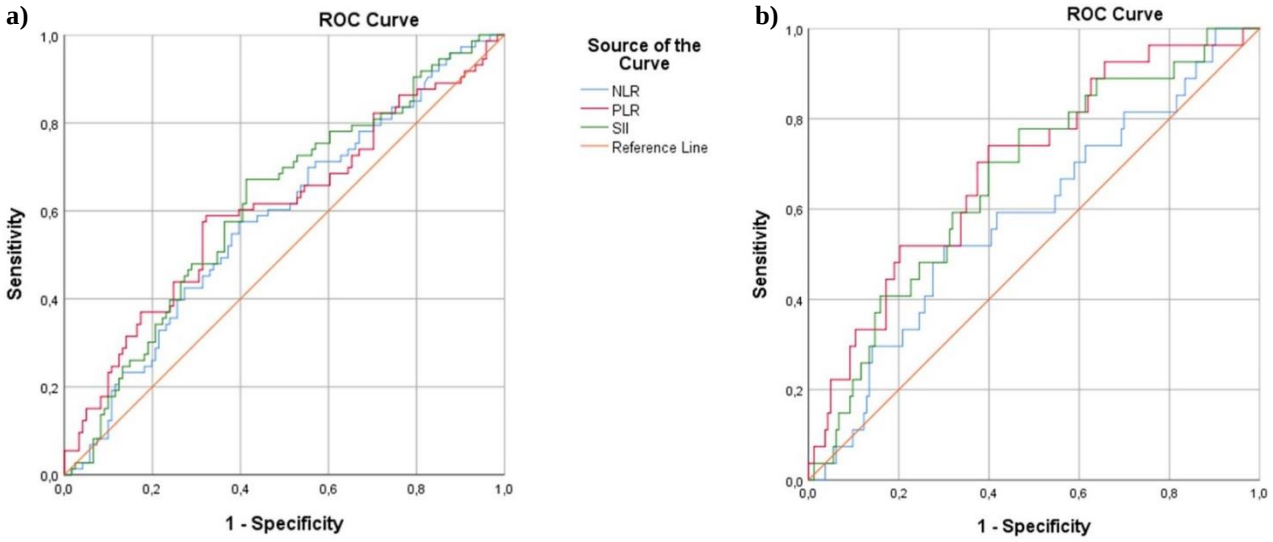

Figure 1. Receiver operating characteristic curve for **a)** composite adverse maternal outcomes, and **b)** composite adverse neonatal outcomes in pregnant women with recurrent urinary tract infection

DISCUSSION

The main finding of this study is that inflammatory indices are elevated in pregnancies with symptomatic RUTI, especially the high platelet count draws attention. There was no significant difference in leukocyte components between the groups. The present findings suggest that inflammatory indices could be used to predict composite adverse maternal and neonatal outcomes in pregnancies with RUTI.

The alterations in the urogenital system associated with pregnancy cause more frequent occurrence of UTIs, easy dissemination, and more frequent recurrence of the infection (22). Previous studies have shown that bacteriuria, whether symptomatic or asymptomatic, is associated with adverse maternal and neonatal outcomes (23-25). RUTIs are associated with an increased incidence of adverse outcomes as bacteriuria persists for a longer period of time

during pregnancy (9,26). In this study, preterm birth, which was seen at a rate of 37.5%, was the most prominent adverse pregnancy outcome caused by RUTIs. Adverse outcomes such as FGR, SGA, preeclampsia, and PPROM were also observed more frequently, but this difference did not gain statistical significance. Upon review of the literature, it is noteworthy that the prevalence of preterm birth and low birth weight is high, although the treatment of UTIs with antibiotics causes a decrease in negative outcomes (27). Inflammation caused by bacterial colonization in the urinary tract plays a role in the etiology of preterm birth. Inflammation leads to the production of cytokines by the leukocytes, resulting in cervical ripening and disrupting myometrial silence (28). Maternal inflammation also affects fetoplacental tissues, disrupting vascular structure and circulation, leading to an increase in adverse neonatal outcomes (4). Consistent with this information, this study shows that composite adverse maternal and neonatal outcomes are more common in RUTIs.

The immune system changes occurring during pregnancy are necessary for normal maternal-neonatal interaction. This change plays an active role in the development and regulation of the fetoplacental component (4). The effects of inflammation during pregnancy on adverse outcomes have been extensively investigated in the field of obstetrics, and its relationship with maternal and neonatal prognosis has been evaluated. NLR, PLR, and SII are inflammatory indices obtained by the combination of FBC components (12-14). When the FBC components were analyzed in pregnancies with RUTI, a significant increase in platelet count was noted in the study group than in the control group. No significant difference was observed in other components. The role of leukocytes in inflammation has been mentioned in previous sections. Along with their hemostatic functions, platelets also play a role in chemotaxis, tissue regeneration, and inflammatory reactions. They are also involved in implantation and vascular development and remodeling in pregnancy (29).

This study shows that inflammatory indices incorporating platelet count in the calculation can be used to predict adverse maternal and neonatal outcomes in pregnant women with RUTI. The cut-off values for PLR and SII in predicting the composite adverse maternal outcomes were found 185.00, and 1210.48, respectively. In the prediction of composite adverse neonatal outcomes, the cut-off values for PLR and SII were found 192.98, and 1339.47, respectively.

Adverse pregnancy outcomes can be reduced by appropriate and effective antibiotic therapy for UTI. In the presence of recurrent infections, preventing bacteriuria is important in reducing morbidity. It has been demonstrated that complications are reduced with continuous or postcoital antibiotic therapy in these pregnant women (1,30). The effects of antibiotic therapy during pregnancy on the fetus, and the infant in the neonatal and childhood periods are still the subject of research (31-33). The concerns of parents regarding drug use during pregnancy reduce adherence to treatment. Therefore, we believe that inflammatory indices can be used to predict adverse pregnancy outcomes in pregnant women with RUTI, as well as to guide prophylactic antibiotic therapy and evaluate treatment adherence.

The limitations of this study are its retrospective nature, small number of patients, and single-center study design. The patients with acute cystitis were also included in this study. Cystitis is a UTI confined to the bladder without signs and symptoms of systemic infection. Because of the discomfort caused by its symptoms, cystitis is often treated early. Although it does not cause a significant increase in pregnancy complications on its own, it causes a predisposition to bacterial colonization (1,34,35). We believe that the low sensitivity and specificity rates found in the present study can be explained by this situation, new cut-off values with higher sensitivity and specificity can be found with the inclusion of an ideal number of patients.

CONCLUSION

PLR and SII are useful in predicting composite maternal and neonatal outcomes in pregnancies with RUTI and can be used to guide patients' management. However, their effectiveness needs to be supported by prospective studies involving a larger number of patients.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Gynecology Training and Research Hospital (26.02.2021, 03/6).

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Polycystic Ovary Syndrome in Adolescent Period: Physical, Metabolic, and Hormonal Features

Adölesan Dönemde Polikistik Over Sendromu: Fiziksel, Metabolik ve Hormonal Özellikler

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ABSTRACT

Aim: The Rotterdam criteria are used in the diagnosis of polycystic ovary syndrome (PCOS). However, since menstrual irregularities and acne are seen more frequently in adolescents, it is theoretically difficult to use the Rotterdam criteria. The aim of this study was to investigate whether the Rotterdam criteria are sufficient for the diagnosis of PCOS in adolescence.
Material and Methods: Thirty patients and thirty healthy individuals between the ages of 16 and 18 years were included in the study. Anthropometric measurements, Ferriman Gallwey score (FGS), ultrasonographic ovarian volumes, androgens, and other hormone levels of the participants were recorded.
Results: There was a statistically significant positive correlation between the homeostatic model assessment of insulin resistance (HOMA-IR) and waist circumference/hip circumference ratio in the patient group ($r=0.656$, $p<0.001$). The mean luteinizing hormone (LH) levels of the patient group were found to be statistically significantly higher than the control group ($p=0.048$). The median FGS of the patient group was statistically significantly higher than the control group ($p<0.001$). Serum total testosterone levels of adolescent girls who have polycystic ovaries were statistically significantly higher than the ones who have not ($p=0.001$). The median FGS of those with polycystic ovarian appearance on ultrasonography was found to be statistically significantly higher than those without ($p=0.034$).
Conclusion: Among the Rotterdam diagnostic criteria, oligoanovulation, acne, and polycystic ovarian appearance on ultrasonography can be seen in healthy adolescents without PCOS. In this study, the most supportive finding for the diagnosis of PCOS was the appearance of polycystic ovaries accompanying moderate hirsutism.
Keywords: Polycystic ovary syndrome; adolescent; free androgen index; HOMA-IR.

ÖZ

Amaç: Polikistik over sendromu (PKOS) tanısı için Rotterdam kriterleri kullanılmaktadır. Bununla birlikte, adolesanlarda adet düzensizlikleri ve sivilceler daha sık görüldüğünden teorik olarak Rotterdam kriterlerini kullanmak zordur. Bu çalışmanın amacı ergenlik çağındaki PKOS tanısı için Rotterdam kriterlerinin yeterli olup olmadığını incelemektir.
Gereç ve Yöntemler: Çalışmaya 16 ve 18 yaş arası 30 hasta ve 30 sağlıklı birey dahil edildi. Çalışmaya dahil edilenlerin antropometrik ölçümleri, Ferriman Gallwey skoru (FGS), ultrasonografik over hacimleri, androjenler ve diğer hormon seviyeleri kaydedildi.
Bulgular: Hasta grubunda insülin direncinin homeostatik model değerlendirmesi (HOMA-IR) ile bel çevresi/kalça çevresi oranı arasında istatistiksel olarak anlamlı pozitif bir korelasyon vardı ($r=0,656$; $p<0,001$). Hasta grubunun ortalama luteinizing hormone (LH) düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı derecede daha yüksek olarak bulundu ($p=0,048$). Hasta grubunda medyan FGS kontrol grubundan istatistiksel olarak anlamlı derecede daha yüksekti ($p<0,001$). Polikistik overi olan adolesan kızların serum total testosteron düzeyleri polikistik overi olmayanlara göre istatistiksel olarak anlamlı derecede daha yüksekti ($p=0,001$). Ultrasonografide polikistik over görünümü olanların ortanca FGS'si polikistik over görünümü olmayanlara göre istatistiksel olarak anlamlı derecede daha yüksek olarak bulundu ($p=0,034$).
Sonuç: Rotterdam tanı kriterlerinden oligoanovulasyon, akne ve ultrasonografide polikistik over görünümü, PKOS olmayan sağlıklı adolesanlarda da görülebilir. Bu çalışmada PKOS tanısını en çok destekleyen bulgu, orta derecede hirsutizme eşlik eden polikistik over görünümü idi.
Anahtar kelimeler: Polikistik over sendromu; ergen; serbest androjen indeksi; HOMA-IR.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrinological disorder characterized by menstrual irregularity and hyperandrogenism (1). At least two of the following Rotterdam 2003 criteria are necessary for diagnosis (2). These criteria include oligo-anovulation over six months, clinical or laboratory findings of hyperandrogenism, and polycystic appearance of ovaries on ultrasonography.

The most common earliest findings of PCOS start with the maturation of the Hypothalamic-hypophyseal-ovarian axis. Due to this axis's developmental delay in some adolescents, it is sometimes difficult to differentiate the menstrual pattern from the anovulation associated with puberty. Although most adolescents start to have regular periods in the second year after the first menstruation, the irregularity may continue without reason (3). Studies showed that the responsible cause is PCOS in 20% of the patients with amenorrhea and 75% of infertile women. Even though 25% of the women of childbearing age have a polycystic appearance on ultrasonography, only 10% of these women have a PCOS diagnosis (4,5).

Excess androgen has an essential role in PCOS pathophysiology. More than 80% of the patients with PCOS have hyperandrogenism (6). Clinical findings of high serum androgen levels, such as acne and hirsutism, may not be optimal for adolescent girls' PCOS evaluation because 90% of the average teenage population has acne. The frequency of acne drops to 23% in adulthood age (7). Hirsutism is a more reliable clinical criterion to predict hyperandrogenism (8). As there is no other scale to evaluate hirsutism, the Ferriman Gallwey score (FGS) is also used in adolescents (9).

PCOS is a disorder characterized by menstrual irregularity and hyperandrogenism, in which Rotterdam criteria are used to diagnose. Since menstrual irregularities and acne are seen more frequently in adolescents, it is theoretically difficult to use the Rotterdam criteria. We examine to show whether the Rotterdam diagnostic criteria are sufficient for the diagnosis of PCOS in adolescence.

MATERIAL AND METHOD

In this study carried out to investigate the parameters used in the diagnosis of PCOS in adolescents, two groups, the PCOS group (16-18 years old, 2 years past the first menstrual period) and the control group (16-18 years old, 2 years past the first menstrual period), were used. The smallest significant difference between the luteinizing hormone (LH) values, which is one of the parameters to be calculated between groups, is 6.2 units of source (IU/L), and it is necessary to study with a minimum of 27 in each group and a minimum of 54 adolescents in total, with 80% power and 5% type I error (10).

We included 30 newly diagnosed patients with PCOS and 30 healthy volunteers without any complaints or diseases aged between 16 and 18 years who were admitted to the Endocrinology outpatient clinic at Mersin University between 01.10.2016 and 01.10.2017.

This age group was included because people over the age of 16 can apply to the adult endocrinology polyclinic. Cases with any chronic disease or using oral contraceptives were excluded. All of the patients had a dermatological examination in terms of acne or acanthosis nigricans. We determined the hirsutism level according to the FGS. Patients who had FGS ≥8 and/or acne were accepted as clinically hyperandrogenic.

We collected 10-15 ml of blood samples from each patient between 3-5 days of the menstrual cycle for biochemical and hormonal tests. Patients not having had menstruation for more than three months were started on 10 mg/day of medroxyprogesterone for seven days. Blood samples of these patients were collected on the third day of menstrual bleeding under medroxyprogesterone treatment. As the test results may vary according to hunger and satiety, the sample collection time was between 8:00-10:00 am. The height, weight, waist circumference (WC), and hip circumference (HC) of all cases were measured and recorded. We used a homeostatic model assessment of insulin resistance (HOMA-IR) to calculate insulin resistance. It is the value obtained by dividing the product of plasma insulin level and plasma glucose level (mg/dl) by 405.

The same gynecologist evaluated all cases' ovaries by ultrasonography between 3-5 days of the menstrual cycle. Length, thickness, width, number of follicles, and parameters of the largest and smallest follicles of patients' ovaries were recorded. Ovarian volume was calculated as the following formula (11-14):

length x thickness x width x pi number / 6 (Figure 1).

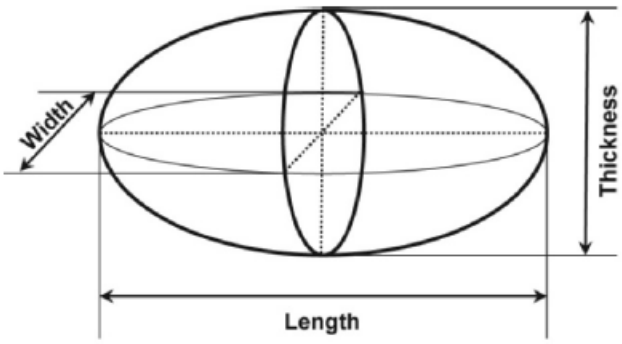

Figure 1. Dimensions of the ellipsoid ovary

The presence of 12 or more antral follicles of 2-9 mm in one or both ovaries; or ovarian volume over 10 cm^3 was evaluated in favor of PCOS (15,16). This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Mersin University Ethics Committee (06.10.2016, 305). Written informed consent of all patients and controls were obtained.

Statistical Analysis

The Shapiro-Wilk test was used for normal distribution control. Numerical variables that provide normality assumption were summarized in terms of mean±standard deviation, and variables that do not provide are outlined in terms of the median (minimum-maximum). In comparing the two groups, the independent sample t-test was used when the normality assumption was provided, and the Mann-Whitney U test was used when it was not provided. Pearson and Spearman correlation coefficient was calculated for the relationship between two continuous variables. $p<0.05$ was taken as the significance level, and statistical analyses were done with SPSS v.20 package.

RESULTS

A total of 60 adolescent girls aged between 16 and 18 years, 30 of whom were in the patient group newly diagnosed with PCOS and 30 in the control group, were included in the study. The menstrual cycle length of the patient group was significantly longer than the control group (p<0.001). While the menstrual period in the patient group was 40-90 days, it was 24-35 days in the control group. One (3.3%) of the cases in the patient group did not have oligomenorrhea. 60% (n=18) of the patients had six or fewer, 23.3% (n=7) had four or fewer, and 13.3% (n=4) had three or fewer menstrual cycles per year. The annual number of menstrual cycles in the control group was ten or more. General anthropometric and ultrasonographic characteristics of the groups were shown in Table 1.

The mean WC (78.93±16.56 cm) of the patient group was found to be statistically significantly higher than the mean WC (68.37±10.33 cm) of the control group (p=0.006). Also, the mean HC (100.75±12.38 cm) of the patient group was found to be statistically significantly higher than the HC (91.23±8.22 cm) of the control group (p=0.001). While the mean WC/HC ratio of the patient group was 0.77±0.08, the mean WC/HC ratio of the control group was 0.75±0.12. The WC/HC ratio of the patient group was not statistically different from the control group (p=0.345). While the mean body mass index (BMI) index of the patient group was 24.00±6.81, the mean BMI of the control group was 20.72±3.92. The BMI of the patient group was found to be statistically significantly higher than the control group (p=0.031).

Acne was detected in 26 (86.7%) adolescents in the patient group, while 14 (46.7%) adolescents in the control group had acne (p=0.001).

When the patient group was compared with the control group in terms of HOMA-IR, there was no statistically significant difference (p=0.871). The median HOMA-IR level was found to be 2.5 (range, 0.6-17) in the patient group, and 2.45 (range, 1-8.7) in the control group. HOMA-IR ≥2.5, as an indicator of insulin resistance, was considered high. The HOMA-IR was found to be high in 13 (43.3%) patients in the patient group and 15 (50.0%) adolescents in the control group (p=0.605).

There was a statistically significant positive correlation between HOMA-IR and WC/HC ratio (r=0.656, p<0.001) in the patient group, also a statistically significant positive correlation was found between HOMA-IR and BMI in the patient group (r=0.645, p<0.001).

The mean LH level (9.43±6.55 IU/L) of the patient group was found statistically significantly higher than the mean LH level (6.61±3.88 IU/L) of the control group (p=0.048). There was no significant difference between the groups in terms of serum sex hormone binding globulin (SHBG) levels and dehydroepiandrosterone sulfate (DHEAS) levels (p=0.987, and p=0.857, respectively). A significant difference was found between the groups of serum total testosterone (p=0.032) and 17-OH progesterone (p=0.039) levels, and both parameters were higher in the patient group. The mean total testosterone level was found to be 0.34±0.18 ng/ml in the patient group and 0.25±0.13 ng/ml in the control group. The median 17-OH progesterone level was 0.56 (range, 0.20-1.62) ng/ml in the patient group, while it was 0.46 (range, 0.20-1.34) ng/ml in the control group (Table 2).

There was no significant difference in the free androgen index (FAI) level between the two groups (p=0.264). FAI reference sample was 1-3%. The number of adolescents with FAI >3% was determined as 8 (26.7%) in the patient group and as 4 (13.3%) in the control group (p=0.197). Although it was not statistically significant the number of adolescents with high FAI was higher in the patient group than in the control group.

The total number of follicles (22.87±7.71) in the patient group was found to be statistically significantly higher than the total number of follicles (13.39±5.59) in the control group (p<0.001). There was no statistically significant correlation between FAI and the total number of follicles in the patient group (r=0.161, p=0.432).

The median FGS (13.5; range, 3-29) of the patient group was found statistically significantly higher than the median FGS (3, range, 1-11) of the control group (p<0.001).

In the patient group, the number of patients with a polycystic ovarian appearance on ultrasonography was 23 (76.7%), while 8 (26.7%) of the control group had a polycystic ovarian appearance in the ovaries on ultrasonography (p<0.001).

An inverse difference was found between polycystic ovarian appearance and HOMA-IR (p=0.020). HOMA-IR was found to be higher in adolescents without polycystic ovarian appearance with a median of 3.2 (range, 1-17) than in adolescents with polycystic ovarian appearance with a median of 2.2 (range, 0.6-8.7). There was no significant difference between adolescents with and without a polycystic ovarian appearance in terms of serum SHBG levels (p=0.743) and DHEAS (p=0.315). Serum total testosterone level (0.36±0.16 ng/ml) of adolescent girls who have a characteristic sonographic appearance of polycystic ovaries were statistically significantly higher than the serum total testosterone level (0.23±0.14 ng/ml) of the ones who had not a characteristic sonographic appearance of polycystic ovaries (p=0.001). Serum 17-OH progesterone levels of adolescent girls who have a characteristic sonographic appearance of polycystic ovaries were statistically significantly higher than the ones who had not (p=0.001). The median FGS of those with a polycystic ovarian appearance on ultrasonography was found to be statistically significantly higher than those without polycystic ovarian appearance (p=0.034, Table 3).

Table 1. Comparison of demographic, anthropometric, and ultrasonographic measurements

	PCOS (n=30)	Control (n=30)	p
Age (year)	17.27±0.83	16.80±0.71	**0.023**
First menstrual age	12.57±0.97	12.80±0.66	0.283
Height (cm)	163.89±5.83	165.03±5.79	0.458
Weight (kg)	66.21±20.96	57.17±10.93	**0.048**
WC (cm)	78.93±16.56	68.37±10.33	**0.006**
HC (cm)	100.75±12.38	91.23±8.22	**0.001**
WC/HC ratio	0.77±0.08	0.75±0.12	0.345
BMI (kg/m^2)	24.00±6.81	20.72±3.92	**0.031**
Right OV (cm^3)	6.48±3.32	5.67±2.99	0.335
Left OV (cm^3)	6.15±3.12	5.07±2.29	0.144

PCOS: polycystic ovary syndrome, WC: waist circumference, HC: hip circumference, BMI: body mass index, OV: ovarian volume

Table 2. Comparison of laboratory and hormonal parameters

	PCOS (n=30)	Control (n=30)	p
Fasting blood glucose (mg/dl)	89.90±6.65	88.37±8.19	0.429
Insulin (IU/ml)	11 (15.8) [3-84]	11 (6) [5-37]	0.982
Homeostatic model assessment for insulin resistance	2.5 (3.9) [0.6-17]	2.45 (1.4) [1-8.7]	0.871
Triglyceride (mg/dl)	86.07±26.83	90.40±30.15	0.563
Total cholesterol (mg/dl)	159.76±26.41	164.00±26.94	0.544
Low density lipoprotein cholesterol (mg/dl)	84.68±22.27	85.00±21.57	0.956
High density lipoprotein cholesterol (mg/dl)	56.62±13.93	60.53±13.60	0.280
Thyroxine (pmol/L)	15.95±3.371	16.02±2.42	0.926
Thyroid stimulating hormone (IU/ml)	1.99±1.21	1.85±0.91	0.608
Follicle stimulating hormone (IU/L)	5.37±1.81	5.30±2.33	0.888
Luteinizing hormone (IU/L)	9.43±6.55	6.61±3.88	**0.048**
Prolactin (ng/ml)	22.44±10.02	19.52±9.41	0.249
Sex hormone binding globulin (mg/L)	4.84 (4.52) [0.76-17.85]	4.78 (2.50) [1.81-8.92]	0.987
Total testosterone (ng/ml)	0.34±0.18	0.25±0.13	**0.032**
17-OH progesterone (ng/ml)	0.56 (0.35) [0.20-1.62]	0.46 (0.18) [0.20-1.34]	**0.039**
Dehydroepiandrosterone sulfate (ug/dl)	313.40±164.95	306.87±108.17	0.857
Free androgen index	2.05 (2.77) [0.12-18.01]	1.22 (1.59) [0.33-6.05]	0.264
Ferriman Gallwey score	13.5 (6) [3-29]	3 (4) [1-11]	**<0.001**

Table 3. Comparison of adolescents with and without a polycystic appearance

	With PA (n=31)	Without PA (n=29)	p
Homeostatic model assessment for insulin resistance	2.2 (2.0) [0.6-8.7]	3.2 (3.4) [1-17]	**0.020**
Sex hormone binding globulin (mg/L)	4.84 (3.08) [0.76-14.72]	4.82 (4.45) [1.29-17.85]	0.743
Total testosterone (ng/ml)	0.36±0.16	0.23±0.14	**0.001**
17-OH progesterone (ng/ml)	0.56 (0.42) [0.26-1.62]	0.44 (0.19) [0.20-1.34]	**0.001**
Dehydroepiandrosterone sulfate (ug/dl)	327.65±154.56	290.75±120.54	0.315
Free androgen index	2.10 (2.44) [0.33-18.01]	1.19 (0.81) [0.12-10.40]	0.266
Ferriman Gallwey score	10 (10) [1-29]	4.5 (10) [1-18]	**0.034**

DISCUSSION

The primary aim of this study was to examine whether the Rotterdam diagnostic criteria are sufficient for PCOS diagnosis in adolescence. The secondary purpose was to establish the relationships of HOMA-IR and FAI with clinical and laboratory findings of PCOS.

One of the clinical signs of hyperandrogenism is acne vulgaris. While 26 (86.7%) of the patients included in this study had acne, 14 (46.7%) of those included in the control group had acne vulgaris. According to the study by Kaewnin et al. (17), the presence of moderate acne in adolescence was determined as the most decisive risk factor for PCOS.

One of the diagnostic criteria for PCOS, according to Rotterdam 2003 consensus, is oligoanovulation. In this study, 26 (86.7%) of the 30 patients had oligoanovulation for at least six months. In a study by van Hooff et al. (18) involving school-age children in adolescence, it was found that oligoanovulation continued at the age of 18 due to unknown reasons in 52% of those aged 15-18 years.

Although a significant positive correlation was found between BMI and HOMA-IR in our study, the detection of insulin resistance in thin individuals shows that this relationship is not clear. Zeng et al. (19), showed that the relationship between obesity and insulin resistance was disproportionate. The BMI was more than 25 kg/m^2 in only 6 (20%) of the patients in the patient group participating in this study. The reason why insulin resistance is not related to polycystic appearance may be due to the lower BMI of the patients included in this study. Again, the presence of insulin resistance in the adolescent group without PCOS may also explain this situation. In this study, no significant relationship was found between HOMA-IR and the total number of follicles. Similarly, in the study of Reid et al. (20), no significant relationship was found between HOMA-IR and the number of follicles.

There was no significant difference between the groups in terms of serum SHBG levels. Many studies are showing that low levels of SHBG are associated with PCOS. Qu et al. (21), reported that the SHBG levels were associated with an increased risk of PCOS.

In this study, no significant difference was found between the groups regarding DHEAS levels. Kumar et al. (22) found the total T, free T, A4, DHEAS, and BMI were higher in women with PCOS than in control women.

Besides, there was no significant difference in FAI and HOMA-IR between both groups, there was a significant difference in FGS. In a comparative study by Hernandez et al. (23), including 25 patients with clinical findings of hyperandrogenism and 21 healthy adolescents, FAI, FGS, free testosterone, and total testosterone levels were significantly higher in the patient group.

Higher levels of total testosterone and 17-OH progesterone were found in patients with a polycystic ovarian appearance on ultrasonography compared to those without a polycystic ovarian appearance on ultrasonography. There was a statistically significant difference between the patient and control group for those with and without a polycystic ovarian appearance on ultrasonography.

There were 23 (76.7%) patients with a polycystic ovarian appearance on ultrasonography, while the number of patients with a polycystic ovarian appearance on ultrasonography was 8 (26.7%) in the control group. The most common PCOS subtype in the literature is the classic type with polycystic ovarian morphology. This group constitutes approximately 2/3 of the cases. In a study by Rosenfield et al. (24), the polycystic ovary rate on ultrasonography was 95%.

Mean FGS was significantly higher in the patient group than in the control group. Likewise, mean FGS was higher in patients with a polycystic appearance on ultrasonography than those without. In a cross-sectional study involving adolescent girls, Ybarra et al. (25), found the high FGS patients rate to be 16.8%.

CONCLUSIONS

In conclusion, among the Rotterdam diagnostic criteria, oligoanovulation, acne, and the polycystic appearance of ovaries on ultrasonography can be seen in healthy adolescents without PCOS. In our study, in terms of PCOS diagnosis in adolescents, polycyclic ovaries accompanying moderate hirsutism are the findings that support the diagnosis most. At the end of the study, some of the Rotterdam diagnostic criteria we used for adolescents can also be seen in healthy adolescents. This makes the diagnosis of PCOS difficult in adolescents. Studies involving more patients and control groups in the adolescent age group are required to obtain more precise results.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Mersin University (06.10.2016, 305).

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Effect of Tocilizumab Use on Mortality in COVID-19 Patients Admitted to Intensive Care Unit

Yoğun Bakım Ünitesine Kabul Edilen COVID-19 Hastalarında Tocilizumab Kullanımının Mortalite Üzerine Etkisi

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ABSTRACT

Aim: Coronavirus disease 2019 (COVID-19) mostly proceeds with mild respiratory symptoms, but sometimes severe pneumonia, cytokine storm, and acute respiratory distress syndrome can develop. Anti-cytokine treatments are being tried for cytokine storm. In this study, we aimed to examine the effect of tocilizumab on mortality associated with COVID-19.
Material and Methods: The study included 146 patients with moderate-to-severe acute respiratory distress syndrome diagnosed with COVID-19. The patients were divided into two groups, receiving only standard treatment (ST group, n=44), and tocilizumab treatment in addition to standard treatment (TCZ group, n=102). Groups were compared in terms of demographic, clinic, and laboratory data. Also, mortality rates were determined to detect the effect of tocilizumab on mortality.
Results: Overall, 36.3% (n=53) of the patients were female, 63.7% (n=93) were male, and the mean age was 69.5±14.2 years. The mortality rate was 29.4% (n=30) in the TCZ group and 52.3% (n=23) in the ST group (p=0.009). While C-reactive protein, fibrinogen, and lactate levels on admission to the intensive care unit (ICU) were similar across the groups, the TCZ group had higher ferritin levels (p=0.006). On discharge from ICU, the TCZ group had a significant decrease in C-reactive protein (p<0.001), while their ferritin levels decreased to levels in the ST group (p=0.134). The absence of tocilizumab in the treatment regimen was associated with a 2.63-fold increase in the mortality risk.
Conclusion: Tocilizumab reduces the mortality in COVID-19 patients in ICU. However, further studies are warranted to better elucidate the efficacy and side effects of tocilizumab.
Keywords: COVID-19; cytokine storm; tocilizumab; mortality; interleukin 6.

ÖZ

Amaç: Koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) çoğunlukla hafif solunum semptomları ile seyreder, ancak bazen şiddetli pnömoni, sitokin fırtınası ve akut solunum sıkıntısı sendromu gelişebilir. Sitokin fırtınası için anti-sitokin tedaviler denenmektedir. Bu çalışmada, tocilizumabın COVID-19 ile ilişkili mortalite üzerindeki etkisinin incelenmesi amaçlandı.
Gereç ve Yöntemler: Çalışmaya, COVID-19'a bağlı orta-şiddetli akut solunum sıkıntısı sendromu olan 146 hasta dahil edildi. Hastalar sadece standart tedavi alanlar (ST grubu, n=44) ve standart tedavi ile birlikte tocilizumab tedavisi alanlar (TCZ grubu, n=102) olmak üzere iki gruba ayrıldı. Gruplar demografik, klinik ve laboratuvar verileri açısından karşılaştırıldı. Tocilizumabın mortalite üzerindeki etkisini tespit etmek için mortalite oranları da belirlendi.
Bulgular: Genel olarak, hastaların %36,3'ü (n=53) kadın, %63,7'si (n=93) erkek ve yaş ortalaması 69,5±14,2 yıl idi. Mortalite oranı TCZ grubunda %29,4 (n=30) ve ST grubunda ise %52,3 (n=23) idi (p=0,009). Hastaların yoğun bakım ünitesine (YBÜ) kabuldeki C-reaktif protein, fibrinojen ve laktat seviyeleri gruplar arasında benzer iken, TCZ grubunda ferritin seviyeleri daha yüksekti (p=0,006). YBÜ'den taburcu olduklarında TCZ grubunda C-reaktif protein'de anlamlı bir düşüş olurken (p<0,001), ferritin seviyeleri ST grubundaki seviyelere geriledi (p=0,134). Tedavi rejiminde tocilizumabın olmaması, mortalite riskinde 2,63 katlık bir artış ile ilişkili bulundu.
Sonuç: Tocilizumab YBÜ'de takip edilen COVID-19 hastalarında mortaliteyi azaltmaktadır. Bununla birlikte, tocilizumabın etkinliğini ve yan etkilerini daha iyi aydınlatmak için daha fazla çalışmaya ihtiyaç vardır.
Anahtar kelimeler: COVID-19; sitokin fırtınası; tocilizumab; mortalite; interlökin 6.

INTRODUCTION

Following the detection of a cluster of pneumonia cases of unknown origin in December 2019 in Wuhan city of the Hubei province of China, a novel β-coronavirus strain was isolated as the responsible causative agent (1). This disease, named as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO), rapidly spread to other countries, resulting in a global pandemic (1,2). Since COVID-19 may lead to severe respiratory failure, which is a major cause of morbidity and mortality, it was also termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (3,4).

The reported mortality rates in severe COVID-19 cases exhibit a wide range from 35% to 50-62% (5-7). As of April 2022, there have been more than 505 million confirmed cases of COVID-19, with the total deaths exceeding 6.2 million (8). Although most patients have mild symptoms, those with comorbid conditions show a particular inclination for more severe disease courses including severe pneumonia and acute respiratory distress syndrome (ARDS), as well as increased mortality (9,10). ARDS represents the most devastating complication of COVID-19 (11). Severe ARDS results from intracellular replication of viruses and inflammatory response of the host (12). The pathogenesis of ARDS is associated with a cytokine storm involving high serum pro-inflammatory cytokine and chemokine levels; interleukins (IL) 1, 6, 8, and 12, tumor necrosis factor-alpha (TNF-α), and interferon (IFN)-γ (4). Certain clinical features of these more severe COVID-19 cases have been found to be akin to those in hemophagocytic lymphohistiocytosis (HLH), a life-threatening condition associated with increased release of cytokines (13). Although HLH may be a familial condition, it can also be acquired following viral infections, and therefore may presumably be triggered by SARS-CoV-2 (14).

Uncontrolled excessive production of pro-inflammatory cytokines such as IL-6 results in epithelial and endothelial apoptosis, abnormal coagulation, pulmonary fibrinolysis, and diffuse alveolar injury (15). The aim of COVID-19 treatment is to strengthen anti-viral immunity and to prevent hyper-inflammatory injury (16). Treatment options generally fall into two categories. The frontline approach involves the prevention of viral replication via antiviral treatments, and second-line treatments should aim to control the cytokine storm caused by IL-1 and IL-6 in patients with more advanced diseases requiring intensive care unit (ICU) admission. The choice and timing of medications to be utilized, particularly in the latter scenario, are of importance (17).

Tocilizumab is a monoclonal human antibody developed against the IL-6 receptor. The central role of IL-6 in the pathogenesis of cytokine release syndrome (CRS) and ARDS in patients with COVID-19 has been firmly established (11). Although many cytokines are involved in CRS, IL-6 is the most important culprit and is associated with poor prognosis (18). In CRS associated with COVID-19, tocilizumab treatment has been shown to stabilize patients by reducing acute phase reactants and to offer an effective treatment option for cytokine storm (19). When the key role of IL-6 in CRS induced by COVID-19 is taken into consideration, it may be assumed that the suppression of the immune responses occurring via IL-6 by tocilizumab may prove to be useful against the hazardous effects of hyperinflammation (20).

In this study, patients with COVID-19 pneumonia admitted to ICU due to moderate to severe ARDS were administered standard treatment with or without tocilizumab and were compared in terms of the changes in clinical, radiological, and laboratory parameters, intubation duration, length of ICU stay, and mortality rates, in order to elucidate the effect of tocilizumab on these parameters. A particular emphasis was placed on the effect of tocilizumab treatment on mortality.

MATERIAL AND METHODS

All patients consecutively admitted to the ICU, Pulmonology Department, Medical Faculty of Erzincan Binali Yıldırım University between April 2020 and March 2021 due to COVID-19 pneumonia were retrospectively screened. Of 229 patients with SARS-CoV-2 detected in a polymerase chain reaction (PCR), 75 were excluded due to the presence of mild ARDS (according to the diagnostic criteria of ARDS Berlin (21), 200< partial pressure of arterial oxygen / fraction of inspired oxygen (PaO_2/FiO_2) ≤300, positive end-expiratory pressure (PEEP), or continuous positive airway pressure (CPAP) ≥5 cm H_2O), 5 due to reasons other than ARDS (3 myocardial infarction, 2 acute renal failure), and 3 due to inability to use tocilizumab (pregnancy, active hepatitis B, fungal infection). Thus, a total of 146 patients participated in the study, including 44 patients with moderate to severe ARDS (according to the diagnostic criteria of ARDS Berlin (21), PaO_2/FiO_2 ≤200 + PEEP ≥5 cm H_2O) who received standard treatment (ST group), and 102 patients with moderate to severe ARDS who received tocilizumab in addition to standard treatment (TCZ group, standard treatment + 4-8 mg/kg tocilizumab with 24h intervals). In the standard treatment, in accordance with the recommendations of the COVID-19 Treatment Guide of the Ministry of Health of the Republic of Turkey; Favipiravir 2×1600 mg loading, 2×600 mg maintenance, enoxaparin 1 mg/kg, 6 mg/day dexamethasone or equivalent glucocorticoids, for example, 0.5-1 mg/kg of prednisolone or 32 mg/day of methylprednisolone and antibiotics (if signs of bacterial infection are present and according to the factor) were given (22). TCZ group patients, of the Scientific Committee of the Ministry of Health of the Republic of Turkey in accordance with the recommendations in the field of high flow oxygen therapy (HFOT) or mechanical ventilation (MV) and abnormal serum level of at least two biomarkers of C-reactive protein (CRP), D-dimer, lactate dehydrogenase (LDH), or ferritin, were patients that received approval by reference tocilizumab treatment (23). Data on demographics, vital parameters on admission, Charlson comorbidity index (CCI) scores, acute physiology and chronic health evaluation II (APACHE II) scores, other treatments, duration of MV, complications, length of hospital and ICU stay, and mortality were retrieved from patient files.

Two groups defined on the basis of the treatments administered, i.e. the ST group, and the TCZ group, were compared to examine the effect of tocilizumab on the clinical course, length of ICU and hospital stay, acute

phase reactants, and most importantly on the mortality rate. The study was approved by the Erzincan Binali Yıldırım University Ethics Committee for Clinical Research (date: 22.02.2021, no: 04/02).

Statistical Analysis

Statistical analyses were performed using IBM SPSS v.22 software (IBM Corp., Armonk, NY). Categorical variables were expressed as number and percentage, and continuous variables as mean±standard deviation, median, 25^{th}-75^{th} percentile, and minimum-maximum values. Chi-square and Fisher's exact tests were used to compare categorical variables between the groups. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables were compared with Student's t-test or Mann-Whitney U test between the two groups. When comparing study parameters between the groups on discharge from the ICU, a correction for baseline measurements was made using ANCOVA. For all tests, a p level of <0.05 was considered for statistical significance.

RESULTS

A total of 146 patients with moderate to severe ARDS due to COVID-19 pneumonia were included. The mean age was 69.5±14.2 years. Of the 146 patients, 36.3% (n=53) were female, and 63.7% (n=93) were male. Two groups were defined based on the treatments administered, the TCZ group (n=102), and the ST group (n=44). The two groups were comparable with respect to gender, cigarette smoking status, APACHE II scores, ARDS stages, and CCI. Patients in the TCZ group had significantly lower mean age than in the ST group (p=0.030, Table 1). Most common comorbid conditions in decreasing order included hypertension (HT, 33%), cardiovascular disease (CVD, 17%), chronic obstructive pulmonary disease (COPD, 16.5%), and diabetes mellitus (DM, 10.8%). A comparison of vital parameters between the study groups showed comparable heart rate and systolic blood pressure (SBP) on admission (p=0.580, and p=0.052, respectively), while patients in the ST group had lower diastolic blood pressure (DBP) and mean arterial pressure (MAP) on admission (p=0.026, and p=0.025, respectively). Regarding hypoxemia, the TCZ group had significantly lower SaO_2, and P/F ratio, and significantly higher respiratory rate (p<0.001, p=0.004, and p<0.001, respectively). The demographic data, clinical features, and vital parameters on admission in study groups were presented in Table 1.

While CRP, fibrinogen, and lactate levels were comparable between the two groups at the time of ICU admission, ferritin levels were higher in the TCZ group. On the other hand, CRP was significantly decreased on discharge in the TCZ group, while ferritin decreased to levels similar to those in the ST group (p<0.001, and p=0.153, respectively, Table 2, Figure 1). Furthermore, patients in the ST group had higher D-dimer levels on admission, while these levels were similar on discharge from the ICU (p=0.033, and p=0.146, respectively, Table 2). While procalcitonin (PCT) was higher on admission to ICU in the ST group, PCT on discharge was comparable in the two groups on discharge (p=0.001, and p=0.540, respectively). Triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol were significantly higher among the patients in the TCZ group than patients in the ST group (Table 2).

While hematologic parameters including lymphocyte, neutrophil count and percentage were comparable on admission, patients in the TCZ group had a significant increase in lymphocyte count on discharge. Also, although neutrophil count showed a similar decrease in both groups, neutrophil percentage showed a significantly more pronounced decline as compared to the ST group (Table 2). On admission, the neutrophil-to-lymphocyte ratio (NLR) was comparable in the groups, while it was significantly lower in the TCZ group on discharge (p=0.532, and p=0.006, respectively, Table 2, Figure 2). LDH levels, which are expected to rise in patients with lung injury, were significantly higher in the TCZ group on admission to ICU, while they were comparable between the two groups on discharge (p=0.004, and p=0.351, respectively). A comparison of laboratory parameters examined in both groups was presented in Table 2.

Table 1. Comparison of disease severity scores, vital parameters, and length of treatment in study groups

	ST Group (n=44)		TCZ Group (n=102)		p
	Mean±SD	**Median (Q_1-Q_3) [Min-Max]**	**Mean±SD**	**Median (Q_1-Q_3) [Min-Max]**	
Age (year)	73.4±15.5	75 (68-84) [18-96]	67.9±13.4	70 (60-78) [26-96]	**0.030**
APACHE II	24.1±6.8	24 (18.5-29) [12-42]	24.4±5.5	24 (20-28) [14-37]	0.952*
CCI	4.2±1.5	4 (3-5) [0-7]	3.5±1.8	4 (2-5) [0-8]	0.260*
SBP (mmHg)	106±23	100 (90-120) [60-170]	115±21	110 (100-130) [65-170]	0.052
DBP (mmHg)	63±11	60 (55-70) [35-90]	67±9.5	70 (60-70) [40-90]	**0.026***
MAP (mmHg)	77.5±14	76.7 (69.2-87.5) [43-110]	83±13	83 (73.3-90) [48-113]	**0.025**
Pulse (bpm)	110±20.5	112 (100-126) [56-148]	109±13	108 (98-116) [88-168]	0.580
SaO_2 (%)	89±6.5	89 (82.5-93) [74-98]	83±6	84 (80-86) [55-90]	**<0.001***
RR (min)	26±6	26 (22.5-32.5) [14-36]	30±3.5	29 (28-32) [23-40]	**<0.001***
P/F ratio	140±37	150 (111-177.5) [70-185]	120±38	120 (90-145) [60-195]	**0.004**
Intubation (days)	8.7±13.1	2.5 (0-13) [0-54]	9.5±12.5	6.5 (0-15) [0-66]	0.658*
ICU (days)	11.3±11.6	8 (4-14) [2-58]	17.6±12.2	14.5 (9-21) [4-75]	**<0.001***
Hospitalization (days)	20±15.5	15 (9.5-22) [3-74]	27±13	24 (18-32) [12-77]	**<0.001***

ST Group: standard treatment group, TCZ Group: tocilizumab in addition to standard treatment group, SD: standard deviation, Q_1-Q_3: 25^{th}-75^{th} percentile, APACHE II: acute physiology and chronic health evaluation II, CCI: Charlson comorbidity index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, RR: respiratory rate, ICU: intensive care unit, *: Mann-Whitney U test was performed; otherwise, independent samples t-test was used

Table 2. Comparison of laboratory parameters on admission and discharge from the ICU in study groups

	ST Group (n=44)		TCZ Group (n=102)		p
	Mean±SD	**Median (Q_1-Q_3) [Min-Max]**	**Mean±SD**	**Median (Q_1-Q_3) [Min-Max]**	
PCT (ng/m)[a]	3.8±5.9	0.8 (0.2-4.8) [0.1-24]	1.2±3.3	0.3 (0.1-0.5) [0.1-20]	**0.001**
PCT (ng/m)[d]	2.1±5.6	0.4 (0.1-1.8) [0.1-35]	1.1±3.3	0.1 (0.1-0.2) [0.1-19]	0.540*
CRP (mg/L)[a]	122±103.2	116 (34-177) [11-489]	110±66.4	104.5 (64-132) [6-444]	0.484
CRP (mg/L)[d]	82±79.3	57 (22-137) [3-327]	33.8±58.1	7 (4-28) [1-289]	**<0.001***
FER (ng/mL)[a]	586±546.8	379 (141-881) [10-1650]	803±532	669 (457-1213) [50-2000]	**0.006**
FER (ng/mL)[d]	669±567.2	426 (172-1050) [16-1650]	658±479	519 (297-924) [40-1800]	0.153*
FIB (mg/dL)[a]	323±91.8	349 (271-374) [103-509]	332±69	348 (292-377) [77-501]	0.546
FIB (mg/dL)[d]	309±70.2	317 (258-364) [156-438]	299.4±89	304.5 (239-354) [45-501]	0.317*
D-dimer (µg/L)[a]	6261±9788	2640 (1276-6064) [236-45000]	4479±8721	1503 (1046-3650) [165-71500]	**0.033**
D-dimer (µg/L)[d]	2839±3077	1874 (968-3706) [421-16250]	3287±5084	1535 (910-3094) [224-36900]	0.146*
bLac (mmol/L) [a]	2.4±1.1	2.6 (1.7-3.1) [0.9-5.3]	2.2±1	2 (1.7-2.5) [1-9.8]	0.090
bLac (mmol/L)[d]	2.6±2.1	1.9 (1.4-3.3) [0.2-11.8]	2±1.6	1.5 (1.1-2.3) [0.5-11.2]	0.204*
Urea (mg/dL) [a]	87±63.7	68 (39-119) [13-278]	70±42.3	56.5 (44-94) [16-250]	0.376
Cr (mg/dL) [a]	1.4±0.9	1 (0.9-2) [0.4-4.9]	1.2±1	1 (0.9-1.3) [0.5-8.5]	0.359
ALT (u/L) [a]	74.5±175	23.5 (15.5-47.5) [5-1047]	57.7±68	41.5 (25-65) [9-567]	**0.004**
AST (u/L) [a]	74.2±118	38 (23.5-66) [10-657]	55.7±52	37 (29-63) [11-345]	0.405
LDH (u/L) [a]	450±232	379 (289-575) [166-1075]	529±188	488 (407-605) [188-1339]	**0.004**
LDH (u/L) [d]	466±379	333 (261-429) [137-2079]	455±341	384 (302-497) [166-3049]	0.351*
TC (mg/dL) [a]	145±48.5	139 (110-165) [49-293]	175.5±55	168 (140-205) [59-351]	**0.003**
HDL (mg/dL) [a]	30.3±8.5	31 (25-36) [12-47]	35.6±15.3	33 (26-44) [11-98]	**0.038**
LDL (mg/dL) [a]	87±34.5	91 (74-110) [8-153]	108±39.7	104 (79-134) [14-232]	**0.016**
TG (mg/dL) [a]	164±117.4	141 (88-203) [36-668]	203±113	177 (126-252) [51-630]	**0.005**
WBC ($\times10^9$/L) [a]	11.6±6.8	9.8 (7.4-13.8) [2.3-35]	10.7±4.7	10.5 (7.6-13.8) [2.1-34.6]	0.361
WBC ($\times10^9$/L) [d]	9.7±6.1	8.5 (6-12.1) [2.4-33.4]	9.6±4.8	9.1 (6.6-12) [1.5-34]	0.953*
LYM ($\times10^9$/L)[a]	0.83±0.70	0.74 (0.46-0.95) [0.14-0.45]	0.66±0.35	0.58 (0.42-0.8) [0.19-2.0]	0.133
LYM ($\times10^9$/L)[d]	0.93±0.63	0.87 (0.43-1.2) [0.16-3.0]	1.2±0.74	1.0 (0.64-1.57) [0.17-3.90]	**0.007***
LYM (%)[a]	8.2±6.6	6.5 (3.9-10.1) [1.2-36.0]	7.0±4.4	6.0 (4.5-8.6) [1.2-27.5]	0.738
LYM (%)[d]	11.3±8.6	10.4 (5-14.7) [1.2-44]	14.8±10.7	13 (7.5-17.8) [1.8-60]	**0.021***
NEU ($\times10^9$/L)[a]	10.1±6.7	8.9 (5.7-11.6) [1.2-34.0]	9.6±4.5	9.3 (6.6-12.4) [1.8-32.7]	0.581
NEU ($\times10^9$/L)[d]	8.1±5.5	6.8 (4.5-10.6) [1.7-32.0]	7.6±4.6	7.1 (4.4-9.3) [0.56-31.6]	0.694*
NEU (%)[a]	83.7±12	87.5 (79-92) [45-96]	88.4±5.5	90 (86-92) [65-96]	0.107
NEU (%)[d]	80.4±12.1	81.5 (72-91) [45-96]	77±12.9	78 (70-86) [34-96]	**0.004***
NLR[a]	17.8±15.2	13.2 (7.4-24.5) [1.4-78.4]	17.5±11.9	14.7 (9.9-20.5) [2.4-79.1]	0.532
NLR[d]	15.2±17.1	7.7 (4.5-17.5) [0.9-77.5]	9.4±8.8	6.2 (4.2-11.7) [0.6-52.6]	**0.006***

ICU: intensive cae unit, ST Group: standard treatment group, TCZ Group: tocilizumab in addition to standard treatment group, SD: standard deviation, Q_1-Q_3: 25^{th}-75^{th} percentile, PCT: procalcitonin, CRP: C-reactive protein, FER: ferritin, FIB: fibrinogen, bLac: blood lactate, Cr: creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: triglyceride, WBC: white blood cell, LYM: lymphocyte, NEU: neutrophil, NLR: neutrophil-to-lymphocyte ratio, [a]: admission, [d]: discharge, *: adjusted for baseline value while comparing groups

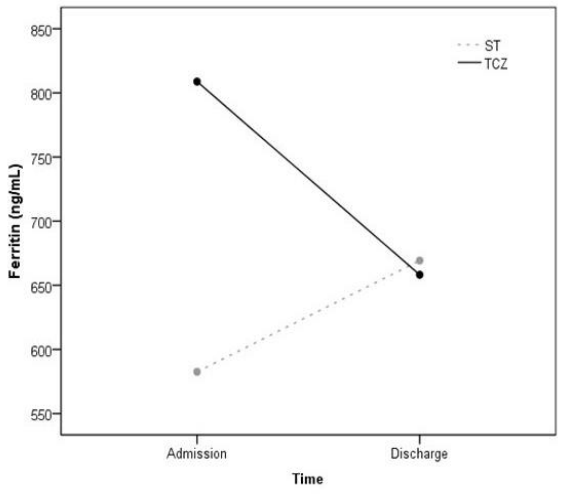

Figure 1. Ferritin on admission and discharge from ICU

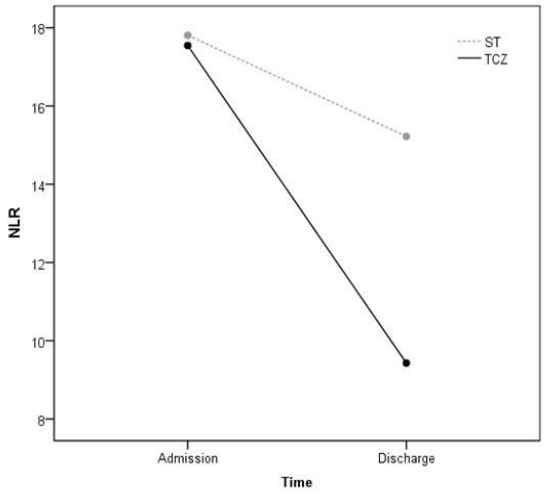

Figure 2. NLR on admission and discharge from ICU

The use of HFOT was significantly more frequent among the patients in the TCZ group (81.4%, n=83) as compared to the ST group (47.7%, n=21, p<0.001). The duration of MV was similar in the two groups (p=0.658), while the TCZ group had significantly longer total hospital stay and ICU stay (both p<0.001, Table 1).

The mortality rate in the ST group was 52.3% (n=23) versus 29.4% (n=30) in the TCZ group, indicating significantly lower mortality in patients receiving tocilizumab treatment (p=0.009) with a 2.63-fold increased risk of mortality in the absence of additional tocilizumab (Table 3).

DISCUSSION

This study analyzed the efficacy of an IL-6 receptor inhibitor, tocilizumab, in the treatment of COVID-19 patients developing moderate to severe ARDS. When the central role of IL-6 is in cytokine storm, it may be presumed that tocilizumab may represent a plausible therapeutic option in cytokine storm induced by COVID-19. Our clinical data showed that tocilizumab treatment was associated with an improved acute phase reactant profile and reduced mortality.

The severity of COVID-19 may range from mild symptoms to ARDS and death. Progression to ARDS and eventual death in COVID-19 patients is thought to result from an HLH-like condition, triggered by excessive release of proinflammatory cytokines (24). Until now, many studies have suggested that tocilizumab has beneficial effects in COVID-19 patients (19,25). In a recent study in Turkey, anakinra (IL-1 receptor antagonist), another anti-cytokine treatment, was compared with tocilizumab. The need for non-invasive MV requirements, HFOT, and the length of stay in the ICU were found to be lower in the group receiving tocilizumab treatment compared to the group receiving anakinra treatment (26). The American Infectious Disease Society guidelines recommend the use of tocilizumab in addition to standard treatment in hospitalized adult COVID-19 patients who have increased systemic inflammatory markers (27). Also, the National Institutes of Health guidelines endorse the use of tocilizumab (single intravenous dose up to 8 mg/kg, max. 800 mg) in conjunction with dexamethasone, in newly hospitalized patients who have rapid respiratory decompensation due to COVID-19 (28).

In a meta-analysis of 7 studies examining the effect of comorbid conditions on the course of COVID-19, a number of conditions such as COPD, CVD, and HT were found to be associated with an increased risk of ICU admission (29). In the current study, patients in the ST group were older, possibly due to the smaller sample size. The most common comorbidities in the patient group included HT, CVD, COPD, and DM. On the other hand, the two study groups were comparable with regard to APACHE II scores, which is a measure of the severity of the condition, as well as with regard to the CCI score, which measures the comorbidities.

Although vital parameters of MAP and DBP were lower in the ST group, this finding may be associated with the low number of patients in that group. On the other hand, although oxygenation parameters (SaO_2 and P/F ratio) were lower and respiratory rate was higher in the TCZ group, the mortality rates were lower, suggesting that tocilizumab was an effective therapeutic option. Furthermore, although the duration of MV was comparable in the two groups, the length of ICU and hospital stay was longer in the TCZ group. This may be due to the fact that lower mortality rates in the TCZ group vs. ST group may be related to a longer need for palliative care following ICU stay among patients receiving tocilizumab treatment.

Previous studies have underscored the prognostic importance of several laboratory parameters, including D-dimer, lymphocyte count, ferritin, CRP, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in COVID-19 (21,33). In Sciascia et al.'s study (19), tocilizumab was shown to stabilize patients by reducing acute phase reactants in the treatment of COVID-19 related CRS, as well as to have efficacy in the treatment of cytokine storm. In the present study, CRP, an acute phase reactant, was similar on admission to ICU in both groups, while it decreased significantly on discharge among patients in the TCZ group. Also, ferritin that has significantly elevated on admission to ICU in the TCZ group was reduced to similar levels to those in the ST group on discharge. In another retrospective, multi-center study involving 150 COVID-19 patients, elevated ferritin and IL-6 levels were found to be associated with poor prognosis, suggesting that hyperinflammation of viral origin could be associated with mortality (32). Lower rates of mortality in the TCZ group of the current study despite higher ferritin levels suggest that tocilizumab may be effective in reducing mortality. In a study, tocilizumab was found to improve patients by reducing CRP, fibrinogen, and ferritin levels, and increasing lymphocyte count, similar to our observations. Again, in that same study, D-dimer levels remained elevated in patients receiving tocilizumab, which was explained on the basis of the lack of an effect of tocilizumab on coagulation (33). In parallel with these findings, patients in the TCZ group did not experience a significant decrease in D-dimer levels as compared to the ST group.

Numerous studies regarding hematological parameters in COVID-19 patients have been performed (36,37). Lymphocytopenia is a common finding in these patients, which has been purported to result from the destructive effect of SARS-CoV-2 on T lymphocytes (36). In a meta-analysis of 37 studies among COVID-19 patients, age was found to correlate negatively with lymphocyte count (37). The main three hematological findings that were associated with poor prognosis included leukocytosis, thrombocytopenia, and lymphopenia (38). In the present study, although patients in the TCZ group were younger, their lymphocyte count on admission was lower than that of patients in the ST group, although the difference was not significant. On the other hand,

Table 3. Mortality according to tocilizumab using

	ST (n=44)	TCZ (n=102)	p
Status, n (%)			
Death	23 (52.3)	30 (29.4)	**0.009**
Surviving	21 (47.7)	72 (70.6)	
OR (95% CI)	2.63 (1.27-5.45)		

ST Group: standard treatment group, TCZ Group: tocilizumab in addition to standard treatment group, OR: odds ratio, CI: confidence interval

lymphocyte count and percentage on discharge were significantly higher in the TCZ group vs. the ST group. Considering the role of lymphopenia in poor prognosis, this observation suggests a positive effect of tocilizumab on COVID-19. Many studies suggested that elevated NLR in COVID-19 may represent an independent biomarker for predicting poor prognosis and may be used to identify high-risk individuals (37,42). In the current study, NLR on admission was similar in both groups, while it was significantly lower in the TCZ group on discharge.

In a retrospective cohort study of 544 patients (TESEO study), intravenous or subcutaneous tocilizumab treatment was found to result in a decrease in the risk of MV and death (40). In the current study, the mortality rate in the ST group was 52.3% (23/44) vs. 29.4% (30/102) in the TCZ group, indicating a significant difference. Thus, lack of tocilizumab treatment was associated with a 2.63-fold increased risk of mortality. In another meta-analysis by Zhao et al. (41), involving a total of 1675 severe COVID-19 cases, 1000 patients received standard treatment and 675 also received tocilizumab. Similar to our observations, the mortality among patients receiving tocilizumab (132/675, 19.5%) was significantly lower than in controls (283/1000, 28.3%).

Conversely, tocilizumab was reported to offer no additional benefits in terms of clinical outcomes in severe COVID-19, based on a collective analysis of seven retrospective studies (42). However, none of these were randomized and controlled studies, and the clinical characteristics in the study groups were not homogeneously distributed. Also, there were significant differences in terms of tocilizumab dose, dose frequency, and anti-viral treatment regimens. Additionally, the delay between disease onset and initiation of treatment was not uniform (42). No consistent findings have been reported in these studies comparing tocilizumab and other treatment regimens. Most of these studies reported worse clinical outcomes with tocilizumab treatment, explaining why tocilizumab was not found to be useful. However, as emphasized earlier, these studies exhibit significant differences in terms of the characteristics of the study and control groups. Also, in a study by Klopfenstein et al. (43), although patients in the tocilizumab group had higher disease severity and lower survival than controls, their CCI was also higher.

The timing of tocilizumab treatment may be of high significance (17). Patients should be closely monitored for progression to ARDS and the development of CRS. Several studies have emphasized the importance of timely administration of tocilizumab with respect to treatment efficacy (17,44). In the present study, initiation of tocilizumab treatment within 48 hours of admission to ICU may have contributed to the observed efficacy.

This study has certain limitations. Firstly, it is a retrospective study. Secondly, the sample size in the ST group was small. Finally, IL-6 levels were not measured.

CONCLUSION

ARDS and CRS represent major causes of mortality in COVID-19. Despite the lack of curative treatment, patients are frequently given anti-viral agents, steroids, and heparin, in addition to anti-inflammatory agents. IL-6 inhibitors may represent a plausible therapeutic option in COVID-19, considering the role of IL-6 in the pathogenesis of CRS and ARDS. In this study, we conclude that tocilizumab can be administered to COVID-19 patients, based on the observed reduction in mortality. Proper selection of patients, the timing of treatment, and safety concerns should be considered when deciding on tocilizumab treatment. For further studies, we recommend the development of a scoring system for establishing treatment indications for tocilizumab that should include disease severity, the burden of lung injury, the presence of risk factors, and the level of inflammatory markers as well as IL-6. Also, further research should better elucidate the tolerability of tocilizumab treatment.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Erzincan Binali Yıldırım University (22.02.2021, 04/02).

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The Effect of Combination of Aerobic and Strengthening Exercise on Muscle Strength, Balance, and Sleep Quality in Individuals with Type 2 Diabetes

Tip 2 Diyabet Olan Bireylerde Aerobik ve Kuvvetlendirme Egzersiz Kombinasyonunun Kas Gücü, Denge ve Uyku Kalitesi Üzerine Etkisi

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ABSTRACT

Aim: The aim of this study was to determine the effects of aerobic and strengthening exercise combination on glycosylated hemoglobin (HbA1c), muscle strength, balance, and sleep quality in individuals diagnosed with type 2 diabetes mellitus (DM).

Material and Methods: Fifty individuals aged between 30 and 65 years and diagnosed with type 2 DM were included in this study. Anthropometric measurements, HbA1c, quadriceps and hamstring muscle strength, balance, and sleep quality of the included individuals were evaluated. Aerobic and strengthening exercises were applied to the participants 3 days a week for 12 weeks. All evaluation measurements were repeated at the end of 12 weeks.

Results: Of the 50 individuals included in this study and diagnosed with type 2 DM, 64% (n=32) were male and 36% (n=18) were female. The mean age of the participants was 50.12±10.81 years, the mean body mass index was 29.97±3.12 kg/m^2, the waist/hip ratio was 0.91±0.08, and the mean HbA1c was 9.19±2.39. When the pre and post-treatment HbA1c, muscle strength, balance, and sleep quality measurements of all participants were compared, there were statistically significant differences ($p<0.001$).

Conclusion: It was determined that the combination of long-term aerobic and strengthening exercise decreased the HbA1c value and also significantly improved muscle strength, balance, and sleep quality in individuals with type 2 DM. A structured exercise program that includes strengthening and aerobic exercises would be beneficial in developing the most effective and appropriate exercise prescriptions in terms of exercise efficiency and sustainability for individuals with type 2 DM.

Keywords: Aerobic exercise; balance; muscle strength; strengthening exercises; type 2 diabetes.

ÖZ

Amaç: Bu çalışmanın amacı, tip 2 diabetes mellitus (DM) tanısı olan bireylerde aerobik ve kuvvetlendirme egzersiz kombinasyonunun glikozile hemoglobin (HbA1c), kas kuvveti, denge ve uyku kalitesi üzerine etkilerini belirlemektir.

Gereç ve Yöntemler: Bu çalışmaya 30 ve 65 yaş arası ve tip 2 DM tanısı olan 50 birey dahil edildi. Dahil edilen bireylerin antropometrik ölçümleri, HbA1c, quadriseps ve hamstring kas gücü, denge ve uyku kalitesi değerlendirildi. Katılımcılara 12 hafta boyunca haftada 3 gün aerobik ve kuvvetlendirme egzersizleri uygulandı. Tüm değerlendirme ölçümleri 12 haftanın sonunda tekrar edildi.

Bulgular: Bu çalışmaya dahil edilen ve tip 2 DM tanısı olan 50 bireyin %64'ü (n=32) erkek ve %36'sı (n=18) kadın idi. Katılımcıların yaş ortalaması 50,12±10,81 yıl, ortalama beden kitle indeksi 29,97±3,12 kg/m^2, bel/kalça oranı 0,91±0,08 ve ortalama HbA1c değeri 9,19±2,39 idi. Tüm katılımcıların tedavi öncesi ve tedavi sonrasındaki HbA1c, kas gücü, denge ve uyku kalitesi ölçümleri karşılaştırıldığında istatistiksel olarak anlamlı farklar vardı ($p<0,001$).

Sonuç: Tip 2 DM olan bireylerde uzun süreli aerobik ve kuvvetlendirme egzersizi kombinasyonunun HbA1c değerini azalttığı ve ayrıca kas gücü, denge ve uyku kalitesini de önemli ölçüde iyileştirdiği belirlendi. Kuvvetlendirme ve aerobik egzersizleri içeren yapılandırılmış bir egzersiz programı, tip 2 diyabet olan bireyler için egzersiz etkinliği ve sürdürülebilirliği açısından en etkili ve uygun egzersiz reçetelerinin geliştirilmesinde faydalı olacaktır.

Anahtar kelimeler: Aerobik egzersiz; denge; kas gücü; kuvvetlendirme egzersizleri; tip 2 diyabet.

INTRODUCTION

Type 2 diabetes mellitus (DM) is the most common metabolic disease characterized by insulin resistance and increased glucose production in the adult population. Type 2 DM, also known as 'non-insulin-dependent diabetes', accounts for more than 90% of all diabetes cases. While type 2 diabetes affects 5-10% of the population in developed countries, it affects 10-25% of the population in developing countries. Although type 2 DM is known as a middle and advanced-age disease, it has also been seen at earlier ages in recent years. In people who are genetically predisposed to type 2 DM, it is observed that insulin resistance increases over time, as obesity increases and physical activity decreases as a result of negative lifestyle changes (1,2).

Muscle atrophy occurs as a result of the loss of strength in the skeletal muscles of individuals with diabetes depending on the duration of diabetes. In addition, deterioration in blood values (especially fasting blood sugar, glycosylated hemoglobin, and cholesterol levels), postprandial hyperglycemia, systolic and diastolic blood pressure problems, deterioration of functionality, loss of balance, and decrease in quality of life occur (3,4).

Physical activity and regular exercise programs, as well as pharmaceutical treatment and diet approaches, are demonstrated to be the most effective in the treatment of type 2 diabetes research. It is known that exercise can prevent or delay type 2 diabetes symptoms and complications (5). Studies have shown that exercise training has beneficial effects on the glycemic profile, such as decreased glycosylated hemoglobin (HbA1c) level, increased maximum oxygen consumption (VO_2 max), and improved insulin sensitivity in diabetic patients (6,7). Exercise in individuals with type 2 diabetes improves glycemic control, lowers blood pressure, reduces abdominal fat mass, reduces cardiovascular morbidity and mortality, and positively affects balance and quality of life. In addition, exercise affects falls, injury, and balance factors by improving insulin sensitivity and function (4,8). Aerobic and strengthening exercises assist individuals with type 2 diabetes control their glucose levels. Aerobic exercises facilitate the uptake and distribution of oxygen to the body. Exercise reduces HbA1c level, lipid level, body fat percentage, blood pressure, insulin resistance, and inflammation parameters. On the other hand, strengthening exercises provide glycemic control and normalize fat mass and blood pressure in individuals with type 2 DM. Combining strengthening and aerobic activities has a two-fold greater impact on metabolic parameters than performing these exercises separately (9). Although combined exercises have been shown to improve HbA1c, fasting and postprandial glucose levels, and insulin sensitivity, studies on the effects on balance, muscle strength, and sleep quality are limited. The impact of the structured versions of both exercises on patients differs based on comorbidities, patient preference, equipment, and clinical availability (10,11).

The effectiveness of different exercise programs has been studied, but studies examining the effects of structured aerobic and strengthening exercises on individuals with type 2 DM seem to be insufficient. Also, it is unclear whether exercise programs improve muscular strength, balance, and sleep quality in people with type 2 DM. We anticipate that combining exercises will be more effective for reducing HbA1c and improving mobility function. The study aimed to examine the effects of structured exercise programs on muscle strength, balance, and sleep quality in individuals with type 2 diabetes.

MATERIAL AND METHODS

Study Design and Participants

The study was conducted with 50 individuals with type 2 diabetes who were followed up in the Department of Endocrine and Metabolism Diseases of Istanbul Medipol University Hospital in Istanbul. All participants were recruited between December 2021 and March 2022. This study was approved by the Non-interventional Clinical Researches Ethics Committee at Istanbul Medipol University (dated: 09.12.2021 and numbered: 1262). All participants signed written information and provided an informed consent form, and the study was conducted by the principles of the Declaration of Helsinki.

The participants who were 30-65 years of age, had body mass index (BMI) of 25-30 kg/m^2, HbA1c ≥6.5%, fasting blood glucose >126 mg/dl, and type 2 DM between 5-10 years were included in the study. The exclusion criteria were defined as having type 1 DM or having any neurologic, cardiac, or orthopedic diseases that would prevent exercise participation.

The sample size was determined using the G* power sample size calculator (G* Power, v.3.1.9.7). The required sample size was calculated as 47 using the "difference between two dependent means (matched pairs)" model with 95% power (δ= 3.35, effect size=0.5, t=1.68) considering the HbA1c value (10).

Intervention

The supervised structured moderate-vigorous intensity aerobic and resistance training was applied to the participants 3 days a week for 12 weeks. Simple to difficult repetitive movements and walking exercises without equipment were used in aerobic exercises. The progression of the exercises was determined by the Borg rating of perceived exertion (RPE) scale. The amount of fatigue felt on the Borg RPE scale increased from 11-12 to 13-14 when moderate-intensity aerobic exercises were performed. Strengthening exercises consisted of exercises for shoulder girdle muscles, flexor and extensor muscles of knee and hip, abdominal muscles, and trunk extensor muscles. Theraband resistance was increased every 12 sessions for the progression of the strengthening exercises in the 36-session exercise program, which has been separated into three parts. Exercises were performed using a red-colored theraband, 8 repetitions, and 1 set in the first 12 sessions. Exercises were repeated with the green-colored theraband in the second 12 sessions, increasing the number of repetitions from 8 to 10, and the number of sets from 1 to 3. The exercises were performed with the blue-colored theraband in the last 12 sessions, with the number of repetitions and sets rising according to the patient's condition.

A dietitian created a diet plan for all of the study participants to maintain a consistent calorie intake. For all participants, a diabetic diet consisting of 1800 calories, 46 percent carbohydrates, 18 percent protein, and 36 percent fat was standardized. The participant's compliance with the diet program was evaluated.

Outcome Measurements

Demographic information and anthropometric measurements such as age, weight, height, waist circumference, waist/hip ratio, BMI, and disease duration of all individuals participating in the study were obtained. During the evaluation process of the participants, HbA1c measurements, muscle strength measurements, balance, and sleep quality evaluations were performed.

Body Composition Assessment

Basic anthropometric measurements included weight, height, waist circumference (level of umbilicus), and hip circumference (largest protrusion of the buttocks), measured to the nearest 0.1 cm. BMI was calculated as kg/m^2.

HbA1c Measurement

HbA1c blood test evaluation of all participants was obtained from the Istanbul Medipol University laboratory.

Muscle Strength Evaluations

Myometer was used to evaluate hamstring and quadriceps muscle strength. Myometer is a device that allows for measuring muscle strength objectively. During muscle strength measurement, the patient is first positioned. Then the myometer is placed on the distal side of the area to be measured. The force applied patient is asked to maintain his position. At the point where the patient cannot continue the movement, the force application is stopped and the maximum force recorded by the device is recorded. Each measurement was repeated 3 times, and the average of the test was recorded as kilograms (12).

Single Leg Stance Test

A single-leg stance test was used for balance assessment. For this test, the participants were asked to stand for 30 seconds without falling, with the non-evaluated side knee in 90° flexion. The stopwatch was started as soon as the foot was lifted off the ground. Each falling move was recorded as a score to keep the individual's balance. The test was repeated 3 times for the right and left leg and the average was taken (13). The validity and reliability of the single leg balance test were done by Sarac et al. (14).

Pittsburgh Sleep Quality Index

It is a scale that provides a quantitative measurement of sleep quality to define good and bad sleep developed by Buysse et al. (15) in 1989. The validity and reliability of the Pittsburgh sleep quality index (PSQI) were done by Agargun et al. (16) in 1996. It contains a total of 24 questions. 19 of these questions are self-evaluation questions, and 5 of them are answered by the spouse or roommate of the individual. The 18 items scored are grouped into 7 components. These seven component scores give the overall score. The total score is between 0-21. A high score indicates low sleep quality (15).

Statistical Analysis

IBM SPSS v.25.0 for Windows was used for statistical analysis. The normal distribution of the variables was tested by the Kolmogorov-Smirnov test. The comparison of the participants' pre and post-treatment evaluations was done with paired samples t-test in dependent samples. The significance value was accepted as $p<0.05$.

RESULTS

The study was completed with 50 participants with type 2 DM. The demographic and clinical characteristics of the participants were shown in Table 1. 32 (%64) of the participants were female and 18 (%36) were male. The mean age of the participants was 50.12±10.81 years, the mean BMI was 29.97±3.12 kg/m^2, and the waist/hip ratio was 0.91±0.08. In addition, 18 (%36) of the participants are type 2 DM between 0-5 years, 12 (%24) of them are type 2 DM between 5-10 years, and 20 (%40) of them are type 2 DM for more than 10 years. 22 (%24) of the participants use drugs, 12 (24%) of them use drugs and insulin, and 16 (32%) of them use insulin. When the participants' pre and post-treatment HbA1c, muscle strength, balance, and sleep quality measurement results were compared, there was a statistically significant difference in all parameters ($p<0.001$, Table 2). HbA1c values statistically significantly decreased after treatment. Muscle strength results of hamstring, quadriceps, and deltoid muscles significantly increased after treatment. Also, balance scores statistically significantly increased.

DISCUSSION

It was aimed to examine the effects of 12 weeks of aerobic and strengthening exercises on HbA1c value, muscle strength, balance, and sleep quality. It was observed that the exercises provided significant improvement on all variables.

Table 1. Demographic and clinical characteristics of the participants with type 2 DM

Age (years), mean±SD (min-max)	50.12±10.81 (30-65)
Gender, n (%)	
Female	32 (64)
Male	18 (36)
Height (cm), mean±SD (min-max)	164.60±9.74 (148-185)
Weight (kg), mean±SD (min-max)	82.28±11.78 (60-105)
WC (cm), mean±SD (min-max)	96.84±14.75 (70-133)
HC (cm), mean±SD (min-max)	106.04±14.42 (90-140)
BMI (kg/m^2), mean±SD (min-max)	29.97±3.12 (23.94-38.30)
WC/HC, mean±SD (min-max)	0.91±0.08 (0.67-1.07)
Age of DM, n (%)	
≤5 years	18 (36)
6-10 years	12 (24)
>10 years	20 (40)
Medical history, n (%)	
Oral drug	22 (44)
Drug+insulin	12 (24)
Insulin	16 (32)

DM: diabetes mellitus, SD: standard deviation, WC: waist circumference, HC: hip circumference BMI: body mass index

Table 2. Comparison of pre and post-treatment results

	Pre	Post	p
HbA1c (%)	9.19±2.39	7.26±1.30	**<0.001**
Right quadriceps MS	67.20±11.81	73.90±10.64	**<0.001**
Left quadriceps MS	64.85±12.59	70.29±11.41	**<0.001**
Right hamstring MS	60.97±12.25	65.52±12.50	**<0.001**
Left hamstring MS	54.82±12.59	60.36±12.56	**<0.001**
Right deltoid MS	57.45±10.22	62.16±9.58	**<0.001**
Left deltoid MS	55.81±11.23	61.91±10.06	**<0.001**
Right single LS	3.24±2.78	1.08±0.86	**<0.001**
Left single LS	3.92±2.64	1.32±0.94	**<0.001**
PSQI	10.16±3.28	6.48±2.67	**<0.001**

MS: muscle strength, LS: leg stance, PSQI: Pittsburgh sleep quality index

In the literature, it is stated that the incidence of type 2 DM is higher in females (1). When the genders of the individuals with type 2 DM who participated in this study were examined, 64% (n=32) were female. As in the results of epidemiological studies conducted in the field of type 2 DM in Turkey, the number of female participants in this study was higher than males (17). Routine doctor and nutritionist controls of all individuals were continued. We consider that these controls, which are repeated at regular intervals increase the effectiveness of the exercise program.

Hormonal and physical changes that occur in individuals with type 2 DM cause changes in body composition, increase the waist/hip ratio, and increase the risk of cardiovascular disease (18). In addition, changes in body composition in individuals with type 2 DM affect the musculoskeletal system, reducing the muscle strength and flexibility of individuals (19). As a result of the loss of strength in the skeletal muscles of individuals with diabetes, muscle atrophy accompanies the picture depending on the duration of diabetes. Changes occur in individuals such as deterioration in fasting blood sugar and glycosylated hemoglobin levels, postprandial hyperglycemia, systolic and diastolic blood pressure problems, and as a result, impairment of functionality, loss of balance, and decrease in sleep quality (3,4).

Exercise is one of the first treatment strategies recommended in the treatment of type 2 DM which has negative effects on many systems (7). In the American Diabetes Association (ADB), American Sports Medicine, and other current guidelines, it is reported that exercise is one of the most important strategies for the prevention and treatment of type 2 diabetes (1,20). Studies have shown that exercise training has beneficial effects on the glycemic profile, such as decreased HbA1c level, increased VO_2 max, and improved insulin sensitivity in diabetic patients. The risk of hypoglycemia increases in cases where the decrease in blood glucose levels cannot be controlled while performing exercise (21). For this reason, it is important to pay attention to all complications while creating an exercise program. In the present study, a structured exercise program specific to type 2 diabetes was created by considering cardiovascular risk factors and possible side effects. As a result, a decrease in the HbA1c value of participants was achieved.

Current national and international guidelines report that aerobic and resistance exercise training is effective in providing glycemic control for type 2 DM (22,23). In systematic reviews, positive effects of aerobic and resistance exercises have been proven on glycemic control, cardiovascular risk factors, and muscle strength in individuals with type 2 DM (23). Studies have shown that combining aerobic and strengthening exercises is more effective than aerobic or resistance exercises alone (24,25). Schwingshackl et al. (26), in a systematic review of 14 randomized controlled studies including 915 individuals with type 2 DM, reported that a combination of aerobic and strengthening exercise resulted in a greater reduction in HbA1c than aerobic or resistance training alone. In another meta-analysis, it was shown that an average of 0.6% reduction in HbA1c level can be achieved with combined aerobic and resistance exercise programs (27). In parallel with the literature, a structured exercise program consisting of aerobic and strengthening exercises was applied to individuals with type 2 DM for 12 weeks in the current study. It was observed that structured combined aerobic and strengthening exercises decreased the HbA1c value by 21%. We consider that the decrease in the HbA1c values of the individuals is related to the increase in blood glucose stores of the increased muscle mass with long-term exercise training under the supervision of a physiotherapist for 12 weeks.

Decreased muscle strength during type 2 DM also affects the physical activity level of individuals causing balance losses (28). In a study by Hameed et al. (29) on individuals with type 2 DM, it was stated that 12-week combined aerobic and progressive resistance exercises provided a statistically significant improvement in lower and upper extremity muscle strength. In individuals with type 2 DM, resistance exercise training increases muscle mass and strength, improves blood pressure, and thus positively affects metabolic control (30). In parallel with the literature, a significant improvement was found in bilateral quadriceps, hamstring, and deltoid muscle strength in this study. We consider that the significant increase in muscle strength is due to the fact that during 12 weeks of diabetes-specific structured aerobic and strengthening exercises increasing the resistance levels by changing the theraband color based on the tolerance of all individuals strengthens the mitochondrial structure in the muscle motor unit and causes changes in the volume of its fibers.

Balance problems also develop depending on the muscle strength and physical performance losses of individuals with type 2 DM (31). In a study, strengthening and sensorimotor exercises were given to individuals with type 2 DM for 4 weeks. At the end of 4 weeks, it was observed that there was a significant improvement in the test results of individuals standing on one leg (32). In the study of Lee et al. (33), a program consisting of stretching, balance, and aerobic exercises given 3 days a week for 6 weeks was applied to individuals with type 2 DM, and it was reported that there was an improvement in balance function as measured by the single-leg standing test and the timed sit and stand test (33). In the current study, the single-leg stance test was used to evaluate balance function, and a significant improvement was noted in balance function with structured aerobic and strengthening exercises. We believe that increasing muscle strength with a 12-week exercise program increases the improvement in balance function. We also propose that balance functions were improved by activating visual, vestibular, and proprioceptive stimuli together with aerobic and strengthening exercises. It is thought that exercises increase the elasticity of the muscles, muscle contraction speed, joint range of motion, the amount of oxygen coming to the muscle, and most importantly, the nerve conduction velocity contributes positively to proprioception, thus improving balance.

Studies have reported that disruptions in the glucose mechanism of individuals with diabetes impair sleep quality by making it difficult to fall asleep (34,35). It has been reported that individuals with type 2 DM have lower sleep and quality of life compared to individuals without diabetes (36). Delevatti et al. (37) reported that there is a relationship between sleep quality and quality of life in individuals with type 2 DM, and exercise training

improves both at a similar rate. A similar increase was found in the PSQI and World Health Organization (WHO) quality of life indexes with aerobic exercises lasting 12 weeks (37). Another study has shown that yoga and aerobic exercises performed for 12 weeks have positive effects on PSQI in individuals with type 2 DM (38). Similar to the literature, sleep quality was also examined in this study and significant results were recorded in sleep quality in individuals with type 2 DM with 12-week exercise training.

The strength of our study is that the exercise program is combined and continued for 12 weeks. It has been proven that combined exercises have positive effects on HbA1c, fasting and postprandial glucose levels, and insulin sensitivity before, but studies examining the effects on balance, muscle strength, and sleep quality are limited. The limitation of our study is the absence of a control group. More meaningful results can be achieved by adding a control group in future studies.

CONCLUSION

It was observed that the combination of long-term aerobic and strengthening exercise improved glucose level, muscle strength, balance, and sleep quality in individuals with type 2 DM. A structured exercise program, which includes strengthening and aerobic exercises would be beneficial in developing the most effective and appropriate exercise prescriptions in terms of exercise efficiency and sustainability for individuals with type 2 diabetes in clinics. It would manage overall symptoms, enhance functionality and quality of life, and reduce disease-related health costs.

Ethics Committee Approval: The study was approved by the Non-invasive Clinical Researches Ethics Committee of İstanbul Medipol University (09.12.2021, 1262).

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The Effect of Preoperative Anemia on Hiatal Hernia Surgery and Postoperative Complications

Preoperatif Aneminin Hiatal Herni Cerrahisi ve Postoperatif Komplikasyonlara Etkisi

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ABSTRACT

Aim: Anemia is associated with increased mortality and morbidity in all patients undergoing surgery. This study aimed to reveal to what extent anemia caused postoperative complications before hiatal hernia surgery.

Material and Methods: One hundred and ninety-two patients operated for hiatal hernia in our clinic between 2015 and 2019 were analyzed retrospectively. Patients were compared according to demographics, preoperative laboratory values, preoperative Charlson comorbidity index score, hiatal hernia type, postoperative intensive care requirement, presence of postoperative complications, Clavien-Dindo score, blood transfusion need, and length of hospital stay.

Results: Of the patients, 109 were female and 83 were male. The mean age was 50.1±9.1 years. Fifty-four (28.2%) of the patients were in the anemic group, and 138 (71.8%) were in the non-anemic group. There was no statistically significant difference between the groups in terms of gender distribution (p=0.663). When the groups were compared in terms of age distribution, it was found that the anemic group was older than the non-anemic group and the difference was statistically significant (p<0.001). There was a significant difference between the groups in terms of the postoperative complications rate and it was more common in the anemic group (p<0.001). There was a significant difference between the groups in terms of the need for intensive care and blood transfusion in the postoperative period (p=0.020, p<0.001, respectively).

Conclusion: Preoperative anemia increases postoperative complications in hiatal hernia surgery. Therefore, the presence of anemia should be investigated before hiatal hernia surgery and patients with anemia should be followed up closely postoperatively.

Keywords: Anemia; hiatal hernia; morbidity; mortality.

ÖZ

Amaç: Anemi, cerrahi geçiren tüm hastalarda artmış mortalite ve morbidite ile ilişkilidir. Bu çalışmada, hiatal herni cerrahisi öncesi aneminin ne ölçüde postoperatif komplikasyonlara neden olduğunu ortaya koymak amaçlandı.

Gereç ve Yöntemler: Kliniğimizde, 2015 ve 2019 yılları arasında hiatal herni nedeniyle ameliyat edilmiş olan hastalar geriye dönük olarak incelendi. Hastalar demografik, preoperatif laboratuvar değerleri, preoperatif Charlson komorbidite indeksi skoru, hiatal herni tipi, postoperatif yoğun bakım gereksinimi, postoperatif komplikasyon varlığı, Clavien-Dindo skoru, kan transfüzyon ihtiyacı ve hastanede kalış süresi açısından karşılaştırıldı.

Bulgular: Hastaların 109'u kadın ve 83'ü erkek idi. Yaş ortalaması 50,1±9,1 yıl idi. Hastaların 54'ü (%28,2) anemik grupta ve 138'i (%71,8) ise anemik olmayan grupta idi. Cinsiyet dağılımı açısından gruplar arasında istatistiksel olarak anlamlı bir fark yoktu (p=0,663). Gruplar yaş dağılımı açısından karşılaştırıldığında, anemik grubun anemik olmayan gruba göre daha yaşlı olduğu ve bu farkın istatistiksel olarak da anlamlı olduğu saptandı (p<0,001). Postoperatif komplikasyon oranı açısından gruplar arasında anlamlı bir fark vardı ve anemik grupta daha sık olduğu görüldü (p<0,001). Postoperatif dönemde yoğun bakım ünitesi ve kan transfüzyonu ihtiyacı açısından da gruplar arasında anlamlı bir fark vardı (sırasıyla p=0,020, p<0,001).

Sonuç: Preoperatif anemi, hiatal herni cerrahisinde postoperatif komplikasyonları arttırır. Bu nedenle, hiatal herni ameliyatı öncesi anemi varlığı araştırılmalı ve anemisi olan hastalar postoperatif dönemde yakın takip edilmelidir.

Anahtar kelimeler: Anemi; hiatal herni; morbidite; mortalite.

INTRODUCTION

Hiatal hernia is a common pathology. In a hiatal hernia, the normal anatomy of the gastroesophageal junction and the diaphragmatic hiatus is disrupted. Hiatal hernia is a predisposing factor for gastroesophageal reflux disease (GERD). According to United States of America statistics, hiatal hernia could be seen between 10% and 80% of adults (1). It is detected with a rate of 0.8-2.9% in patients who have undergone endoscopy (2,3).

The cause of anemia in the hiatal hernia is an iron deficiency caused by Cameron's ulcer due to hernia (4,5). However, Cameron ulcer was seen in only 29-42% of anemic hiatal hernia patients. Furthermore, when patients were operated on for hiatal hernia that caused anemia, it was observed that their anemic status improved (6). It has been shown that anemia and malnutrition increase mortality and morbidity in all patients undergoing surgery (7). Another cause of morbidity is postoperative complications. These complications are seen with a rate of 3-45% after hiatal hernia surgery (8). Complications are divided into those related to the patient and those that are not. Therefore, anemia is a factor that causes complications depending on the patient's status in hiatal hernia surgery.

The aim of this study was to reveal the effect of anemia existing before hiatal hernia surgery on perioperative complications.

MATERIAL AND METHODS

Patients who were operated for hiatal hernia in the General Surgery Clinic of Okmeydanı Training and Research Hospital between January 2015 and January 2019 were retrospectively analyzed. Patients who were operated for hiatal hernia, who had no history of abdominal surgery, whose hiatal hernia type was determined by preoperative endoscopy, who had preoperative blood tests, and whose data were complete, were included in the study. Patients who had surgery with the diagnosis of recurrent hiatal hernia, who underwent additional surgery together with the hiatal operation, who had a history of abdominal surgery, and whose preoperative blood tests and endoscopy data were missing were excluded from the study. Physical examination, blood tests (complete blood count, iron, ferritin, serum iron-binding capacity), chest radiography, and upper gastrointestinal endoscopy were routinely performed on all patients before surgery.

Data Collection

Demographics, complaints at the time of admission, preoperative laboratory values, preoperative upper gastrointestinal endoscopy reports, hiatal hernia type, operation timing (emergency-elective), intraoperative or postoperative blood transfusion, perioperative mortality, and morbidity, the length of hospital stay was recorded.

Study Design and Evaluation

According to the World Health Organization, anemia is defined as serum hemoglobin levels below 12 mg/dL in female and 13 mg/dL in male (10). Hemoglobin value was determined from the complete blood count of the patients within one month before surgery. According to their serum hemoglobin level, the patients were divided into two groups: anemic and the group without anemia. In our clinic, the presence of iron deficiency anemia is investigated in patients with endoscopic pathology. Iron, ferritin, and serum iron binding capacity tests were performed in all patients preoperatively, along with routine laboratory tests. The patient's preoperative status and comorbid diseases were examined according to the Charlson comorbidity index (CCI), and each patient's CCI score was determined (10,11). Postoperative complications were graded according to their severity using the modified Clavien-Dindo (CD) scale (12). The group with and without anemia was compared according to demographics, preoperative laboratory values, preoperative CCI score, hiatal hernia type, postoperative intensive care requirement, postoperative complications, CD degree, need for blood transfusion, and length of hospital stay.

All patients were evaluated preoperatively by the Anesthesia and Reanimation Clinic. According to the hemoglobin >10 mg/dl in patients with additional disease in elective surgery, and hemoglobin >7 mg/dl in patients without the additional disease, general anesthesia was approved for use in elective surgery (13).

The local ethics committee approval was obtained from the Clinical Researches Ethics Committee of Okmeydanı Training and Research Hospital (20.02.2019, 57).

Statistical Analysis

The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Mean and standard deviation (SD) were used in the analysis of continuous data. Median, minimum, maximum, and interquartile range (IQR) were used in the analysis of ordinal data. Frequency (n) and percentage values (%) were used when defining categorical variables. The Fisher's exact and Fisher-Freeman-Halton tests were used to evaluate the relationship between categorical variables. The Independent samples t-test and the Mann-Whitney U test were used to compare the two independent groups. The statistical significance level of the data was taken as $p<0.05$. Statistical analyzes were performed using the MedCalc v.19.5.1 program and www.e-picos.com (New York).

RESULTS

During the study, 215 patients were operated on with the diagnosis of hiatal hernia in our clinic. One patient with the diagnosis of recurrent hiatal hernia, six patients who underwent hiatal surgery and cholecystectomy, five patients with a history of abdominal surgery, eight patients with missing preoperative blood tests, and three patients with missing preoperative endoscopy data were excluded from the study. A total of 192 patients who met the inclusion criteria were included in the study. Of the 192 patients, 109 (56.8%) were female, and 83 (43.2%) were male. The mean age of the patients was 50.1±9.1 years.

Patients with hemoglobin levels below 12 mg/dL in female and 13 mg/dL in male were considered anemic. While the hemoglobin value was below 12 mg/dL in 32 (29.4%) female patients, it was above the reference value in 77 (70.6%) patients. While the hemoglobin value was below 13 mg/dL in 22 (26.5%) male patients, it was above the reference value in 61 (73.5%) patients. 54 (28.1%) of the patients were in the anemic group, and 138 (71.9%) were in the non-anemic group (Table 1).

In the anemic group, of the 54 patients, 32 (59.3%) were female, and 22 (40.7%) were male; in the non-anemic group, of the 138 patients, 77 (55.8%) were female, and 61 (44.2%) were male. There was no statistically significant difference between the groups regarding gender distribution (p=0.663). The mean age was 56.4±8.5 years in the anemic group and 47.7±8.1 years in the non-anemic group. When the groups were compared in terms of age distribution, it was detected that the anemic group was older than the non-anemic group and the age difference was statistically significant (p<0.001, Table 1).

Type 1 hiatal hernia was detected in 39 (20.3%) patients, type 2 in 23 (11.9%) patients, type 3 in 118 (61.5%) patients and type 4 in 12 (6.3%) patients. There is a difference between the groups in terms of hernia types and this difference is due to the distribution of Types II, III, and IV (p=0.001, Table 1).

The CCI was used to evaluate the preoperative clinical conditions of the patients. The mean CCI score of the patients in the anemic group was 3.24±0.78, while that of the non-anemic group was 2.25±0.64. Thus, there was a significant difference in comparing the groups using the CCI score (p<0.001, Table 1).

According to serum iron, ferritin, and serum iron-binding capacity values, chronic disease anemia was not found in any of the patients. While there was a positive correlation between hemoglobin, serum iron, and ferritin levels of the patients, a negative correlation was found between serum iron-binding capacity and other values (p<0.001, Table 2).

Elective surgery was performed on all patients. All patients were initiated with laparoscopic technique, but four patients had laparotomy due to insufficient laparoscopic exploration. The rate of transition from laparoscopic surgery to conventional surgery was 2.1%. All of these four patients were in the anemic group.

The postoperative median time of hospital stay of the patients was 3 (range, 1-7) days. The median length of hospital stay was 3 (range, 2-7) days in the anemic group and 2 (range, 1-5) days in the non-anemic group. Thus, there was a statistically significant difference between the anemic and non-anemic groups regarding the length of hospital stay (p<0.001, Table 3).

Intraoperative bleeding was detected in five (2.6%) patients in total. Three (5.6%) of these patients were from the anemic group, and two (1.4%) patients were from the non-anemic group. Intraoperative blood transfusion was applied to four (7.4%) patients from the anemic group and two (1.4%) from the non-anemic group. When intraoperative bleeding and subsequent blood transfusion were compared, there was no statistically significant difference between the groups (p=0.136, and p=0.054, respectively, Table 3).

In the postoperative period, complications were observed in 35 (64.8%) patients in the anemic and 40 (28.9%) patients in the non-anemic group. Of the 35 patients in the anemic group who had complications, 22 (62.9%) had CD class I, 11 (31.4%) had class II, and 2 (5.7%) had class III complications. Class I complications were detected in 30 (75%), and class II complications were detected in 10 (25%) of the 40 patients who had complications in the non-anemic group. Thus, there was a significant difference between the groups regarding the proportion of postoperative complications, and it was found to be more common in the anemic group (p<0.001, Table 3).

Of the seven (3.6%) patients who needed postoperative intensive care, five (9.3%) patients were in the anemic group, and two (1.4%) were in the non-anemic group. Eight (14.8%) of ten (5.2%) patients who received a blood transfusion in the postoperative period were in the anemic group, and two (1.4%) patients were in the non-anemic group. Thus, there was a significant difference between the groups regarding the need for intensive care and blood transfusion in the postoperative period (p=0.020, and p<0.001, respectively, Table 3).

Table 1. Patients' demographics and preoperative factors

	Anemic (n=54)	Non-anemic (n=138)	p	All Patients (n=192)
Age (year), mean±SD	56.4±8.5	47.7±8.1	**<0.001**	50.1±9.1
Charlson comorbidity index, mean±SD	3.24±0.78	2.25±0.64	**<0.001**	2.53±0.81
Gender, n (%)				
Female	32 (59.3)	77 (55.7)	0.663	109 (56.8)
Male	22 (40.7)	61 (44.3)		83 (43.2)
Hernia Type, n (%)				
Type I	9 (16.7)	30 (21.7)	**<0.001**	39 (20.3)
Type II	11 (20.4)	12 (8.7)		23 (11.9)
Type III	26 (48.1)	92 (66.7)		118 (61.5)
Type IV	8 (14.8)	4 (2.9)		12 (6.3)

Table 2. Comparison of laboratory values

	Anemic (n=54)	Non-anemic (n=138)	p	All Patients (n=192)
Hemoglobin (mg/dL)	10.8±1.1	14.2±1.3	**<0.001**	13.2±1.9
Hematocrit (%)	81.5±10.5	84.9±6.8	**<0.001**	83.9±8.1
MCV (fl)	32.7±3.7	42.3±4.1	**<0.001**	39.6±5.9
Serum Iron (mg/dL)	51.2±12.9	121.3±34.9	**<0.001**	101.6±43.9
Ferritin (mg/L)	41.7±30.4	224.7±18.1	**<0.001**	173.2±130.7
FIBC (mg/dL)	318.5±50.2	212.9±76.1	**<0.001**	242.6±84.4

MCV: mean corpuscular volume, FIBC: free iron-binding capacity

Table 3. Perioperative and postoperative results

	Anemic (n=54)	Non-anemic (n=138)	p	All Patients (n=192)
Hospitalization time (day)	3 (1) [2-7]	2 (1) [1-5]	**<0.001**	3 (1) [1-7]
Intraoperative bleeding, n (%)	3 (5.6)	2 (1.4)	0.136	5 (2.6)
Intraoperative blood transfusion, n (%)	4 (7.4)	2 (1.4)	0.054	6 (3.1)
Postoperative complication, n (%)	35 (64.8)	40 (28.9)	**<0.001**	75 (39.1)
Clavien-Dindo scale, n (%)				
Stage I	22 (40.7)	30 (21.7)		52 (27.1)
Stage II	11 (20.4)	10 (7.2)	0.228	21 (10.9)
Stage III	2 (3.7)	0 (0.0)		2 (1.1)
Postoperative intensive care unit, n (%)	5 (9.3)	2 (1.4)	**0.020**	7 (3.6)
Postoperative blood transfusion, n (%)	8 (14.8)	2 (1.4)	**<0.001**	10 (5.2)

DISCUSSION

Sliding hiatal hernia, which is common in adults, is a result of weakened muscles and fascial tissues in the hiatal region (1). Conditions such as obesity, pregnancy, chronic cough, vomiting, and retching that increase positive intraabdominal pressure also predispose to hiatal hernia. When the hiatal hernia is symptomatic, an operation is required (2).

Although hiatal hernia surgery is mostly elective and clean field surgery; rarely, emergency operations may be required. For these reasons, complications during the perioperative period are desired to be prevented. Stauder et al. (14) reported that anemia is most common in advanced age. Anemia prevalence was found at 17% in a cohort case series of older adults over 65 years old, although it was lower at younger ages. Bleeding in patients with hiatal hernia is primarily chronic and leads to anemia. Bleeding may occur in 1/4 of the patients with hiatal hernia and GERD. Bleeding due to these diseases constitutes 10% of all acute and chronic upper gastrointestinal bleeding. The most common causes of bleeding related to hiatal hernia and GERD are gastric ulcers, erosive esophagitis, esophageal ulcers, peptic strictures, and Barrett's esophagus (15). In the present study, the patients were adults between the ages of 4 and 6 decades; the rate of anemia is 28.1%. Therefore, it was considered that the increase in the incidence of anemia was due to hiatal hernia. Although anemia was more common in females in this study, gender was not effective in anemia.

In a study conducted by Cheverie et al. (16), it was reported that hiatal hernia causes anemia, and anemia disappears at a rate of 50% after repair. Besides, Addo et al. (17) reported that Cameron ulcer healed, anemia disappeared, and the quality of life improved after hiatal hernia repair in patients with secondary chronic anemia. We investigated the extent of complications caused by anemia after hiatal hernia surgery, but the presence of stomach ulcers or anemia that stomach ulcers may cause was not discussed. However, it was reported by Hourani et al. (18) that this pathology, defined as Cameron ulcer, may cause anemia and even regress after the operation. The current study is a retrospective study, and the data are limited. Therefore, the presence of ulcers and the location of the ulcers in patients is not known. Furthermore, it has been shown that as the ulcer regresses with hiatal hernia repair, anemia can also be treated, and complications that may be caused by hiatal hernia can be prevented (19,20).

There is other literature showing the effect of the hiatal hernia type on the prevalence of anemia. While Hourani et al. (18) reported that the type of hiatal hernia affects the frequency of anemia, notably paraesophageal hernia, which causes anemia more frequently. Yu et al. (1) showed that there is no relationship between hernia type and anemia.

The present study considered that other causes of comorbidity might be together with anemia as the leading cause of comorbidity in patients. The patients were evaluated with CCI, and it was detected that there were other causes of comorbidity together with anemia. In a study by Che et al. (21), it was shown that more than one comorbid disease might coexist in patients with hiatal hernia. Also, it has been shown that the coexistence of more than one comorbid disease after hiatal hernia surgery may cause increased morbidity. In the study conducted by Chevrollier et al. (22), the CCI score was high in anemic patients. Similar results were obtained in terms of postoperative intensive care requirement, postoperative complication risk, and blood transfusion need both in the current study and in the study of Chevrollier et al. (22). It was detected higher in anemic patients.

In a study conducted by Trastek et al. (23), postoperative complications were observed in 18 (36.7%) patients after hiatal hernia repair, nine were significant, and nine were minor complications. In the current study, postoperative complications were observed in 65% of patients with preoperative anemia, 94% of these were class I and II, and 6% were class III. Thus, in anemic patients, it was observed that the rate of complication and the severity of complications support the literature, but the distribution of complications may vary.

After hiatal hernia repair, chronic anemia negatively affects the length of stay in the hospital and the need for intensive care (1). While preoperative anemia is a risk factor in increasing hospitalization duration after hiatal hernia surgery, it does not significantly affect intensive care needs.

This study has some limitations. Some studies on different gastrointestinal diseases other than hiatal hernia, preoperative anemia, and malnutrition data were collected together, and postoperative complications were evaluated accordingly (24). In their hiatal hernia study, Clark et al. (25) reported that anemia and malnutrition had separate effects on postoperative complications. Malnutrition

was not evaluated in this study, and only anemia was evaluated. Besides, we could not evaluate the relation of preoperative anemia with intraoperative bleeding, intraoperative and postoperative blood transfusion needed due to limited data. Due to the limited number of studies in the literature, prospective randomized controlled studies with more extensive patient series are needed to reach more precise and detailed data on this subject.

The limitations of the current study are its retrospective design, the small number of patients, the lack of a clear algorithm for surgical indications, the effect of intraoperative bleeding, and the lack of postoperative endoscopic recovery data.

CONCLUSIONS

Anemia, sometimes, might be the only finding for a hiatal hernia. Cameron ulcer, which is caused by a hiatal hernia and is the cause of anemia, regresses after the hernia repair. Anemia before hiatal hernia repair increases postoperative complications. When surgery is planned to treat asymptomatic hiatal hernia, anemia, or ulcers, care should be taken for postoperative complications caused by anemia.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Okmeydanı Training and Research Hospital (20.02.2019, 57).

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Comparison of Asymptomatic Bacteriuria Agents and Antimicrobial Susceptibility in Term and Preterm Pregnancies

Term ve Preterm Gebelerde Asemptomatik Bakteriüri Etkenleri ve Antimikrobiyal Duyarlılığın Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to compare the resistance to asymptomatic bacteriuria (ABU) between term and preterm pregnancies.
Material and Methods: Pregnant women aged 17-41 years who were followed up in the obstetrics department of our hospital and whose urine cultures were sent to the laboratory for analysis and reported as ABU were included in the study. Patients were divided into two groups according to gestational weeks as preterm (<37 weeks) and term (≥37 weeks). The results were compared between the two groups.
Results: From among a total of 123 pregnant women, 29 (23.6%) delivered at preterm and 94 (76.4%) at term. The most frequently identified pathogen throughout the study group was 52.0% (n=64) *Escherichia coli* (*E. coli*), followed by 16.3% (n=20) *Streptococcus agalactiae* (*S. agalactiae*). There was no statistically significant difference in terms of the distribution of *E. coli* and *S. agalactiae* between the term and preterm groups (p=0.698 and p=0.930). *E. coli* was resistant to ampicillin 56.3% (n=36), to cefuroxime 40.6% (n=26). While fosfomycin resistance was 1.6% (n=1), nitrofurantoin resistance was not found. Extended-spectrum beta-lactamase positivity was 23.4% (n=15) in *E. coli* strains. No statistically significant difference was found in antibiotic resistance rates of *E. coli* strains between the term and preterm groups.
Conclusion: The most commonly isolated pathogen was *E. coli* which was highly resistant to beta-lactams. Screening of pregnant women for ABU and treatment with appropriate antibiotics; is the most effective way to prevent both maternal and fetal complications and antimicrobial resistance.
Keywords: Pregnancy; asymptomatic bacteriuria; antibiotic resistance.

ÖZ

Amaç: Bu çalışmanın amacı, asemptomatik bakteriüri (ASB) etkenlerinin direnç paternlerini term ve preterm gebelikler arasında karşılaştırmaktır.
Gereç ve Yöntemler: Hastanemiz kadın doğum bölümünde takip edilen ve idrar kültürleri laboratuvarda incelenmek üzere gönderilen ve ASB olarak rapor edilen 17-41 yaş arası gebeler çalışmaya dahil edildi. Hastalar gebelik haftalarına göre preterm (<37 hafta) ve term (≥37 hafta) olmak üzere iki gruba ayrıldı. Sonuçlar iki grup arasında karşılaştırıldı.
Bulgular: Toplam 123 gebeden 29'u (%23,6) preterm, 94'ü (%76,4) term doğum yaptı. Tüm çalışma grubunda en sık izole edilen patojen %52,0 (n=64) ile *Escherichia coli* (*E. coli*) idi ve bunu %16,3 (n=20) ile *Streptococcus agalactiae* (*S.agalactiae*) izlemekteydi. Term ve preterm grupları arasında *E. coli* ve *S. agalactiae* dağılımı açısından istatistiksel olarak anlamlı bir fark yoktu (p=0,698 ve p=0,930). *E. coli* suşları ampisiline %56,3 (n=36) oranında, sefuroksime %40,6 (n=26) oranında dirençliydi. Fosfomisin direnci %1,6 (n=1) olarak belirlenirken, nitrofurantoin direnci saptanmadı. *E. coli* suşlarında genişletilmiş spektrumlu beta-laktamaz pozitifliği %23,4 (n=15) olarak tespit edildi. Term ve preterm grupları arasında *E. coli* suşlarının antibiyotik direnç oranları bakımından istatistiksel olarak anlamlı fark saptanmadı.
Sonuç: En sık izole edilen patojen bakteri, beta laktamlara oldukça dirençli olan *E. coli* idi. Gebelerin ASB için taranması ve uygun antibiyoterapi ile tedavisi; maternal ve fetüste gelişecek komplikasyonları ve antimikrobiyal direnci önlemede etkili bir yoldur.
Anahtar kelimeler: Gebelik; asemptomatik bakteriüri; antibiyotik direnci.

INTRODUCTION

Pregnancy is a specific state in which both anatomical and physiological alterations reversibly occur in the urinary tract, giving a conducive environment for bacterial proliferation (1). During pregnancy, several alterations including ureter dilatation, decreased urethral peristalsis and bladder tonus, increased plasma volume and urine estrogen increase the risk of developing bacteriuria (2). Asymptomatic bacteriuria (ABU) is defined as the presence of at least 10^5 organisms/mL in the urine culture without any systemic or local symptoms in the urinary tract (3,4). The global prevalence of ABU was estimated between 2-15%, although its frequency depends on geographic regions (4).

The most commonly isolated bacteria in ABU are *Escherichia coli* (*E. coli*) and some other bacteria including *Enterobacteriaceae, Streptococcus agalactiae* (*S. agalactiae*), *Pseudomonas aeruginosa*, *Enterococcus* spp. and *Staphylococcus aureus* may cause ABU among pregnant women, while the isolated bacteria widely vary according to geographical areas (1,2,5,6). Studies have reported that ABU during pregnancy increases the risk of developing pyelonephritis and obstetric outcomes that may result in adverse events such as premature delivery and low birth weight (7-9). Possible outcomes of untreated pyelonephritis are increased maternal and fetal morbidity and mortality. In addition, it might lead to acute respiratory distress, acute renal failure, preterm delivery, and maternal fever might as well (10). Some studies have proposed that antimicrobial treatment of ABU improves fetal outcomes and decreases the frequency of low-birth-weight infants and preterm delivery (8,11). Although the adverse effects of antibiotic therapy associated with the treatment of ABU have been described in a limited number of studies, there is sufficient evidence that pyelonephritis in pregnancy is associated with adverse maternal outcomes and that ABU is detected by screening to reduce the incidence of the disease. ABU is recommended to be tested in pregnant women by urine culture (12).

In recent years, rising antimicrobial drug resistance has been observed in ABU cases. As a consequence, the significance of performing antibiotic sensitivity tests and routine urine cultures with the aim of determining the resistance patterns of isolated uropathogens and detecting ABU should be taken into consideration. Therefore, the objective of this study was to compare the resistance to ABU between term and preterm pregnancies.

MATERIAL AND METHODS

Ethics committee approval for the study was obtained from the clinical research ethics committee of the İstanbul Medeniyet University Göztepe Training and Research Hospital (numbered 154, dated 24.02.2021). Moreover, the study was conducted in accordance with the principles of the Declaration of Helsinki. In this study, urine samples of 1710 pregnant women were analyzed and 123 pregnant women between the ages of 17-41 years were diagnosed with ABU, in our hospital between January 2018 and February 2020. Patients with, dysuria, pollakiuria, complaint of suprapubic pain, vaginal discharge, malodor, those who were using antibiotics, and a history of renal disease or renal calculi were excluded from the study. Patients' demographic such as maternal age, gestational week, mode of delivery, and birth weight of the infant were recorded. Patients were divided into two groups according to gestational weeks, preterm (<37 weeks) and term (≥37 weeks).

Medium-flow urine samples were collected from the patients in a sterile vial after verbal instructions and a culture test was performed within two hours. Urine samples were inoculated on Chrom agar medium (bioMérieux, France). Culture outcomes were read 24 hours following incubation at 37° C. An amount of organism $>10^5$/mL was reported as ABU. Growing microorganisms were defined as matrix-assisted laser desorption ionization time-lapse light mass spectrometry (MALDI-TOF MS). VITEK-2 (bioMérieux, Marcy l'Etoile, France) compact automated systems were used to examine the antimicrobial susceptibility of microorganisms. The results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (13).

Statistical Analysis

Categorical variables were presented as numbers and percentages, while continuous variables were expressed as mean±standard deviation and median (minimum-maximum). Categorical variables were compared using the Pearson chi-square or Fisher's exact test. Normal distribution was tested using the Shapiro-Wilk test. Non-normal distributed variables were compared with the Mann-Whitney U test. SPSS v.25.0 (Statistical Package for Social Sciences, IBM Inc., Armonk, NY, USA) was used for statistical analysis. Two-sided p-values of less than 0.05 were considered statistically significant.

RESULTS

Significant growth was detected in 123 (7.2%) of a total of 1710 urine samples (10^5 organisms/mL). The mean age was found as 28.4±7.2 (range, 17-41) years. No statistically significant difference was found between the term and the preterm groups in terms of age (p=0.671, 28.90±6.87 vs 28.19±7.36, respectively). The mean gestational week was found to be 37.4±2.9 (range, 26-40) weeks. Of the 123 pregnancies, 29 (23.6%) were preterm births.

The most commonly isolated pathogen in entire study group was *E. coli* at 52.0% (n=64) followed by *S. agalactiae* at 16.3% (n=20), *Klebsiella pneumoniae* at 11.4% (n=14), *Enterococcus* spp. at 10.6% (n=13), *Candida* spp. at 3.3% (n=4), *Proteus* spp. at 2.4% (n=3), *Staphylococcus* spp. at 2.4% (n=3), and *Enterobacter cloaca* %1.6 (n=2).

Table 1 and Figure 1 present the distribution of pathogens between term and preterm pregnancies. There was no statistically significant difference in terms of the distribution of *E. coli* (p=0.698) and *S. agalactiae* (p=0.930) between the term and preterm groups.

All *Candida albicans* strains were susceptible to fluconazole. Considering the beta-lactam antibiotic resistance that can be safely used in pregnant women, among the gram-negative causative microorganisms, ampicillin resistance was 51/83 (61.5%), amoxicillin-clavulanate resistance was 32/83 (38.6%), cefuroxime resistance was 28/83 (33.7%), ceftriaxone resistance was 20/83 (24.1%), and ceftazidime was resistance 15/83 (18.1%). While fosfomycin resistance was 6/83 (7.2%), nitrofurantoin resistance was 4/83 (4.8%). *E. coli* strains, which were the

most common causative microorganism, were resistant to ampicillin 36/64 (56.3%), amoxicillin-clavulanate 24/64 (37.5%), cefuroxime 26/64 (40.6%), and ceftriaxone 20/64 (31.3%). While fosfomycin resistance was 1/64 (1.6%), no nitrofurantoin resistance was found. *Klebsiella pneumoniae* was the second most common gram-negative bacterium and resistance was found in 12/14 (85.7%) of the strains to ampicillin, 5/14 (35.7%) to amoxicillin-clavulanate, 4/14 (28.6%) to fosfomycin, 1/14 (7.1%) to nitrofurantoin, while all strains were susceptible to second and third-generation cephalosporins. Carbapenem resistance was not detected in any of the gram-negative agents. Fifteen of the cases had extended-spectrum beta-lactamase (ESBL) positivity and this resistance was only in *E. coli* strains (23.4%, 15/64). ESBL was not positive in *Klebsiella pneumoniae*, *Proteus* spp., or *Enterobacter cloaca* strains.

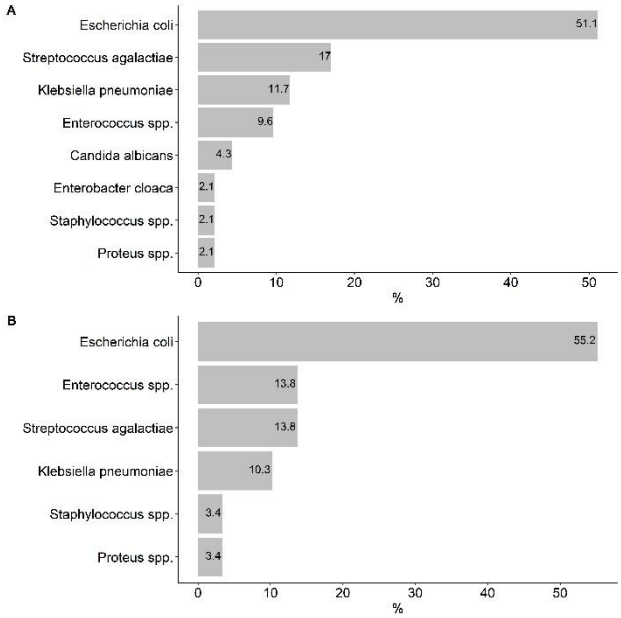

Figure 1. Percentages of the isolated microorganisms, **A)** term pregnancies (n=94), **B)** preterm pregnancies (n=29)

Table 1. Distribution of pathogens between term and preterm pregnancies

	Term (n=94)	Preterm (n=29)	p
Gram-negative, n (%)	63 (67.0%)	20 (69.0%)	0.845
Escherichia coli	48 (51.1%)	16 (55.2%)	0.698
Klebsiella pneumoniae	11 (11.7%)	3 (10.3%)	>0.999
Proteus spp.	2 (2.1%)	1 (3.4%)	>0.999
Enterobacter cloaca	2 (2.1%)	0 (0.0%)	>0.999
Gram-positive, n (%)	27 (28.7%)	9 (31.0%)	0.811
Streptococcus agalactiae	16 (17.0%)	4 (13.8%)	0.930
Enterococcus spp.	9 (9.6%)	4 (13.8%)	0.731
Staphylococcus spp.	2 (2.1%)	1 (3.4%)	>0.999
Fungi, n (%)			
Candida albicans	4 (4.3%)	0 (0.0%)	0.672

Table 2. Comparison of antimicrobial resistance of isolated *Escherichia coli* strains between term and preterm births

	Term (n=48)	Preterm (n=16)	p
Ampicillin, n (%)	27 (56.3%)	9 (56.3%)	>0.999
Ceftriaxone, n (%)	14 (29.2%)	6 (37.5%)	0.533
Cefuroxime, n (%)	18 (37.5%)	8 (50%)	0.382
Ceftazidime, n (%)	11 (22.9%)	4 (25%)	>0.999

In Table 2, antibiotic resistance frequencies of *E. coli* strains were compared in the term and preterm groups. In *E. coli* strains, ESBL positivity was 22.9% (11/48) in the term group and 25% (4/16) in the preterm group. However, there was no statistically significant difference between the groups in terms of ESBL positivity (p>0.999).

All strains of *S. agalactiae*, which was the most growing gram-positive agent, were susceptible to benzylpenicillin. *Enterococcus* spp. ampicillin and ampicillin-sulbactam resistance were found in 1/13 (7.7%) and 1/13 (7.7%) of the strains, respectively. Cefoxitin resistance was not detected in any staphylococcal strains.

DISCUSSION

ABU requires particular attention during pregnancy due to the absence of symptoms and related maternal and fetal consequences (14). In the present study, among 1710 urine samples cultured from asymptomatic pregnant women, 123 samples yielded uropathogens, showing a prevalence of 7.2%. This is consistent with the prevalence of ABU seen in other studies by Aktun et al. (2), Tokak et al. (5), and Aşgın et al. (6), respectively, 7.1%, 7.8%, and 10%. The occurrence of significant bacteria in the lower urinary tract with no symptoms makes the condition more susceptible in pregnant women. Several factors play a role in the development of ABU such as the number of pregnancies, physiological and anatomical changes, and poor genital hygiene. Being isolated from their samples to stop fetal and maternal morbidities, bacteria should be taken into consideration with its antimicrobial sensitivity patterns while treating the positive urine cultures of women (15).

In pregnant women, progesterone relaxes uteretic smooth muscle, which causes dilatation of the uterus caused by the pressure from the enlarging uterus. These factors lead to urinary stasis, dysfunctional urethral valves, and vesicoureteral reflux that assists bacterial colonization (16). Several studies have reported that the geographic variability of pathogens in the case of ABU is limited with the predominance of gram-negative, particularly *E. coli* (2,5,6,17,18). We also detected gram-negative isolates as the causative agent at a higher rate, 67.5%. However, there are studies reporting converse outcomes. Gram-positive isolates were reported as 63.8% by Ali et al. (16) and 64% by Dange et al. (19).

In the present study, the most commonly isolated pathogen was *E. coli* at 52.0% followed by *S. agalactiae* at 16.3%, *Klebsiella pneumoniae* at 11.4%, and *Enterococcus* spp. at 10.6% in all participants. The most common two isolates in the term group were *E. coli* with 51.1% and *S. agalactiae* with 17%. Whereas the most common two isolates in the preterm group were *E. coli* with 55.2% and *S. agalactiae* with 13.8%. There was no

statistically significant difference in terms of the distribution of *E. coli* and *S. agalactiae* between the term and preterm groups.

Andabati et al. (20) and Enayat et al. (21) reported that *E. coli* was the most commonly isolated organism with 51% and 58.96%, respectively. Aktun et al. (2) reported that the organism with the highest incidence was *E. coli* with 69%, while this rate was reported as 61.1% by Sonkar et al. (22) Nteziyaremye et al. (1) reported that the incidence of *E. coli* being 46.4%. Although different rates have been reported in previous studies, *E. coli* was the most commonly isolated pathogen in these studies. While Onu et al. (23) detected *Staphylococcus aureus* as the most common causative microorganism in their study, 45.9%; Ali et al. (16) found *E. coli* and *Staphylococcus aureus* strains to be equal in etiology at a percent of 31%. The variation between the rates of organisms might have resulted from the differences in geographic locations, socioeconomic state, and time of the study.

Therapeutic options are very limited and challenging due to different bacterial agents and their different resistance patterns in ABU (24). A concerning point, which limits therapeutic options in ABU is the emergence of significantly high drug resistance and over or misuse of antibacterial agents (25). What is more effective than treatment is ABU with some points of view such as monitoring, prevention, health condition, and cost. Antimicrobial drugs are frequently prescribed in pregnant women, and the increasing drug resistance must be taken into account (7). In this study considering the beta-lactam antibiotic resistance that can be safely used in pregnant women, *E. coli* is resistant to ampicillin 56.3%, amoxicillin-clavulanate 37.5%, cefuroxime 40.6%, ceftriaxone 31.2%. While fosfomycin resistance was 1.6%, nitrofurantoin resistance was not found. In the study of Aktun et al. (2), ampicillin resistance was 46%, cefuroxime resistance was 15%, ceftriaxone resistance was 10%, and nitrofurantoin resistance was 6.3%, while fosfomycin resistance was found below 1% in *E. coli* strains. In the study of Aşgın et al. (6), ampicillin resistance of *E. coli* isolates was 53%, and cefuroxime resistance was 18%, while fosfomycin resistance was 3%. In the same study, ESBL rates were found to be 8% in *E. coli* strains and 13% in *Klebsiella pneumoniae* strains (6). While we detected ESBL positivity in 23.4% of *E. coli* strains, we did not detect ESBL positivity in *Klebsiella pneumoniae*, *Proteus* spp., or *Enterobacter cloaca* strains. In the present study, pregnant women with ABU were treated with appropriate antibiotics and resistance rates in *E. coli* strains were compared between women with term and preterm deliveries. However, there was no statistically significant difference between the groups.

However, it is well known that the resistance patterns of these organisms vary significantly between countries and even among healthcare centers (26). It has been reported that in the prevention of ABU, the gold standard for treatment options and prevention of serious complications is screening the patients with urine culture and prescribing antibiotics correlated to the urine culture (7). Preterm birth incidence is indicated to be approximately 11% (27). A study by Uncu et al. (28) showed culture-negative group has 9.3% preterm birth, compared to the ABU group has 26% preterm birth. In addition, Lallar et al. (29) provided data that the preterm birth rate is 30% in the ABU group. The present study detected 23.6% preterm birth among pregnant women with ABU. Although mechanisms behind ABU causing preterm labor and ABU's independency as a risk factor are unclear (30), the statistical relationship between preterm birth and ABU distinct. Therefore, ABU should be diagnosed and treated early in pregnant women. In this way, the incidence of acute symptomatic urinary tract infections can be reduced by up to 70% (7,17).

The major limitations of this study are its retrospective design, relatively small sample size, and being conducted in a single center. We believe that our results would be encouraging for further extensive studies to be performed in the future.

CONCLUSION

The most commonly isolated pathogen was *E. coli* and no statistically significant difference was found between the term and preterm pregnancies in terms of the resistance in pregnant women with ABU. Considering undesired complications of ABU for the mother and fetus, screening at certain intervals for bacteria seems a better strategy than treating with antibiotics most of which are resistant to multiple therapeutic agents.

Ethics Committee Approval: The study was approved by the ethics committee of İstanbul Medeniyet University Göztepe Training and Research Hospital (24.02.2021, 154).

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Retrospective Analysis of Metastatic Bone Tumors

Metastatik Kemik Tümörlerinin Retrospektif Değerlendirmesi

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ABSTRACT

Aim: Bone tissue is the primary organ of hematopoiesis and osteogenesis in healthy individuals. Bone tissue is one of the most frequently metastasized organs. In addition, for all types of cancer, it is an indication that cancer has reached a level where it can no longer be cured and the patient's life expectancy is reduced. The aim of this study was to retrospectively evaluate the cases operated for bone metastasis.
Material and Methods: A total of 67 patients who underwent a biopsy or operation due to bone tumors at Eskişehir Osmangazi University Medical Faculty Hospital between January 2020 and January 2022 were included in this study.
Results: Of the metastatic cases, 49 (73.1%) were male and 18 (26.9%) were female. The mean age of the patients was 61.7±19.8 (range, 9-88) years. The most frequently metastasized tumors were lung carcinoma and tumor of unknown primary in males, while lung and breast in females. Tumors most frequently metastasize to the spine region. There were 6 (9.0%) cases, 1 (1.5%) of which was mesenchymal, in which the primary origin couldn't be detected in metastatic tumors.
Conclusion: Bone pain is always a symptom that is suspicious for metastasis in a patient followed up with a diagnosis of cancer and requires further investigation. Treatment is more palliative after bone metastasis. There is a need for targeted studies to prevent metastasis. It should be kept in mind that the primary focus may not be detected in all clinical and imaging methods in a group of patients.
Keywords: Bone neoplasms; neoplasm metastases; unknown primary neoplasm metastases.

ÖZ

Amaç: Kemik dokusu sağlıklı bireylerde hematopoez ve osteogenezisin primer olarak yapıldığı organdır. Kemik dokusu en sık metastaz alan organlardan biridir. Ek olarak, tüm kanser türleri için kanserin artık iyileşemeyecek bir düzeye ulaştığının ve hastanın beklenen yaşam süresinin kısaldığının bir göstergesidir. Bu çalışmanın amacı, kemik metastazı nedeniyle ameliyat edilmiş olan olguların geriye dönük olarak değerlendirilmesidir.
Gereç ve Yöntemler: Ocak 2020 ve Ocak 2022 tarihleri arasında Eskişehir Osmangazi Üniversitesi Tıp Fakültesi Hastanesi'nde kemik tümörü nedeniyle biyopsi yapılan veya ameliyat edilen toplam 67 hasta bu çalışmaya dahil edilmiştir.
Bulgular: Metastatik olguların 49 (%73,1)'u erkek ve 18 (%26,9)'i kadın idi. Hastaların ortalama yaşı 61,7±19,8 (aralık, 9-88) yıl idi. En sık metastaz yapan tümörler erkeklerde akciğer karsinomu ve primeri bilinmeyen tümör iken, kadınlarda ise akciğer ve meme karsinomu olarak saptanmıştır. Tümörlerin en sık omurga bölgesine metastaz yaptığı tespit edilmiştir. Metastatik tümörlerin içinde primer odağın saptanamadığı 1'i (%1,5) mezenkimal olmak üzere toplam 6 (%9,0) olgu vardır.
Sonuç: Kemik ağrısı kanser tanısı ile takip edilen bir hastada her zaman metastaz açısından şüpheli olan ve ileri tetkik yapılması gereken bir semptomdur. Kemik metastazı sonrasında tedavi daha çok palyatif olmaktadır. Metastazı önlemeye yönelik hedefe yönelik çalışmalara ihtiyaç vardır. Ayrıca bir grup hastada tüm klinik ve görüntüleme yöntemlerine rağmen primer odağın tespit edilemeyebileceği de akılda tutulmalıdır.
Anahtar kelimeler: Kemik neoplazileri; tümör metastazı; bilinmeyen primer neoplazm metastaz.

INTRODUCTION

Bone tissue is the primary site of hematopoiesis and osteogenesis in healthy individuals. Bone tissue has a microenvironment called the bone niche, supported by stem cells, progenitor cells, and immune system cells (1).

Metastatic cancer is an indication that cancer has become incurable. In addition, the most common cause of cancer-related death is untreatable metastatic cases (2). In metastatic cancer cases, treatment is mostly palliative. However, studies on this subject now show that this situation may change. As the bone metastasis mechanism of cancers is understood, new treatment modalities have begun to emerge (3). Before cancer cells metastasize to bone tissue, they must first be extravasated from their primary environment. As a second step, as a result of the mutual communication of cancer cells and the metastatic bone environment with mediators, cancer cells circulating in the bone are seen first. Then there is a period when cancer stays in a state of silence to survive. When it is reactivated, it first appears with micrometastasis and then with macro metastasis (4). Bone marrow tissue is a very suitable environment for tumor cells to come and settle. Cell types, connective tissue, and signaling mechanisms that create the appropriate environment constitute the "metastatic bone niche" (3). Recent studies are investigating treatments that target signaling mechanisms and support the metastatic bone niche (5).

It was first put forward in the study of Paget in 1889 in breast cancer cases that some tumors show tropism to some organs (6). Breast and prostate cancers show a greater affinity for bone and account for approximately 70% of all cases (7,8).

The most common cause of morbidity in cancer patients is bone metastasis. Complications such as fracture, spinal cord compression, nerve paralysis, hypercalcemia, and suppression of bone marrow function can be seen in cases with bone metastases (9-11). The most common reasons for patients to come to the hospital are bone pain, pathological fracture, and nerve compression (12).

This study, it was aimed to evaluate the cases presenting with metastasis to the bone of known or unknown primary, in the light of clinical and demographic findings.

MATERIAL AND METHODS

In this study, diagnostic biopsy, curettage, or surgical excision materials of the cases who applied to the clinic of Eskişehir Osmangazi University Medical Faculty Hospital with pathological fracture, bone pain, or incidentally detected bone mass between January 2020 and January 2022 were included. Between these dates, incisional or excisional biopsy was applied to a total of 116 cases for definitive diagnosis. Among these cases, 67 (57.8%) cases diagnosed with metastatic tumors were included in the study. Cases diagnosed as primary bone tumors were not included in the study. Metastatic tumors were divided into epithelial, mesenchymal, and hematological malignancies according to the cell of origin. Epithelial, mesenchymal, and hematological malignancies other than primary bone tumors were included in the study. All neoplasms with the known or unknown primary focus, metastasizing to the bone, were evaluated in the study. Biopsy was not taken from the cases that were clinically and radiologically certain for metastasis. Therefore, they were not included in this study. Demographic data of the cases included in the study were obtained from the pathology reports. The clinical data of the cases were obtained from the patient files of the Orthopedics and Traumatology department. The clinical pre-diagnosis of the cases, whether the primary focus was known during the operation, and whether the primary focus was detected later were recorded.

This study was approved by the Eskişehir Osmangazi University Non-Invasive Clinical Research Ethics Committee with decision number 18 on 26.04.2022.

Statistical Analysis

Data were recorded in the Microsoft Excel program. Gender, location of metastasis, type of metastatic tumor, and presence or absence of primary focus were evaluated. Continuous data were summarized as mean±standard deviation. Categorical data were reported as numbers and percentages.

RESULTS

Of the 116 bone tumor materials evaluated, 49 (42.2%) were primary and 67 (57.8%) were metastatic tumors. Most metastatic bone tumors presented with bone pain (40 of the 67 patients, 59.7%). 52 of the metastatic tumor cases were cases with a previous diagnosis of tumor and presenting with the diagnosis of metastasis. Other cases were referred with the suspicion of primary bone tumor and without a previous diagnosis of other organ malignancy. Forty of the metastatic bone disease cases presented with bone pain. 49 (73.1%) of the cases were male and 18 (26.9%) were female. The mean age of the patients was 61.7±19.8 (range, 9-88) years. The mean age for males was 61.9±20.1 and for females 58.1±18.8 years. The most common age ranges for metastases were 60-69 and 50-59 years.

The majority of metastatic cases were tumors of epithelial origin. Of the cases, 54 (80.6%) were epithelial, 8 (11.9%) were hematological, and 5 (7.5%) were mesenchymal neoplasms. The most common metastatic primary origin was lung carcinomas (Figure 1). While breast and lung tumors were most common in female patients, respectively, lung tumors, tumors of unknown origin, kidney, and urothelial carcinomas were most

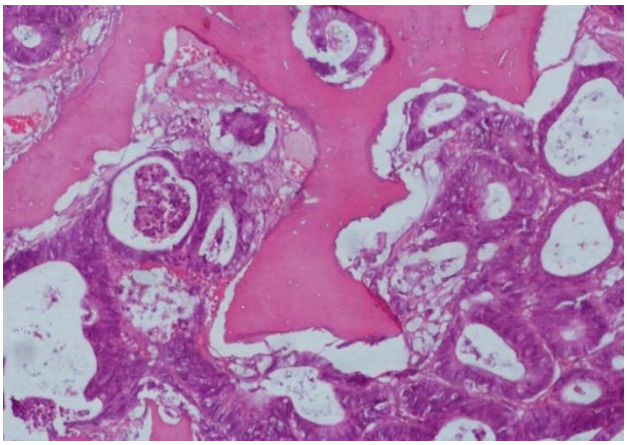

Figure 1. Metastatic lung adenocarcinoma (H&E, x100)

common in male patients, respectively (Table 1). The mean age of breast tumors, which is the most common metastatic carcinoma in females, was 65.8±5.9 years. The mean age of lung tumors, which is the most common metastasis in males, was 62.1±6.9 years.

Twenty-five (37.3%) of the tumors were in the vertebrae, 18 (26.9%) in the lower extremity, 9 (13.4%) in the upper extremity, 4 (6.0%) in the ribs, 3 (4.5%) in the cranial bones, 6 (9.0%) in the pelvic bones, 1 (1.5%) in the scapula, and 1 (1.5%) in the clavicle. Among the most frequently metastasizing carcinomas, primary lung carcinomas were most found to metastasize to the vertebrae and lower extremities, and breast carcinomas to the vertebrae most frequently. One of the prostate carcinoma metastases was to the lower extremity and one was to the cranial bones. Renal carcinomas most commonly metastasize to the vertebrae. The region with the most metastasis was the vertebral region, of which 19 (76%) were epithelial, 4 (16%) were hematological, and 2 (8%) were mesenchymal tumors.

The localization of tumors of hematological and mesenchymal origin was shown in Table 2.

Of the hematological origin metastases, 5 were plasma cell neoplasia, 2 were high-grade B-cell lymphoma, and 1 was Langerhans cell histiocytosis. Of the metastases of mesenchymal origin, 2 were Ewing sarcoma, 1 was monophasic synovial sarcoma, 1 was an undifferentiated solitary fibrous tumor (Figure 2), and 1 was a mesenchymal tumor of unknown primary.

Of the 8 cases of unknown primary, 1 was mesenchymal and reported as metastatic sarcoma. Of the 7 epithelial metastatic tumors unknown histopathologically, 2 were squamous cell carcinomas (Figure 3). There was no additional investigation to determine the squamous cell carcinoma primary histopathologically. For this reason, in metastatic squamous cell carcinoma cases, pathological interpretation cannot be made in terms of primary focus, and clinical radiological correlation is recommended. One of the primary foci in our cases was evaluated as lung and the other as skin squamous cell carcinoma.

As a result, there were 6 (9%) cases, 1 of whom was mesenchymal, whose primary focus could not be detected after the scans. Of the epithelial tumors, 2 were reported as poorly differentiated adenocarcinoma and 3 as poorly differentiated carcinoma.

Table 1. The most common bone metastasis epithelial tumors in male and female patients (n=54)

Female (n=11)		**Male (n=43)**	
Breast	5 (45.4)	Lung	14 (32.6)
Lung	3 (27.3)	UP Origin	5 (11.6)
GIS	1 (9.1)	Kidney	4 (9.3)
Other	2 (18.2)	Other	20 (46.5)

GIS: gastrointestinal system, UP: unknown primary

Table 2. Localization of mesenchymal and hematological malignancies (n=13)

Mesenchymal (n=5)		**Hematological (n=8)**	
Lower extremity	1 (20)	Lower extremity	2 (25)
Costa	1 (20)	Cranium	1 (12.5)
Pelvic bone	1 (20)	Upper extremity	1 (12.5)
Vertebra	2 (40)	Vertebra	4 (50)

Figure 2. Metastatic dedifferentiated solitary fibrous tumor. A gray-white mass with a pathological fracture in the femoral diaphyseal region is seen

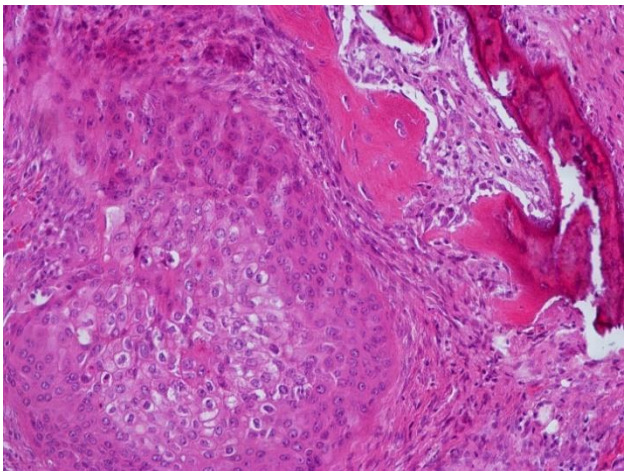

Figure 3. Metastatic squamous cell carcinoma (H&E, x200)

DISCUSSION

Bone is the most frequently metastasized organ after the lung and liver (13). The metastatic bone disease disrupts the process of balanced osteoblastic/osteoclastic activity of bone, resulting in skeletal complications resulting in an unbalanced bone turnover (14).

Bone pain in cancer follow-up cases is a finding that should be evaluated primarily in terms of metastatic bone disease. Bone pain is primarily due to the secretion of pain-inducing mediators such as bradykinin and Substance P by tumor cells, as well as the formation of microfractures in the trabecular bone and stimulation of the periosteum (15). The majority of the cases in this series presented with clinical bone pain.

Consistent with the literature, the majority of the cases were male. This may be because cancer rates are generally higher in males.

Lung, breast, prostate, and kidney tumors constitute the majority of metastatic bone tumors. The condition for all cancer types are as follows; bone metastasis is an indication that the tumor is advanced and the survival time is shortened (16-18). Even in cases with only bone metastases, life expectancy is less than 5 years (19).

Of breast carcinomas, 75% metastasize to bone in the late stage. As with other types of cancer, the disease becomes incurable in bone metastasis of breast cancer. In the literature, there are studies in terms of agents that can predict bone metastasis of breast cancer and that can be used in treatment (20,21). In one of these studies, it was determined that both breast cancer cells and the tumor microenvironment secrete interleukin 1B, and it was reported that agents against this molecule can be used in targeted therapy (22).

Bone metastases are seen at a similar rate to the contralateral lung and liver metastases in lung carcinomas. Compared to breast carcinoma, treatments for bone metastases are used less often because the overall survival effect is not great. However, there are also studies reporting positive effects on mean survival in the literature. Metastases in lung carcinomas are frequently encountered with multiple bone metastases. The region where they metastasize most is the vertebral region, which is consistent with the present study (23-25).

In cases where the primary origin cannot be detected, clinical imaging methods such as thorax CT, PET-CT, mammography, as well as endoscopic examination, and tumor markers in the blood can be examined. In addition, a histopathological examination is necessary. However, after all these examinations, the primary origin may still not be detected. In literature, this rate varies between 2.3% and 11% (19,26,27). In the current study, this rate was 9%. Morphologically, most of these tumors were reported as poorly differentiated adenocarcinoma or poorly differentiated carcinoma. In the literature, carcinomas of unknown origin are reported as well-moderately differentiated adenocarcinoma, poorly differentiated carcinoma, squamous cell carcinoma, and undifferentiated carcinoma, respectively. Solitary bone metastases have a relatively better prognosis in metastatic cases where the primary origin cannot be detected, compared to other organ metastases (28).

Axial skeletal metastases are more common than appendicular skeletal metastases (13). Similarly, in cases included in the present study, metastasis was most frequently seen in the vertebrae. In the appendicular skeleton, metastases were most common in the femur. In another study, the most common metastasis was found in the femur, followed by the vertebra (13,25). In the literature, lung, breast, and kidney cancers are most frequently encountered with vertebral metastases, which is consistent with this study (16,29,30). Although the primary treatment of vertebral metastases is radiotherapy, surgery is performed in case of treatment failure. Because according to the literature, surgery has no superiority over radiotherapy. In the case series in this study, most of the vertebral surgeries were performed for decompression of the vertebrae or definitive diagnosis (31).

The most important limitation of this study is that sampling was not made from all tumor cancer cases that metastasized to the bone. Palliative therapies are applied in cases with multiple organ metastases and biopsy is not needed. We think that this is the most important reason for the low number of cases in this study.

CONCLUSION

Bone pain is always a symptom that should be considered in a patient followed up with a diagnosis of cancer. The data in this study were generally compatible with the literature. It should be kept in mind that the primary origin may not be detected in a group of patients despite all clinical and imaging methods. We have discussed all types of cancer in this study, but there is a need for more specific studies on the type of cancer and its localization at the molecular level, which may affect the treatment, based on such studies.

Ethics Committee Approval: The study was approved by the Non-Invasive Clinical Researches Ethics Committee of Eskişehir Osmangazi University (26.04.2022, 18).

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Clinical Comparison of Patients Undergoing Anterior Cruciate Ligament Reconstruction Using an Allograft or Autograft

Allogreft ve Otogreft Kullanılarak Ön Çapraz Bağ Rekonstrüksiyonu Yapılan Hastaların Klinik Karşılaştırması

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ABSTRACT

Aim: The purpose of this study was to examine the clinical results of individuals who underwent an autograft or allograft repair of the anterior cruciate ligament (ACL).
Material and Methods: Retrospective analysis was done on the patient files of patients who underwent ACL reconstruction between 2014 and 2020 using semitendinosus-gracilis tendon autografts (SGT-A) and tibialis anterior tendon allografts (TAT-A). In this study, the data of 30 patients in each group were included. Knee laxity tests, the Lysholm knee grading system, the Tegner activity score, and the International Knee Documentation Committee (IKDC) score were used to compare patient results.
Results: While there was no significant difference in the Tegner activity score between the preoperative and the final measurement (p=0.241), the IKDC scores and the Lysholm knee ratings changed statistically significantly between the preoperative measurement and the last control visit (p=0.020, and p=0.038, respectively) for both groups in this study. The SGT-A group's Lysholm knee score had a preoperative value of 60.97% and a final control value of 90.48%. The preoperative Lysholm knee score for the TAT-A group was 61.31%, and the final control value was 95.03%. The anterior drawer and Lachman test findings showed statistically significant intergroup (autograft and allograft) alterations in both the autograft and allograft groups (both p<0.001).
Conclusion: In terms of knee function and laxity, this study achieved a better clinical outcome in the allograft group compared to the autograft group.
Keywords: Anterior cruciate ligament; allograft; arthroscopy.

ÖZ

Amaç: Bu çalışmanın amacı, ön çapraz bağın (ÖÇB) otogreft veya allogreft onarımı yapılmış olan bireylerin klinik sonuçlarının incelenmesidir.
Gereç ve Yöntemler: 2014 ve 2020 yılları arasında semitendinosus-gracilis tendon otogreftleri (semitendinosus-gracilis tendon autografts, SGT-A) ve tibialis anterior tendon allogreftleri (tibialis anterior tendon allografts, TAT-A) kullanılmak suretiyle ÖÇB rekonstrüksiyonu yapılmış olan hastaların dosyaları üzerinde geriye dönük olarak analiz yapıldı. Bu çalışmaya her iki grupta da 30 hastanın verileri dahil edildi. Hastaların sonuçlarının karşılaştırılması amacıyla diz laksite testleri, Lysholm diz skorlama sistemi, Tegner aktivite skoru ve Uluslararası Diz Dokümantasyon Komitesi (International Knee Documentation Committee, IKDC) skoru kullanıldı.
Bulgular: Preoperatif ölçüm ile son ölçüm arasında Tegner aktivite skoru bakımından anlamlı bir fark yokken (p=0,241), IKDC skorları ve Lysholm diz skorlamaları, bu çalışmadaki her iki grup için de preoperatif ölçüm ve son kontrol ziyareti arasında istatistiksel olarak anlamlı bir şekilde değişti (sırasıyla, p=0,020 ve p=0,038). SGT-A grubu Lysholm diz skorunun preoperatif değeri %60,97 ve son kontrol değeri ise %90,48 idi. TAT-A grubu için preoperatif Lysholm diz skoru değeri %61,31 ve son kontrol değeri ise %95,03 idi. Ön çekmece ve Lachman test bulguları, hem otogreft hem de allogreft gruplarında gruplar arası (otogreft ve allogreft) istatistiksel olarak anlamlı şekilde değişiklikler gösterdi (her iki p<0,001).
Sonuç: Bu çalışmada diz fonksiyonu ve laksite açısından, otogreft grubuna kıyasla allogreft grubunda daha iyi klinik sonuç elde edilmiştir.
Anahtar kelimeler: Ön çapraz bağ; allogreft; artroskopi.

INTRODUCTION

Injuries to the anterior cruciate ligament (ACL) affect 75/100,000 people annually in the general population (1,2). If left untreated, ACL tears may result in meniscal tears, knee instability, and cartilage abnormalities (3). The two basic methods for treating ACL ruptures are ACL reconstruction or repair. Results from ACL reconstruction were superior to those from ACL repair (4). The choice of graft, or whether to employ autograft or allograft, is one of the critical factors in the effectiveness of the reconstruction. Numerous studies have been carried out to find the most effective graft, and their findings have been documented in the literature (5,6).

Commonly used autograft choices include hamstring tendons, quadriceps tendons, and bone-patellar tendon-bone (BPTB). The anterior and posterior tibial tendons, the Achilles tendon, and the peroneal tendon are available as allograft possibilities. Despite the fact that ACL reconstruction treatments have a success record of greater than 90%, the topic of graft selection is still up for dispute (5,6). Although hamstring tendon utilization has increased recently, quadriceps tendon autografts have also become more common (7).

Numerous research on the choice of autografts or allografts for ACL restoration has been undertaken (5,6). These research studies investigated the benefits and drawbacks of both autografts and allografts (5,6). This study investigated the differences between the functional outcomes in patients undergoing ACL reconstruction surgery using semitendinosus and gracilis tendon autografts (SGT-A) or tibialis anterior tendon allografts (TAT-A).

MATERIAL AND METHODS

Study Design and Participants

A retrospective analysis was performed on patient files of patients who underwent SGT-A- and TAT-A-based ACL reconstruction between 2014 and 2020. Patients under the age of 18 years, those who had surgery in the same knee before, those who had ACL tears along with traumatic fractures or avulsion fractures, those who had surgery in the same extremity, those who did not adhere to the treatment and rehabilitation plan, those who did not finish a 24-month follow-up period, and/or those who lacked the necessary documentation were excluded from the study. It was noted that 51 patients had operations using TAT-A and 72 patients had operations using SGT-A between 2014 and 2020. Six patients in the TAT-A group failed to complete the 24-month follow-up, and 15 patients in the TAT-A group were eliminated from the study because their files were missing. In the SGT-A group, seven patients had previously undergone surgery on the same knee, 15 patients did not complete their 24-month follow-up, and 20 patients were not included in the study because their files were missing. In the final sample, the data of 30 patients in each group were analyzed. The methods for the scientific investigation were explained to the patients, and their consent was obtained. Documents were examined. The Malatya Turgut Özal University Faculty of Medicine's ethical committee granted the study permission on August 20, 2021 (decision number 2021/58). The informed consent form was signed by all patients involved in the study.

Surgical Technique

Spinal anesthesia was administered to all patients. The anesthesia was followed by the application of a tourniquet. In the TAT-A group, an allograft of a freeze-dried, radioirradiated tibialis anterior tendon was utilized. In the SGT-A group, semitendinosus and gracilis tendons were utilized. The semitendinosus and gracilis tendons in the SGT-A group were cut through an oblique incision made about 2 cm medial to the ipsilateral knee's tibial tuberosity. The two groups of tendons were doubled, and the grafts were produced. A femoral tunnel was first made once the tendon’s thickness was established, and then a tibial tunnel with the same diameter as the tendon was made. The Endobutton loop device was then used to attach the tendons to the femoral cortex. A bioabsorbable screw was used in the tibial fixation, and the remaining tendon was fixed with a staple screw (U screw).

Postoperative Follow-up

Exercises and a rehabilitation program were initiated by the Physical Therapy and Rehabilitation Department of our hospital on the first day after the operation. A hinged brace was placed so that the patient could fully extention and flexion the knee at 90°. The patients were allowed to walk using double crutches by bearing as much weight as they could on their knee, and were then discharged after a mean period of five days. The braces were removed after three weeks. The patients started flat racing approximately four months later, and they were advised to return to active sports activities six months after surgery. The study analyzed patients who underwent control visits for at least 24 months following surgery. Both active and passive flexion and extension movements were evaluated. The Lachman test, the anterior drawer test, and the pivot-shift test were all given a positive or negative evaluation.

The performance of the knee was assessed using the Lysholm knee score. A score of 91 to 100 was considered exceptional, 84 to 90 was considered decent, 65 to 83 was considered average, and 65 was considered low (8). A score of 0 on the Tegner activity scale implied eligibility for sick leave or a disability pension due to knee issues, whereas a score of 10 showed eligibility for professional sports participation (2). The knee exam employed the International Knee Documentation Committee (IKDC) score system. The IKDC score is a crucial factor in determining if post-injury care was successful. The existence of mobility restrictions (restricted flexion and/or limited extension) was assessed in the patients.

Sample Size

According to the calculation made using the G*power 3.1 program, the required sample size was determined to be 58 (where each group consisted of 29 patients) with an effect size of 0.80, a margin of error of 0.05, a confidence level of 0.95, and a population representation of 0.90 (9).

Statistical Analysis

The IBM SPSS Statistics for Windows, version 25.0 software program was used for data analysis. In statistical comparisons, the level of statistical significance was accepted as 0.05. The mean, standard deviation, number, and percentage were used as descriptive statistics for the variables. The chi-square test was used to evaluate independent categorical data. The McNemar chi-square and marginal homogeneity tests were used for dependent group categorical data. The two-sample t-test (t-test) of the

difference between the two means was used in the comparison of two independent groups. The multivariate normal distribution and homogeneity of variance were controlled. Parametric analysis methods were used because the distributions were provided. In repeated measurements, the two paired samples t-test was used in pairwise comparisons within the group. Two-way ANOVA was used to compare changes over time between the groups.

RESULTS

At the clinic, 30 patients received ACL reconstruction using semitendinosus and gracilis tendon autografts and 30 patients underwent ACL reconstruction using tibialis anterior tendon allografts throughout the designated study period. In terms of age, follow-up time, or injury-surgery duration, there was no statistically significant difference between the autograft and allograft groups (Table 1).

Both the etiology and intraarticular pathologies that could accompany an ACL tear, such as a medial meniscus tear, a lateral meniscus tear, and cartilage abnormalities, did not show any significant intergroup differences (Table 2). Meniscorraphy, cartilage abnormalities, or microfracture applications did not significantly differ during the surgical intervention (Table 3). All meniscus tears were repaired. Meniscectomy was not performed. Arthroscopic irrigation surgery was performed in one patient from each group because of high C-reactive protein (CRP) levels and sedimentation. Patients recovered after treatment with the provided therapy. One patient with an infection in the autograft group suffered from an ACL rupture owing to trauma at 20 months, and this patient underwent corrective surgery. One patient in the allograft group experienced an ACL rupture after a fall and subsequently underwent corrective surgery. Regarding postoperative infections and re-rupture, there was no discernible difference between the autograft and allograft groups (Table 3). Participants from the SGT-A and TAT-A groups experienced no limited extension at the most recent control visit. Twenty-four (80.0%) and six (20.0%) patients in the SGT-A group displayed limited flexion, compared to the other patients. Limited flexion was present in 25 (83.3%) and 5 (16.7%) of the TAT-A group patients, respectively (Table 3).

The mean Tegner activity score and the mean IKDC score were compared between intra- and intergroup (autograft and allograft) to determine the changes over time (Table 4). Between the autograft and allograft groups, there was no discernible difference in that the Tegner activity score changed over time between the preoperative and the final measurement (p=0.241). Between the autograft and allograft groups, there were substantially different changes in terms of the IKDC score over time between the preoperative and the final measurement (p=0.020).

The results of comparing intra- and intergroup (autograft and allograft) changes in the Lysholm knee score over time were shown in Table 4. The SGT-A group Lysholm score had a preoperative value of 60.97% and a final control value of 90.48%. The preoperative Lysholm score for the TAT-A group was 61.31%, while the control value at the end was 95.03%. Between the autograft and allograft groups, there were substantially different changes in the Lysholm score over time between the preoperative and the final measurement (p=0.038, Table 4).

Table 2. Comparison of the etiology, presence of medial meniscus tear, lateral meniscus tear, cartilage defect

	SGT-A (n=30)	TAT-A (n=30)	p
Etiology, n (%)			
Sports	16 (53.3)	16 (53.3)	
Falls	9 (30.0)	12 (40.0)	0.415
Trauma	5 (16.7)	2 (6.7)	
Medial meniscus tear, n (%)	9 (30.0)	10 (33.3)	0.781
Lateral meniscus tear, n (%)	4 (13.3)	3 (10.0)	0.687
Cartilage defect, n (%)	3 (10.0)	4 (13.3)	0.687

SGT-A: semitendinosus and gracilis tendon autografts, TAT-A: tibialis anterior tendon allografts

Table 3. Comparison of the meniscorraphy, cartilage defect, microfracture, postoperative infections, re-rupture

	SGT-A (n=30)	TAT-A (n=30)	p
Meniscorraphy, n (%)	11 (36.7)	10 (33.3)	0.787
Microfracture, n (%)	3 (10.0)	4 (13.3)	0.687
Post-op infections, n (%)	1 (3.3)	1 (3.3)	0.754
Re-rupture, n (%)	2 (6.7)	1 (3.3)	0.754
Limitation, n (%)	6 (20.0)	5 (16.7)	0.500

SGT-A: semitendinosus and gracilis tendon autografts, TAT-A: tibialis anterior tendon allografts

Table 4. Intragroup and intergroup comparison of Tegner activity score, IKDC score, and Lysholm knee score

	SGT-A (n=30)	TAT-A (n=30)	p^2
Tegner, mean±SD			
Preoperative	5.20±1.30	5.60±1.33	0.241
24-month control	4.97±1.30	5.53±1.41	
p^1	**0.032**	0.489	
IKDC, mean±SD			
Preoperative	31.01±3.79	29.40±3.36	**0.020**
24-month control	91.01±4.88	92.94±3.75	
p^1	**<0.001**	**<0.001**	
Lysholm score, mean±SD			
Preoperative	60.97±6.61	61.31±8.38	**0.038**
24-month control	90.48±1.51	95.03±2.92	
p^1	**<0.001**	**<0.001**	

IKDC: international knee documentation committee, SGT-A: semitendinosus and gracilis tendon autografts, TAT-A: tibialis anterior tendon allografts, SD: standard deviation, p^1: within-group pairwise comparison (two paired samples t-test), p^2: between groups repeated measures two-way ANOVA

Table 1. Homogeneity of distribution for age, duration of follow-up, and injury-surgery time in the groups

	SGT-A (n=30)	TAT-A (n=30)	test value	p
Age (years)	25.50±4.96 [18-34]	25.20±5.65 [18-37]	0.218	0.828
Duration of follow-up (months)	23.87±0.57 [23-25]	23.67±0.61 [23-25]	1.315	0.194
Time from injury to surgery (weeks)	7.87±2.53 [4-12]	8.10±3.26 [4-14]	-0.310	0.758

SGT-A: semitendinosus and gracilis tendon autografts, TAT-A: tibialis anterior tendon allografts, test value: independent samples t-test value, descriptive statistics were reported as mean±standard deviation [minimum-maximum]

The pivot-shift, anterior drawer, and Lachman test final follow-up results were compared within and between groups (autograft and allograft), and the results were shown in Table 5. Due to an equal number of patients in the autograft and allograft groups, both intragroup and intergroup assessments of the pivot-shift test findings did not reveal any statistically significant differences (p=1.000, Table 5). Results of the anterior drawer test revealed statistically significant differences between the autograft and allograft groups in both intragroup and intergroup analyses (p=0.001, Table 5). Results of the Lachman test revealed statistically significant differences in the autograft and allograft groups in both intragroup and intergroup analyses (p=0.001, Table 5).

DISCUSSION

The selection of grafts is a topic of discussion due to the rise in surgical procedures for ACL injuries. The choice of graft, the patient's age, the existence of additional diseases, the amount of activity, and the patient's compliance with the treatment all contribute to the success of ACL reconstruction surgery (10). In this study, it was observed that the IKDC and Lysholm scores exhibited better clinical results in terms of knee function in the allograft group, and the anterior drawer and Lachman test results exhibited better clinical results in terms of knee laxity for this group. Hamstring and BPTB grafts are the most frequently used grafts in ACL restoration procedures, followed by quadriceps tendons (11). The use of BPTB grafts has some drawbacks, including patellar and knee pain, patellar fractures, patellar tendonitis, a loss of complete extension, a weakening of the quadriceps muscle, and a decrease in extensor mechanism strength (12). Reduced adaption time due to bone-to-bone union in the tunnel and usability of the graft in stiff fixations are benefits of using BPTB grafts. Due to their firmness, strength, and low rate of morbidity at the donor site, four-strand gracilis and semitendinosus tendon autografts are frequently employed in orthopedic procedures (13). The benefits of allografts include quick recovery times, low rates of surgical complications, and easy limitless access to grafts of the required length and thickness (14,15). In both laboratory and clinical tests, autografts outperformed allografts, according to Lin et al. (16). In terms of knee function and laxity, this study achieved a better clinical outcome in the allograft group compared to the autograft group.

An autograft was used in 32 patients and an allograft was used in 29 patients in a study by D'Ambrosi et al. (17) that evaluated the use of autografts and allografts in patients having ACL restoration. There was no significant difference in the Tegner activity score, the subjective IKDC score, or the Lysholm score between the two groups. No statistically significant difference between the mean subjective IKDC score and the allograft and autograft groups was reported in the study by Razi et al. (18). In a study by Yang et al. (19), the Lysholm knee scores were 90.9 in the allograft group and 91.8 in the autograft group at the last control visit. In terms of the Lysholm knee score, the subjective IKDC score, and the Tegner activity score, the study by Cengiz et al. (20) study found no evidence of a significant difference between the groups. According to certain research, there was no discernible difference between the autograft and allograft groups in terms of the IKDC, Tegner activity, or Lysholm knee scores (21,22). In this study, according to the Tegner activity scale, patients returned to their prior activity levels in both groups. In terms of knee functions as measured by the IKDC and Lysholm knee scores, the allograft group did better than the autograft group.

The patients' knee laxity tests were assessed. There was no statistically significant difference between the autograft and allograft groups in the study by Cengiz et al. (20) and the intergroup evaluations of the pivot-shift test results. There was a statistically significant difference between the autograft and allograft groups in both the intra- and intergroup assessments of the anterior drawer and Lachman tests. In this study, it is believed that the most important factors affecting success in ACL reconstruction are proper graft selection according to the clinical experience of the surgeon and the physical activity level of the patient, correct implementation of the surgical procedure, postoperative patient compliance, and an appropriate rehabilitation program (20). In the trial by Yang et al. (19), the Lachman test resulted in a negative result in 76.5% of patients receiving allografts and a positive result in 23.5% of patients at the last control visit. The Lachman test was negative in 77.8% of the autograft group and positive in 22.2% at the most recent control visit. The pivot-shift test was negative in the allograft group in 81.3% of cases and positive in 18.7% of cases at the most recent control visit. The pivot-shift test in the autograft group was negative in 96.7% of cases and positive in 3.3% at the last control visit (19). Similar objective and subjective findings were seen in patients who underwent repair using allografts and hamstring tendon autografts in this investigation during the long-term

Table 5. Intragroup and intergroup comparison of pivot-shift, anterior drawer, and Lachman test results

			SGT-A (n=30)		**TAT-A (n=30)**		**p²**
			24-month control		24-month control		
			Negative	Positive	Negative	Positive	
Pivot-Shift, n (%)	Preoperative	Negative	27 (90.0)	0 (0.0)	25 (83.3)	0 (0.0)	1.000
		Positive	3 (10.0)	0 (0.0)	5 (16.7)	0 (0.0)	
		p¹	0.250[a]		0.063[a]		
Anterior Drawer, n (%)	Preoperative	Negative	7 (25.9)	0 (0.0)	8 (27.6)	0 (0.0)	**<0.001**
		Positive	20 (74.1)	3 (100)	21 (72.4)	1 (100)	
		p¹	**<0.001**[a]		**<0.001**[a]		
Lachman, n (%)	Preoperative	Negative	13 (48.1)	0 (0.0)	6 (23.1)	0 (0.0)	**<0.001**
		Positive	14 (51.9)	3 (100)	20 (76.9)	3 (100)	
		p¹	**<0.001**[b]		**<0.001**[b]		

SGT-A: semitendinosus and gracilis tendon autografts, TAT-A: tibialis anterior tendon allografts, p¹: within-group pairwise comparison ([a]: McNemar, [b]: marginal homogeneity test), p²: between groups comparison

follow-up period (19). In the Bistolfi et al. (23) trial, patients who underwent either autografts or allografts and were followed up with for an average of 10 years experienced nearly the same functional results. Freshly frozen allografts were demonstrated in this study to be a viable alternative for ACL restoration. Autografts and nonirradiated allografts for primary ACL reconstruction showed equal patient-reported clinical results and graft failure rates in the study by Dhillon et al. (24). In terms of knee laxity, this study was found to be more successful in the autograft group compared to the allograft group (19). In this study, knee laxity results were found to be more successful in the allograft group.

After ACL reconstruction, a restriction in the range of motion of the knee joint was noted. Potential causes of the restricted range of motion include the patient's noncompliance with the rehabilitation program, failure to place the graft in the proper position and tone, infections that developed after the surgery, and the patient's preoperative range of joint motion. Studies in the literature established the criteria for arthrofibrosis, which has a reported frequency range of 614%, as a limited extension larger than 10° and a limited flexion less than 125° (25-28). According to studies in the literature, the rates for loss of extension greater than 6° and the loss of flexion greater than 16° are 12-15% and 8-50%, respectively (29,30). In the current investigation, neither the SGT-A group nor the TAT-A group experienced any limited extension during the final control visit. Six (20%) patients in the SGT-A group and five (16.7%) patients in the TAT-A group had limited flexion at the most recent control visit. No discernible difference was reported in the study by Yang et al. (19) between patients who employed autografts or allografts in terms of extension and flexion. In this study, there was no discernible difference in the range of joint mobility limitation between the autograft and allograft groups.

CONCLUSION

In terms of knee function and laxity, this study achieved a better clinical outcome in the allograft group compared to the autograft group. The study's shortcomings included the patients' low average age, the absence of body mass indices, the exclusion of graft thicknesses, and the short follow-up period (less than 25 months).

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Malatya Turgut Özal University (20.08.2021, 58).

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Comparison of Two Different Concentrations for Foot and Ankle Surgeries During Intravenous Regional Anesthesia (IVRA): A Randomized Cohort Study

İntravenöz Rejyonel Anestezi (IVRA) Sırasında Ayak ve Ayak Bileği Ameliyatları için İki Farklı Konsantrasyonun Karşılaştırılması: Randomize Kohort Çalışma

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ABSTRACT

Aim: Intravenous regional anesthesia (IVRA) is not commonly preferred in the lower extremity because of the toxic risks of high-dose local anesthetics. This study aimed to compare the use of two different concentrations of anesthetics with additional tourniquet application to reduce local anesthetics amount during the IVRA method for short-term foot and ankle surgeries.
Material and Methods: In this prospective study, 40 patients were allocated to two groups with different concentration formulations of 200 mg lidocaine hydrochloride (Group 30 and Group 20). The groups were compared in terms of demographic data, tourniquet pain, operation time, hemodynamic indicators, and sedo-analgesia needs.
Results: Demographic data were similar in the two groups. The mean tourniquet pain time was 41.66±6.61 minutes in Group 20 (n=9) and 36.76±7.17 minutes in Group 30 (n=13) (p=0.120). Perioperative sedo-analgesia consumptions were similar between the groups: weight-adjusted before/after tourniquet pain (p=0.390, p=0.207, p=0.536, and p=0.176), weight-adjusted/none total amount (p=0.425, p=0.578, p=0.268, and p=0.612), per minute before/after tourniquet pain (p=0.075, p=0.506, p=0.354, and p=0.055), for propofol and remifentanil, respectively. There was a significant difference between the propofol and remifentanil consumption per minute before and after the tourniquet pain in both groups: 5.61±1.67 and 14.58±6.62 mg/min propofol (p=0.001), and 4.79±1.69 and 7.86±1.55 mcg/min remifentanil (p=0.001), respectively. No patient had signs of local anesthetic toxicity.
Conclusion: Low-dose sedo-analgesia can be used by a modified IVRA method in the management of tourniquet discomfort that may occur until the tourniquet pain develops.
Keywords: intravenous regional anesthesia, additional tourniquet, short-term foot and ankle surgery, tourniquet pain, lower extremity.

ÖZ

Amaç: Intravenöz rejyonel anestezi (IVRA), yüksek doz lokal anesteziklerin toksik riskleri nedeniyle alt ekstremitede yaygın olarak tercih edilmemektedir. Bu çalışmada, kısa süreli ayak ve ayak bileği ameliyatlarında IVRA yöntemi sırasında lokal anestezik miktarını azaltmak için iki farklı anestezik konsantrasyonunun ek turnike uygulaması ile karşılaştırılması amaçlandı.
Gereç ve Yöntemler: Bu prospektif çalışmada, 40 hasta 200 mg lidokain hidroklorürün farklı konsantrasyon formülasyonlarına sahip iki gruba ayrıldı (Grup 30 ve Grup 20). Gruplar demografik veriler, turnike ağrısı, operasyon süresi, hemodinamik göstergeler ve sedo-analjezi ihtiyaçları açısından karşılaştırıldı.
Bulgular: Demografik veriler her iki grupta da benzerdi. Turnike ağrı süresinin ortalaması Grup 20'de (n=9) 41,66±6,61 dakika ve Grup 30'da (n=13) 36,76±7,17 dakika idi (p=0.120). Perioperatif sedo-analjezi tüketimleri gruplar arasında benzerdi: propofol ve remifentanil için sırasıyla, kiloya göre turnike ağrısı önce/sonra (p=0,390; p=0,207; p=0,536 ve p=0,176), kiloya göre/yok toplam miktar (p=0,425; p=0,578; p=0,268 ve p=0,612), dakika başına turnike ağrısı önce/sonra (p=0,075; p=0,506; p=0,354 ve p=0,055). Her iki grupta da turnike ağrısı öncesi ve sonrası dakika başına propofol ve remifentanil tüketimi arasında önemli bir fark vardı: sırasıyla, 5,61±1,67 ve 14,58±6,62 mg/dk propofol (p=0,001) ve 4,79±1,69 ve 7,86±1,55 mcg/dk remifentanil (p=0,001). Hiçbir hastada lokal anestezik toksisite bulguları yoktu.
Sonuç: Düşük doz sedo-analjezi, turnike ağrısı gelişene kadar oluşabilecek turnike rahatsızlığının tedavisinde modifiye bir IVRA yöntemi ile kullanılabilir.
Anahtar kelimeler: intravenöz rejyonel anestezi, ek turnike, kısa süreli ayak ve ayak bileği cerrahisi, turnike ağrısı, alt ekstremite.

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INTRODUCTION

Intravenous regional anesthesia (IVRA) is regional anesthesia that can be created by eliminating nerve conduction and pain sensation due to the tourniquet. First, the tourniquet is placed on the proximal extremity, keeping to a constant pressure above the systemic arterial pressure, then local anesthetic agents are administered into the venous system. IVRA is a preferable method because it is easy to apply in upper extremity surgeries, its effect starts and ends quickly, it provides effective anesthesia, and, finally, it has low preoperative morbidity and short postoperative hospital stay (1).

IVRA is more advantageous than general anesthesia since the airway remains open during the application, airway reflexes are protected, and the risk of aspiration is reduced in emergency patients whose fasting state is not suitable. It is frequently used in outpatient surgeries of the upper extremities due to its low cost, high (95%) chance of success, and low risk of complications and mortality (2).

Although the IVRA technique is similar in principle at the upper and lower extremities, the local anesthetic dose required for the lower extremity block is approximately twice more than that required for the upper extremity. The risk of local anesthetic toxicity increases if tourniquet leakage occurs or the tourniquet is deflated at the end of the short surgical period. Due to the need for a higher dose of local anesthetic, IVRA is rarely preferred in lower extremities to avoid the postoperative toxicity findings linked to higher doses. Modifications to the traditional IVRA method and changes to the amount of local anesthetics have been tried to reduce such side effects of local anesthetics (3).

We aimed to compare the use of two different regimens in the modified IVRA method with a tourniquet placed under the knee in addition to the traditional IVRA method to reduce the amount of local anesthetics used in foot and ankle surgeries.

MATERIAL AND METHODS

Patients

This prospective study was conducted in 2016 with the approval of the local ethics committee (approval numbers: 2015/351 and 2016/272). The patients, in whose cases a single-cuff proximal tourniquet was routinely used for a bloodless surgical field by an additional tourniquet in foot and ankle surgeries. The primary population for analysis is the so-called modified IVRA population. In the analysis, patients were grouped into two based on the concentration of anesthetics according to the modified IVRA principle: 200 mg lidocaine hydrochloride in 20 mL (Group 20, n=24) and 30 mL (Group 30, n=27) saline.

The primary outcome of the study was weight-adjusted analgesic consumption with a specific sedo-analgesia protocol before tourniquet pain. The secondary outcome of the study was weight-adjusted analgesic consumption with a specific sedo-analgesia protocol after tourniquet pain. Weight-adjusted sedo-analgesia consumption was quantified in propofol and remifentanil equivalents. This study was planned to compare the two groups where the anticipated difference was 30% in the hemodynamic values. Accordingly, the sample size of 20 patients in each group was required for a type I error (α) of 0.050 and a power of 80% in a two-sided design.

Patients were excluded if they met any of the following criteria: chronic disease associated with the liver, peripheral vascular diseases, neuromuscular diseases, bleeding disorder, over one-hour surgery time, American Society of Anesthesiologists (ASA) physical status III-IV, being younger than 16, and older than 60 years of age. The patients who had cognitive impairment or developmental delay, the non-cooperative patients with debile or senile dementia or head trauma, the foreign-speaking patients, the patients with preoperative pain scores greater than 0, and the patients with a history of fibromyalgia syndrome were excluded as well. A total of 11 patients were excluded from the study sample due to these criteria. The patients were randomly allocated into two groups in a way to include twenty patients in each one. The groups were compared in terms of the hemodynamic effects.

Measurements

Age, sex, weight, body mass index (BMI), and the ASA classification of the patients included in the study were recorded. Heart rate (HR), mean arterial pressure (MAP), and peripheral oxygen saturation (SpO_2) were recorded in the patients who were routinely monitored, and these values were saved as basal values. Intraoperative measured values were recorded every 5 minutes.

Anesthesia Management

Patients were given the infusion of fluid with a 20 or 22-gauge cannula from the back of the hand as 0.9% NaCl 4-6 mL/kg/h. Patients were prepared for the operation by applying premedication with 0.03 mg/kg midazolam. Patients were given 40% O_2 3 L/min from a nasal cannula with the help of an anesthesia machine (GE Datex-Ohmeda S5 Avance). A 22-gauge cannula was used to establish vascular access from the operated extremity, and a single cuff pneumatic tourniquet was placed on the proximal side of the extremity. After keeping the extremity above the head level for 3 minutes, it was started being wrapped with an Esmarch bandage starting from distal to proximal. 1 mcg/kg fentanyl was administered as standard. The single-cuff proximal tourniquet was inflated to maintain a value that is 100 mmHg higher than systolic arterial pressure or a maximum value of 300 mmHg. After dissolving the Esmarch bandage, dorsalis pedis and under nail capillary fillings were controlled to assess the adequacy of the tourniquet pressure. Lipid Rescue Kit was kept ready for possible local anesthetic toxicity, based on the protocol determined by the American Society of Regional Anesthesia and Pain Medicine (ASRA).

An additional tourniquet was applied under the knee level in the non-circulating extremity after proximal tourniquet inflation. The local anesthetic solution was administered by giving 1 mL in 3 seconds. The additional distal tourniquet was held at a pressure of 100 mmHg above the systolic value, following the injection of the local anesthetic solution. Surgery was started after an adequate anesthesia level was reached, being checked with the pinprick test. Perioperative nausea and vomiting, skin rash, headache, dizziness, tinnitus, metallic taste, and numbness in the tongue were also evaluated for local anesthetic toxicity.

Various operation time intervals were determined based on certain milestones. The duration from the administration of local anesthetic solutions to the surgical incision was

considered the time to start the surgery. The duration from the surgical incision to the proximal tourniquet deflation was considered as the surgery time. The duration from the proximal tourniquet inflation to the proximal tourniquet deflation was considered the tourniquet time. The duration from the proximal tourniquet inflation to the time when tourniquet pain developed was considered the tourniquet pain time.

Procedure

The pain status of patients was evaluated using the visual analogue scale (VAS). No additional analgesic drug was administered to the patients with a VAS score between 0 and 2. In the patients with a VAS score between 3 and 5, tourniquet-related discomfort was considered, and 3 mg/kg/h propofol and 3 mcg/kg/h remifentanil infusion were initiated. Tourniquet pain was thought to occur when severe, blunt pain occurs in the tourniquet area or just distal to the cuff, or the VAS score was above 5 despite an adequate anesthesia level or the patient's being uncomfortable despite the infusion. In these patients, 1 mg/kg propofol and 0.25 mcg/kg remifentanil were started for induction, and 3 mg/kg/h propofol and 6 mcg/kg/h remifentanil for infusion. Before the induction, the patient was observed every 5 min with simultaneous bispectral index score (BIS) and Ramsey sedation scale (RSS). Propofol was given in 0.4 mg/kg bolus dose in 3 minutes. The infusion rate was increased to 0.5 mg/kg/h until a target BIS level of 60 to 75 was reached in the two groups. At levels below the target values, the propofol infusion rate was reduced to 0.5 mg/kg/h in 5 min. The sedo-analgesia needs used in our clinic-specific protocol were compared with the help of BIS monitoring.

Tramadol citrate 100 mg was routinely administered to all patients just before the end of the operation. At the end of the operation, a local anesthetic was given to the surgical incision line just before the proximal tourniquet was deflated. Bupivacaine hydrochloride 2.5 mg/mL was applied to help postoperative pain control.

Vital signs, toxic symptoms, and postoperative pain status were evaluated postoperatively for two hours. After the deflation of the proximal tourniquet at the end of the operation, patients were transferred to the post-anesthesia care unit (PACU) after VAS and RSS scoring. Fentanyl 1 mcg/kg was given as a rescue analgesic to the patients with pain during observation. After PACU, patients with an RSS score of 2-3 were referred to their services for standard follow-up and treatment by nurses who were not associated with the study.

Statistical Analysis

The Shapiro-Wilk test was used to check the conformity of continuous variables to normal distribution. The Student's t-test was used to compare normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. The relationships between categorical variables were tested with the chi-square test. To compare the numerical measurements obtained at different times, a repeated measurement variance analysis was used for variables with normal distribution, and Freidman tests were used for variables that were not normally distributed. For statistical analysis, the IBM SPSS Statistics for Windows, version 22.0, statistical software was used, and a p-value <0.050 was considered statistically significant.

RESULT

Demographic data and operation times were shown in Table 1. The distribution of patients numbers in terms of the surgical operations for Group 20-Group 30, were benign soft-tissue mass excision (Morton neuroma, glomus tumor, ganglion cyst, etc.) 1-4, deformity surgery (hallux valgus, hammer finger, claw toe, etc.) 2-2, small bone fracture fixation 4-3, isolated lateral 3-1 and medial 2-3 malleolus fracture fixation (plate, screw, etc.), foot 3-4 and ankle 5-3 implant removal (plate, screw, etc.), respectively.

It was seen that the proximal tourniquet was not deflated even though the operation was completed within 30 minutes after the injection of local anesthetic solution in the patients who received IVRA.

No tourniquet pain was seen in the two groups before 30 minutes. No tourniquet pain was seen in Group 20 at 30 minutes, but two patients developed tourniquet pain at the 35th minute. In Group 30, tourniquet pain developed in three patients at the 30th minute and the 35th minute in six patients. Tourniquet pain was more frequent in Group 30 than in Group 20 in earlier periods (Figure 1).

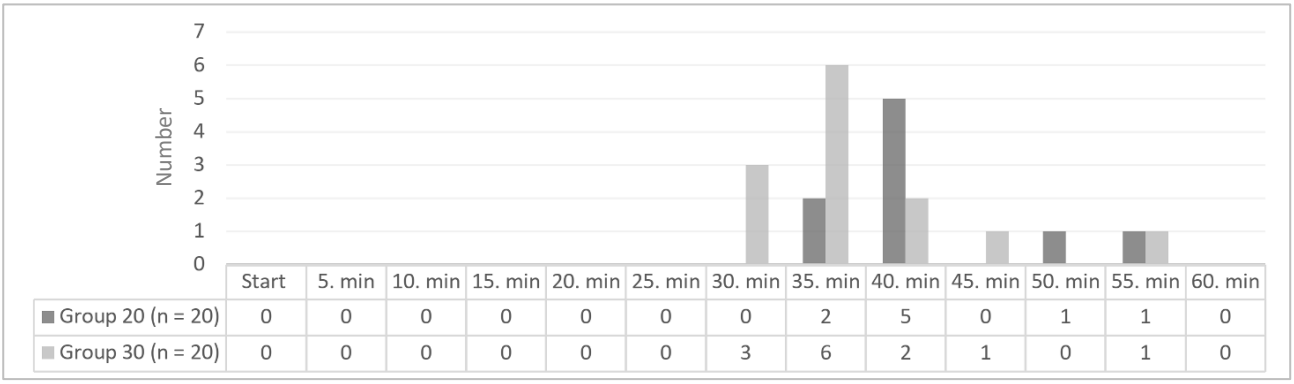
The mean tourniquet pain time was 41.66±6.61 minutes in Group 20 (n=9) and 36.76±7.17 minutes in Group 30 (n=13). There was no significant difference between the groups in terms of tourniquet pain (p=0.120).

In Group 30, a significant MAP increase occurred at the 35th minute (p=0.022). No significant difference was found between the groups in terms of MAP values in other periods. The difference between the groups in terms of hemodynamic parameters was shown in Figure 2.

Table 1. Demographic data and operation times

	Group 20 (n=20)	Group 30 (n=20)	p
Age (years), mean±SD	33.85±10.94	33.30±14.90	0.894
Gender (male/female), n (%)	12 (60) / 8 (40)	13 (65) / 7 (35)	0.744
Weight (kg), mean±SD	77.20±12.42	78.20±15.73	0.825
Height (cm), mean±SD	171.40±10.99	169.50±9.70	0.566
BMI (kg/m^2), mean±SD	25.95±3.53	26.90±4.50	0.463
ASA (I/II), n (%)	8 (40) / 12 (60)	11 (55) / 9 (45)	0.537
Time to start surgery (min), mean±SD	17.00±7.67	14.90±6.62	0.711
Surgery time (min), mean±SD	28.00±8.64	29.35±13.61	0.360
Tourniquet time (min), mean±SD	45.00±10.13	44.25±13.69	0.845
Tourniquet pain time* (min), mean±SD	41.66±6.61	36.76±7.17	0.120

SD: standard deviation, BMI: body mass index, ASA: American Society of Anesthesiologists, min: minute, *: Group 20 (n=9), Group 30 (n=13)



	Start	5. min	10. min	15. min	20. min	25. min	30. min	35. min	40. min	45. min	50. min	55. min	60. min
Group 20 (n = 20)	0	0	0	0	0	0	0	2	5	0	1	1	0
Group 30 (n = 20)	0	0	0	0	0	0	3	6	2	1	0	1	0

Figure 1. Time of tourniquet pain and distribution of patient numbers according to the groups

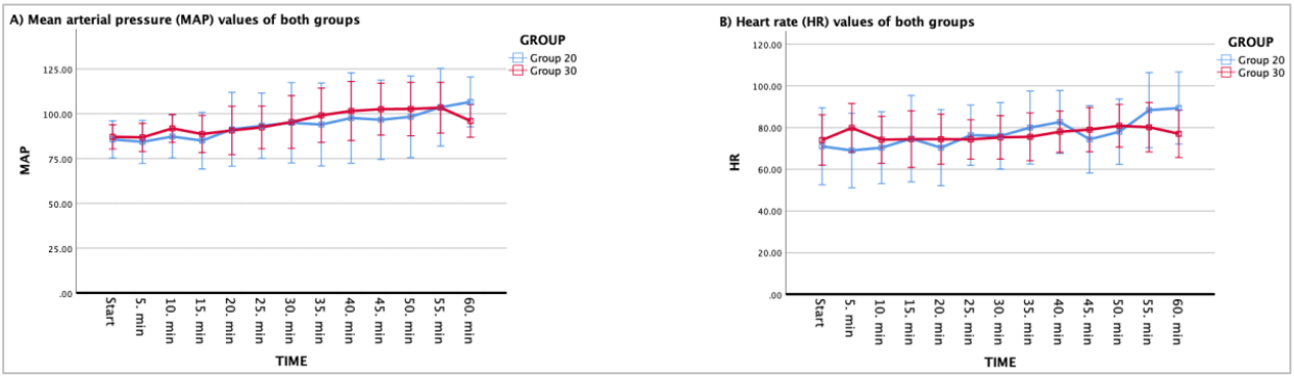


Figure 2. Mean arterial pressure (MAP) and heart rate (HR) values of both groups

Perioperative weight-adjusted sedo-analgesia consumption with a specific protocol before tourniquet pain was 2.76±0.58 mg/kg propofol and 2.51±0.88 mcg/kg remifentanil in Group 20 and 2.58±0.73 mg/kg propofol and 2.16±0.83 mcg/kg remifentanil in Group 30 (p=0.390, p=0.207, propofol, and remifentanil, respectively). Perioperative weight-adjusted sedo-analgesia consumption with a specific protocol after tourniquet pain was 2.00±0.81 mg/kg propofol and 1.03±0.81 mcg/kg remifentanil in Group 20 and 2.28±1.16 mg/kg propofol and 1.51±0.77 mcg/kg remifentanil in Group 30 (p=0.536, p=0.176, propofol, and remifentanil, respectively). There was no significant difference between the two groups in terms of perioperative weight-adjusted sedo-analgesia consumption before and after tourniquet pain (Table 2).

Perioperative weight-adjusted total sedo-analgesia consumption with a specific protocol was 3.66±1.28 mg/kg propofol and 2.97±0.95 mcg/kg remifentanil in Group 20 and 4.06±1.79 mg/kg propofol and 3.19±1.47 mcg/kg remifentanil in Group 30 (p=0.425, p=0.578, propofol, and remifentanil, respectively). Perioperative total sedo-analgesia consumption with a specific protocol was 278.91±95.55 mg propofol and 231.06±82.50 mcg remifentanil in Group 20 and 329.90±177.88 mg propofol and 249.22±135.21 mcg remifentanil in Group 30 (p=0.268, p=0.612, propofol, and remifentanil, respectively). There was no significant difference between the groups in terms of the total amount of perioperative sedo-analgesia consumption (Table 2).

Perioperative sedo-analgesia consumption per minute with a specific protocol before tourniquet pain was 5.08±1.43 mg/min propofol and 5.30±2.90 mcg/min remifentanil in Group 20 and 5.94±1.53 mg/min propofol and 4.82±1.41 mcg/min remifentanil in Group 30 (p=0.075, p=0.506, propofol, and remifentanil, respectively). Perioperative sedo-analgesia consumption per minute with a specific protocol after tourniquet pain was 16.45±9.21 mg/min propofol and 7.08±1.52 mcg/min remifentanil in Group 20 and 13.28±3.97 mg/min propofol and 8.40±1.39 mcg/min remifentanil in Group 30 (p=0.354, p=0.055, propofol, and remifentanil, respectively). There was no significant difference between the groups in terms of perioperative sedo-analgesia consumption per minute before and after tourniquet pain (Table 2).

Table 2. Propofol and remifentanil consumption (mean±SD)

	Group 20	Group 30	p
W-A Before TP			
Propofol (mg/kg)	2.76±0.58	2.58±0.73	0.390
Remifentanil (mcg/kg)	2.51±0.88	2.16±0.83	0.207
W-A After TP*			
Propofol (mg/kg)	2.00±0.81	2.28±1.16	0.536
Remifentanil (mcg/kg)	1.03±0.81	1.51±0.77	0.176
W-A Total Amount			
Propofol (mg/kg)	3.66±1.28	4.06±1.79	0.425
Remifentanil (mcg/kg)	2.97±0.95	3.19±1.47	0.578
Per Minute Before TP			
Propofol (mg/min)	5.08±1.43	5.94±1.53	0.075
Remifentanil (mcg/min)	5.30±2.90	4.82±1.41	0.506
Per Minute After TP*			
Propofol (mg/min)	16.45±9.21	13.28±3.97	0.354
Remifentanil (mcg/min)	7.08±1.52	8.40±1.39	0.055
Total Amount			
Propofol (mg)	278.91±95.55	329.90±177.88	0.268
Remifentanil (mcg)	231.06±82.50	249.22±135.21	0.612

SD: standard deviation, W-A: weight-adjusted, TP: tourniquet pain, *: Group 20 (n=9), Group 30 (n=13)

In Group 20, perioperative sedo-analgesia consumption per minute with a specific protocol was 5.61±1.67 mg/min propofol before tourniquet pain and 14.58±6.62 mg/min propofol after tourniquet pain. In Group 30, perioperative sedo-analgesia consumption per minute with a specific protocol was 4.79±1.69 mcg/min remifentanil before tourniquet pain and 7.86±1.55 mcg/min remifentanil after tourniquet pain. There was a significant difference between propofol and remifentanil consumption per minute before and after the tourniquet (p=0.001) (Table 3). Postoperative additional fentanyl was required in two patients in Group 20 and three patients in Group 30. None of the patients had signs of local anesthetic toxicity.

Table 3. Propofol and remifentanil consumption per minute before and after the tourniquet pain (mean±SD)

Consumption	Before (n=22)	After (n=22)	p
Propofol (mg/min)	5.61±1.67	14.58±6.62	**0.001**
Remifentanil (mcg/min)	4.79±1.69	7.86±1.55	**0.001**

DISCUSSION

IVRA is less preferred in lower extremity surgeries compared to upper extremity surgeries due to the high amount of local anesthetic used, which may be toxic. Although IVRA has lost its popularity with the use of several other regional approaches today, it is still considered an alternative due to its advantages such as ease of application, early recovery time, and low side effect profile. It can be used in cases with high comorbidity and where applying other regional approaches is risky (4).

The factors that limit the use of IVRA are toxic complications related to the local anesthetic agent, tourniquet pain, and postoperative pain management. In successful IVRA management, these parameters are chosen as the primary target (5). We aimed to compare two different concentrations that were used as part of the standard practice at our institution for foot and ankle surgeries with additional tourniquet application and the IVRA method supported by sedo-analgesia.

An increased likelihood of toxicity due to the high local anesthetic amount hampered the use of traditional IVRA in lower extremity operations (6). The Fellowship of the British Royal College of Anaesthetists (FRCA) recommended a dose of 200 mg of a 5% lidocaine solution for IVRA (7). In addition, no complications were observed in the use of the traditional IVRA method at a dose of 3 mg/kg in lower extremity surgeries (8). IVRA method between two tourniquets, first described by Bier in 1908, was reorganized for knee anesthesia as an inter-cuff technique (9). Arslan et al. (10) have found that the addition of ketamine to the traditional IVRA procedure in knee arthroscopy shortened the onset of sensory block and prolonged the initial analgesic requirement duration. Similar to our study, reducing the total amount of local anesthetic was also aimed by the modification and sedo-analgesia compared to the traditional IVRA in the lower extremity, which requires a high amount of local anesthetic in routine use.

It was reported that the distribution of the local anesthetic agent could be limited with the use of a modified IVRA technique using additional or temporary tourniquets in the upper extremity surgeries, resulting in lower local anesthetic use, and shorter duration of action, and higher quality of anesthesia (11). It has been shown that low anesthetic doses allowed the tourniquet to be deflated as early as 10 minutes (12). The use of a tourniquet under the knee did not increase the risk of local anesthetic leakage through the intraosseous space; in fact, it has been shown to provide a lower dose required to achieve a comparable level of anesthesia. Therefore, an under-knee tourniquet is as safe as the use of an above-knee tourniquet (13).

The main goal of the traditional double-cuff IVRA method, which requires a high amount of local anesthetics, is to reduce the tourniquet pain. Tourniquet pain is one of the most critical complications of tourniquet use and has two important components: tourniquet-related discomfort and real tourniquet pain. Tourniquet-related discomfort can be kept under control with low sedo-analgesia. The sedo-analgesic requirements of the use of a single-cuff tourniquet or double-cuff tourniquet for up to 40 minutes are similar. The required sedo-analgesic consumption also increases in direct proportion with time (14). In our study, although no difference was found between the groups, the sedo-analgesic consumption significantly increased after tourniquet pain developed in patients. Tourniquet pain time was 41.66±6.61 minutes in Group 20 and 36.76±7.17 minutes in Group 30.

The hypertensive tendency is linearly correlated with the increasing levels of tourniquet discomfort over time and this tendency may reach an uncontrollable level. The tourniquet pain can be observed for 60 minutes along with a hypertensive tendency that has an unknown cause and may develop even under general anesthesia depending on insufficient tourniquet tolerance (15). The hypertensive tendency might be prevented with the sedo-analgesia protocol in our study. There was a significant difference between the groups in terms of MAP values at around 35 minutes, but there was no significant difference at other time points. It may have resulted from the fact that tourniquet pain was more frequent in Group 30 than in Group 20 in earlier periods.

BIS monitoring with a hemodynamic follow-up is recommended in patients to avoid unwanted pain. The BIS monitoring has been reported to provide a more balanced anesthesia depth in terms of the required sedo-analgesic consumption and contribute to recovery from anesthesia, postoperative recovery, and orientation (16). We also benefited from BIS monitoring support in the detection and effective management of tourniquet pain and hypertensive tendency with the help of propofol and remifentanil infusion. No patient had any sign of local anesthetic toxicity.

The agent used for the infiltration of local anesthetic should have a slow transition to the plasma so that it has a long postoperative effect. Bupivacaine stays longer and provides long-term analgesia with more lipid-solubility and strong protein dependence in nerves (17). Bupivacaine hydrochloride 2.5 mg/mL was added to the incision line just before the skin and subcutaneous suturing in our study. This infiltration allowed early deflation of the tourniquet, as well as contributed to the control of postoperative analgesia where IVRA was insufficient. Two patients had postoperative additional fentanyl requirements, and there

was no significant difference between the groups in terms of postoperative analgesic need.

This study has several limitations. Firstly, a limiting factor was that only two different concentrations were compared. Another limitation was that there was no control group for comparison.

CONCLUSION

We can also use a modified IVRA approach with low-dose sedo-analgesia support in patient management until the tourniquet pain develops. We also think that the modified IVRA approach can be considered as an alternative method to other anesthetic approaches in high-risk patients by limiting the side-effect profile with a controlled amount of local anesthetic in short-term orthopedic surgery. We believe that the optimal approach can be found with modifications to the IVRA method applied in the lower extremity.

Main points:

- The use of an additional tourniquet reduces the local anesthetic amount.
- Reduced risk of local anesthetic toxicity.
- IVRA creates an alternative to lower extremity surgeries.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Gaziantep University (14.12.2015, 351, and 31.10.2016, 272).

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Author Contributions: Idea/Concept: EM, EŞ, MC; Design: EM, EŞ, MC, SG; Data Collection/Processing: EM, HG, YE; Analysis/Interpretation: EM, EŞ, MC, HG, YE, SG; Literature Review: EM, EŞ, MC, HG, YE, SG; Drafting/Writing: EM, EŞ, MC; Critical Review: EŞ, MC, SG.

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The Relationship Between Body Mass Index and Mononeuropathies

Vücut Kitle İndeksi ve Mononöropatiler Arasındaki İlişki

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ABSTRACT

Aim: The study aimed to find out whether there is a relationship between the mononeuropathies of the median, ulnar, radial, peroneal, and sciatic nerves and body mass index (BMI).
Material and Methods: Patients whose clinical and electrodiagnostic findings were compatible with carpal tunnel syndrome (CTS), ulnar neuropathy at the elbow (UNE), radial neuropathy at the spiral groove (RNS), peroneal neuropathy at the fibular head (PNFH), and sciatic injury due to intramuscular injection (SNIII) were included in this retrospective cohort study. In addition, controls whose clinical and electrodiagnostic features were not compatible with mononeuropathy were included in the study. The BMI values of all participants were analyzed.
Results: One hundred thirty-one CTS patients, 53 UNE patients, 6 RNS patients, 25 PNFH patients, 72 SNIII patients, and 53 controls were included in the study. The BMI of CTS patients was higher than the BMI of controls ($p<0.001$), PNFH patients ($p<0.001$), and SNIII patients ($p<0.001$). The BMI of SNIII patients was lower than the BMI of controls ($p<0.001$), CTS patients ($p<0.001$), and UNE patients ($p<0.001$). The BMI of PNFH patients was lower than that of CTS patients ($p<0.001$) and UNE patients ($p=0.004$). No significant correlation was found between BMI values and electrodiagnostic classification of mononeuropathies in the groups.
Conclusion: This study showed that high BMI is a risk factor for CTS and low BMI is a risk factor for SNIII. There may also be a relationship between BMI and PNFH, but this should be confirmed by further studies.
Keywords: Body mass index; carpal tunnel syndrome; entrapment neuropathy; mononeuropathy; sciatic nerve injury.

ÖZ

Amaç: Bu çalışmada medyan, ulnar, radyal, peroneal ve siyatik sinirlerin mononöropatileri ile vücut kitle indeksi (VKİ) arasında bir ilişki olup olmadığının araştırılması amaçlandı.
Gereç ve Yöntemler: Klinik ve elektrodiagnostik bulguları karpal tünel sendromu (KTS), dirsekte ulnar nöropati (DUN), spiral olukta radiyal nöropati (RNS), fibula başında peroneal nöropati (PNFB) ve intramüsküler enjeksiyona bağlı siyatik yaralanması (İMESY) ile uyumlu olan hastalar bu geriye dönük kohort çalışmasına dahil edildi. Ayrıca klinik ve elektrodiagnostik özellikleri mononöropati ile uyumlu olmayan kontroller çalışmaya dahil edildi. Tüm katılımcıların VKİ değerleri analiz edildi.
Bulgular: Yüz otuz bir KTS hastası, 53 DUN hastası, 6 RNS hastası, 25 PNFB hastası, 72 İMESY hastası ve 53 kontrol bu çalışmaya dahil edildi. KTS hastalarının VKİ değeri kontrollerin ($p<0,001$), PNFB hastalarının ($p<0,001$) ve İMESY hastalarının ($p<0,001$) VKİ değerinden daha yüksekti. İMESY hastalarının VKİ değeri kontrollerin ($p<0,001$), KTS hastalarının ($p<0,001$) ve DUN hastalarının ($p<0,001$) VKİ değerinden daha düşüktü. PNFB hastalarının VKİ değeri KTS hastalarından ($p<0,001$) ve DUN hastalarından ($p=0,004$) daha düşüktü. Gruplarda VKİ değerleri ile mononöropatilerin elektrodiagnostik sınıflandırması arasında anlamlı bir korelasyon bulunmadı.
Sonuç: Bu çalışma, yüksek VKİ değerinin KTS için bir risk faktörü olduğunu ve düşük VKİ değerinin ise İMESY için bir risk faktörü olduğunu göstermiştir. VKİ ve PNFB arasında da bir ilişki olabilir, ancak bu daha ileri çalışmalarla doğrulanmalıdır.
Anahtar kelimeler: Vücut kitle indeksi; karpal tünel sendromu; tuzak nöropati; mononöropati; siyatik sinir hasarı.

INTRODUCTION

Mononeuropathies are disorders that can lead to disability. They can occur as a result of trauma or compression of the nerve while passing through a narrow anatomical canal. Injury of the nerve as a result of increased pressure in the narrow canal is known as entrapment mononeuropathy (1-3). The most common entrapment mononeuropathy is carpal tunnel syndrome (CTS). Other common entrapment mononeuropathies include ulnar neuropathy at the elbow (UNE), radial neuropathy at the spiral groove (RNS), and peroneal neuropathy at the fibular head (PNFH) (1-3). Disorders such as diabetes mellitus or thyroid disease, activities during daily life or business life, and factors such as age or body mass index (BMI) may be risk factors for these entrapment mononeuropathies (4-9). The relationship between BMI and entrapment mononeuropathies has been analyzed in many studies. Most of the research was conducted on CTS and UNE (4-11). Although BMI is known to be a risk factor for CTS, there were conflicting results regarding the association between UNE and BMI (4,5,10-12). It has also been reported that sciatic nerve injury due to intramuscular injection (SNIII) is seen in thin patients due to a low amount of gluteal muscle mass (13-15).

In this study, it was aimed to find out whether SNIII and entrapment neuropathies are associated with BMI.

MATERIAL AND METHODS

Study Design

Individuals aged over sixteen years who applied to Adana City Training and Research Hospital (ACTRH) Clinical Neurophysiology Laboratory between September 2018 and October 2020, with both clinical and electrodiagnostic characteristics compatible with entrapment mononeuropathy or SNIII, and controls were included in this retrospective study. Individuals with the following conditions were excluded from the study: disease that could cause neuropathy, such as diabetes mellitus; polyneuropathy; clinical, electrodiagnostic, and imaging test findings consistent with cervical/lumbosacral radiculopathy or plexopathy. In addition, individuals with two or more different entrapment mononeuropathies were not included. The age, gender, and BMI of the patients were analyzed. It was also recorded whether the patients had weight loss or not. Individuals with a BMI of <18.5 kg/m^2 were classified as underweight, individuals with a BMI of 18.5 to <25 kg/m^2 as normal, and those with a BMI of ≥25 kg/m^2 as overweight (16). Ethics committee approval was received from the ACTRH Ethics Committee (date: 20.05.2020, and number: 57/869).

Electrodiagnostic Tests

The nerve conduction studies and the needle electromyography (EMG) were performed with a Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). Electrodiagnostic tests were performed if the temperature of the patient's extremities was ≥32 °C. Recording and stimulation were made with surface electrodes. Supramaximal nerve stimulation was applied. For sensory and motor nerve conduction studies, band filters are set to 20 Hz-2 kHz and 20 Hz-10 kHz, respectively. For the sensory nerve conduction study, the sensitivity and sweep speed were 10 uV/division and 1 ms/division, respectively. Sensitivity and sweep speed for motor nerve study were 2 mV/division and 5 ms/division, respectively. If the patient had mononeuropathy in the upper extremity, nerve conduction studies were performed in the bilateral upper extremities and one lower extremity. If the patient had PNFH or SNIII, nerve studies were performed in bilateral lower extremities and one upper extremity. A nerve conduction study was performed in one upper and one lower extremity of the controls. Protocols for the median, ulnar, peroneal, posterior tibial, superficial peroneal, and sural nerve conduction studies were made using conventional methods (17-19). Reference values of ACTRH Clinical Neurophysiology Laboratory were used for routine nerve conduction studies (18,19). Reference values for routine motor and sensory nerve conduction studies are shown in Table 1 and Table 2, respectively. Unlike other nerve conduction studies, radial nerve compound muscle action potential (CMAP) was recorded from the extensor indicis proprius muscle with a concentric needle electrode (20). The lower reference limit for the radial motor nerve conduction velocity (NCV) across the forearm-above spiral groove segment was 49.8 m/s (20). A superficial radial sensory nerve conduction study was made with surface electrodes using conventional methods (17). The lower reference limit for superficial radial nerve sensory nerve action potential (SNAP) amplitude and upper reference limit for superficial radial SNAP peak latency were 11 μV and 2.8 ms, respectively. The high pass and low pass band filters for needle EMG were 10 Hz and 10 kHz, respectively. Needle EMG was performed visually. Active denervation was carefully analyzed. Motor unit action potential (MUAP) analysis was performed during light muscle contraction. The sweep speed was 10 ms/division for both active denervation and MUAP analysis. Sensitivity for active denervation and MUAP analysis was 100 uV/division and 200-500 uV/division, respectively. Ten to twenty MUAPs were analyzed. If the MUAP amplitude was >4 mV and the MUAP duration was >15 ms, the MUAP was considered neurogenic.

Subjects

Carpal Tunnel Syndrome (CTS) Patients

Patients should have one of the following criteria clinically (21,22): *i)* paresthesia in the first three fingers or one of the first three fingers, *ii)* in neurological examination, sensory abnormality in the first three fingers or one of the first three fingers and/or weakness in hand muscles innervated by the median nerve. Patients should have one of the following characteristics in electrodiagnostic tests (21,22): *i)* slowing of median sensory NCV across second finger-wrist/first finger-wrist/third finger-wrist/palm-wrist segments (mild CTS), *ii)* in addition to the first electrodiagnostic criterion, the delay of the median nerve CMAP latency (moderate CTS), *iii)* delay of median nerve CMAP latency and absence of median nerve SNAP across first/second/third finger-to-wrist segments (severe CTS). Since the lesion localization could not be determined clearly, patients whose median nerve CMAP could not be obtained were not included in the study. Needle EMG was applied to abductor pollicis brevis, pronator teres, and first dorsal interosseous muscles of all CTS patients. Patients with

needle EMG abnormalities in the first dorsal interosseous and pronator teres muscles were not included in the study.

Ulnar Neuropathy at the Elbow (UNE) Patients

Patients with one of the following abnormalities in their neurological examination were included in the study (23,24): *i)* sensory abnormality in the fourth/fifth finger or in the medial palm or dorsomedial of the hand, *ii)* weakness of the muscles innervated by the ulnar nerve. If one of the following features was present in the nerve conduction study, the patient was considered to have UNE (18,23,24): *i)* slowing of ulnar motor NCV across below elbow-above elbow segment, *ii)* motor conduction block (decreasing the CMAP amplitude obtained by stimulation of the ulnar nerve above the elbow by more than 50% compared to that obtained by stimulation of the ulnar nerve below the elbow) across below elbow-above elbow segment, *iii)* abnormal latency difference in short segment (2 cm) ulnar motor nerve conduction study. UNE patients were divided into three groups according to electrodiagnostic classification (25): *i)* mild UNE: slowing of the ulnar motor NCV across the below elbow-above elbow segment or motor conduction block across this segment, *ii)* moderate UNE: in addition to the first criterion, reduction of SNAP amplitude across the 5th finger-wrist segment, *iii)* severe UNE: in addition to the first criterion, the absence of SNAP across the 5th finger-wrist segment. Since the location of the lesion could not be determined clearly in patients whose ulnar nerve CMAP could not be obtained, these patients were not included in the study. Needle EMG was applied to the abductor digiti minimi, first dorsal interosseous, flexor carpi ulnaris, flexor digitorum profundus (ulnar), and abductor pollicis brevis muscles in patients with UNE. Patients with needle EMG abnormalities in the abductor pollicis brevis muscle were not included in the study (26). Patients whose electrodiagnostic findings were compatible with ulnar neuropathy in the wrist were excluded (27).

Radial Neuropathy at the Spiral Groove (RNS) Patients

Patients with one of the following neurological examination findings were included in the RNS group (28,29): Weakness in the dorsiflexion of the fingers or wrist. In addition to this criterion, there may be an abnormality in the sensory area innervated by the superficial radial nerve. Patients should have radial motor nerve conduction block (CMAP amplitude reduction >50%) across the below spiral groove-above spiral groove segment. The electrodiagnostic classification of RNS was as follows: *i)* mild RNS: radial motor conduction block, *ii)* moderate RNS: motor conduction block and reduced superficial radial nerve SNAP amplitude, *iii)* severe RNS: motor conduction block and absence of superficial radial nerve SNAP. Patients whose radial nerve CMAP could not be obtained were excluded from the study. The needle EMG findings of the triceps, abductor pollicis brevis, and first dorsal interosseous muscles of these patients should have been normal (28,29).

Peroneal Neuropathy at the Fibular Head (PNFH) Patients

PNFH was considered if one of the following features was present in the neurological examination of the patients (30,31): *i)* weakness of the muscles with peroneal

Table 1. Reference values for motor nerve conduction studies

Motor nerve conduction study parameter	Reference value
Median nerve terminal CMAP amplitude (mV) / latency (ms)	3.7 / 4.3
Median motor NCV across wrist - elbow segment (m/s)	49.1
Ulnar nerve terminal CMAP amplitude (mV) / latency (ms) ADM	8.0 / 2.9
Ulnar nerve terminal CMAP amplitude (mV) / latency (ms) FDI	6.4 / 4.9
Ulnar motor NCV across wrist - below elbow segment ADM / FDI	52.0 / 50.9
Ulnar motor NCV below elbow - above elbow segment ADM / FDI	43.0 / 45.7
Latency difference in short segment ulnar motor nerve conduction study (ms)	0.7
Posterior tibial nerve CMAP amplitude (mV) / latency (ms)	4.2 / 5.8
Posterior tibial motor NCV across ankle - popliteal fossa segment (m/s)	41.0
Peroneal nerve terminal CMAP amplitude (mV) / latency (ms) (EDB)	3.7 / 5.2
Peroneal nerve terminal CMAP amplitude (mV) (TA)	3.9
Peroneal motor NCV across ankle - below fibular head segment (m/s)	43.9
Peroneal motor NCV below fibular head - popliteal fossa segment EDB / TA (m/s)	40.1 / 41.0

CMAP: compound muscle action potential, NCV: nerve conduction velocity, ADM: abductor digiti minimi, FDI: first dorsal interosseous, EDB: extensor digitorum brevis, TA: tibialis anterior, CMAP amplitudes were measured from peak to peak

Table 2. Reference values for sensory nerve conduction studies

Sensory nerve conduction study parameter	Reference value
Median nerve	
2nd digit-wrist SNAP amplitude (uV) / sensory NCV (m/s)* / sensory NCV (m/s)**	10.3 / 40.9 / 44.6
1st digit-wrist SNAP amplitude / sensory NCV (m/s)*	4.3 / 34.0
3rd digit-wrist SNAP amplitude / sensory NCV (m/s)*	2.8 / 39.5
Palm-wrist SNAP amplitude / sensory NCV (m/s)*	14.7 / 37.5
Ulnar nerve 5th digit-wrist SNAP amplitude / sensory NCV (m/s)* / sensory NCV (m/s)**	7.1 / 38.8 / 42.0
Superficial peroneal nerve SNAP amplitude / sensory NCV (m/s)	5.3 / 37.0
Sural nerve SNAP amplitude / sensory NCV (m/s)	5.1 / 33.2

SNAP: sensory nerve action potential, NCV: nerve conduction velocity, *: Sensory NCV was calculated using peak latency, **: Sensory NCV was calculated using onset latency, SNAP amplitudes were measured from peak to peak

nerve innervation, *ii)* sensory abnormality in the skin area supplied by the peroneal nerve. Electrodiagnostic tests should have one of the following findings (17,32,33): *i)* slowing of the peroneal motor NCV across the below fibular head-popliteal fossa segment, *ii)* peroneal motor nerve conduction block (CMAP amplitude reduction >25%) across the below fibular head-the popliteal fossa segment (17). PNFH classification was as follows: *i)* mild: motor conduction block or slowing of motor NCV across the below fibular head-popliteal fossa segment, *ii)* moderate: reduced superficial peroneal nerve SNAP amplitude in addition to the first item, *iii)* severe: in addition to the first item, absence of the superficial peroneal SNAP. Patients whose peroneal nerve CMAP could not be obtained from both the tibialis anterior and extensor digitorum brevis muscles were not included in the study. The needle EMG of the medial gastrocnemius, biceps femoris (short head), and vastus lateralis muscles of the patients should have been normal (30,31,33).

Sciatic Nerve Injury due to Intramuscular Injection (SNIII) Patients

The complaints of the patients must have been related to intramuscular injection. If the patient had one of the following neurological examination findings, it was considered that the diagnosis was compatible with SNIII (15,19,34): *i)* weakness in the muscles innervated by the tibial, peroneal, or sciatic nerves, *ii)* sensory abnormality in the skin area supplied by the sciatic nerve branches. SNIII was considered if one of the following findings was present in the electrodiagnostic tests: *i)* abnormality in motor and sensory nerve conduction studies of the sciatic nerve branches, *ii)* needle EMG abnormality in the muscles innervated by the sciatic nerve or its branches. Electrodiagnostic classification of SNIII was as follows: *i)* mild: normal sural and superficial peroneal nerve SNAPs, *ii)* moderate: reduced sural or superficial peroneal nerve SNAP amplitude, *iii)* severe: absence of sural or superficial peroneal nerve SNAP. Needle EMG was performed on the tibialis anterior, medial gastrocnemius, peroneus longus, and biceps femoris (short head) muscles of the patients. In order to exclude lumbosacral radiculopathy/plexopathy, needle EMG was applied to the vastus lateralis, gluteus maximus, L3, L4, L5, and S1 paraspinal muscles (13-15).

Controls

The individuals who applied to the clinical neurophysiology laboratory and whose clinical and electrodiagnostic findings were not compatible with mononeuropathy or polyneuropathy or radiculopathy or plexopathy were included in the control group. Electrodiagnostic tests for controls were performed for joint, bone, or muscle pain. Neurological examinations of the controls were normal. In addition, individuals with diseases that could cause neuropathy and neurodegenerative diseases such as diabetes mellitus were excluded. Fifty-three healthy participants were analyzed.

Statistical Analysis

Categorical variables were summarized as percentage and frequency. The mean, standard deviation, and minimum and maximum of the numeric data were calculated for descriptive statistics. Pearson's chi-square and Fisher's exact tests were used to analyze categorical variables. The Shapiro-Wilk test was used to determine the distribution of the data. Kruskal-Wallis and Mann-Whitney U tests were used in the analysis of quantitative data. Bonferroni correction was used for post hoc analysis and multiple comparisons. Spearman's rank correlation coefficient test was used for the correlation analysis. If p value was <0.05, it was considered statistically significant. The Statistical Package for the Social Sciences (SPSS IBM Corp; Armonk, NY, USA) 22.0 was used for statistical analysis.

RESULTS

One hundred thirty-one CTS patients, 53 UNE patients, 6 RNS patients, 25 PNFH patients, 72 SNIII patients, and 53 controls were included in the study. 19 (14.5%) of the CTS patients, 37 (69.8%) of the UNE patients, 5 (83.3%) of the RNS patients, 21 (84.0%) of the PNFH patients, 52 (72.2%) of the SNIII patients, and 30 (56.6%) of the controls were male. The rate of females in CTS patients was significantly higher than the rate of females in all other groups ($p<0.001$). In addition, the rate of males in PNFH patients was higher than the control group ($p=0.022$).

Age, height, weight, and BMI values among the groups were shown in Table 3. BMI values and BMI classification among groups were shown in Figure 1 and Figure 2, respectively. There was a history of weight loss in eleven PNFH patients and five SNIII patients. Six of the eleven PNFH patients with weight loss also had a history of prolonged repetitive leg posture, such as crossing the legs. The mean amount of weight loss in PNFH patients was 4.7±2.3 (range, 2.5 to 8) kg/month. Except for PNFH and SNIII patients, none of the participants had a history of weight loss. Eleven PNFH patients had only a history of prolonged repetitive leg posture, four had only a history of prolonged sleep (two had a history of alcohol intake before sleep). In all RNS patients, RNS occurred after prolonged sleep (history of alcohol intake in two patients, history of heroin use in one patient). The side of mononeuropathies and the electrodiagnostic classification of mononeuropathies among the groups were shown in Table 4. There was no correlation between BMI values and electrodiagnostic classification of mononeuropathies in the groups (Table 5).

DISCUSSION

Different risk factors have been identified in different mononeuropathies (4-8,11,12). Disorders such as diabetes mellitus or thyroid disease, age, gender, activities during daily life or work, and BMI can be risk factors for some mononeuropathies (4-8). It is known that high BMI is a risk factor for CTS (4-7). This can be explained by the increased pressure in the carpal tunnel as a result of the increase in adipose tissue due to obesity and the subsequent injury to the median nerve (1,2,4,7,35). On the other hand, although there are studies showing that low BMI is a risk factor for UNE (10,11), there are also authors who report otherwise (12). It is thought that thin people have less amount of tissue protecting the ulnar nerve at the elbow, and consequently, the ulnar nerve in thin people may become more susceptible to compression (11). Although there was no difference between the BMIs of the control group and the UNE group in this study, the fact that most of the UNE patients had a BMI of <30 kg/m^2 may mean that UNE is less common in obese patients. However, it should be kept in mind that the distance can be measured more than it should be in nerve conduction studies in overweight patients (11). Future studies are needed to reveal whether there is a relationship between UNE and BMI.

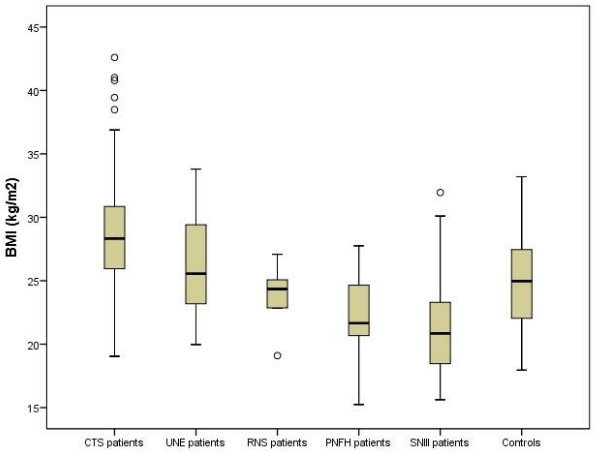

Figure 1. BMI among groups
BMI: body mass index, CTS: carpal tunnel syndrome, UNE: ulnar neuropathy at the elbow, RNS: radial neuropathy at the spiral groove, PNFH: peroneal neuropathy at the fibular head, SNIII: sciatic nerve injury due to intramuscular injection

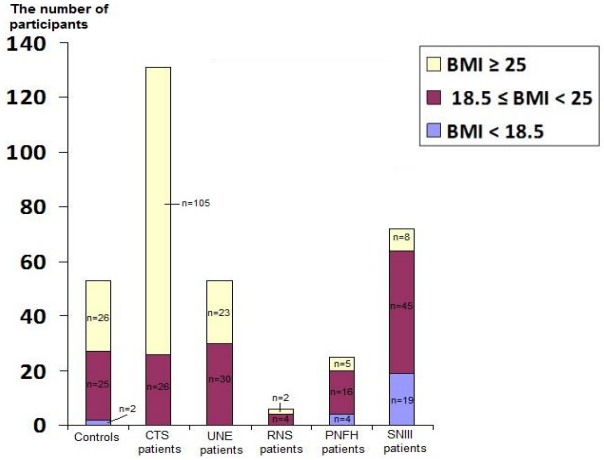

Figure 2. BMI classification among groups
BMI: body mass index, CTS: carpal tunnel syndrome, UNE: ulnar neuropathy at the elbow, RNS: radial neuropathy at the spiral groove, PNFH: peroneal neuropathy at the fibular head, SNIII: sciatic nerve injury due to intramuscular injection

Table 3. Age, weight, height, and BMI among groups, mean±SD, median (IQR_{25-75}) [min-max]

	CTS (n=131)	UNE (n=53)	RNS (n=6)	PNFH (n=25)	SNIII (n=72)	Controls (n=53)	p*
Age (years)	47.3±12.7 46 (38.0-56.0) [23-83]	42.0±14.9 42 (28.5-53.5) [18-77]	41.3±17.3 41 (22.8-59.8) [22-62]	32.8±16.0 30 (18.0-41.5) [18-82]	41.3±16.2 35 (30-54) [19-79]	38.8±12.8 38 (30.0-46.5) [18-69]	**<0.001**
Height (cm)	162.8±5.9 162 (159-165) [150-186]	172.1±7.9 173 (168-178) [150-191]	169.8±6.1 171 (164-175) [161-176]	176.2±7.6 176 (172-180) [160-191]	171.1±8.4 170 (165-177) [155-196]	170.1±7.9 172 (164-176) [150-185]	**<0.001**
Weight (kg)	76.5±12.3 76 (68.0-84.5) [54-110]	77.7±11.9 76 (68.0-86.0) [58-112]	68.8±10.0 70 (61.8-76.0) [52-82]	68.9±12.7 70 (59.0-80.0) [40-85]	62.0±8.9 60 (56.0-66.8) [47-87]	73.0±12.5 73 (63.5-84.0) [50-97]	**<0.001**
BMI (kg/m^2)	28.7±4.4 28.3 (25.7-30.9) [19.1-42.6]	26.3±4.1 25.6 (23.2-29.4) [19.9-33.8]	23.8±2.7 24.4 (21.9-25.6) [19.1-27.1]	22.1±3.4 21.7 (20.5-24.7) [15.2-27.8]	21.3±3.5 20.9 (20.5-24.7) [15.6-31.9]	25.1±3.9 24.9 (22.0-27.5) [17.9-33.2]	**<0.001**

BMI: body mass index, CTS: carpal tunnel syndrome, UNE: ulnar neuropathy at the elbow, RNS: radial neuropathy at the spiral groove, PNFH: peroneal neuropathy at the fibular head, SNIII: sciatic nerve injury due to intramuscular injection, SD: standard deviation, IQR_{25-75}: interquartile range (25^{th}-75^{th} percentile), *: Kruskal-Wallis test was used, Bonferroni correction was used for post hoc analysis and multiple comparisons: The ages of CTS patients were higher than that of PNFH patients (p<0.001), SNII patients (p=0.012), and controls (p=0.005); The heights of CTS patients were lower than that of controls (p<0.001), SNIII patients (p<0.001), UNE patients (p<0.001), and PNFH patients (p<0.001); The weights of SNIII patients were lower than that of controls (p<0.001), UNE patients (p<0.001), and CTS patients (p<0.001); The BMI of CTS patients was higher than that of controls (p<0.001), UNE patients (p<0.001), and SNIII patients (p<0.001); The BMI of SNIII patients was lower than that of controls (p<0.001), CTS patients (p<0.001), and UNE patients (p<0.001); The BMI of PNFH patients was lower than that of CTS patients (p<0.001), and UNE patients (p=0.004).

Table 4. Side and electrodiagnostic classification of mononeuropathies

Mononeuropathy	CTS (n=131)	UNE (n=53)	RNS (n=6)	PNFH (n=25)	SNIII (n=72)
Side of the mononeuropathy					
Right	25	38	3	18	22
Left	5	15	3	7	50
Bilateral	101	0	0	0	0
Electrodiagnostic classification					
Mild	51	36	6	20	7
Moderate	72	13	0	3	22
Severe	8	4	0	2	43

CTS: carpal tunnel syndrome, UNE: ulnar neuropathy at the elbow, RNS: radial neuropathy at the spiral groove, PNFH: peroneal neuropathy at the fibular head, SNIII: sciatic nerve injury due to intramuscular injection

Table 5. Correlation between BMI and electrodiagnostic classification among groups

Electrodiagnostic classification	BMI	
	r_s	p
CTS	0.098	0.413
UNE	0.219	0.186
PNFH	0.028	0.894
SNIII	-0.127	0.404

BMI: body mass index, r_s: Spearman correlation, CTS: carpal tunnel syndrome, UNE: ulnar neuropathy at the elbow, PNFH: peroneal neuropathy at the fibular head, SNIII: sciatic nerve injury due to intramuscular injection

In this study, the BMI of PNFH patients were less than the BMIs of CTS and UNE patients, although they were not different from the BMI of the controls. However, some of the patients had a history of weight loss. It is known that long-term repetitive leg postures and weight loss are associated with PNFH (30-32). This situation can be explained by the reduction of the tissues protecting the peroneal nerve due to weight loss and the peroneal nerve becoming more susceptible to injury due to compression, as we mentioned earlier (36). However, some patients with

PNFH associated with weight loss did not have a history of prolonged repetitive leg postures or prolonged sleep. For this reason, the reduction of nerve protective tissue may not be the only cause of PNFH. Metabolic changes due to weight loss may also have caused PNFH (37,38). These findings and the fact that the BMI of PNFH patients was lower than that of CTS and UNE patients found in this study may indicate that thin individuals are more likely to develop PNFH.

We found that the BMIs of SNIII patients were lower than the BMIs of controls, CTS patients, and UNE patients. This can be explained by the fact that the sciatic nerve becomes more sensitive to trauma as a result of the reduction of the tissue in the gluteal region that protects the sciatic nerve (13,15,39). Therefore, factors such as the length of the needle and the position of the patient should be taken into consideration when administering an intramuscular injection to individuals with low BMI (15). Intramuscular injection into the gluteus medius muscle instead of the gluteus maximus muscle can also inhibit SNIII (40).

Two RNS patients had a BMI of >25 kg/m^2 but less than 30 kg/m^2. The BMI of other RNS patients was normal. Although the findings in this study may indicate that BMI is not a risk factor for RNS, it should be noted that the number of patients is low. Further studies on BMI and RNS patients are needed.

Although one study found a relationship between CTS severity and BMI (41), there is also a study by Kouyoumdjian, which showed that there was no relationship between severely affected median nerve SNAP latency and high BMI (6). In our study, no correlation was found between the electrodiagnostic classification of CTS and BMI. In addition, a similar finding was found in patients with UNE, PNFH, and SNIII. These findings may suggest that factors other than BMI affect the severity of mononeuropathy after the development of mononeuropathy associated with BMI. We think that neurological examination and electrodiagnostic tests to be performed in patients with mononeuropathy after weight gain or weight loss will have interesting results.

This study had some limitations. First, the proportion of females in the CTS group was high. This was an expected result. CTS is known to be more common in women (9,42). In addition, there are studies reporting that UNE is more common in men (12,43). Given that gender is a risk factor for some mononeuropathies, this may have influenced our results. But it should be noted that we are making a comparison in a heterogeneous group. The male-gender ratio was higher in patients with PNFH. This finding may be important and needs to be confirmed by further studies. Second, the age of CTS patients was different from the participants in other groups. This finding supported the conclusion found in previous studies that age is a risk factor for CTS (9,44). Third, as we mentioned earlier, the numbers of RNS patients were low. Finally, the controls did not consist of completely healthy individuals. However, it should be noted that his neurological examinations were normal, and he had no neurological symptoms.

Despite these limitations, this study also had its strengths. We used both clinical and electrodiagnostic criteria for mononeuropathy. Thus, it can be said that the diagnoses are more precise. In addition, neurological examinations and nerve conduction studies were performed in the upper and lower extremities of all participants, as a result, the possibility of more than one mononeuropathy was eliminated clinically or subclinically. We did not include patients with only the motor and sensory nerve conduction studies of the median and ulnar nerves, and all patients had nerve conduction studies of the upper and lower extremities.

CONCLUSION

This study showed that high BMI is a risk factor for CTS and low BMI is a risk factor for SNIII. There may also be a relationship between BMI and PNFH, but this should be confirmed by further studies.

Ethics Committee Approval: The study was approved by the clinical research ethics committee of the Adana City Training and Research Hospital (20.05.2020, 57/869).

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Evaluation of Patients Presenting to the Pediatric Emergency Department with Carbon Monoxide Poisoning

Çocuk Acile Karbonmonoksit Zehirlenmesi ile Başvuran Hastaların Değerlendirilmesi

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ABSTRACT

Aim: Carbon monoxide poisoning is one of the significant causes of intoxication and presentation to pediatric emergency departments, especially in winter. The primary aim of this study was to evaluate the demographic characteristics, and clinical and laboratory findings of pediatric patients who presented to the pediatric emergency department with carbon monoxide poisoning, and the secondary aim was to examine the laboratory values of the patients with impaired consciousness.
Material and Methods: The demographic and clinical characteristics and laboratory values of 162 patients presented to the pediatric emergency department due to carbon monoxide poisoning between 2017 and 2020 were retrospectively analyzed. The laboratory parameters of the patients with and without symptoms of impaired consciousness were compared.
Results: The mean age of the patients was 8.94±5.33 years, and the gender distribution was homogenous. The highest frequency of presentation was during winter. Nausea, vomiting, and headache were the most common symptoms. Fifteen of the patients had impaired consciousness. While the laboratory values of the patients were generally within normal ranges, patients with high carboxyhemoglobin, lactate, and troponin values, and low pH were encountered. The carboxyhemoglobin and lactate levels of patients with impaired consciousness were found to be significantly higher than the patients without impaired consciousness ($p<0.001$ and $p=0.019$, respectively).
Conclusion: Elevated carboxyhemoglobin and lactate levels were associated with impaired consciousness. Although carboxyhemoglobin levels are important for diagnosis and clinical follow-up, they should not be used as the only marker. High lactate and troponin levels, and low pH should also be taken into account.
Keywords: Carbon monoxide; children; impaired consciousness; poisoning.

ÖZ

Amaç: Karbonmonoksit zehirlenmeleri özellikle kış mevsiminde zehirlenme ile çocuk acil servisine başvuruların önemli nedenlerinden biridir. Bu çalışmanın birincil amacı çocuk acil servisine karbonmonoksit zehirlenmesi ile başvuran çocuk hastaların demografik özellikleri ile klinik ve laboratuvar bulgularını değerlendirmek, ikincil amacı ise karbonmonoksit zehirlenmesine bağlı bilinç değişikliği gelişen hastaların laboratuvar değerlerini incelemektir.
Gereç ve Yöntemler: 2017 ve 2020 yılları arasında çocuk acil servisine karbonmonoksit zehirlenmesi nedeniyle başvurmuş olan 162 hastanın demografik ve klinik özellikleri ile laboratuvar değerleri geriye dönük olarak incelendi. Bilinç değişikliği semptomu olan ve olmayan hastaların laboratuvar parametreleri karşılaştırıldı.
Bulgular: Hastaların ortalama yaşı 8,94±5,33 yıl idi ve cinsiyet dağılımları homojendi. En yüksek başvuru sıklığı kış mevsimindeydi. Bulantı, kusma ve baş ağrısı en sık görülen semptomlardı. Hastaların on beşinde bilinç değişikliği vardı. Hastaların laboratuvar değerleri genel olarak normal aralıklarda iken karboksihemoglobin, laktat ve troponin değerleri yüksek olan ve pH değeri düşük olan hastalar da olduğu tespit edildi. Bilinç değişikliği olan hastaların karboksihemoglobin ve laktat düzeyi, bilinç değişikliği olmayan hastalara göre anlamlı şekilde daha yüksek bulundu (sırasıyla $p<0,001$ ve $p=0,019$).
Sonuç: Yüksek karboksihemoglobin ve laktat seviyesi bilinç değişikliği ile ilişkili bulunmuştur. Karboksihemoglobin düzeyi tanı ve klinik takip için önemli olmakla birlikte tek başına bir belirteç olarak kullanılmamalıdır. Yüksek laktat ve troponin değerleri ve düşük pH değeri de dikkate alınmalıdır.
Anahtar kelimeler: Karbonmonoksit; çocuk; bilinç değişikliği; zehirlenme.

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INTRODUCTION

Carbon monoxide (CO) is a toxic gas called the "silent killer". This non-irritant gas is formed as a result of the incomplete combustion of carbon-containing materials (1,2). CO poisoning is an important public health problem in Turkey and across the world, especially in the winter months. Smoke from fires, inadequate maintenance of heating systems, and vehicle exhaust gases are the most common causes of CO poisoning. The most common reason encountered in Turkey is the inadequacy of waste gas removal in heating and hot water sources (3).

CO causes toxicity through hypoxic and inflammatory processes and can affect many systems. CO poisoning may present with non-specific symptoms, such as headache, nausea, vomiting, abdominal pain, respiratory distress, weakness, and chest pain, and it is a significant cause of morbidity, coma, and death in severe cases (4). In a study evaluating 20 years of data from Turkey, it was found that 1.42% of forensic deaths were caused by CO poisoning (5). In the United States, the Centers for Disease Control and Prevention reported that approximately 21,000 patients were admitted to the emergency department annually due to non-fire carbon monoxide poisoning, and 450 patients died (6).

The diagnosis of CO poisoning is made based on the medical history, clinical examination, and carboxyhemoglobin (COHb) levels of patients. However, the clinical symptoms and severity of acute CO poisoning are not always related to COHb concentrations at presentation (7-9). In such cases, markers including increased lactate and troponin levels as indicators of tissue hypoxia and the presence of metabolic acidosis may be useful in determining the severity of poisoning.

The primary aim of this study was to evaluate the demographic characteristics, clinical findings, and laboratory findings of pediatric patients who presented to the pediatric emergency department with carbon monoxide poisoning, and the secondary aim was to examine the laboratory values of the patients with impaired consciousness.

MATERIAL AND METHODS

In this study, patients aged one month to 18 years, who were brought to the pediatric emergency department of the University of Health Sciences Gülhane Training and Research Hospital between 1 January 2017 and 31 December 2020 due to CO poisoning, were retrospectively evaluated. The diagnosis of CO poisoning was made according to a history of exposure to CO, clinical findings, and high COHb levels. A COHb level above 5% was considered to be high (4). Patients with a history of chronic diseases (pulmonary, cardiovascular, and neurological diseases) were excluded from the study. The patients' demographic characteristics (age and sex), signs and symptoms, laboratory values (COHb, blood pH, lactate, troponin I, leukocyte, and platelet counts), treatments, and follow-up data were evaluated. A lactate level of >2 mmol/l was considered significant (10,11). There are insufficient data on reference ranges for cardiac Troponin I levels indicating cardiac injury in children (12). In this study, a significant elevation was accepted based on the reference upper Troponin I level of our hospital laboratory. Normobaric oxygen (NBO) therapy was given to all patients. If patients had acute cardiac or neurological findings, metabolic acidosis, or high COHb values (>25%), hyperbaric oxygen (HBO) therapy was given (13). We compared the laboratory results of the patients with or without impaired consciousness at the time of admission to the hospital. Impaired consciousness was assessed using the Glasgow coma scale (GCS).

To collect data, approval was obtained from the Clinical Research Ethics Committee of the University of Health Sciences, Gülhane Training and Research Hospital with the decision dated 17 June 2021 and numbered 2021/226, and official permission was received from the Medical Specialization Education Board. The study was carried out according to the principles of the Declaration of Helsinki.

Statistical Analysis

The data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23.0. The data obtained by measurements were expressed as mean±standard deviation, median, interquartile range, and minimum-maximum values, and the grouped data are expressed as frequencies (percentages). The normality of the distribution of the data was checked by the Kolmogorov-Smirnov test, and the assumption of normality was violated for all quantitative variables. The Mann-Whitney U test was used to compare the quantitative data for the variables that did not show normal distribution between the two groups. The categorical data were analyzed using the chi-square test and Fisher's exact test. The level of statistical significance was accepted as $p<0.05$.

RESULTS

During the period covered in the study, 162 patients were diagnosed with CO poisoning in the pediatric emergency department. The mean age of the patients was 8.94±5.33 years (1 month-17 year), and 81 (50%) were female. Seventeen patients were foreign nationals. The times of the day with the highest frequency of admissions were between 00:01 and 06:00 in the winter months (December and January). Among the patients, 53.1% (n=86) were brought to the emergency department by the 112 ambulance service. More than half (n=107) of the patients had no complaints and were brought to the hospital with the suspicion of poisoning because they had a family history of CO poisoning. Percentages for symptoms are given over the total number of patients. The most common symptoms during admission were nausea (43.6%), vomiting (32.7%), and headache (32.7%). Twenty-five patients had multiple symptoms. Fifteen (27.3%) cases had a history of impaired consciousness (sleepiness in nine patients, syncope in four, and convulsions in two). In the physical examinations, three patients had a GCS score of 14 and had only a tendency to sleep. Other systemic examinations were normal in all patients. The demographic and clinical characteristics of the patients were given in Table 1. The electrocardiograms of all patients were within normal limits.

COHb levels at the time of admission were 20% or higher in 48 (29.6%) of the patients, lactate levels in blood gas tests were high in 76 (46.9%), and troponin I levels were high in 12 (7.4%). The laboratory values of the patients are presented in Table 2.

Table 1. Demographic and clinical characteristics of patients

Age (years), mean±SD (min-max)	8.94±5.33 (1-17)
Gender, n (%)	
Female	81 (50.0)
Male	81 (50.0)
Admission time, n (%)	
00:01-06:00	46 (28.4)
06:01-12:00	36 (22.2)
12:01-18:00	38 (23.5)
18:01-00:00	42 (25.9)
Means of transport to the hospital, n (%)	
112 ambulance service	86 (53.1)
By own means	76 (46.9)
Admission season, n (%)	
Winter	94 (58.0)
Spring	44 (27.2)
Summer	8 (4.9)
Fall	16 (9.9)
Has symptoms, n (%)	
Yes	55 (34.0)
No	107 (66.0)
Signs and symptoms at admission[#], n (%)	
Nausea	24 (43.6)
Vomiting	18 (32.7)
Headache	18 (32.7)
Impaired consciousness	15 (27.3)
Dizziness	14 (25.4)
Cough	2 (3.6)
Fatigue	2 (3.6)
Shortness of breath	1 (1.8)

SD: standard deviation, min: minimum, max: maximum, #: twenty-five patients had multiple symptoms, percentages for symptoms were given over the total number of patients

Table 2. Laboratory parameters of patients

	Mean±SD	Median (IQR) [min-max]
COHb (%)	13.08±10.23	13.6 (19.1) [0.2-38.9]
pH	7.38±0.05	7.40 (0.05) [7.08-7.50]
Lactate (mmol/L)	2.42±1.42	2.0 (1.4) [0.8-11.5]
Troponin-I (pg/mL)	11.36±22.96	4.5 (5.8) [0.9-150]
WBC ($x10^3/mm^3$)	10.01±3.23	9.5 (4.3) [2.2-21.2]
PLT ($x10^3/mm^3$)	314.48±8.24	304 (108) [181-753]
Hemoglobin (g/L)	13.12±1.46	13.1 (1.7) [9.0-17.6]
Urea (mg/dL)	25.95±7.18	25.5 (10.0) [8-45]
Creatinine (mg/dL)	0.60±0.18	0.56 (0.23) [0.33-1.50]
AST (IU/L)	29.58±13.61	28 (15) [4-114]
ALT (IU/L)	17.83±14.12	14 (8) [4-108]

SD: standard deviation, IQR: interquartile range, min: minimum, max: maximum, COHb: carboxyhemoglobin, WBC: white blood cell, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase

The demographic characteristics and laboratory findings of the patients with and without impaired consciousness were given in Table 3. The mean ages of the patients with and without impaired consciousness were 11.73±5.44 and 8.66±5.25 years, respectively, and a statistically significant difference was found between the two groups in terms of age (p=0.027). The COHb and lactate levels of the patients with impaired consciousness were significantly higher than those of the patients without impaired consciousness (p<0.001 and p=0.019, respectively). Although troponin I levels were higher in the patients with impaired consciousness, this difference was not statistically significant (p=0.796). There was also no significant difference between the groups with and without impaired consciousness in terms of their leukocyte counts (p=0.944), while platelet counts were significantly higher in the group without impaired consciousness (p=0.047).

All patients were followed up in the pediatric emergency department with 100% oxygen therapy. Additionally, HBO therapy was applied to 30 (18.5%) patients.

The mean hospital stay duration of the patients was 6.5±4.6 hours. The patients with impaired consciousness were followed up statistically significantly longer in the hospital compared to the group without impaired consciousness (p<0.001). All patients were discharged from the pediatric emergency department with recovery.

DISCUSSION

Carbon monoxide poisoning is one of the most common causes of pediatric emergencies presenting with intoxication, in both Turkey and the world. The causes of CO poisoning vary according to geographical regions, as well as social and cultural factors (14).

CO poisoning often develops due to acute accidents in childhood. It has been stated that CO poisoning is generally caused by fire and exhaust fumes in developed countries. In Turkey, it has been reported that the sources of poisoning are coal stoves and water heaters working with gas cylinders or natural gas (3,15,16). In this study, 55.6% of the patients presented to the hospital with CO poisoning due to smoke inhalation, and 44.4% presented due to cylinder gas and natural gas exposure. In the literature, it has been shown that CO poisoning is frequently seen in the winter months (9,17,18). In the present study, we determined that the patients were brought to the emergency department most frequently in the winter season and at night (00:01-06:00).

Table 3. Characteristics of patients with and without impaired consciousness due to carbon monoxide poisoning

	With IC (n=15)		Without IC (n=147)		
	Mean±SD	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	**p**
Age (years)	11.73±5.44	14 (10) [2-17]	8.66±5.25	9 (9) [1-17]	**0.027**
COHb (%)	22.76±8.91	23.8 (8.9) [3.0-38.9]	12.10±9.86	12.0 (18.5) [0.2-36.3]	**<0.001**
pH	7.34±0.10	7.38 (0.10) [7.08-7.44]	7.39±0.40	7.40 (0.06) [7.27-7.50]	0.130
Lactate (mmol/L)	3.60±2.73	2.5 (2.3) [1.7-11.5]	2.30±1.17	2.0 (1.3) [0.8-6.8]	**0.019**
Troponin-I (pg/mL)	20.85±43.79	3.3 (6.8) [1.8-150]	10.22±19.13	4.6 (5.8) [0.9-141]	0.796
WBC ($x10^3/mm^3$)	9.93±2.98	7.8 (3.6) [5.9-18.0]	10.02±3.27	9.5 (4.4) [2.2-21.2]	0.944
PLT ($x10^3/mm^3$)	275.40±6.37	229 (79) [186-381]	319.13±8.37	307 (110) [181-753]	**0.047**
Length of hospital stay (hours)	12.13±5.40	12 (4) [6-24]	5.93±4.10	6 (2) [2-34]	**<0.001**

IC: impaired consciousness, SD: standard deviation, IQR: interquartile range, min: minimum, max: maximum, COHb: Carboxyhemoglobin, WBC: white blood cell, PLT: platelet

Poisoning develops with the release of CO formed by the incomplete combustion of carbon-containing compounds. Clinical findings in CO poisoning may be non-specific; however, these cases can cause symptoms through damage to many organs that need more oxygen, especially the brain and the heart. The most common symptoms have been reported as nausea, vomiting, and headache (15,19,20).

In the present study, the most common signs and symptoms were nausea, vomiting, and headache. CO poisoning should be considered in the differential diagnosis of patients presenting to the emergency department with unexplained nausea, vomiting, and headache, especially in the winter months.

Severe CO poisoning can cause neurological symptoms such as seizures, syncope, or impaired consciousness, as well as cardiac symptoms such as myocardial ischemia and ventricular arrhythmias, and metabolic findings related to lactic acidosis (21).

In this study, myocardial ischemia and arrhythmias did not develop in any of the patients during follow-up. Impaired consciousness (sleepiness, syncope, and convulsions) was observed in fifteen patients.

Hemogram parameters can also be affected in cases of CO poisoning. Ertekin et al. (22) determined that leukocyte and platelet values increased in patients with CO poisoning in comparison to healthy controls. In another study, platelet and leukocyte counts did not significantly differ between CO poisoning cases of mild and moderate severity (23). In the study conducted by Coşkun et al. (24), patients were divided into 2 groups as those with severe poisoning and those without severe poisoning, and leukocyte values were found to be high in the severe poisoning cases. In two studies, leukocyte values were found to be high in CO poisoning cases that required HBO treatment (25,26). In the study carried out by Bağcı et al. (27), who divided patients presenting with CO poisoning into two groups as mild-moderate and severe, no difference was found between the two groups in terms of platelet counts. In the present study, there were patients with leukocytosis and thrombocytosis. The platelet counts were significantly higher in the group without impaired consciousness. There was no statistically significant difference between the groups in terms of their leukocyte counts. These results may be due to the limited number of patients included in the study.

Previous studies have shown that patients with severe system involvement have higher COHb levels (7,9,28). When COHb levels rise above 20%, the heart and brain are severely affected (29). In a study conducted in Turkey, it was found that a COHb level of >20% measured at the time of admission in pediatric CO poisoning cases can be used to predict target organ damage (30). Similarly, in this study, the mean COHb level was found to be above 20% in the patients with impaired consciousness at presentation, which was significantly higher than in the patients without impaired consciousness.

In CO poisoning, lactate levels increase due to tissue hypoxia and cytotoxicity, and metabolic acidosis is observed (31,32). However, the relationship between a high lactate level and the severity of clinical findings is debated. Many studies have shown that morbidity and mortality rates are higher in patients with lactate levels of >2 mmol/L (10,11).

In the literature, lactate levels have been found to be significantly higher in patients with neurological symptoms related to CO poisoning (33,34). In this study, the lactate levels of the patients with impaired consciousness at the time of their presentation were found to be significantly higher than those without impaired consciousness, but no significant difference was found between the groups in relation to pH levels. In patients presenting with CO poisoning, there is a need to closely monitor serum lactate levels.

In cases of CO poisoning, the risk of developing toxicity is high in tissues that are more sensitive to hypoxia, such as the central nervous system and the cardiovascular system. Therefore, in patients admitted to the hospital with suspected CO poisoning, troponin I levels should be measured as an important variable in demonstrating myocardial injury. Studies have shown that troponin I levels increase in CO poisoning cases (35,36). In the present study, the mean troponin level of the entire patient group (11.36±22.96 pg/mL) was within normal limits, and troponin I elevation was detected in only 12 patients. Troponin I levels were found to be higher in the patients with impaired consciousness, but there was no statistically significant difference compared to the patients without impaired consciousness. We consider that in pediatric CO poisoning cases, cardiac enzyme levels should be monitored closely during both admission and follow-up.

HBO therapy is applied in the presence of cardiac and neurological involvement, end-organ damage, metabolic acidosis, worsening or no regression of symptoms despite four-hour NBO therapy, and a COHb level above 25% (13). In this study, 30 patients were treated with HBO therapy in accordance with these criteria. All patients were followed up in the emergency department and were discharged without any problem.

This study had certain limitations. The small number of patients was one of these limitations. Moreover, the duration of the CO exposure of the patients and the time of arrival at the hospital could not be evaluated. Additionally, the data reflected the experience of a single center, and the study had a retrospective design. There is a need for prospectively designed, larger multicenter case series on CO poisoning.

To sum up, in the present study, the patients with impaired consciousness due to CO poisoning had lower serum pH and higher COHb, lactate, and troponin I levels, and these patients were followed up in the emergency department for a longer period. Despite this, the COHb levels of three of our patients with neurological involvement were below 20%.

CONCLUSION

Although high COHb levels are important for the diagnosis of CO poisoning, low values do not exclude the possibility of this condition. COHb levels alone should not be used as a marker for treatment and follow-up. Lactate, troponin I, and pH levels should also be evaluated in pediatric patients presenting to the hospital with CO poisoning.

Ethics Committee Approval: The study was approved by the Ethics Committee of the University of Health Sciences, Gülhane Training and Research Hospital (17.06.2021, 226).

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Maternal and Fetal Outcomes of Gestational Thrombocytopenia

Gestasyonel Trombositopeninin Maternal ve Fetal Sonuçları

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ABSTRACT

Aim: The study aimed to evaluate maternal and fetal outcomes of gestational thrombocytopenia according to platelet levels.
Material and Methods: The cases who were followed up in our clinic between January 2017 and December 2018, who had no additional diseases, who had term deliveries, and who had gestational thrombocytopenia, were screened retrospectively. The pregnant women included in the study were divided into two groups according to their platelet values, ≤70 x10^3/mm^3 and >70 x10^3/mm^3, and the subgroups were analyzed among themselves. The demographic, clinical, and laboratory data of the patients were also compared between the groups.
Results: Among the patients with gestational thrombocytopenia, it was found that the birth week was significantly earlier in the group with platelet ≤70 x10^3/mm^3 (p=0.002). When perinatal characteristics were compared between the groups, the birth weight of the infants in the group with platelet ≤70 x10^3/mm^3 was found to be significantly lower than in the other group (p=0.033). APGAR 1st-minute score was found to be significantly decreased in the group with platelet ≤70 x10^3/mm^3 when compared to the other group (p=0.039). Single and multiple regression analyzes were performed on pregnant women with gestational thrombocytopenia. No risk factors that were associated with adverse maternal and perinatal outcomes were detected in the group with platelet values ≤70 x10^3/mm^3.
Conclusion: Fetal growth retardation is seen in patients diagnosed with gestational thrombocytopenia and with platelet values below 70 x10^3/mm^3 and their APGAR scores are lower. The premature birth rate is higher in the same patient group.
Keywords: Gestational thrombocytopenia; pregnancy; maternal outcomes; fetal outcomes.

ÖZ

Amaç: Bu çalışmanın amacı gestasyonel trombositopeninin maternal ve fetal sonuçlarının trombosit seviyelerine göre değerlendirilmesidir.
Gereç ve Yöntemler: Ocak 2017 ve Aralık 2018 tarihleri arasında kliniğimizde takip edilen, ek bir hastalığı olmayan, miad doğum yapmış olan ve gestasyonel trombositopeni saptanmış olan olgular geriye dönük olarak tarandı. Çalışmaya dahil edilen gebeler trombosit değerlerine göre ≤70 x10^3/mm^3 ve >70 x10^3/mm^3 şeklinde iki alt gruba ayrıldı ve bu alt gruplar kendi arasında analiz edildi. Ayrıca, hastaların demografik, klinik ve laboratuvar verileri de gruplar arasında karşılaştırıldı.
Bulgular: Gestasyonel trombositopeni tanısı olan hastalardan; trombosit değeri ≤70 x10^3/mm^3 olan grupta doğum haftasının anlamlı olarak daha erken olduğu görüldü (p=0,002). Gruplar arasında perinatal özellikler karşılaştırıldığında ise, trombosit değeri ≤70 x10^3/mm^3 olan gruptaki bebeklerin doğum ağırlıklarının diğer grupta olanlara göre anlamlı şekilde daha düşük olduğu bulundu (p=0,033). APGAR 1. dakika skoru trombosit değeri ≤70 x10^3/mm^3 olan grupta diğer grup ile karşılaştırıldığında anlamlı şekilde azalmış olarak bulundu (p=0,039). Gestasyonel trombositopenisi olan gebelerde tekli ve çoklu regresyon analizi yapıldı. Trombosit değerleri ≤70 x10^3/mm^3 olan grupta olumsuz maternal ve perinatal sonuçlar ile ilişkili olan bir risk faktörü saptanmadı.
Sonuç: Gestasyonel trombositopeni tanısı alan ve trombosit değerleri 70 x10^3/mm^3 altında olan hastalarda fetal gelişme geriliği görülmekte ve APGAR skorları daha düşük olmaktadır. Aynı hasta grubunda erken doğum oranı daha yüksektir.
Anahtar kelimeler: Gestasyonel trombositopeni; gebelik; maternal sonuçlar; fetal sonuçlar.

INTRODUCTION

Thrombocytopenia is among the most common hematological problems in pregnancy and is defined as a platelet count of less than 150 $x10^3/mm^3$ (1). Considering the etiological causes, gestational thrombocytopenia is the most common. Thrombocytopenia can be detected because of many reasons such as immune thrombocytopenia (ITP), pregnancy-specific hypertensive diseases (preeclampsia, eclampsia, hemolysis, elevated liver enzymes, and low platelets, HELLP), connective tissue diseases (systemic lupus erythematosus, SLE, antiphospholipid syndrome), drug interactions, and infections (2). Depending on the severity and cause of thrombocytopenia, the follow-up and treatment protocols of pregnant women might vary (3). Gestational thrombocytopenia accounts for approximately 80% of thrombocytopenia cases during pregnancy (4) and there is no specific test for its diagnosis. It is detected in a routine blood count during pregnancy, diagnosed by excluding other diseases, and the platelet count is typically above 70 $x10^3/mm^3$. The platelet count reaches normal values approximately 12 weeks after delivery (5-7). Maternal and fetal prognosis is generally good in cases with thrombocytopenia (8).

Thrombocytopenia is classified as mild, moderate, and severe thrombocytopenia according to platelet values (9). Approximately 8% of pregnant women have mild thrombocytopenia. Moderate and severe thrombocytopenia might accompany other diseases (9,10). Considering the neonatal outcomes of pregnant women with moderate and severe thrombocytopenia, it was detected that the risk of low 5th-minute APGAR score, intrauterine growth restriction (IUGR), and stillbirth is high (11).

In previous studies, thrombocytopenia cases that were detected generally during pregnancy and maternal and fetal complications that might be related to additional diseases in its etiology were evaluated together (9). Studies conducted on whether gestational thrombocytopenia causes an increased risk of maternal and fetal complications according to platelet levels in pregnant women are limited. For this reason, in the present study, the patients diagnosed with gestational thrombocytopenia were divided into subgroups according to their platelet levels. The purpose of the present study was to evaluate maternal and fetal outcomes and investigate their relationship with the degree of thrombocytopenia.

MATERIAL AND METHODS

The present study was a hospital-based case-control study that included pregnant women, who applied to the Health Sciences University Konya Training and Research Hospital, Gynecology and Obstetrics Clinic, and who were followed up with the diagnosis of gestational thrombocytopenia and was conducted between January 2017 and December 2018. The study was approved with the decision of the Konya Training and Research Hospital ethics committee with the numbers 01-13 on 03.01.2019 and 13826 on 29.04.2019.

The information of the pregnant women who applied to our clinic between January 2017 and December 2018 and were followed up because of thrombocytopenia was obtained from the hospital information management system and patient files. The demographic data of the patients, routine blood test results, prenatal-postnatal follow-ups, and fetal results were examined and recorded.

Term (>37) and late-preterm (34-36 6/7) pregnant women who were aged 20-40 years were included in the study as those with thrombocyte count of <150 $x10^3/mm^3$ as a result of complete blood counts performed 3 times during pregnancy. Platelet counts were divided into two groups as below or above 70 $x10^3/mm^3$, and it was planned to analyze whether there were significant differences between the subgroups in terms of maternal and fetal outcomes.

The patients who were not followed up because of thrombocytopenia and patients who did not give birth in our hospital were not included in the study. Those who were under the age of 20 and over the age of 40 were not included in the study. The diagnosis of thrombocytopenia before pregnancy, bleeding profile disorder, ITP, use of acetic salicylic acid and/or low molecular weight heparin or unfractionated heparin, hepatic or renal dysfunction, venous thrombosis, hepatitis, sepsis, viral and/or bacterial infection, gestational diabetes, autoimmune disease, drug use that would affect platelet functions or structure, hereditary factor deficiency such as von Willebrand factor (vWF), patients with bone marrow disease such as leukemia, lymphoma, patients receiving intravenous immune globulin (IVIG) and/or steroids, pregnancy-related preeclampsia, pregnancy cholestasis, HELLP, disseminated intravascular coagulation (DIC), those with diseases affecting liver and kidney functions were not included in the study.

Spontaneous single pregnancies without fetal ultrasonographic and/or cytogenetic fetal anomalies were included in the study and patients who were <34 early preterm and those who had multiple pregnancies with fetal anomalies, intracranial hemorrhage detected on ultrasound and/or fetal MR, and intrauterine fetal interventions were not included in the study.

Statistical Analysis

The IBM SPSS Statistics for Windows, Version 22.0 and PASTE programs were used for data analysis. The Kolmogorov-Smirnov test and Shapiro-Wilk test were used to determine the conformity of the data to normal distribution. A parametric test (independent sample t-test) was used for the analysis of the normally distributed variables, and a non-parametric test (Mann-Whitney U) was used for those that were not. The descriptive statistics for normally distributed variables were expressed as mean±standard deviation, and median, interquartile range, and minimum-maximum were used for non-normally distributed variables. The categorical data were analyzed with Pearson's chi-square test and represented by numbers and percentages. Univariate and multiple logistic regression analysis was used to identify the risk factors that were associated with adverse maternal outcomes and adverse perinatal outcomes in pregnant women. The data were analyzed at a 95% confidence interval and a p-value of <0.050 was accepted as statistically significant.

RESULTS

A total of 200 patients with gestational thrombocytopenia were included in the study. The demographic and clinical characteristics of the cases included in the study and

laboratory data evaluated in routine follow-ups were examined. It was found that the pregnant women included in the study did not have any additional problems other than thrombocytopenia (Table 1).

Since it was reported that especially platelet levels below 70 $x10^3/mm^3$ are rarely seen in the definitions for gestational thrombocytopenia, the pregnant women were divided into two subgroups according to their platelet counts as below 70 $x10^3/mm^3$ and above, and the groups were compared among themselves. In the comparison made according to demographic characteristics, the week of delivery was statistically significantly earlier in the group with platelet value ≤70 $x10^3/mm^3$ than in the other group (p=0.002). No significant differences were detected in terms of other parameters (Table 2).

When both subgroups were evaluated in terms of laboratory parameters, urea (p<0.001), creatinine (p=0.008), prothrombin time (p=0.011), and activated partial thromboplastin time (p=0.008) were statistically significantly higher in the platelet ≤70 $x10^3/mm^3$ group, and the hemoglobin level was found to be significantly lower (p<0.001) compared to the other group (Table 3).

When ultrasonographic and perinatal characteristics were compared between the groups, the birth weights of the infants in the group with platelet ≤70 $x10^3/mm^3$ were significantly lower than in the other group (p=0.033). The 1st-minute APGAR score was found to be significantly lower in the group with platelet ≤70 $x10^3/mm^3$ when compared to the other group (p=0.039). Neonatal intensive care hospitalization rates of the infants were not found to be different between the groups (Table 4).

After the subgroup analysis, it was planned to analyze whether there was an increased risk of adverse maternal, and fetal outcomes, especially in pregnant women who had platelet count ≤70 $x10^3/mm^3$, and possible risk factors were evaluated. According to pregnancy results, the presence of any of the postpartum complications, and/or admission to the intensive care unit, and/or any two of the patients who received massive blood transfusion were determined as adverse maternal outcomes. Admission to the neonatal intensive care unit, and/or patients with an APGAR score of <7 and/or any two of those with postnatal complications (postpartum death, intracranial bleeding, fetal growth restriction, FGR, prematurity) were considered adverse perinatal outcomes (11,12).

As a result of the analysis, no relationship was detected between the parameters examined here and negative maternal outcomes and negative perinatal outcomes in pregnant women with gestational thrombocytopenia and platelet counts ≤70 $x10^3/mm^3$ (Table 5).

Table 1. The demographic and clinical characteristics of pregnant women with gestational thrombocytopenia

Age (years)	29.41±5.93
Gravida	3 (2) [1-10]
Living	2 (1) [0-9]
BMI (kg/m^2)	30.16±4.74
Weight gained during pregnancy (kg)	12.61±4.57
Gestational week (week)	38.03±2.44
Platelet ($x10^3/mm^3$)	101.68±24.81
Hemoglobin (g/dl)	10.77±1.36
Urea (mg/dl)	17.57±9.54
Creatinine (mg/dl)	0.63±0.10
AST (U/L)	20.65±7.53
ALT (U/L)	11.01±4.64
INR	0.92±0.58
PT (s)	8.28±4.96
aPTT (s)	24.21±2.77

BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, INR: international normalized ratio, PT: prothrombin time, aPTT: activated partial thromboplastin time, mean±standard deviation or median (interquartile range) [min-max]

Table 3. The comparison of intragroup laboratory findings

	Platelet ≤70 ($x10^3/mm^3$)	**Platelet >70 ($x10^3/mm^3$)**	**p**
Hemoglobin (g/dl)	9.91±1.29	10.90±1.32	**<0.001**
Urea (mg/dl)	24.96±2.46	16.46±5.36	**<0.001**
Creatinine (mg/dl)	0.66±0.17	0.62±0.08	**0.008**
AST (U/L)	19.50±4.27	26.91±5.65	0.058
ALT (U/L)	16.73±2.40	19.63±6.83	0.066
INR	0.87±0.07	0.92±0.62	0.665
PT (s)	10.57±3.66	7.94±0.75	**0.011**
aPTT (s)	25.54±3.63	24.01±2.57	**0.008**

AST: aspartate aminotransferase, ALT: alanine aminotransferase, INR: international normalized ratio, PT: prothrombin time, aPTT: activated partial thromboplastin time, the results were reported with mean±standard deviation

Table 4. The comparison of perinatal characteristics

	Platelet ≤70 ($x10^3/mm^3$)	**Platelet >70 ($x10^3/mm^3$)**	**p**
Birth weight (g)	2820.58±810.06	3102.43±594.14	**0.033**
APGAR, n (%)			
<7	4 (15.4)	7 (4)	**0.039**
≥7	22 (84.6)	167 (96)	
Newborn ICU, n (%)			
Yes	5 (19.2)	31(17.8)	0.521
No	21 (80.8)	143 (82.2)	

ICU: intensive care unit

Table 2. Comparison of intragroup demographic and clinical characteristics

	Platelet ≤70 ($x10^3/mm^3$)	**Platelet >70 ($x10^3/mm^3$)**	**p**
Age (years), mean±SD	28.85±5.95	29.49±5.93	0.604
Gravida, median (IQR) [min-max]	3 (2) [1-10]	3 (2) [1-8]	0.854
Living, median (IQR) [min-max]	1 (1) [0-9]	1 (1) [0-5]	0.771
BMI (kg/m^2), mean±SD	28.85±3.59	30.35±4.87	0.133
Weight gained during pregnancy (kg), mean±SD	12.12±3.27	12.68±4.74	0.556
Gestational week (week), mean±SD	36.65±2.96	38.24±2.29	**0.002**

BMI: body mass index, SD: standard deviation, IQR: interquartile range

Table 5. Risk factors associated with adverse maternal and perinatal outcomes in pregnant women with platelet ≤70 (x10^3/mm^3)

	Adverse Maternal Outcomes		Adverse Perinatal Outcomes	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Age	1.077 (0.937-1.239)	0.296	1.020 (0.865-1.204)	0.814
Gravida	1.185 (0.736-1.907)	0.485	1.126 (0.678-1.870)	0.647
BMI	1.175 (0.920-1.502)	0.197	1.013 (0.769-1.333)	0.928
Weight gained during pregnancy	1.075 (0.841-1.375)	0.562	0.866 (0.623-1.205)	0.395
Gestational week	0.714 (0.499-1.021)	0.065	0.256 (0.065-1.019)	0.053
Birth weight	0.999 (0.998-1.000)	0.060	0.993 (0.986-1.000)	0.063
Hemoglobin	0.499 (0.242-1.028)	0.059	0.741 (0.341-1.613)	0.450
Urea	0.990 (0.949-1.033)	0.639	1.016 (0.978-1.056)	0.413
Creatinine	2.877 (0.120-6.874)	0.229	13.145 (0.591-29.263)	0.068
AST	1.003 (0.997-1.010)	0.269	1.008 (0.999-1.017)	0.081
ALT	1.007 (0.997-1.016)	0.178	1.014 (1.001-1.026)	0.040
INR	0.037 (0.001-2.684)	0.563	0.010 (0.001-12.439)	0.263
PT	1.160 (0.353-3.814)	0.804	0.118 (0.006-2.265)	0.156
aPTT	0.886 (0.701-1.121)	0.315	1.372 (0.985-1.912)	0.061

OR: odds ratio, CI: confidence interval, BMI: body mass index, Hgb: hemoglobin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, INR: international normalized ratio, PT: prothrombin time, aPTT: activated partial thromboplastin time

DISCUSSION

There might be a physiological tendency to thrombocytopenia in the usual course of pregnancy. Increased destruction, decreased platelet production, or dilutional causes during pregnancy explain the decreased physiological limits (2). Gestational thrombocytopenia is the most common cause of thrombocytopenia during pregnancy (3). It usually occurs in the last trimester and the platelet count is typically above 70 x10^3/mm^3, maternal and fetal outcomes are milder. It is diagnosed by excluding other diseases causing low platelet values, and there is usually no need for additional treatment (5,6,8). When the etiology of thrombocytopenia in pregnancy is evaluated, gestational hypertensive diseases and ITP, another common group, have a higher risk of fetal and maternal complications (9). For this reason, when pregnancy thrombocytopenia cases are evaluated holistically, the rate of negative results also increases (5). Since the risk of additional disease increases, especially in patients with moderate and severe thrombocytopenia in cases diagnosed with thrombocytopenia during pregnancy, other diagnoses should be excluded (5). In the present study, it was planned to examine the pregnancy outcomes of patients diagnosed with gestational thrombocytopenia by excluding these groups, according to their platelet levels. It was also planned to investigate whether there is a significant difference in terms of maternal and fetal outcomes in gestational thrombocytopenia cases with moderate and severe thrombocytopenia.

In the study conducted by Artunç Ülkümen et al. (13), the rate of low birth weight (<2500 g) and very low birth weight infants (<1500 g) in thrombocytopenic pregnant women were found to be significantly more frequent than in the control group. When newborn results were compared, a decrease was detected in the 5th-minute APGAR score in the group with thrombocytopenia, and the stillbirth and intrauterine growth retardation rates were found to be significantly higher (9). In the present study, birth weight and APGAR scores were found to be lower in the group with severe thrombocytopenia. Unlike the results of the present study, maternal and fetal adverse outcome rates were found to be higher in previous studies because gestational thrombocytopenia was not evaluated alone.

In the study of Parnas et al. (9), when the newborns with thrombocytopenia were compared with the healthy group, the 5th-minute APGAR scores were found to be lower in the thrombocytopenia group, and the rates of stillbirth and intrauterine growth retardation were found to be significantly higher. Similarly, in the present study, birth weights and weeks were found to be lower in the group that had lower platelet levels. When evaluated in terms of maternal and fetal negative outcomes, the rate of negative outcomes was found to be higher in pregnant women diagnosed with HELLP syndrome and preeclampsia than in other causes of thrombocytopenia. Since hypertensive diseases of pregnancy among the causes of thrombocytopenia are a group with maternal and fetal risks, it is known that premature birth and stillbirth rates increase relatively (6,7). Since conditions complicating pregnancy such as gestational hypertensive diseases were excluded in the present study, no adverse maternal and fetal outcomes were observed.

In the study that was conducted by Wang et al. (14), when evaluating thrombocytopenia in pregnancy, the patients were grouped according to etiology and the groups were compared. Postpartum hemorrhage rate and newborn 5th-minute APGAR scores were found to be similar in the three groups. No adverse maternal and fetal outcomes were detected in the group diagnosed with gestational thrombocytopenia.

In the study conducted by Elveđi-Gašparović et al. (15), gestational thrombocytopenia was analyzed retrospectively against the control group and it was found that thrombocytopenia did not have a significant effect on the mode of delivery, and preterm delivery. The need for neonatal intensive care, low 1st-minute APGAR score, and fetal growth retardation were found to be significantly higher in the thrombocytopenic group than in the control

group. The present study had similar results to the literature in this regard. Fetal weight, week of birth, and APGAR scores were significantly lower during delivery in groups with severe thrombocytopenia. After evaluating the subgroups, univariate and multivariate regression analyzes were made in terms of the parameters considered in terms of negative maternal and fetal outcomes, and as a result, no negative maternal and fetal outcomes were detected.

It was detected that 3% of the cases included in the study were followed up and treated for reasons such as the threat of premature birth or vaginal bleeding during pregnancy. FGR was observed in 4.5% and amniotic fluid anomalies in 4%. After the delivery, 5% of patients required additional intervention because of bleeding. These results obtained with the available data were relatively high in terms of isolated gestational thrombocytopenia results in the literature. Since the patients in the present study were examined retrospectively based on the files and systems, it was not known whether the additional disease was diagnosed in the long-term postpartum results. For this reason, we think that the presence of possible diseases that might cause these outcomes should be evaluated with other studies that include long-term results.

The results of the present study were compatible with general literature data. The study was conducted in a single center with a sufficient number of patients for power analysis. On the other hand, since it was a retrospective study based on the patient files and system, we do not have data on maternal and fetal outcomes other than routine follow-ups. We think that clearer results will be obtained by examining and evaluating long-term maternal and fetal outcomes, especially in the postpartum period.

CONCLUSION

Gestational thrombocytopenia has significant effects on fetal birth weight, APGAR scores, and gestational week, especially in low platelet levels; however, it was found in the present study that it did not cause adverse maternal and fetal outcomes. We think that prospective studies to be conducted with large case series and long-term postpartum follow-ups are needed to elucidate the maternal and fetal effects of gestational thrombocytopenia.

Ethics Committee Approval: The study was approved by the Ethics Committee of Konya Training and Research Hospital (03.01.2019, 01-13, and 29.04.2019, 13826).

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Effect of Hysteroscopic Surgery Before Frozen Embryo Transfer on Patients with Previous Implantation Failure

İmplantasyon Başarısızlığı Olan Hastalarda Donmuş Embriyo Transferi Öncesi Histeroskopik Cerrahinin Etkisi

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ABSTRACT

Aim: The aim of this study was to evaluate the benefit of hysteroscopy (HS) before single frozen-thawed embryo transfer (sFET) on patients with previous implantation failure.
Material and Methods: A total of 1352 infertile women with a previous implantation failure who underwent their first sFET treatment between January 2015 and December 2017 were included in this study. The patients were classified into two main groups in which HS was omitted (Group 1), and who underwent HS (Group 2). Furthermore, Group 2 was classified into two subgroups as patients without any intrauterine pathology (Group 2a), and those with intrauterine pathology (Group 2b). sFET was performed on all patients within 50 days of hysteroscopy. The major outcome measure was the clinical pregnancy rate.
Results: The mean number of mature oocytes and fertilization rates were similar between groups. The clinical pregnancy rate was found to be 33.3% (n=70) in Group 1. Comparatively this rate was statistically significantly higher in patients in Group 2. The clinical pregnancy rate was 44.2% (n=378) in Group 2a, and 44.4% (n=127) in Group 2b (p=0.014). There was a significant difference between Group 1 and Group 2a (OR: 1.58, 95% CI: 1.15-2.17, p=0.004), and also Group 2b (OR: 1.59, 95% CI: 1.10-2.31, p=0.013). However, no significant difference was observed between Group 2a and Group 2b (p=0.896).
Conclusion: Our findings demonstrate that HS surgery increases the probability of pregnancy rate at least by 1.58 times in patients having previous implantation failure when the hysteroscopic procedure is followed by sFET.
Keywords: Hysteroscopy; infertility; sFET; blast; implantation failure.

ÖZ

Amaç: Bu çalışmanın amacı, daha önce implantasyon başarısızlığı olan hastalarda tek donmuş çözülmüş embriyo transferi (single frozen-thawed embryo transfer, sFET) öncesi histeroskopinin faydasını değerlendirmektir.
Gereç ve Yöntemler: Bu çalışmaya, Ocak 2015 ile Aralık 2017 tarihleri arasında daha önce bir implantasyon başarısızlığı olan ve ilk kez sFET tedavisi yapılan toplam 1352 infertil kadın dahil edildi. Hastalar HS yapılmayanlar (Grup 1) ve HS uygulananlar (Grup 2) olmak üzere iki ana gruba ayrıldı. Ayrıca, Grup 2, intrauterin patolojisi olmayan hastalar (Grup 2a) ve intrauterin patolojisi olanlar (Grup 2b) olmak üzere iki alt gruba ayrıldı. Tüm hastalara histeroskopi prosedüründen sonraki 50 gün içinde sFET uygulandı. Ana sonuç ölçütü klinik gebelik oranları idi.
Bulgular: Ortalama olgun oosit sayısı ve fertilizasyon oranları gruplar arasında benzerdi. Grup 1'de klinik gebelik oranı %33,3 (n=70) olarak bulundu. Karşılaştırıldığında bu oran Grup 2'deki hastalarda istatistiksel olarak anlamlı şekilde daha yüksekti. Klinik gebelik oranı, Grup 2a'da %44,2 (n=378) ve Grup 2b'de ise %44,4 (n=127) idi (p=0,014). Grup 1 ile Grup 2a arasında (OR: 1,58; %95 GA: 1,15-2,17; p=0,004) ve aynı zamanda Grup 1 ile Grup 2b arasında (OR: 1,59; %95 GA: 1,10-2,31; p=0,013) istatistiksel olarak anlamlı fark vardı. Ancak Grup 2a ve Grup 2b arasında anlamlı bir fark gözlenmedi (p=0,896).
Sonuç: Bulgularımız, histeroskopik prosedürü sFET ile takip edildiğinde, daha önce implantasyon başarısızlığı olan hastalarda HS cerrahisinin gebelik olasılığını en az 1,58 kat artırdığını göstermektedir.
Anahtar kelimeler: Histeroskopi; infertilite; sFET; blast; implantasyon başarısızlığı.

INTRODUCTION

Hysteroscopy (HS) is a surgical procedure for diagnosing and simultaneously treating intrauterine pathologies. In order to increase pregnancy rates, HS can be utilized either before the embryo transfer or after a failed embryo transfer attempt. Recent advances in reproductive medicine have propagated higher pregnancy rates, however, many women still suffer from recurrent implantation failure (1). For these individuals, treatment approaches vary according to the patient and the individual clinical protocols. Many factors may contribute to implantation failure such as endometrial receptivity disorders, low embryo quality, intrauterine pathologies, anatomical uterine abnormalities, thrombophilias, endometritis, male and female reproductive cell problems, and immunological and chromosomal anomalies (2,3). When we evaluate these causes, it is important to choose the most appropriate action; in order to lower the economic costs and increase the success of future treatments. Currently, 2D transvaginal ultrasonography (2D-TVUSG) and hysterosalpingography (HSG) are recommended as first-line management steps in the detection of intrauterine pathologies (4). However, HS is the gold standard for the assessment of the uterine cavity. It provides an opportunity for concomitant treatment of intrauterine pathologies (5). In patients with recurrent in vitro fertilization (IVF) failure, intrauterine pathology has been identified in 25-50% of cases (6). Furthermore; published studies have reported that office HS including endometrial scratching (ES) increases pregnancy success while other randomized controlled studies have not found a beneficial effect of local endometrial injury before IVF (7-9).

There is a Cochrane review published in 2019 (10). One of the key results of this Cochrane review is that in women undergoing IVF, performing an HS first, improved the chances of live birth or clinical pregnancy (10). We conducted this study, prior to the publication of this review and we wanted to evaluate the effectiveness of HS before the second embryo transfer in order to increase pregnancy rates. The aim of this study was to evaluate the effectiveness of HS in increasing pregnancy rates before single frozen-thawed embryo transfer (sFET) in patients with previous implantation failure.

MATERIAL AND METHODS

Study Groups

This is a retrospective study conducted between January 2015 and December 2017 at Private Prof. Dr. Mehmet Turan Çetin IVF Center. During this period, 2012 patients underwent their first sFET treatment. Among these, 1430 who had first implantation failure were evaluated. Seventy-eight patients who were lost to follow-up were excluded. A total of 1352 infertile women were included in the study. The patients were classified into two groups: Group 1 consisted of 210 patients who did not undergo HS and group 2 consisted of 1142 patients who underwent HS. Group 2 was further classified into 2 subgroups: Group 2a consisted of 856 patients without any diagnosed intrauterine pathology. In Group 2a bilateral vertical incision was performed on the lateral walls of the uterus during HS until tubal ostiums were observed. Group 2b consisted of 286 patients with intrauterine pathology diagnosed and/or treated during HS (Figure 1).

Most patients had normal findings on 2D-TVUSG and HSG with the exception of 48 patients who had an arcuate uterus and 32 patients with uterine subseptus. After giving detailed information about the potential benefits of HS, all patients with subseptus and arcuate uterus and no suspicion of uterine pathology decided to undergo HS. The rest proceeded directly to the second transfer treatment. HS was performed within 50 days before the beginning of the second sFET treatment.

Endometrial polyps, filmy adhesions, chronic endometritis, and arcuate and subseptate uterus were considered as intrauterine pathologies. Operative intervention was performed when pathology was detected. Chronic endometritis was suspected by the appearance of the endometrial lining, the presence of stromal edema, focal or diffuse periglandular hyperemia, and micropolyps of <1 mm in size as described previously (11). Endometritis diagnosis was confirmed by histological examination of the punch biopsies taken during the procedure, using hysteroscopic instruments. When chronic endometritis was diagnosed, patients were treated with estrogen and progesterone for 21 days together with 14 days of doxycycline 100 mg twice daily before the initiation of the second sFET cycle.

Hysteroscopy Procedure

Outpatient HS was done in the early follicular phase of the cycle (cycle days 6-11) under intravenous sedation with midazolam. The operating device was a 5 mm office hysteroscope with a 30-degree angle and 1.5 mm operative channel (Bettocchi Office Hysteroscope, Karl Storz GmbH & Co Tuttlingen, Germany). Uterine distention was performed with 0.9% normal saline using an electronic

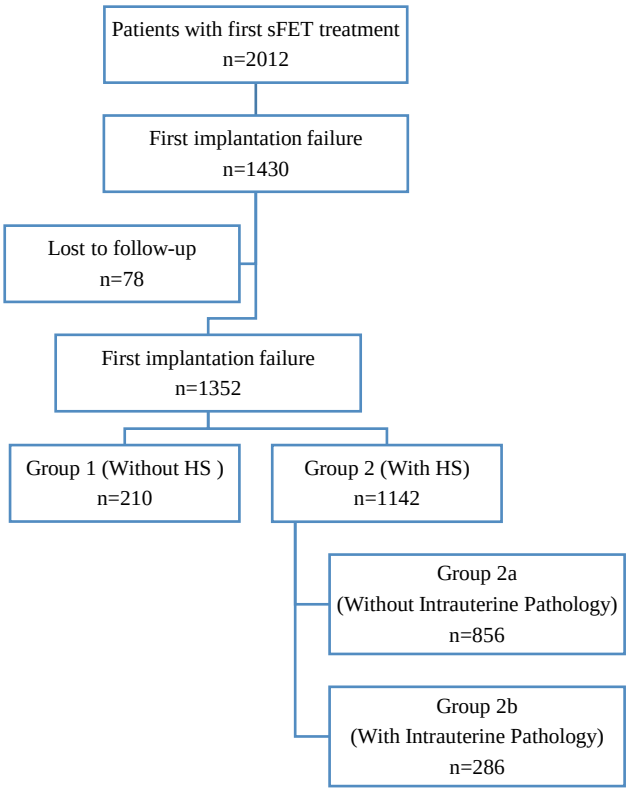

Figure 1. Flow chart of the study

pump (Hysteromat, Karl Storz GmbH & Co Tuttlingen, Germany). Uterine cavity pressure during the operation was between 80-100 mmHg. Standard gynecologic surgical procedures were used to treat the recognized pathologies. Scissors were used for filmy adhesiolysis. Grasping forceps and Twizzle tip of Versapoint bipolar electrosurgical system (Versapoint Electro-Surgical System; Gynecare Inc, Menlo Park, CA, USA) were used for the removal of polyps and for the incision of the arcuate uterus and uterine subseptum. A highly experienced single physician (MTÇ) performed all the procedures and each procedure was digitally recorded. If no pathology was detected during the procedure, a vertical incision was made on the lateral uterine walls until the internal os by Twizzle tip of Versapoint.

ICSI-sFET Protocol

An antagonist protocol with follitropin alfa (GONAL-f, Merck, Germany) and cetrorelix (Cetrotide, Merck, Germany) were used in all of the patients for ovulation stimulation. Vitrification was used for the cryopreservation of all embryos. Endometrium was prepared with estradiol (Estrofem, Novo Nordisk) 2 mg orally, 3 times a day starting from day 3 of the menstrual cycle. Patients who had an endometrial thickness of more than 8 mm started to receive vaginal progesterone (Crinone vaginal gel, Merck Serono) twice a day (days 17-20) for 6 days. All cycles were frozen-thawed cycles. Day 5 single frozen-thawed grade 1 quality embryo transfers were performed on days 17-20 of menstruation. After the transfer, estrogen and progesterone administration was continued for 12 days until the beta-hCG test. If the beta-hCG test was positive, treatments were continued until the 10^{th} week of pregnancy. Clinical pregnancy was defined as the visualization of an intrauterine fetus plus fetal heart activity with TVUSG at 7 weeks of pregnancy.

Ethical committee approval was obtained from the Ethics Committee of Çukurova University (Report no: 76/26 date: 13.04.2018) and all subjects provided informed consent for the utilization of their clinical data.

Statistical Analysis

The descriptive statistics of the qualitative variables in the study were given as numbers and percentages, and the descriptive statistics of the quantitative variables were given as mean and standard deviation. Relationships between qualitative variables were examined by Pearson chi-square analysis. In post hoc evaluations, Bonferroni corrections were made and group differences were determined in detail. The conformity of the quantitative variables to the normal distribution was examined with the Kolmogorov-Smirnov test. An independent sample t-test was used to compare the mean of quantitative variables. A one-way analysis of variance was used to compare the mean of more than two groups. Tukey test was used as a post hoc test in pairwise comparisons of the groups with differences. The level of statistical significance was defined as $p<0.05$. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0.

RESULTS

There were 1352 patients with one failed implantation. Among these, 210 (15.5%) of the participants did not undergo HS (Group 1), and the other 1142 (84.5%) underwent HS (Group 2a+Group 2b). The mean age of the patients in groups 1, 2a, and 2b were 33.02±4.40, 32.51±4.90, and 33.01±4.12 years, retrospectively. The duration of infertility and causes of infertility were demonstrated in Table 1. There were no statistically significant differences between the groups with regard to demographics, infertility etiology, or duration of infertility. Following HS surgery, 25% (n=286) of the patients in Group 2 were discovered to have an intrauterine pathology (Group 2b), which was surgically or medically treated. The most frequently diagnosed intrauterine pathology was a single endometrial polyp at 8.1% (n=93). The median polyp size was 7 mm (range, 5-10 mm). Chronic endometritis was diagnosed in 5.3% (n=61) of patients. There were 5.8% (n=66) patients with an arcuate uterus and 2.8% (n=32) uterine subseptum (Table 2). No complications occurred during any procedure.

The mean number of mature oocytes, fertilization rates, and sFET quality were similar between groups (Table 3). There was a significant difference in clinical pregnancy rate between the three groups (p=0.014). The clinical pregnancy rate was found to be 33.3% (n=70) in patients without HS (Group 1), 44.2% (n=378) in patients without intrauterine pathology (Group 2a), and 44.4% (n=127) in patients with intrauterine pathology (Group 2b). When subgroup analysis was performed, a statistically significant difference was found between Group 1 and Group 2a (OR: 1.58, 95% CI: 1.15-2.17, p=0.004), and also Group 2b (OR: 1.59, 95% CI: 1.10-2.31, p=0.013). However, no significant difference was observed between Group 2a and Group 2b (p=0.896).

Table 2. Findings of hysteroscopy (n=1142)

Without Intrauterine Pathology, n (%)	**856 (75)**
With Intrauterine Pathology, n (%)	**286 (25)**
Endometrial polyp	93 (8.1)
Uterus arcuatus	66 (5.8)
Endometritis	61 (5.3)
Filmy Adhesions	34 (3.0)
Uterine subseptum	32 (2.8)

Table 1. Clinical characteristics and infertility causes of patients

	Group 1 (n=210)	Group 2a (n=856)	Group 2b (n=286)	p
Age (years), mean±SD	33.02±4.40	32.51±4.90	33.01±4.12	0.160
Duration of infertility (years), mean±SD	5.15±0.92	5.30±0.97	5.20±0.95	0.070
Causes of infertility, n (%)				
Male	63 (30.0)	274 (32.0)	89 (31.1)	
Tubal-Ovulatory	59 (28.1)	248 (29.0)	77 (26.9)	0.874
Unexplained	88 (41.9)	334 (39.0)	120 (42.0)	

Group 1: without hysteroscopy, Group 2a: hysteroscopy without intrauterine pathology, Group 2b: hysteroscopy with intrauterine pathology, SD: standard deviation

Table 3. Results of intracytoplasmic sperm injection-embryo transfer cycle and clinical pregnancy rates

	Group 1 (n=210)	Group 2a (n=856)	Group 2b (n=286)	p
Number of mature oocytes, mean±SD	7.9±1.3	8.0±2.0	7.5±1.5	0.278
Fertilization rate, n (%)	149 (70.9)	645 (75.4)	210 (73.4)	0.653
Clinical pregnancy rate, n (%)*	70 (33.3)	378 (44.2)	127 (44.4)	**0.014**

Group 1: without hysteroscopy, Group 2a: hysteroscopy without intrauterine pathology, Group 2b: hysteroscopy with intrauterine pathology, SD: standard deviation

DISCUSSION

The major finding in this study was that HS increased the probability of pregnancy rate at least by 1.58 times in patients with or without intrauterine pathology. In the literature, when HS was performed in asymptomatic patients in whom no intrauterine pathology was detected with 2D-TVUSG and/ or HSG, intrauterine pathologies were identified in 11-22.9% of the cases (12,13). Even higher rates (51.2%) were reported in patients with previous IVF failure (14). The most important causes of implantation failure are poor embryo quality, problems with embryo transfer techniques, and chromosomal and intrauterine pathologies. The first step during infertility evaluation for intrauterine pathology is 2D-TVUSG in many centers. However, the gold standard for the detection of intrauterine pathology is HS. HSG has a lower positive predictive value (PPV) and specificity than HS for intrauterine pathology (5). In a study with routine HS before IVF 26% of patients were shown to have common intrauterine pathologies including endometrial polyp, arcuate and subseptus uterus (15,16). In this study, intrauterine pathology was detected in 286 (25%) patients. Therefore we can strongly argue that HS is of upmost importance prior to IVF trials. In this study, a vertical incision was performed to the sidewalls of the uterus. A vertical incision was preferred because we wanted to avoid intrauterine adhesions and we did not want to decrease the chances of embryo implantation.

The most common hysteroscopic pathology in this study group was endometrial polyps in 8.1% (n=93) of the cases. We demonstrated improved clinical pregnancy rates after the polyp resection, which is consistent with prior literature (17). In addition, the pregnancy rate was increased by 10% with the HS procedure in patients with or without intrauterine pathology. Hysteroscopic injury may also increase the pregnancy rate, even in cases with a normal uterine cavity (18). Likewise, we performed vertical incisions on 856 patients who had no intrauterine pathology (Group 2a). When subgroup analysis was performed, there was a statistically significant difference between Group 1 and Group 2a. Several explanations have been suggested to explore the benefit of HS in patients without any intrauterine pathology (19). First, washing of the cavity with saline may provide mechanical cleaning and removal of harmful anti-adhesive glycoprotein molecules on the endometrial surface (20). Second, office HS can aid the clinician as "try-on transfer" and improve the results of easy embryo transfer. In addition to all the aforementioned mechanisms, endometrial injury (EI) may induce a change in the endometrial ultrastructure by the following mechanisms: i) facilitating regulation of the inflammatory reactions of macrophages, ii) decidualization and preparation of the endometrium to receive the transferred embryo (21,22). In a meta-analysis, it was reported that EI intervention is 70% more likely to achieve clinical pregnancy compared to no intervention (23). Nastri et al. (24) reported that if 30% of women become pregnant without EI, 33-48% will conceive if the hysteroscopic intervention takes place. On the other hand, anti-biotherapy was used in all HS groups in the present study. This may also have added accessive benefits by healing undiagnosed endometritis of uterobiata. Cicinelli et al. (25) demonstrated that women who received adequate antibiotic treatment for endometritis had a significantly higher rate of successful pregnancy compared with women who were not treated with persistent disease. In a randomized controlled study involving 171 patients, Ben Abid et al. (26) compared the groups who underwent HS before the first IVF with those who did not. The authors did not detect a statistically significant difference between the groups in pregnancy rate and live birth rate (LBR) at 12-month follow-up. In a retrospective cohort study by Eserol et al. (27) in which 765 patients were included, it was shown that performing HS before IVF had no effect on pregnancy rates in both groups who underwent fresh cycle and FET cycle.

There is still controversy about the efficacy and the optimal timing of HS in sFET cycles. In this study, a consecutive approach was preferred. Although a systematic review and meta-analysis showed that HS prior to the first IVF cycle may improve treatment outcomes, some prospective randomized studies concluded that it does not improve the results (12,18,28). The TROPHY study, a multicenter, randomized controlled trial reported that, especially for women with recurrent, unsuccessful implantation following IVF, HS had no effect on the LBR (16). However, this contrasts with the results of a prior systematic review suggesting that routine HS improved the LBR for women with recurrent unsuccessful IVF cycles (29). The problem with large multicenter trials such as the TROPHY or SCRATCH trial is that the techniques are not consistent and the laboratory performance of embryologists differ. Consequently, multicenter trial results should be interpreted with caution. Marking our point in a single-center randomized controlled trial Gurgan et al. (18) showed that endometrial injury is beneficial in recurrent implantation failure. In conclusion, consistent with prior other studies, we demonstrated that HS with a 5 mm office hysteroscope can be done with sedation in the office environment for diagnostic and operative procedures utilizing electrosurgery in experienced hands with no complications.

Strengths and Limitations

The major limitation of this study was the retrospective design. The strengths of this study were embryo transfer was with a frozen-thawed single blast in all cases, and

there was a large sample size. Additionally, all the hysteroscopies were performed by a single, experienced surgeon.

CONCLUSION

HS after the first failed embryo transfer may improve the pregnancy rates regardless of intrauterine pathology. In conclusion, our findings clearly show that HS surgery increases the probability of pregnancy rate at least by 1.58 times compared to the cases when hysteroscopic surgery was omitted.

Ethics Committee Approval: The study was approved by the Non-invasive Clinical Research Ethics Committee of Çukurova University (13.04.2018, 76/26).

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Fixed-Bearing versus Mobile-Bearing Unicondylar Knee Arthroplasty: Comparison of Patients with Similar Component and Mechanical Axis Alignment

Sabit-İnsörtlü ve Mobil-İnsörtlü Unikondiler Diz Artroplastisi: Benzer Komponent ve Mekanik Eksen Dizilimine Sahip Hastaların Karşılaştırması

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ABSTRACT

Aim: Unicondylar knee arthroplasty (UKA) is among the treatment options for patients with arthritis limited to one compartment of the knee. Fixed-bearing (FB) and mobile-bearing (MB) inserts are present. This study aimed to compare functional and clinical outcomes and revision rates of patients operated with FB-UKA and MB-UKA.

Material and Methods: A total of 131 knees of 118 patients underwent cemented UKA, with a mean follow-up period of 80.58±31.31 months for FB-UKA and 97.66±29.24 months for MB-UKA. Clinical and functional evaluation was performed by the Knee Society Score (KSS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) score, at the last follow-up visit. The factors affecting the radiological and functional results, complication, and revision rates were examined under three main titles; i) surgeon-related, ii) patient-related, and iii) component alignment-related factors.

Results: There was no significant difference between the groups in terms of age, gender, body mass index, and side. Regarding the KSS scores, 9 (6.87%) knees were within acceptable limits, 62 (47.32%) knees were found to be good, and 60 (45.80%) knees were found to be excellent. No statistically significant difference was found between groups (p=0.497). Regarding the WOMAC scores, the MB-UKA group had significantly lower pain (p=0.049) and stiffness (p=0.014), but similar functional (p=0.591) scores. There was no statistically significant difference regarding revision rates (p=0.931).

Conclusion: Similar clinical, functional, and radiological results and low revision rates were found. In terms of pain and joint stiffness, a significant difference was found between groups, in favor of MB-UKA.

Keywords: Knee; fixed-bearing; mobile-bearing; unicondylar.

ÖZ

Amaç: Unikondiler diz artroplastisi (UDA), dizin bir kompartmanı ile sınırlı artritli hastalar için tedavi seçenekleri arasındadır. Sabit-insört (Sİ) ve mobil-insört (Mİ) eki mevcuttur. Bu çalışmada, Sİ-UDA ve Mİ-UDA ile ameliyat edilen hastaların fonksiyonel ve klinik sonuçlarının ve revizyon oranlarının karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Toplam olarak, 118 hastanın 131 dizine çimentolu UDA uygulandı ve ortalama takip süresi Sİ-UDA için 80,58±31,31 ay ve Mİ-UDA için 97,66±29,24 ay oldu. Klinik ve fonksiyonel değerlendirme, Knee Society Skor (KSS) ve Western Ontario and McMaster Universitesi Artrit Indeks (WOMAC) skoru ile son takip ziyaretinde yapıldı. Radyolojik ve fonksiyonel sonuçlar, komplikasyon ve revizyon oranlarını etkileyen faktörler üç ana başlık altında incelendi; i) cerrahla ilgili, ii) hastayla ilgili ve iii) bileşen hizalamayla ilgili faktörler.

Bulgular: Gruplar arasında yaş, cinsiyet, vücut kitle endeksi ve yan açısından anlamlı bir fark yoktu. KSS skorlarına göre 9 (%6,87) diz kabul edilebilir sınırlar içinde, 62 (%47,32) diz iyi olarak ve 60 (%45,80) diz ise mükemmel olarak bulundu. Gruplar arasında istatistiksel olarak anlamlı bir fark bulunmadı (p=0,497). WOMAC skorları ile ilgili olarak, Mİ-UDA grubu anlamlı olarak daha düşük ağrı (p=0,049) ve sertlik (p=0,014), ancak benzer fonksiyonel skorlara (p=0,591) sahipti. Revizyon oranları açısından istatistiksel olarak anlamlı bir fark yoktu (p=0,931).

Sonuç: Benzer klinik, fonksiyonel ve radyolojik sonuçlar ve düşük revizyon oranları bulundu. Ağrı ve eklem sertliği açısından gruplar arasında Mİ-UDA lehine anlamlı bir fark bulundu.

Anahtar kelimeler: Diz; sabit-insörtlü; mobil-insörtlü; unikondiler.

Presented partially at the Global Conference on Rheumatology and Orthopedics (March 14, 2022; Online Conference).

INTRODUCTION

Unicondylar knee arthroplasty (UKA) is among the treatment options of patients with end-stage knee osteoarthritis (OA) more than in the past, particularly in patients where the arthritis is limited to one compartment of the knee. Although other comparable treatment options in the treatment of single-compartment arthritis are high tibial osteotomy (HTO) and total knee arthroplasty (TKA), advantages of this technique, particularly over TKA, include a smaller incision, less blood loss, greater range of motion (ROM), lower perioperative morbidity, and greater preservation of anatomy and kinematics (1-6). Minimally invasive UKA (MIUKA), on the other hand, is performed with a smaller incision and approach that protects the suprapatellar extensor mechanism compared to the conventional UKA approaches.

Two different polyethylene insert designs are present depending on the surgeon's preference; fixed-bearing (FB) and mobile-bearing (MB) designs (7-9). Good clinical outcomes have been reported in the literature for both concepts. While MB designs are known with a more congruent weight bearing, therefore less contact stresses and polyethylene wear (10-13), FB designs have superior long-term clinical outcomes (7,14). The choice of UKAs with MB design has gained popularity. But according to the results of a recent meta-analysis comparing these two design concepts, sufficient long-term clinical, radiological, and kinematic outcome results and a strong consensus are not present in the literature (15).

In this study, it was aimed to compare functional and clinical outcomes and revision rates of patients operated with MB-UKA and FB-UKA while considering post-operative radiological alignment factors, patient and surgeon-related variables. Our hypothesis was to generally obtain good radiological and functional results in both groups, as well as to find better functional results in patients operated with MB-UKA.

MATERIAL AND METHODS

The approval of the institutional ethics committee was obtained (Medical Park Bursa Hospital, 22.05.2020, 223). The study was carried out retrospectively in line with the principles of the Declaration of Helsinki. 150 knees of 132 patients who underwent cemented MIUKA were included. While Journey Uni™ (Smith and Nephew) and Triathlon® PKR (Stryker Orthopedics, Mahwah, NJ) were preferred for FB-UKA, Uniglid® Mobile Bearing (Corin Ltd, Cirencester, UK) and Unicompartmental High Flex Knee System™ (ZUK; Zimmer, Winterthur, Switzerland) were preferred for the MB-UKA. All surgeries were performed by a single orthopedic surgeon at Bursa Bahar Hospital and Bursa Medical Park Hospital, between 2008 and 2018.

14 patients (19 knees) were excluded from the study because 3 patients died during the follow-up period, 3 had revision surgery after a traumatic injury to the knee and 8 patients left the follow-up. While 81 of the remaining 131 knees (118 patients) were reconstructed with a FB-UKA, 50 knees were reconstructed with an MB-UKA.

Inclusion criteria were; i) radiologically diagnosed anteromedial knee OA (Ahlback (16) grade 3 or higher), ii) 15-degree or less varus deformity, iii) patients ≤65 years of age, iv) active and passive knee flexion greater than 90 degree, v) fixed flexion contracture less than 10 degree.

Patients with patellofemoral joint symptoms, full thickness chondral damage on the patellar or trochlear surface, absence of an intact anterior cruciate ligament (ACL) or posterior cruciate ligament (PCL), lateral compartment pathologies (any chondral or meniscal pathology), avascular necrosis of tibia or femur and valgus alignment were excluded. Those with a follow-up period of less than 24 months were not included in the study.

Surgical Method

All cases were performed under spinal or epidural anesthesia and tourniquet hemostasis. A 6-8 cm anteromedial longitudinal incision covering 1/3 of the distal patella was performed. By preserving the vastus medialis, the patella was shifted laterally and the joint was reached. After ACL integrity was evaluated, the medial meniscus was completely excised. The tibial block incision was made by removing a 7 mm thick bone block using an extramedullary guide. A vertical incision was made in the sagittal plane. The tibial component size was determined by measuring the cut area of the removed bone block. Femoral cuts were made under the guidance of the tibial cuts while the knee flexed to 90°. Trial components and insert were placed to control the alignment and gaps in flexion and extension. Components were cemented in all cases. In the FB-UKA, the tibia and insert were first placed in one piece, then the femoral component; in the MB-UKA, the tibial component, insert and femoral component were placed respectively (Figure 1).

Rehabilitation

All patients underwent an intensive rehabilitation program that allows mobilization and full weight-bearing on the day of surgery, aiming to quickly achieve maximum flexion, full extension, and increase quadriceps muscle strength.

Pre-operative radiological evaluation was a routine with an orthoroentgenogram, weight-bearing lateral radiographs, and magnetic resonance imaging (MRI) to rule out any lateral compartment or cruciate ligament pathology.

Preoperative clinical scores were not available due to the retrospective nature of the study. Clinical and functional evaluation was performed at the last follow-up visit, with the Knee Society Score (KSS) (17), and Western Ontario and McMaster Universities Arthritis Index (WOMAC) score (18),

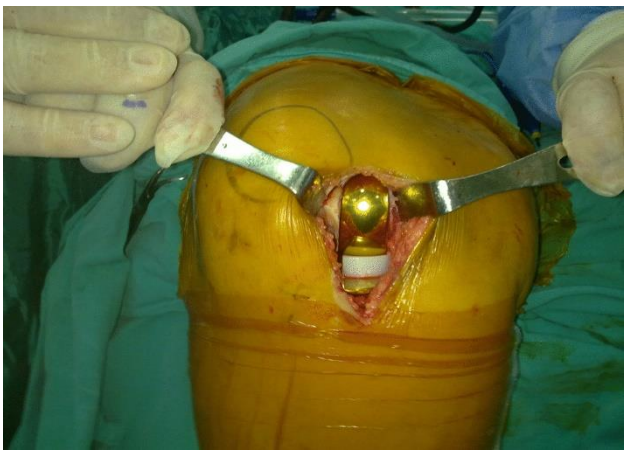

Figure 1. 6-8 cm anteromedial longitudinal incision was sufficient for MIUKA surgery, starting from 1/3 of the distal patella and preserving the vastus medialis muscle

which consists of three parts questioning pain, stiffness, and physical function. Complications (such as polyethylene wear, insert dislocation, progression in OA, aseptic loosening, and postoperative pain) and revisions during the follow-up were all noted. The factors affecting the radiological and functional results, complication, and revision rates of patients underwent MB- and FB-UKA were examined under three main titles: *i) Surgeon-related factors:* Given that the surgeon's experience will increase over the 10-year study period, the distribution of MB- and FB-UKA preferences in MIUKA patients were compared. *ii) Patient-related factors:* Age, gender, and body mass index (BMI). *iii) Component alignment-related factors:* Femoral component alignment (FCA), tibial component alignment (TCA), and mechanical axis angle (MAA) on post-operative orthoroentgenogram, anteroposterior (AP), and lateral radiographs (Figure 2) according to 5 (femoral component coronal and sagittal alignment, tibial component coronal and sagittal alignment, and mechanical axis) of the 17 Oxford alignment criteria (Table 1, 19).

Statistical Analysis

Statistical analyzes were performed using SPSS v.18.0 for Windows (SPSS Inc., Chicago, IL, USA) package program. Normal distribution was verified using the Kolmogorov-Smirnov test. Skewness and kurtosis values were controlled. Data were expressed as mean±standard deviation. According to the distribution of variables, independent t-test and Mann-Whitney U tests were used to compare quantitative data. Categorical data were compared with Chi-square and Fisher's exact tests. For all analyses, statistical significance was set at $p<0.05$.

RESULTS

There was no significant difference between the groups in terms of age, gender, BMI, and side variables (Table 2). The MB-UKA group had a longer mean follow-up time than the group with FB-UKA (97.66±29.24 vs 80.58±31.31, p=0.002). Considering the surgeon's experience factor, the distribution of the two groups in the first five years and the last five years were compared. While 32 MB- and 42 FB-UKAs were operated in the first five years, 18 MB- and 39 FB-UKAs were operated in the last five years, without a significant difference (p=0.210). Post-operative radiological evaluation of the FCA, TCA, and MAA did not show any significant difference between the groups (Table 3). Based on these findings, the patient, surgeon, and component alignment related factors were all found to be similar for both groups.

Regarding the postoperative KSS scores, 9 (6.87%) knees were within acceptable limits, 62 (47.32%) knees were found to be good and 60 (45.80%) knees were found to be excellent. However, when the groups were compared, no statistically significant difference was found (p=0.497). Regarding the postoperative WOMAC scores, MB-UKA

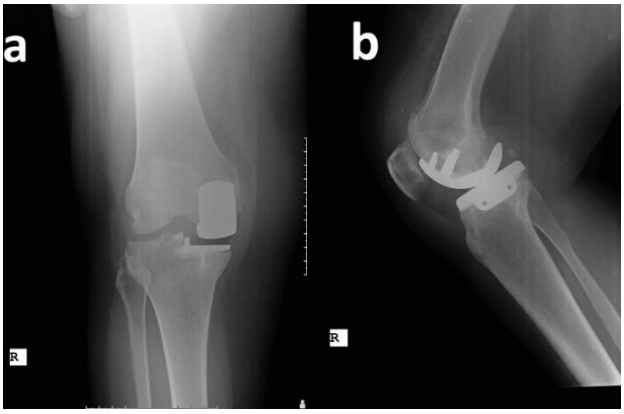

Figure 2. Postoperative **a)** anteroposterior and **b)** lateral radiography of a patient with MB-UKA

Table 1. Radiological evaluation of femoral and tibial components, and mechanical axis alignment according to the Oxford alignment criteria (20)

	Good	Medium	Bad
Femoral component	<10° varus/valgus **and** <5° flexion/extension angle	<10° varus/valgus **or** <5° flexion/extension angle	≥10° varus/valgus **and** ≥5° flexion/extension angle
Tibial component	<10° varus/valgus **and** 5-7° posterior slope	<10° varus/valgus **or** 5-7° posterior slope	≥10° varus/valgus **and** ≥7° posterior slope
Mechanical Axis	170-180°	5-10° deviation	≥10° deviation

Table 2. Demographic characteristics of the groups

	FB-UKA (n=81)	MB-UKA (n=50)	p
Age (years), mean±SD [min-max]	57.51±5.91 [44-65]	55.68±5.16 [46-65]	0.084
Gender, n (%)			
Male	10 (12.35)	9 (18.00)	0.372
Female	71 (87.65)	41 (82.00)	
Side, n (%)			
Right	52 (64.20)	30 (60.00)	0.631
Left	29 (35.80)	20 (40.00)	
Follow-up period (months), mean±SD [min-max]	80.58±31.31 [58-107]	97.66±29.24 [75-123]	**0.002**
BMI group, n (%)			
Normal	26 (32.10)	24 (48.00)	0.262
1st degree obese	33 (40.74)	13 (26.00)	
2nd degree obese	3 (3.70)	2 (4.00)	
Overweight	19 (23.46)	11 (22.00)	

FB-UKA: fixed-bearing unicondylar knee arthroplasty, MB-UKA: mobile-bearing unicondylar knee arthroplasty, SD: standard deviation, BMI: body mass index

Table 3. Clinical and radiological results, and revision rates of the groups

	FB-UKA (n=81)	MB-UKA (n=50)	p
KSS, mean±SD [min-max]	78.56±6.68 [75.1-82.4]	79.4±7.22 [75.0-86.7]	0.497
WOMAC Pain Score, mean±SD [min-max]	2.7±1.38 [2-3.5]	2.32±1.24 [2-3.3]	**0.049**
WOMAC Functional Score, mean±SD [min-max]	6.05±4.27 [1.9-12.1]	5.06±2.16 [2.4-8.3]	0.591
WOMAC Stiffness Score, mean±SD [min-max]	2.07±1.47 [1.0-4.3]	1.46±1.09 [0.2-2.9]	**0.014**
Mechanical Axis Alignment, n (%)			
Medium	8 (9.88)	4 (8.00)	0.718
Good	73 (90.12)	46 (92.00)	
Femoral Component Alignment, n (%)			
Bad	3 (3.70)	0 (0.00)	
Medium	44 (54.32)	20 (40.00)	0.074
Good	34 (41.98)	30 (60.00)	
Tibial Component Alignment, n (%)			
Medium	5 (6.17)	1 (2.00)	0.406
Good	76 (93.83)	49 (98.00)	
Revision to TKA, n (%)			
No	78 (96.30)	48 (96.00)	0.931
Yes	3 (3.70)	2 (4.00)	

FB-UKA: fixed-bearing unicondylar knee arthroplasty, MB-UKA: mobile-bearing unicondylar knee arthroplasty, KSS: knee society score, WOMAC: Western Ontario and McMaster Universities Arthritis Index, TKA: total knee arthroplasty, SD: standard deviation

patients had significantly lower pain (p=0.049) and stiffness (p=0.014), but similar functional (p=0.591) scores with the FB-UKA patients (Table 3).

A revision was necessary for only 5 of the 132 UKA patients and performed with a TKA procedure without the need for stem or augmentation. Two (4.0%) patients with MB-UKA and three (3.7%) with FB-UKA were revised. There was no statistically significant difference in revision rates between the groups (p=0.931). While the reason for the revisions in the MB-UKA group was insert dislocation (Figure 3) after a hyperflexion episode; of the 3 patients in the FB-UKA group, 2 had aseptic loosening of the femoral component and one had ongoing pain without radiological signs of loosening (Table 3).

DISCUSSION

While the long-term successful results of UKA are known in the literature, the importance of patient, surgeon, and implant related factors has been reported (20-24). In this study, we found similar good clinical, functional, and radiological results, and low revision rates in both designs. MB-UKA showed superior clinical results than FB-UKA in the assessment of joint stiffness and pain, supporting our hypothesis.

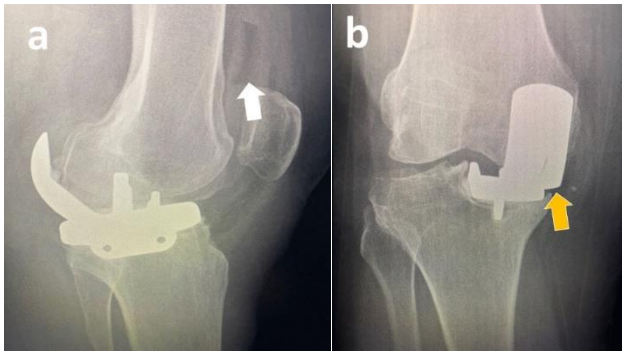

Figure 3. In a patient with MB-UKA, a complication of insert dislocation developed in the postoperative period. **a)** The insert has migrated into the suprapatellar area (white arrow) **b)** Since the insert is not in place, an incongruent tibiofemoral joint is present (orange arrow)

Post-UKA functional outcome, pain, and stiffness assessment have been a constant research topic. While UKA is compared in itself as MB and FB design prostheses, as well as with TKA. However, the evaluation of UKA by comparing it with TKA is controversial. Although UKA seems to be minor surgery and provides faster recovery than TKA, the revision was reported to be three times higher than TKA in the literature (20). Even if there is no mechanical or radiological problem in patients with intense pain after UKA, revision surgery (TKA) can be decided quickly (19). UKA tends to be performed on younger and more active patients than TKA, which is why functional comparison of UKA and TKA may be misleading (24). For these reasons, we compared UKA patients among themselves, as MB-UKA versus FB-UKA. It has been shown in various in-vivo and in-vitro studies in the literature that MB-UKA reconstructs the natural knee kinematics more closely and reduces contact stresses when compared to FB-UKA (10-13). The less joint stiffness and less pain we found in our results might be due to these known biomechanical benefits of MB-UKA design. Contrarily, in a recent meta-analysis made on the data of 1861 patients, it was shown that patients who operated with FB-UKA had better clinical and functional scores and greater ROM measurements (15). However, the shortcoming of this meta-analysis was that it did not take into account the component alignment and the mechanical axis alignment of the leg. After the UKA surgeries, problems in this regard can be encountered frequently, and this may adversely affect long-term functional results. The most common incorrect component placement we detected radiologically in the post-operative period of MIUKA surgery in our previous study were as follows; placement of the femoral component in flexion, presence of a gap in the posterior wall of the femoral component, and posterior protrusion of the tibial component (25). Kennedy et al. (26) reported that superior clinical results were obtained when the mechanical axis fell in the center of the knee or slightly medial to the center. Therefore, in our study, we compared

the postoperative mechanical axis distribution of the groups, as well as the femoral and tibial component alignments, and we found that the groups had an equal distribution in terms of these parameters. We think that these data we determined are a very strong aspect of our study.

Studies evaluating the need for revision after UKA, reported the aseptic loosening and OA progression as the most common causes (9,27). van der List et al. (28), in their systematic review comparing MB- and FB-UKA in this respect, found aseptic loosening in the MB-UKA group and OA progression in the FB-UKA group as the most common cause of revision. However, a more recent meta-analysis study reported high aseptic loosening in FB-UKAs, similar to our results (15). Similarly in a meta-analysis study by Barret et al. (29), which included 96 294 knees, they found that aseptic loosening of the tibial component occurred at a lower rate in UKA patients who underwent cementless robotic-assisted surgery with an MB insert.

Insert dislocation, an urgent complication, was the only cause of revision surgery in the MB-UKAs. This is a unique complication to MB-UKAs. Undersized bearing, medial collateral ligament over-release, component malalignment, and flexion-extension imbalance were blamed as the most likely causes. The rate of development of these complications in MB-UKAs has been reported between 0.64% and 6.5% in the literature (15), consistent with our rates (4.0%). Because of the insert dislocation risk in MB-UKA, Kuyucu et al. (30) reported that FB-UKA should be preferred primarily in obese patients. In our study, the mean BMI of patients who underwent revision was found to be high without a significant difference.

The revision rate for surgeons who apply 12 to 30 UKA per year is 1.5% per year, while the same rate is 1% per year for surgeons who perform more than 30 UKA per year. According to these results, the expected result has been shown, and an inversely proportional relationship between experience and revision rate is present (31). Here, we performed TKA for revision surgery in 4 of our patients in the first five years. The need for only one revision in the last five years of the 10-year follow-up period emphasizes the importance of the surgeon's experience. Because of these findings, the size of the groups operating in the first five years (32 MB-, and 42 FB-UKAs) and the last five years (18 MB-, and 39 FB-UKAs) were compared in order not to cause a bias in the surgeon's experience factor, but no significant difference was found.

We think that our strengths are the similar group distribution, long follow-up period, and high sample size. However, our study also has some weaknesses. One of the main limitations of our study is that it has a retrospective study design. Since there are similar retrospective studies in the literature, prospective randomized controlled studies are needed on this subject. Another limitation is the wide time distribution in the follow-up periods. Besides, groups are significantly different regarding the follow-up period. Since long-term results are very important in prosthetic surgeries, that difference may have been effective in our clinical/functional results and the number of revisions. Lastly, although all surgeries were performed by a single surgeon, the preferred prostheses within the groups had the same type of insert but were not from a single company.

CONCLUSION

In conclusion, MB- and FB-UKAs have both similar good clinical, functional, and radiological results, and low revision rates. A significant difference was found in favor of MB-UKA in terms of pain and joint stiffness. Although similar revision rates were found, insert dislocation for MB-UKA and loosening of the femoral component for FB-UKA were the main revision causes. Obesity seemed to be an important parameter for revision surgery risk.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Medical Park Bursa Hospital (22.05.2020, 223).

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Hypomagnesemia as a Predictor of Early Liver Dysfunction in Critically Ill Patients with Sepsis

Sepsisli Kritik Hastalarda Erken Karaciğer Disfonksiyonunun Bir Göstergesi Olarak Hipomagnezemi

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ABSTRACT

Aim: Liver dysfunction is an early finding caused by the inflammation and hypoperfusion developed in sepsis. Magnesium deficiency may contribute to an excessive response to immune stress and inflammatory tissue damage in sepsis. This study aimed to evaluate the relationship between serum magnesium levels and early liver dysfunction (ELD) in patients with sepsis.
Material and Methods: 142 patients who developed sepsis were divided into two groups according to their liver function, as sequential organ failure assessment (SOFA) hepatic subscore <2 (Non-ELD, n=72) and SOFA hepatic subscore ≥2 (ELD, n=70). The disease severity, including the acute physiology and chronic health evaluation (APACHE) II score and the SOFA score, biochemical determination, and microbiological cultures were evaluated.
Results: ELD patients presented APACHE II and total SOFA scores higher than Non-ELD patients, while PaO_2/FiO_2 ratios were significantly lower (both p<0.001). Hypomagnesemia and hypoalbuminemia were independently associated with ELD (OR: 6.55, 95% CI: 2.62-16.36, and OR: 4.62, 95% CI: 1.35-15.84, respectively). To predict ELD, the area under the curve was 0.81 (95% CI: 0.74-0.89, p<0.001) and 0.70 (95% CI, 0.61-0.79; p<0.001) for serum magnesium and albumin, respectively. The mortality rate in all septic patients was 35.0% for hypomagnesemia and 25.6% for normomagnesemia (p=0.065). The mortality rate in ELD patients was 34.1% for hypomagnesemia and 30.7% for normomagnesemia (p=0.415).
Conclusion: The reduction of magnesium levels was associated with increased rates of ELD in critically ill patients with sepsis. Admission hypomagnesemia did not adversely affect mortality neither in all sepsis patients nor in those who developed ELD.
Keywords: Critically ill; hypomagnesemia; liver; mortality; sepsis.

ÖZ

Amaç: Karaciğer disfonksiyonu, sepsiste gelişen inflamasyon ve hipoperfüzyonun neden olduğu erken bir bulgudur. Magnezyum eksikliği, immün strese karşı aşırı bir yanıta ve sepsiste inflamatuar doku hasarına katkıda bulunabilir. Bu çalışmada, sepsisli hastalarda serum magnezyum düzeyleri ile erken karaciğer disfonksiyonu (early liver dysfunction, ELD) arasındaki ilişkinin değerlendirilmesi amaçlandı.
Gereç ve Yöntemler: Sepsis gelişen 142 hasta karaciğer fonksiyonlarına göre ardışık organ yetmezliği değerlendirme (sequential organ failure assessment, SOFA) karaciğer alt skoru <2 olanlar (Non-ELD, n=72) ve SOFA karaciğer alt skoru ≥2 olanlar (ELD, n=70) olmak üzere iki gruba ayrıldı. Akut fizyoloji ve kronik sağlık değerlendirmesi (acute physiology and chronic health evaluation, APACHE) II skoru ve SOFA skoru dahil olmak üzere hastalık şiddeti, biyokimyasal belirleme ve mikrobiyolojik kültürler değerlendirildi.
Bulgular: ELD hastalarının APACHE II ve toplam SOFA skorları ELD olmayan hastalara göre daha yüksek iken, PaO_2/FiO_2 oranları anlamlı derecede düşüktü (her ikisi için p<0,001). Hipomagnezemi ve hipoalbüminemi ELD ile bağımsız şekilde ilişkiliydi (sırasıyla, OR: 6,55; %95 GA: 2,62-16,36 ve OR: 4,62; %95 GA: 1,35-15,84). ELD'yi öngörmede, serum magnezyum ve albümini için eğri altındaki alan sırasıyla 0,81 (%95 GA: 0,74-0,89; p<0,001) ve 0,70 (%95 GA: 0,61-0,79; p<0,001) idi. Tüm septik hastalardaki ölüm oranı, hipomagnezemi için %35,0 ve normomagnezemi için %25,6 idi (p=0,065). ELD hastalarındaki ölüm oranı hipomagnezemi için %34,1 ve normomagnezemi için %30,7 idi (p=0,415).
Sonuç: Magnezyum düzeylerindeki azalma, sepsisli kritik hastalarda artmış ELD oranları ile ilişkiliydi. Kabuldeki hipomagnezemi ne tüm sepsisli hastalarda, ne de ELD gelişenlerde mortaliteyi olumsuz etkilemedi.
Anahtar kelimeler: Kritik hasta; hipomagnezemi; karaciğer; mortalite; sepsis.

INTRODUCTION

Sepsis is known as life-threatening organ dysfunction resulting from an uncontrolled host response to infection (1). In addition to playing an important role in the defense against microorganisms, the liver is also frequently exposed to dysregulated inflammation (2). The development of liver dysfunction and failure, which is a serious complication due to the ongoing inflammation and hypoperfusion in sepsis, directly promotes the severity of the disease and death (3). Mortality rates in sepsis patients with liver dysfunction or failure are between 54% and 68%, and these mortality rates are higher than the dysfunction or failure of the lung, which is the most commonly affected organ in sepsis (4).

The incidence of hypomagnesemia in critically ill patients reaches up to 65%. The predisposition of critically ill patients to symptomatic or asymptomatic magnesium deficiency may lead to important clinical conditions such as hypokalemia, hypocalcemia, cardiac arrhythmias, neurotoxicity, sepsis, and psychiatric problems, resulting in increased morbidity and mortality (5,6).

In experimental models, magnesium deficiency causes inflammation, and latent magnesium deficiency is associated with chronic low-grade inflammation (7). Moreover, magnesium deficiency shows pro-oxidant effects by increasing lipid peroxidation in liver mitochondria (8). Dietary magnesium intake is inversely related to serum C-reactive protein (CRP) levels (9), and magnesium supplementation reduces CRP levels in individuals with inflammation (10).

It has been shown that treatments aimed at reducing the inflammation process can alleviate sepsis-induced liver injury (11). Magnesium supplementation inhibits the upregulation of inflammatory molecules in cells treated with endotoxin (12) and also provides significant protection against lipopolysaccharide (LPS) induced liver injury (13). To date, evidence that low magnesium levels may facilitate sepsis-induced liver dysfunction has lacked. On this basis, we conducted this study with the hypothesis that a subclinical magnesium deficiency exacerbates sepsis-induced inflammation and could be associated with early liver dysfunction (ELD) in critically ill patients suffering from sepsis.

MATERIAL AND METHODS

Study Design

In this cohort study of prospective character, adult patients (age of ≥18 years) admitted consecutively to a 42-bed department of the medical-surgical intensive care unit (ICU) of Konya Numune Hospital diagnosed with sepsis during a 16-month period from March 2021 to July 2022 were included. It was made in accordance with the Declaration of Helsinki and approved by the ethics committee of Necmettin Erbakan University (approval date/no: 2021/3083). The patients participating in the study or their relatives provided written informed consent.

Inclusion and Exclusion Criteria

Inclusion criteria were the recent onset of sepsis and/or septic shock (at diagnosis or within the first 24 h) according to the SEPSIS-3 definition (1). Exclusion criteria were documented hypomagnesemia or magnesium supplementation prior to the diagnosis of sepsis, hypermagnesemia, preexisting liver disease, hemolytic disease, bilirubin ≥2 mg/dL in the previous 30 days, previous immunodeficiency, history of daily cortisol medication >5 mg, blood product transfusion, malignant disease of any origin, seizures, and pregnancy.

Definitions

The ELD was defined as serum bilirubin ≥2 mg/dL (a SOFA hepatic subscore ≥2) within 48 hours after the onset of sepsis (14). The normal range for magnesium concentration in our laboratory was 1.80-2.60 mg/dL. A magnesium level in serum of <1.80 mg/dL was considered hypomagnesemia, and ≥2.61 mg/dL hypermagnesemia. Magnesium-lowering drugs included mannitol, diuretics, aminoglycosides, amphotericin B, proton pump inhibitors, and digoxin. The requirement for mechanical ventilation was recorded if initiated within the first 48 hours after admission. The main site of causative infection was determined by microbiological results or clinical suspicions and signs of infection.

For the outcomes, death from any cause occurring within 30 days after the onset of sepsis was defined as 30-day mortality. Sepsis-related mortality was considered when the patient died without definitive infection control.

Study Protocol

One hundred and forty-two patients who developed sepsis were divided into two groups according to their liver functions: sequential organ failure assessment (SOFA) hepatic subscore <2 (Non-ELD, n=72) and SOFA hepatic subscore ≥2 (ELD, n=70). The disease severity assessment, including the acute physiology and chronic health evaluation (APACHE) II score and the SOFA score, biochemical determination, and microbiological cultures were evaluated within 24 hours after ICU admission and before the initiation of antimicrobial therapy against sepsis. Serum magnesium levels were measured with the original reagents using AU5800 biochemical analyzer (Beckman Coulter, California, USA). The levels of the other parameters were measured according to routine laboratory working procedures at the local institute. In this study, no interference was made in the routine treatment process in the ICU and appropriate magnesium replacement was given to all patients in the ELD and Non-ELD groups with low magnesium levels (n=44, and n=16, respectively). Additionally, only admission magnesium levels were examined in detail and post-replacement magnesium values were not evaluated. Routine sepsis therapy (fluids, antibiotics, catecholamines, surgery) according to the current sepsis guidelines was administered to all the patients (15). The primary endpoints of this study were the relation between serum magnesium levels and the occurrence of ELD in critically ill patients affected by sepsis. The secondary outcome measures were the impact of low magnesium levels on all-cause ICU mortality within 30 days.

Sample Size

The G*Power v.3.1 software was used for the sample size calculation. This analysis was performed according to the results of a similar study by Vatsalya et al. (16). In order to detect a significant difference in magnesium level among the two groups, a power analysis was carried out using a 2-sided independent samples t-test with a confidence level of 95% ($p<0.050$), an effect size of 49%,

and a power of 80%. Considering a 5% dropout rate, the sample size was finally calculated as at least 141 patients.

Statistical Analysis

For the statistical analysis, IBM SPSS v.21.0 and MedCalc v.14.12 software were used. Mean±standard deviation or median, interquartile range, min-max were chosen to express continuous variables and numbers and percentages for categorical data. The distribution of the continuous variables was evaluated by the Kolmogorov-Smirnov test. For comparing continuous variables between patients with and without ELD, for normally distributed data, the independent samples t-test was used, and for non-normal data, the Mann-Whitney U test. Categorical variables were compared with the Chi-square test or Fisher's exact test. A logistic regression model was used to examine the ionic factors associated with the development of ELD. Based on the results of the multivariate analysis, the predictive capacity of serum magnesium and albumin levels in the differentiation of ELD was evaluated by the receiver operating characteristic (ROC) curve analysis. The correlations were evaluated using Spearman's correlation test. The Cox regression analysis and the Kaplan-Meier method were performed for time-to-event analyses. A p value of lower than 0.050 was defined as statistically significant.

RESULTS

In the study period, 191 critically ill and with new-onset sepsis patients were enrolled. The leading criteria for exclusion were preexisting liver disease or hyperbilirubinemia (n=16), immunodeficiency or cortisol medication (n=15), hypermagnesemia (n=13), and preexisting hypomagnesemia or magnesium supplementation (n=5). Finally, 142 intensive care patients were incorporated into the study for analysis. The baseline traits of patients at admission were shown in Table 1. The most common systemic disease in septic patients was cardiovascular disease, with 27%. ELD patients had higher APACHE II and total SOFA scores than Non-ELD patients, while the PaO_2/FiO_2 ratios were significantly lower (both $p<0.001$). The mortality rates were higher in ELD patients, but this difference was not statistically significant. No statistically significant difference was observed between ELD and Non-ELD patients in terms of other variables.

When the laboratory parameters were evaluated, ELD patients had higher CRP, procalcitonin (PCT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, international normalized ratio (INR), lactate, and lactate/albumin levels, while hemoglobin, magnesium, and albumin levels were lower than Non-ELD patients (Table 2). The microbiological profile was similar in both groups (Table 3).

The risk of ELD associated with ionic disturbance was analyzed using logistic regression. After adjustment according to age, gender, and APACHE II scores, the multivariate regression analysis describing hypomagnesemia and hypoalbuminemia as independently associated with ELD (Odds ratio, (OR): 6.55, 95% CI: 2.62-16.36, p=0.001, and OR: 4.62, 95% CI: 1.35-15.84, p=0.015, respectively) showed the important effect of the decrease

Table 1. Baseline characteristics of patients

	Non-ELD (n=72)	ELD (n=70)	p	Total (n=142)
Age (year), median (IQR) [min-max]	66 (60-74) [17-87]	70 (61-78) [21-82]	0.066	67.5 (60-76) [17-87]
Gender, (male), n (%)	40 (55.6)	38 (54.3)	0.879	78 (54.9)
Cause of ICU admission, n (%)				
Medical	59 (81.9)	52 (74.3)	0.269	111 (78.2)
Trauma/Surgery	13 (18.1)	18 (25.7)		31 (21.8)
APACHE II score, mean±SD	23.2±3.2	25.2±3.3	**<0.001**	24.2±3.4
SOFA score, median (IQR) [min-max]	6 (5-8) [4-11]	8 (7-9) [4-12]	**<0.001**	7 (5-9) [4-12]
Diabetes mellitus, n (%)	13 (18.1)	15 (21.4)	0.614	28 (19.7)
Cardiovascular disease, n (%)	20 (27.8)	18 (25.7)	0.781	38 (26.8)
Neurologic disease, n (%)	12 (16.7)	10 (14.3)	0.695	22 (15.5)
Pulmonary disease, n (%)	13 (18.1)	16 (22.9)	0.478	29 (20.4)
Renal disease, n (%)	14 (19.4)	12 (17.1)	0.723	26 (18.3)
At least one co-morbid illness, n (%)	47 (65.3)	48 (68.6)	0.677	95 (66.9)
Body temperature (°C), mean±SD	37.8±1.0	37.5±0.9	0.069	37.7±1.0
MAP (mmHg), mean±SD	75.1±12.7	71.5±11.4	0.084	73.3±12.1
Heart rate (bpm)	89 (67-116) [49-150]	95 (63-125) [51-152]	0.710	91 (66-120) [49-152]
Respiratory rate (bpm)	18 (15-24) [12-35]	21 (17-26) [13-37]	0.057	20 (16-25) [12-37]
Worst PaO_2/FiO_2 at admission	301 (295-331) [282-380]	284 (280-298) [275-351]	**<0.001**	295 (284-312) [275-380]
Antibiotic exposure, n (%)	50 (69.4)	52 (74.3)	0.521	102 (71.8)
Magnesium lowering drugs, n (%)	51 (70.8)	54 (77.1)	0.392	105 (73.9)
Total parenteral nutrition, n (%)	8 (11.1)	11 (15.7)	0.421	19 (13.4)
Mechanical ventilation, n (%)	21 (29.2)	24 (34.3)	0.512	45 (31.7)
Septic shock, n (%)	20 (27.8)	27 (38.6)	0.172	47 (33.1)
Time from sepsis to death (days)	10 (6-14) [3-28]	9 (6-13) [4-29]	0.844	9.5 (6-13) [3-29]
ICU length of stay (days)	17 (10-27) [3-37]	14.5 (7-23) [3-38]	0.121	16 (8-25) [3-38]
Sepsis-related mortality, n (%)	14 (19.4)	17 (24.3)	0.485	31 (21.8)
30-day mortality, n (%)	19 (26.4)	23 (32.9)	0.398	42 (29.6)

ELD: early liver dysfunction, ICU: intensive care unit, APACHE: acute physiological and chronic health evaluation, SOFA: sequential organ failure assessment, MAP: mean arterial pressure, PaO_2/FiO_2: ratio of arterial oxygen concentration to the fraction of inspired oxygen, IQR: interquartile range (25^{th}-75^{th} percentile), SD: standard deviation

Table 2. Laboratory parameters according to liver dysfunction

	Non-ELD (n=72)	ELD (n=70)	p	Normal Values
White blood cell count (mm^3)	14.8±5.7	16.0±5.2	0.190	3.91-10.9
Hemoglobin (g/dL)	12.4±2.7	11.1±2.4	**0.003**	13.5-16.9
Platelet count (10^9/L)	132 (104-266) [85-479]	125 (95-269) [81-466]	0.225	166-308
Erythrocyte sedimentation rate (mm/h)	93.5 (56-106) [16-144]	89.5 (52-106) [14-121]	0.329	0-20
C-reactive protein (mg/dL)	143.0±33.6	167.9±36.6	**<0.001**	0-8
Procalcitonin (ng/mL)	17.4 (9-23) [0.1-49.0]	24.3 (19-30) [0.1-39.0]	**0.002**	0-0.5
Aspartate aminotransferase (IU/L)	38 (28-57) [10-267]	50 (32-64) [15-273]	**0.043**	3-50
Alanine aminotransferase (IU/L)	36.5 (27-55) [9-259]	40 (31-63) [16-266]	0.177	3-50
Alkaline phosphatase (IU/L)	109.4 (84-124) [30-200]	121 (86-167) [32-211]	**0.032**	30-120
Blood urea nitrogen (mg/dL)	31.9 (26-42) [19-114]	38.9 (28-49) [12-110]	0.083	17-43
Creatinine (mg/dL)	1.2 (1.0-1.5) [0.5-2.6]	1.5 (0.9-2.2) [0.4-2.9]	0.294	0.67-1.17
Bilirubin (mg/dL)	1.6 (1.3-1.8) [0.6-1.9]	2.6 (2.3-2.8) [2.0-5.5]	**<0.001**	0-1.2
International normalized ratio	1.4±0.4	1.5±0.3	**0.043**	0.8-1.2
Sodium (mmol/L)	139.1±10.9	137.2±11.3	0.305	136-145
Potassium (mmol/L)	4.0±0.9	4.2±0.9	0.276	3.5-5.1
Calcium (mg/dL)	8.6 (8.1-9.3) [6.9-11.0]	8.4 (7.3-9.4) [6.8-10.4]	0.073	8.8-10.6
Ionized Ca^{2+} (mmol/L)	1.2 (1.1-1.3) [1.0-1.4]	1.2 (1.1-1.2) [1.0-1.5]	0.112	1.15-1.33
Magnesium (mg/dL)	2.1 (1.9-2.4) [1.5-2.6]	1.6 (1.5-1.8) [1.5-2.5]	**<0.001**	1.8-2.6
Phosphate (mg/dL)	3.7±0.7	3.9±0.8	0.090	2.5-4.5
Chlorine (mmol/L)	103 (100-109) [90-122]	105 (101-118) [93-131]	0.077	101-109
Lactate (mmol/L)	2.2±0.8	2.7±0.9	**<0.001**	0-2
Albumin (g/dL)	3 (2.7-3.6) [2.5-5.0]	2.7 (2.5-3.1) [2.4-4.6]	**<0.001**	3.5-5.2
Lactate/Albumin ratio	0.7 (0.5-0.9) [0.2-1.4]	1.0 (0.7-1.3) [0.3-1.5]	**<0.001**	

ELD: early liver dysfunction, descriptive statistics were shown as mean±standard deviation or median (interquartile ranges, 25^{th}-75^{th} percentile) [minimum-maximum]

in magnesium levels on the development of liver dysfunction in septic patients (Figure 1). As these serum biomarkers have been demonstrated to be significantly associated with liver dysfunction in septic patients, the levels of magnesium and albumin were evaluated by ROC analysis as predictive indicators of ELD. The area under the curve (AUC) was 0.81 (95% CI: 0.74-0.89, p<0.001) and 0.70 (95% CI: 0.61-0.79, p<0.001) for serum magnesium and albumin, respectively. The sensitivities of magnesium and albumin were 80% and 50%, while the specificity was 77.8% and 87.5%, respectively (Figure 2). The pairwise comparison of ROC curves for predicting liver dysfunction showed significant differences in the AUC values of magnesium and albumin (difference between areas: 0.11, SE: 0.05, 95% CI: 0.02-0.21, z=2.31; p=0.020). Therefore, serum magnesium may have a higher predictive value than serum albumin in the evaluation of developing liver dysfunction in septic patients.

The relationship between the SOFA score, by which the organ dysfunction was evaluated, and magnesium was measured by bivariate analysis. Although there was no significant correlation between the SOFA score and magnesium in the Non-ELD group (r=-0.214, p=0.074), there was a weak inverse correlation between the SOFA score and magnesium in the ELD group (r=-0.252, p=0.032).

Table 3. Comparison of microbiological profile between patients with and without early liver dysfunction

	Non-ELD (n=72)	ELD (n=70)	p
Microbiologically documented infection, n (%)	53 (73.6)	49 (70.0)	0.632
Blood culture positive, n (%)	28 (38.9)	24 (34.3)	0.569
Source of Infection	**Non-ELD (n=53)**	**ELD (n=49)**	**p**
Respiratory, n (%)	21 (39.6)	18 (36.7)	0.764
Urinary tract, n (%)	12 (22.6)	11 (22.4)	0.981
Skin/soft tissues, n (%)	5 (9.4)	6 (12.2)	0.647
Intraabdominal, n (%)	4 (7.5)	6 (12.2)	0.515
Other, n (%)	11 (20.8)	8 (16.3)	0.566
Identified Microorganisms	**Non-ELD (n=53)**	**ELD (n=49)**	**p**
Gram-negative, n (%)	20 (37.7)	21 (42.9)	0.598
Gram-positive, n (%)	15 (28.3)	12 (24.5)	0.663
Polymicrobial, n (%)	6 (11.3)	9 (18.4)	0.315
Atypical, n (%)	2 (3.8)	1 (2.0)	1.000
Fungal, n (%)	4 (7.5)	3 (6.1)	1.000
Viral, n (%)	6 (11.3)	3 (6.1)	0.491

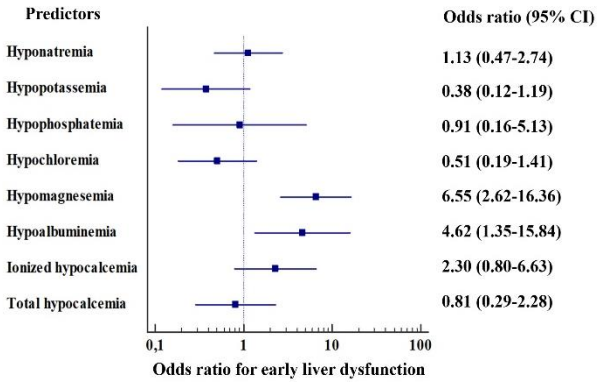

Figure 1. Ionic factors were possibly associated with ELD. All ionic parameter covariate calculations were adjusted for age, sex, and APACHE II score.

The comparison of the effect of liver dysfunction on the mortality of septic patients was shown in Table 1. Although the sepsis-related and 30-day mortality rates were higher in patients in the ELD group than in the Non-ELD group, this difference was insignificant (p=0.485, p=0.398, respectively). We performed an interaction analysis to determine whether ELD carries a high risk of mortality in patients with sepsis and whether this risk is associated with the inflammatory and pro-oxidant function of low magnesium levels. We tested the interaction between bilirubin below or above 2 mg/dL and magnesium levels for 30-day mortality. But no interaction was found (p=0.654). Also, in the Cox regression analysis of septic patients without liver dysfunction, low magnesium levels were not associated with ICU mortality (p=0.183).

To further investigate the impact of hypomagnesemia on the clinical outcome of septic patients, we performed a time-dependent mortality analysis. The Kaplan-Meier curves for normomagnesemic and hypomagnesemic patients were shown in Figure 3. The mortality rate for all septic patients was 25.6% for normomagnesemia vs 35.0% for hypomagnesemia (p=0.065, log-rank test). The mortality rate in ELD patients was 30.7% for normomagnesemia vs 34.1% for hypomagnesemia (p=0.415, log-rank test). Finally, the risk of mortality associated with hypomagnesemia remained unchanged in both septic patients and those who developed liver dysfunction, as it was shown by the survival curve analysis.

DISCUSSION

In this study, the relationship between low magnesium levels in sepsis patients with the development of ELD and its effects on ICU mortality were investigated. Low magnesium levels in critically ill septic patients were associated with increased liver dysfunction. The hypomagnesemia at admission had no effect either on mortality in all the sepsis patients or in those who developed ELD.

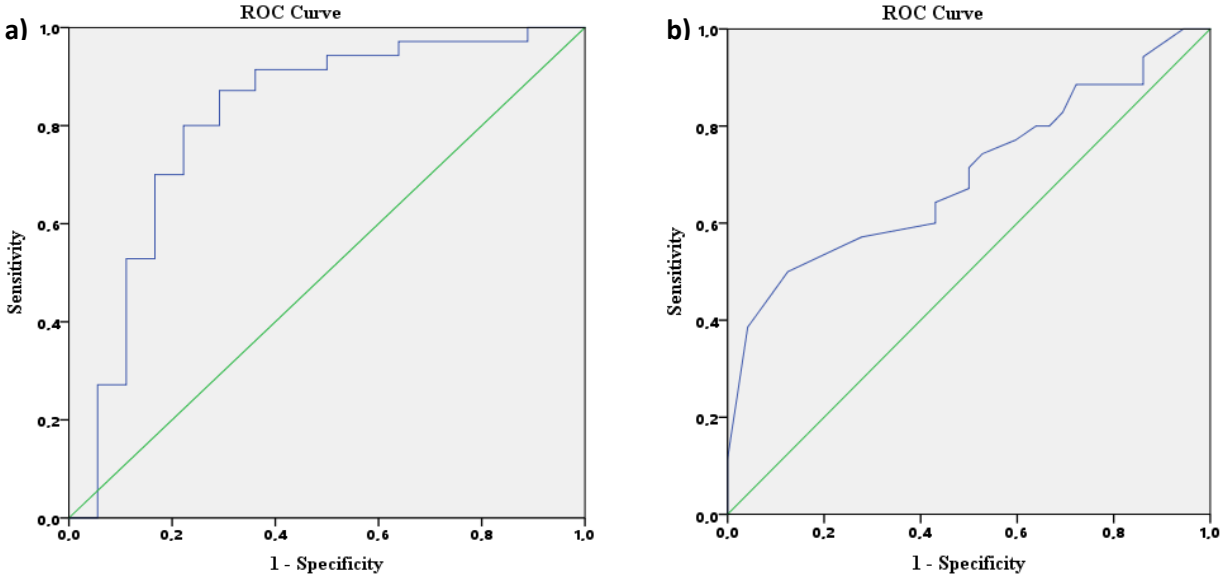

Figure 2. Receiver operating characteristic curves for **a)** magnesium and **b)** albumin to predict early liver dysfunction

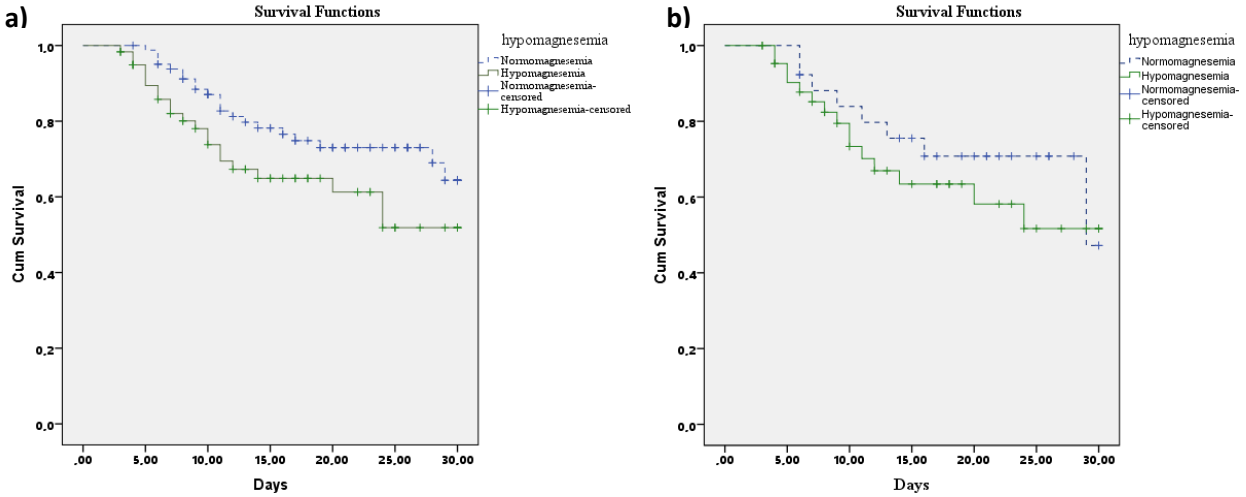

Figure 3. The survival after the onset of sepsis in patients with normomagnesemia and hypomagnesemia was estimated using the Kaplan-Meier method. **a)** Probability of survival in all septic patients with normomagnesemia (n=82; 21 death) and hypomagnesemia (n=60; 21 death) (p=0.065, log-rank test). **b)** Probability of survival in ELD patients with normomagnesemia (n=26; 8 death) and hypomagnesemia (n=44; 15 death) (p=0.415, log-rank test)

The mediators released by the innate immune cells in sepsis and their effects on endothelial cells lead to endothelial leakage, extravascular migration of neutrophils and inflammatory mediators, vasodilation, and coagulation activation, resulting in organ dysfunctions (17). Factors such as steatosis, cholestasis, hepatocellular injury, hepatic mitochondrial respiration, and the disruption of cellular regeneration play a role in clinical septic hepatic dysfunction (18). Despite the increase in cardiac output and hepatic perfusion, hepatocellular function has been shown to decrease in the early period after the onset of sepsis (19). Inflammatory jaundice may occur due to direct bacterial products or due to the response of the host to infection. Cholestatic liver dysfunction is, through hepatocellular and ductular cholestasis mechanisms, directly related to the immunological processes occurring during sepsis and systemic inflammation in the liver (20). Sepsis jaundice, which is usually cholestatic, may emerge before other clinical signs of the underlying infection are seen, with mild increases in serum alkaline phosphatase and aminotransferases (21). Although there are no standard diagnostic criteria and terminology, the limited increases in alkaline phosphatase and liver enzymes, along with the mild deviation in coagulation accompanying the increase in total bilirubin in our study, is consistent with the cholestatic form of sepsis-associated liver dysfunction (22).

The incidence of electrolyte disturbances, sepsis, and septic shock is increased in patients with hypomagnesemia (23). Magnesium modulates the immune response, including macrophage activation, lymphocyte proliferation, and increased production of reactive oxygen species, and magnesium deficiency might increase the production of inflammatory cytokines (24). In the experimental endotoxemia study of Lee et al. (25), magnesium sulfate was shown to dose-dependently reduce the inflammatory response, oxidative stress, and acute injury in the lung.

However, there have not been enough clinical studies on the role of magnesium in the liver. Hernandez et al. (26) studied the effects of magnesium supplementation on hepatic inflammation in non-hypertensive obese women. It showed that oral magnesium supplementation decreased serum ALT levels in hypomagnesemic obese women. In another randomized study, Karandish et al. (27) investigated the effect on liver enzymes of magnesium supplementation in patients with nonalcoholic fatty liver disease. Unlike the previous study, it was reported that magnesium supplementation did not affect liver enzymes. However, this study was conducted in normomagnesemic patients and used lower doses of magnesium.

In an experimental endotoxic model, Sayeed et al. (28) showed that the hepatic Mg^{2+} content of septic rats was significantly lower than non-septic controls. Gunther et al. (8) suggested that the non-Hb Fe increase induced by the magnesium deficiency in the liver cell fractions may play a role in the increase of lipid peroxidation in liver mitochondria. Calviello et al. (29) found that in magnesium deficiency, liver glutathione, CuZn-superoxide dismutase, and vitamin E decreased, while lipid peroxidation increased in liver microsomes, and this was associated with oxidative stress. George et al. (30) showed that a primary disorder in the energy metabolism of the liver mitochondria is produced in magnesium deficiency and that, secondary, protein synthesis is inhibited. In our study, it was found that hypomagnesemia is related to an increased incidence of ELD in septic patients. Consistent with previous studies, these findings might explain the higher incidence of liver dysfunction in patients with hypomagnesemia.

Hypomagnesemia may cause inflammatory tissue damage by increasing acute phase proteins, cytokine biosynthesis, and the production of reactive oxygen species (7,24). Since the severity of the disease in septic patients can be considered a direct indicator of multiple organ dysfunction in response to excessive systemic inflammation (31), in our study, the significant correlations between the SOFA score and magnesium levels in patients in the ELD group suggested that our results are consistent with findings proving the correlation between increased inflammatory response and serum magnesium. Theoretically, the severity of the organ dysfunction assessed by the SOFA score is, as in other inflammatory conditions, an important indicator of acute phase changes in the inflammatory mediators of septic patients. Therefore, inverse correlations between magnesium and the SOFA score can be expected, similar to the inverse relationships between acute phase reactants and cytokines and magnesium.

In liver dysfunction, subtle changes are observed in the hepatocellular functions due to the clearance functions or the decreased synthesis (4). In the absence of underlying liver disease, the correlation between mild to moderate liver dysfunction to mortality is not clear enough. Kramer et al. (14) showed that liver dysfunction is an early sign of sepsis and that ELD is associated with a bad prognosis in septic critically ill patients. In a recent multi-center study, Jensen et al. (32) evaluated the prognostic impact on critically ill patients with mild to moderate liver impairment. It was found that sepsis-related acute liver impairment was seen in the early period of ICU admission and was associated with high mortality. However, liver impairment had no effect on mortality in patients without severe infection. Nesseler et al. (33) evaluated the association of liver dysfunction with long-term mortality in patients with septic shock. Although during the septic shock, there was a significant correlation between new-onset or worsening liver dysfunction and mortality up to 6 months, the same correlation was not observed for baseline liver dysfunction on days 28, 90, and 180. In the present study, the fact that liver dysfunction does not contribute to an increase in the risk of death in patients with sepsis suggests that ELD may be an indicator of more severe disease but is not associated with increased mortality.

Several possibilities can explain the divergent results in the studies evaluating the effects of septic liver dysfunction on mortality. First, the studies have been conducted at a high rate in patients with sepsis without signs of shock, different definitions for liver injury have been used, and short-term mortality has been evaluated (34). Second, patients with more severe liver dysfunction were included in the evaluation due to the restrictive definitions of liver dysfunction (35). The increase in serum bilirubin and alkaline phosphatase are early signs of hepatic dysfunction in patients with sepsis (4). However, although the increase of liver enzymes (aspartate and alanine aminotransferase) is associated with liver injury, it is not an indicator of a decrease in the active hepatocellular function that precedes

hepatocyte injury (36). Despite the fact that bilirubin does not have enough specificity as the only indicator of liver dysfunction, the broader definition of liver dysfunction as SOFA hepatic subscore ≥2 in our study enabled the inclusion of those with subtle septic liver injury and to evaluate the possible major effects of a low-grade injury. Third, ELD lacking symptoms in routine clinical parameters during sepsis is more common (37). Fourth, although the increased bilirubin levels-defining liver dysfunction is an independent predictor of mortality (15,32), there may be a significant time delay between liver damage and the development of hyperbilirubinemia (20).

The studies evaluating the relationship between hypomagnesemia and mortality provided differing results. In the study of Chen et al. (6) in which the 1st-day magnesium levels of critically ill patients were evaluated, when compared to normomagnesemic patients, no difference in age, gender, APACHE II scores, and other electrolyte levels was observed in the hypomagnesemic patients. However, hypomagnesemia was associated with an increased risk of mortality. In the study of Soliman et al. (38), in which the levels of ionized magnesium of critically ill patients were evaluated, although admission hypomagnesemia was not associated with clinical outcomes, ionized hypomagnesemia developing during ICU stay was associated with poor prognosis. This was explained by the strong correlation between ionized hypomagnesemia and sepsis and septic shock. In the study of Chernow et al. (39), in which they evaluated the relationship between magnesium levels and clinical outcomes in postoperative intensive care patients, mortality in patients with admission hypomagnesemia was similar to that in patients with normomagnesemia. However, the increase in mortality was evident in patients with severe hypomagnesemia. Kvarantan et al. (40) studied patients with severe hypomagnesemia upon admission to the ICU. It was shown that although the incidence of hypomagnesemia is high in ICU patients, severe hypomagnesemia is unusual and, unlike the literature, not associated with mortality. In our study, similarly to previous results, admission hypomagnesemia had no effect on the mortality of critically ill septic patients. This may be related to the mild hypomagnesemia of our patients and the administration of magnesium replacement therapy in the intensive care unit.

This study had some limitations. First, disease severity showed baseline differences between the study groups. Although we tried to control for this by covariate inclusion of APACHE II scores in the regression analysis, unmeasurable differences between groups may not have been accounted for. This might lead to variation in the magnitude of the effects observed. Second, we sampled serum magnesium levels only at admission to the ICU. Therefore, the concentrations of magnesium and their effects on septic liver dysfunction could not be evaluated during the course of the disease. Third, septic patients with hypermagnesemia were excluded from the study in line with the procedure in similar studies (23). Although hypermagnesemia occurred in only 13 of the otherwise available 191 patients, this may cause selection bias. Fourth, the assessment of magnesium status in critically ill patients was limited to serum levels. Since magnesium deficiency may occur even when magnesium levels are normal (24), misclassifying patients with hypomagnesemia may lead to bias. However, in this study, the fact that the mild to moderate hypomagnesemia present in hypomagnesemic patients was mostly associated with a true magnesium deficiency. Finally, although a risk factor that may cause confusion was excluded by the nonadmission of patients with preexisting liver disease, the deterioration in liver function, which we identified with static tests in ICU admission, may be caused by different motives, including undiagnosed liver disease. Despite these limitations, our results suggest that hypomagnesemia in septic patients might be an independent liver dysfunction risk factor. Further randomized controlled trials are needed to determine whether magnesium supplementation for the correction or prevention of hypomagnesemia reduces the development of ELD by attenuating sepsis-induced inflammation with potential immunomodulatory effects.

CONCLUSION

Hypomagnesemia was found to be associated with an increased risk of developing liver dysfunction in critically ill patients with sepsis. Admission hypomagnesemia was in association with mortality neither in all septic patients nor in those who developed ELD.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Necmettin Erbakan University (05.02.2021, 3083).

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Enquiring into Experiences of Fear, Posttraumatic Stress and Nutritional Habits of Medical Students during the COVID-19 Pandemic

Tıp Fakültesi Öğrencilerinin COVID-19 Pandemisi Sırasında Korku, Travma Sonrası Stres ve Beslenme Alışkanlıklarının İrdelenmesi

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ABSTRACT

Aim: This study aimed to determine the effects of fear of coronavirus disease 2019 (COVID-19) and post-traumatic stress disorder on eating disorders and eating habits.
Material and Methods: A cross-sectional descriptive study employed 562 (167 males, and 395 females) medical students, utilizing the fear of COVID-19 scale, COVID-19 peritraumatic distress index (CPDI), SCOFF (REZZY) eating disorders scale and Mediterranean diet adherence screener (MEDAS) scale.
Results: Of the participants, 292 (52.0%) had an adequate/balanced diet, and 339 (60.3%) students had an average sleep time of <8 hours. The REZZY scale score showed 198 (35.2%) students had an eating disorder risk, and MEDAS scores exhibited 320 (56.9%) students had incompatible diets. A significant correlation was detected between the REZZY score and the fear of COVID-19 scale score (p=0.003) and CPDI score (p<0.001). The CPDI scores of the underweight participants and those with normal body mass index (BMI) class were significantly higher (p=0.009). A significant difference was spotted in respecting REZZY scores according to BMI classes (p<0.001). The ones with normal BMI class had significantly higher MEDAS score (p=0.031). Females were 2.315 times more likely to develop eating disorders. BMI value affected the risk of eating disorders (p<0.001). When BMI (kg/m^2) increased by 1 unit, the risk of eating disorders would increase by 1.220 times. Adequate and balanced nutrition had a significant impact on the risk of eating disorders (p=0.018).
Conclusion: Assuring adequate and balanced nutrition and eliminating fears and stresses experienced during epidemics are essential.
Keywords: COVID-19 fear; eating disorders; medical students; post-traumatic stress.

ÖZ

Amaç: Bu araştırmanın amacı, koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) korkusu ve posttravmatik stres bozukluğunun yeme bozuklukları ve yeme alışkanlıkları üzerindeki etkilerinin belirlenmesidir.
Gereç ve Yöntemler: Kesitsel tanımlayıcı çalışma, COVID-19 korku ölçeği, COVID-19 peritravmatik stres indeksi (COVID-19 peritraumatic distress index, CPDI), REZZY yeme bozuklukları ölçeği ve Akdeniz diyetine uyum izleme (Mediterranean diet adherence screener, MEDAS) ölçeği kullanılarak 562 (167 erkek ve 395 kadın) tıp öğrencisiyle gerçekleştirildi.
Bulgular: Katılımcıların 292 (%52,0)'sinin yeterli/dengeli beslendiği ve 339 (%60,3) öğrencinin <8 saat ortalama uyku süresi olduğu belirlendi. REZZY ölçeği puanı 198 (%35,2) öğrencinin yeme bozukluğu riski taşıdığını gösterirken MEDAS puanı ise 320 (%56,9) öğrencinin uyumsuz diyet yaptığını ortaya koydu. REZZY puanı ile COVID-19 korku ölçeği puanı (p=0,003) ve CPDI puanı (p<0,001) arasında anlamlı bir korelasyon bulundu. Zayıf olan katılımcılarla normal vücut kitle indeksi (VKİ) sınıfında olanların CPDI puanları anlamlı derecede yüksekti (p=0,009). VKİ sınıflarına göre REZZY puanlarında anlamlı bir farklılık tespit edildi (p<0,001). Normal VKİ sınıfında olanlar anlamlı derecede yüksek MEDAS puanına sahipti (p=0,031). Kadınların yeme bozukluğu ortaya çıkma riski 2,315 kat daha fazlaydı. VKİ değeri yeme bozukluğu riskini etkilemekteydi (p<0,001). VKİ (kg/m^2) 1 birim arttığında yeme bozukluğu riski 1,220 kat artmaktadır. Yeterli ve dengeli beslenmenin yeme bozukluğu riski üzerine anlamlı bir etkisi vardı (p=0,018).
Sonuç: Salgınlarda, yeterli ve dengeli beslenmenin sağlanması ve deneyimlenen korku ve stresin bertaraf edilmesi elzemdir.
Anahtar kelimeler: COVID-19 korkusu; yeme bozukluğu; tıp öğrencileri; posttravmatik stres.

INTRODUCTION

The current day holds the fact that in particular with the rapid increase in the emergence of the relevant outcomes of globalization, an epidemic that breaks out in any part of the globe will spread in a considerably fast fashion constituting a severe threat to the whole world, evolving into a pandemic. With that being said, healthcare workers arguably suffer from the heaviest consequences of such epidemics. It is a predictable result that hundreds of thousands of individuals who will have lost their lives due to the coronavirus disease 2019 (COVID-19) pandemic all over the world will be mostly healthcare workers who are at the forefront during the fight against the pandemic (1,2). Healthcare workers are indeed concerned about infecting both themselves and their loved ones during the course of a post-apocalyptic-like environment, and thence the stress they are exposed to has both individual and societal effects, which carry the potential to cause disruptions in the(ir) service quality whilst trying to serve under that intense stress (3,4). The fact that the death rate as a result of COVID-19 among healthcare workers is higher than the mortality rate in society further reinforces the level of anxiety and fear, at the same time enhancing the perceived individual risk of COVID-19, affecting the societies and leading to anxiety, fear, and stress (5-7). A fair number of preventive measures have been ensured with a view to halting the spread of the virus, shifting the everyday lives of individuals in a rather sudden and unexpected manner. These very alterations have put medical students into one of those groups most impacted by what the pandemic brought (8). It would be fair to accentuate that those uncertainties pertaining to the fight against the disease lead to considerable fear and anxiety. Interns and trainee students, who are prospective medical doctors, have also tried to struggle against the psychological effects of all the processes in question. Verily, the educators of those medical students are doctors who are fighting the pandemic on the front line and who are psychologically affected by the unstable atmosphere and the rather hazy future (9). Thereupon, it turns out that medical students who do their practical training amidst the pandemic inescapably experience anxiety and fear. This is owing to the fact that these students who continue their medical education in the midst of the COVID-19 pandemic are a part of the group with the highest risk of transmission, like onsite healthcare workers, while endeavoring to embrace the ever-changing nature of their education (10). The ongoing apprehension and anxiety of being in this group with a high risk of contagion caused these students to have difficulty in concentrating and to be negatively influenced by psychological means (11). In addition to these, boosted levels of stress and anxiety the pandemic yielded are mirrored in the nutritional habits, particularly in those of the involved individuals. On the other hand, it goes without saying that providing adequate and balanced nutrition is one of the main determinants of health for any group and in particular for those who have the risk of experiencing stress and several sorts of difficulties in their everyday lives. On top of these, since an effective treatment for COVID-19 is not yet available as of the year 2022, escalating the immune response of individuals in the asymptomatic stage of the disease is deemed vital for the protection of health. On the flip side, maintaining an unhealthy diet and lifestyle can cause non-communicable diseases with adverse effects on COVID-19 (12). Certain emotions, to wit, stress, anxiety, and depression can be related to eating, likewise, boredom can trigger eating, elevate fat and calorie consumption, alongside one's resorting to unhealthy snacks, and may precipitate shifts in eating habits. Thereby food consumption and meal patterns of individuals may tend to become unhealthier (13). Albeit there was an observed rise in body weight in numerous individuals during the pandemic period, an inclination in embracing the Mediterranean diet, in healthy eating obsession, and even anorexia were witnessed as well (14).

When it comes to students at the tertiary level, it is apparent that the COVID-19 pandemic has caused severe stress as well as modified eating habits among these groups. In spite of the growing number of studies on eating disorders and stress in university students during the COVID-19 pandemic, it has been detected that research on medical students has not attained sufficing levels as of the year 2022. The situation with medical students all through the COVID-19 pandemic as a group with the higher risks of transmission becomes worthy of delving into for the relevant parties viz. educators, health professionals, and policymakers.

The aim of this study was then to be able to reveal the effects of fear, post-traumatic stress, and nutritional habits experienced by medical students during the COVID-19 pandemic.

MATERIAL AND METHODS

Participants and Procedures

A descriptive cross-sectional study was deployed and a total of 562 medical students, 167 males, and 395 females, aged between 22-26 years, were reached within 3 months, namely, from February to April 2021. The participants reached were students of medical faculties of various universities i.e., Ankara University, Gazi University, Ankara Medipol University, and Hacettepe University located in the capital. The snowball sampling method allowing for reaching other participants once the first participants are found was referred to in the selection of the sample. Following the construction process of the questionnaire forms, they were uploaded to Google Forms and delivered to the volunteered participants, with whom the researchers communicated through individual relationships via social media channels (i.e., WhatsApp, Facebook, Twitter, Instagram, and alike). It took approximately 20 minutes for the participants in question to complete the questionnaire.

The research protocol obtained from the ethics committee of Ankara Medipol University (14.01.2021/53), where the study was designated and carried out, is in accordance with the provisions of the Declaration of Helsinki. In addition, the participants were informed about the purpose of the present research and shared their electronic informed consent as a requirement for participation. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Instruments

In total 4 scales were determined so as to investigate the relevant perceptions as well as attitudes toward the

COVID-19 pandemic together with dietary habits, mindful eating, and tendencies of intuitive eating of the participating individuals. The aforementioned instruments made use of in the current research are classified under seven chief parts: *i)* Information that is pertinent to demographics (3 questions); *ii)* Anthropometric measurements (3 questions); *iii)* Information respecting dietary habits and health (6 questions); *iv)* Fear of COVID-19 Scale (7 items); *v)* COVID-19 Peritraumatic Distress Index (CPDI) (24 items); *vi)* SCOFF (REZZY) Eating Disorders Scale (5 items); and *vii)* Mediterranean Diet Adherence Screener (MEDAS) Scale (14 items).

The participants completed the questionnaire within around 20 minutes using digital devices, that is, PCs, tablets, or mobile phones. Upon the completion of each tool, the related tool was submitted to Google Forms and finally, the latest version of the report was imported as a Microsoft Excel file.

Fear of COVID-19 Scale

The fear of COVID-19 scale which was developed in 2020 by Ahorsu et al. (6), includes a single dimension with 7 items and there are not any reverse items. It constitutes the form of a 5-point Likert (1: strongly disagree to 5: strongly agree) and in light of the given responses, the possible scores may range from 7 to 35. The final score to be received from all of the items indicates the level of fear of COVID-19 experienced by the relevant participant. A higher score herein points to a higher level of fear of COVID-19 (6).

COVID-19 Peritraumatic Distress Index (CPDI)

The COVID-19 peritraumatic distress index (CPDI) is composed of 24 items. CPDI inquired about the frequency of anxiety, depression, specific phobias, cognitive change, avoidance and compulsive behavior, physical symptoms, and loss of social functioning in the former week. The items may be categorized further under four domains (negative mood, changes in behavior and cognitive abilities, tiredness and hyperactivity, and somatization) concerning the COVID-19 outbreak. Scale items are rated on a 5-point scale, ranging from Never: 0 to Often: 4. The total score ranges from 0 to 96. A score between 28 and 51 shows mild to moderate distress whereas a score that is ≥52 implies severe distress (15).

SCOFF (REZZY) Eating Disorders Scale

SCOFF eating disorders scale which was developed in 2010 by Hill et al. (16) allows detailed examination and analysis by screening the eating disorder risk. The scale, which mainly aims to question eating control, eating out, and body dissatisfaction, involves 5 questions. Individuals scoring 2 or more points on the scale, where 1 point is given to each item, are evaluated as the ones with the risk for eating disorders (16).

Mediterranean Diet Adherence Screener (MEDAS)

To measure individuals' adaptation to the Mediterranean diet, Martinez-Gonzalez et al. (17) came up with a scale in 2012 that has 14 questions. The sort of basic oil used by individuals in meals, the amount of olive oil consumed daily, fruit and vegetable portions, margarine-butter and red meat consumption, weekly consumption of wine, pulses, fish-sea products, nuts, cakes, tomato sauce with olive oil, and the ratio of white meat to red meat are investigated through this scale. There exist 14 questions questioning preferences in this regard. 1 or 0 points can be received for each question and the total score is calculated accordingly. Scores are evaluated as ≤5: low agreement, 6-9: moderate agreement, and ≥10: high agreement (17).

Dieter Habits Information

The number of main and snack meals consumed, the status of skipping main or snack meals, the reasons behind skipping meals, daily consumption of water, tea, and coffee, sleep duration, and activity for 30 minutes or more in the previous week were inquired into in this sense.

In this study, the information about measurements belonging to height and body weight was obtained according to the self-reports of the participants since the data were collected during the COVID-19 pandemic. Put differently, the pandemic did not let the researchers meet the participants in person or face-to-face. The body mass index (BMI) of the individuals was assessed using their height (cm) and body weight (kg). Relatively younger individuals were grouped into three as to their BMI, as underweight: <18.5 kg/m^2, normal weight: 18.5-24.9 kg/m^2, and overweight: ≥25.0 kg/m^2 (18).

Statistical Analysis

Statistical analyses were carried out utilizing a package program entitled IBM SPSS Statistics v.24. Frequency tables and descriptive statistics were turned to for the assessment of the results. Non-parametric methods were made use of for the values which did not conform to a normal distribution as per the Kolmogorov-Smirnov test. Mann-Whitney U and Kruskal-Wallis tests were applied in non-parametric tests. In the Kruskal-Wallis test, the post hoc Mann-Whitney U test was used to determine the group that caused the difference. The correlation between the scales was analyzed with the Spearman correlation test. Determination of the factors influencing the risk status of eating disorders was identified by binary logistic regression analysis by applying the backward LR model. A p value of <0.05 was considered significant.

RESULTS

The mean age of the students was 23.81±1.11 years and 55.0% (n=309) of them were ≥24 years old. 70.3% (n=395) of them were female, 97.0% (n=545) were single, and 66.5% (n=374) had normal BMI. 28.8% (n=162) of the participants pronounced that there was no change in their body weight, and 45.2% (n=254) uttered that there was an increase in body weight in the previous year. Addedly, 52.0% (n=292) reported that they had an adequate/balanced diet, 61.4% (n=345) consumed two main meals, 60.3% (n=339) slept <8 hours on average, and 55.0% (n=309) did ≥30 minutes of activity in the last one week (Table 1).

According to the REZZY scale score, 35.2% (n=198) of the participants had an eating disorder risk, and the other 64.8% (n=364) were normal. According to the MEDAS scores, 56.9% (n=320) of them were not compatible with their diets. It was figured out that 27.6% (n=155) of them had an acceptable level and 15.5% (n=87) of them had a tight fit (Table 2).

Considering the REZZY scale score, a positive, weak, and statistically significant correlation was found between the fear of COVID-19 scale score (p=0.003), and also between the CPDI scores (p<0.001), negative mood, changes in behavior and cognitive abilities, tiredness and hyperactivity, and somatization scores (Table 3).

According to the presented model, gender was found to be a parameter of a salient sort influencing the risk of eating disorders (p<0.001). It was known that females were 2.315 times more likely to have an eating disorder than males. When BMI (kg/m^2) increases by one unit, so does the risk of eating disorders, to be more specific, 1.220 times. Adequate and balanced nutritional status was determined as an eminent parameter impacting the risk of eating disorders (p<0.001). The risk of eating disorders was 1.600 times higher for those who do not have an adequate and balanced diet than those with an adequate and balanced diet. It was found that CPDI value was a pivotal factor impacting the risk of an eating disorder (p=0.018). When the CPDI value increases by 1 unit, the risk of eating disorders will do so 1.049 times (Table 4).

CPDI, REZZY, MEDAS, and fear of COVID-19 scale scores belonging to the females were significantly higher than those of the males (p<0.001). The CPDI scores of those who were underweight and with normal BMI classes were significantly higher than the ones in the overweight group (p=0.009). What is more, a statistically significant difference was detected vis-à-vis of REZZY scores according to BMI classes (p<0.001). REZZY scores of those in the underweight BMI class were significantly higher than those pertaining to the normal weight and overweight. The participants in the normal BMI class had significantly higher MEDAS scores than those who were overweight (p=0.031). The individuals in the underweight BMI class had significantly higher fear of COVID-19 scale scores than those who were overweight (p=0.026). The CPDI scores of those whose weight diminished and raised in the previous year were significantly higher than those who did not experience any changes (p=0.001). Those with reduced and raised weight had significantly higher REZZY scores than the ones who did go through any changes (p<0.001). The MEDAS scores of those with lessened and unchanged weights were identified to be statistically significantly higher than the others with lifted weights (p=0.006). No significant difference was found in fear of COVID-19 scores according to the change in weight in the last year (Table 5).

DISCUSSION

The global scale of the COVID-19 epidemic, which indeed makes it a pandemic, interferes with human health at both individual and societal levels, yielding fear and anxiety for varying groups (19). High case numbers, deaths, and fear of quarantine have all impacted health workers as members of society, who are working hard to resist the pandemic. These factors generate issues alongside unwanted consequences such as anxiety, depression, and stress for all, again exclusively for these healthcare workers (20). Healthcare workers are faced with a heavier burden in their front-line fight against COVID-19 in relation to the fact that they are most at risk of being infected, with the realms of overworking and burnout, isolation from society, and even with exposure to discrimination (21).

At this point, it is worthwhile to note that there is a significant relationship between burnout and insomnia (22). As a matter of fact, good sleep quality is invaluable for anyone and to a great extent for healthcare professionals assisting in their working better and supporting immune

Table 1. General characteristics of the participants (n=562)

Age (year), mean±SD	23.81±1.11
Age group, n (%)	
<24	253 (45.0)
≥24	309 (55.0)
Gender, n (%)	
Male	167 (29.7)
Female	395 (70.3)
Marital status, n (%)	
Single	545 (97.0)
Married	17 (3.0)
BMI (kg/m^2), mean±SD	22.63±3.82
BMI group, n (%)	
Underweight (<18.5 kg/m^2)	59 (10.5)
Normal (18.5-24.9 kg/m^2)	374 (66.5)
Overweight-Obese (≥25 kg/m^2)	129 (23.0)
Change in weight in the last year, n (%)	
Decreased	146 (26.0)
No change	162 (28.8)
Increased	254 (45.2)
Sleep time (hour), mean±SD	7.30±1.03
Sleep time, n (%)	
<8 hours	339 (60.3)
8 hours	163 (29.0)
>8 hours	60 (10.7)
≥30 minutes activity in the last week, n (%)	
Yes	309 (55.0)
No	253 (45.0)

BMI: body mass index, SD: standard deviation

Table 2. Distribution of the scale scores

Scale	Mean±SD	Median [min-max]
Fear of COVID-19	15.9±5.10	16 [7-35]
CPDI	27.1±15.24	25 [0-96]
Negative mood	7.4±3.97	8 [0-20]
Changes in BCA	7.3±4.25	12 [0-28]
TH	9.2±6.39	14 [0-28]
Somatization	3.2±3.45	13 [0-20]
REZZY	1.1±1.16	3 [0-5]
MEDAS	6.2±2.14	6 [1-13]

COVID-19: coronavirus disease 2019, CPDI: COVID-19 peritraumatic distress index, BCA: behavior and cognitive abilities, TH: tiredness and hyperactivity, REZZY: SCOFF eating disorders, MEDAS: Mediterranean diet adherence screener, SD: standard deviation

Table 3. Correlation between the scale scores

	REZZY		MEDAS	
	r_s	p	r_s	p
Fear of COVID-19	0.124	**0.003**	0.014	0.735
CPDI	0.334	**<0.001**	-0.044	0.301
Negative mood	0.234	**<0.001**	-0.020	0.637
Changes in BCA	0.331	**<0.001**	-0.042	0.319
TH	0.319	**<0.001**	-0.027	0.529
Somatization	0.211	**<0.001**	-0.056	0.183

COVID-19: coronavirus disease 2019, CPDI: COVID-19 peritraumatic distress index, BCA: behavior and cognitive abilities, TH: tiredness and hyperactivity, REZZY: SCOFF eating disorders, MEDAS: Mediterranean diet adherence screener, r_s: Spearman's rho

Table 4. Logistic regression model based on eating disorder

	β	SE	p	OR	95% CI
Gender (female)	0.840	0.253	**<0.001**	2.315	1.411-3.801
BMI (kg/m^2)	0.199	0.031	**<0.001**	1.220	1.147-1.298
Adequate diet (no)	0.470	0.199	**0.018**	1.600	1.084-2.363
CPDI	0.048	0.007	**<0.001**	1.049	1.035-1.064

BMI: body mass index, CPDI: COVID-19 peritraumatic distress index, SE: standard error, OR: odds ratio, CI: confidence interval, CCR=78.4%, $\chi^2_{(8)}$=10.184, p=0.252

Table 5. Comparison of the scale scores of the participants according to gender, BMI group, and change in weight

Gender	Male (n=167)		Female (n=395)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Fear of COVID-19	14.12±5.05	14 (7) [7-30]	16.73±4.93	16 (7) [7-35]	**<0.001**
CPDI	22.73±13.94	19 (17) [1-71]	28.92±15.40	27 (21) [0-96]	**<0.001**
REZZY	0.65±1.06	1 (2) [0-5]	1.18±1.20	1 (2) [0-5]	**0.047**
MEDAS	5.66±2.04	6 (3) [1-13]	6.43±2.14	6 (3) [1-13]	**<0.001**

BMI Group	Underweight (n=59)		Normal (n=374)		Overweight-Obese (n=129)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Fear of COVID-19	17.09±4.96[a]	17 (6) [7-33]	16.09±5.03[ab]	16 (6) [7-35]	15.03±5.28[b]	14.5 (8) [7-28]	**0.026**
CPDI	30.78±15.73[a]	31 (29) [10-74]	27.48±14.93[a]	26 (19) [0-96]	24.37±15.53[b]	19 (17.5) [1-71]	**0.009**
REZZY	0.51±1.02[a]	0 (1) [0-5]	1.14±1.18[b]	1 (2) [0-5]	1.30±1.09[b]	1 (2) [0-4]	**<0.001**
MEDAS	6.03±2.20[ab]	5 (4) [2-12]	6.34±2.17[a]	6 (3) [2-13]	5.83±1.95[b]	6 (2) [2-11]	**0.031**

Change in Weight in the Last Year	Decreased (n=146)		Unchanged (n=162)		Increased (n=254)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Fear of COVID-19	16.31±5.20	12.5 (6.5) [7-33]	15.64±5.49	16 (8) [7-35]	15.94±4.79	17.5 (9) [7-28]	0.678
CPDI	29.27±15.02[a]	21 (15.5) [0-74]	24.32±15.97[b]	20 (20.5) [2-96]	27.58±14.68[a]	27 (32.5) [2-65]	**0.001**
REZZY	1.39±1.21[a]	2 (1.75) [0-4]	0.69±1.00[b]	0 (1) [0-4]	1.22±1.16[a]	2.5 (1.75) [1-3]	**<0.001**
MEDAS	6.56±1.90[a]	7 (2.75) [2-11]	6.36±2.39[a]	6 (3.25) [1-13]	5.89±2.05[b]	4 (3.5) [1-12]	**0.006**

COVID-19: coronavirus disease 2019, CPDI: COVID-19 peritraumatic distress index, REZZY: SCOFF eating disorders, MEDAS: Mediterranean diet adherence screener, BMI: body mass index, SD: standard deviation, IQR: interquartile range, [a,b]: different superscripts denote the significant difference according to the post hoc test results

function (23). Reversing it, negative factors such as anxiety, stress, and insomnia are acknowledged to prompt an unhealthy lifestyle. Long-term stress heightens the secretion of the hormone cortisol, which in return erects the feeling of hunger in the body (24). In a study conducted with healthcare professionals, it was declared that individuals whose unhealthy eating behaviors were on the increase during the pandemic experienced a higher rate of mental health problems (25). Stress and psychological problems on account of what the pandemic effectuates can also incite sleep disturbances that worsen stress, upturn food intake, clearing the way for a vicious circle (26). Manifestly, healthcare workers are a risky group for insomnia problems. It has been announced that most of the professionals that care for COVID-19 patients experience insomnia and psychological stress symptoms (3). Through the line of relevant literature, it has been underpinned that individuals whose unhealthy eating behaviors increase during the COVID-19 pandemic are more likely to experience negative mental health symptoms like stress burden, trauma, burnout, anxiety, sleep problems, and fatigue (25). Another study pinpointed that emotional eating was common among females during the COVID-19 pandemic and the main predictors for emotional eating were dietary energy, number of meals, sugar, fat, and fast food (27). In this mentioned study, 28.8% of the participants had no change in body weight during the COVID-19 process, 45.2% had an addition in body weight in the previous year, 52.0% had an adequate/balanced diet, and 61.4% consumed two main meals. It was adjudged that 55% of them had an average of 8 hours of sleep and did ≥30 minutes of activity in the former week. The results of our study appear to support the results of this research.

Checking the REZZY scale score, 35.2% of the participants were revealed to carry the risk of eating disorders, 64.8% were normal, and according to the MEDAS scores 56.9% had incompatible diets, 27.6% had an acceptable level, and 15.5% had a tight fit. Howbeit, it is punctuated that the Mediterranean diet, with its properties of anti-inflammatory and immunomodulatory nature, might be beneficial to block or deplete the acuteness of the infection in individuals who have been negatively influenced by COVID-19. It is also proven to be a diet rich in phenolic compounds. Bearing in mind these, one could comfortably put forth that the Mediterranean diet could potentially be beneficial against infections such as COVID-19 by reason of its effects on immunity (28). In a study conducted with individuals aged 18-30 during the COVID-19 pandemic, it was unearthed that 73.5% of these participants were with moderate adherence to the Mediterranean diet, whereas the remaining adherence to the Mediterranean diet was found to be high according to the MEDAS scale (29). Speaking of the REZZY score and fear of COVID-19 and CPDI, a positive, weak, and statistically significant correlation was found between negative mood, changes in behavior and cognitive abilities, tiredness and hyperactivity, and somatization scores. As for the fear of COVID-19 scale and sub-dimensions of CPDI, it is understood that as negative mood, changes in behavior and cognitive abilities, tiredness and hyperactivity, and somatization score increased, REZZY eating disorder score increased.

In this study, those with underweight and with normal BMI had higher CPDI scores than those with overweight. The CPDI scores of those who did not have adequate and balanced nutrition were found to be higher than those who had a balanced diet. This draws attention once again to the role and place of adequate and balanced nutrition, which was constantly emphasized during the course of the pandemic. One study proclaimed that the strongest predictor of dietary restriction and/or a binge eating event was among concomitant eating disorder symptoms. It has

been disclosed that persons having pre-existing eating concerns are at risk of being engaged in problematic eating behaviors throughout the time of an emerging issue such as COVID-19 (30). A possible eating disorder was spotted in 26.5% of the participants who made at least one dietary alteration in view of the pandemic. It has been promulgated that the participants with eating disorders have significantly higher levels of depression and anxiety symptoms yet lower levels of psychological well-being (31). In another study conducted with university students, the prevalence of REZZY was 20.5%. This rate was spotted to be higher in females, in overweight and obese groups. Allegedly, this situation might be related to stress and depression (32). In this study, the prevalence was found to be 35.2%. The fact that the faculty of medicine offers a long and difficult education process, the reality of meeting with patients one-on-one as well as the long working hours of night shifts may be the reasons for the higher frequency of eating disorders. The psychological distress experienced by healthcare professionals in the wake of working under risky conditions around the time of the pandemic also upraises by virtue of the uncertain nature of the disease, the rate of transmission, and also the related measures applied (33). In a study, it was seen that health workers developed post-traumatic stress disorder during the period when they were exposed to infected people in epidemics (34). One remarkable note to share is the reason why females get COVID-19 less than males is that their immune systems are stronger than males and they are less affected by viral infections (35). Advanced age, which is a risk factor, is influential for males more, and the loss of function in the immune system occurs more rapidly in males than females (36). Depression and academic stress related to COVID-19 have been associated with eating disorders (32). In this study, a statistically significant positive result was spotted between the REZZY score and fear of COVID-19 and CPDI scores. As the scores obtained from the scales get higher, the REZZY score increases. A study designated in Croatia underlined moderate adherence to the Mediterranean diet during the COVID-19 pandemic, with an increase in MEDAS scores after closure. The majority of the participants with higher dietary adherence were female, with the highest education level and with normal BMI reported (37). Along similar lines, in our study, compliance was discovered to be higher in females, those who had an adequate and balanced diet, those with normal BMI, and those who did physical activity. In the presence of other factors required for a healthy life, greater compliance with the Mediterranean diet is an expected result. The higher compliance in females may be on the basis of the fact that they attach more importance to body appearance and healthy life. In a study, adherence to the Mediterranean diet was found to be inversely proportional to increasing (38). Other studies have pointed out that obesity is also ascribed to the augmented severity of COVID-19 (39). It has also been annunciated that each unit expansion in BMI is attributed to a 12% extension in the risk of serious COVID-19 disease (40). In a study carried out in France, excess body weight (BMI ≥25 kg/m2) was also discerned to be significantly linked to COVID-19 severity (41). In our study, it was concluded that those who did not comply with the Mediterranean diet were in the obese group. With that being said, the socioeconomic status of the students participating in our study and if they live with their families or not is not known are the two major limitations. In this study, among the factors influencing the risk status of eating disorders, gender, BMI, and adequate and balanced nutrition come to the fore. The instinct of females to struggle to cater to all of their family members and carry too many responsibilities can make them more anxious during the pandemic period. In this direction, it may be necessary to provide comforting psychological and social support to females. The low CPDI scores of those with high BMIs also raise the question of whether they pay enough attention to epidemic measures. In this context, it is essential to plan novel research with these groups and to take protective measures in line with the results. The findings of this study seem to be consistent with some other studies showing that the COVID-19 outbreak causes more fear and psychological effects in females (42,43). Research denotes that females scored significantly higher than males regarding the fear of COVID-19 (44) and being a female is an indicator of moderate to high fear of COVID-19 (42). Correspondingly, anxiety rates were found to be approximately 3 times higher (45) and mild/moderate and severe peritraumatic distress was located in one-third of the participants. Those in quarantine shared less distress than those who were not (46). In a study conducted using the CPDI and the fear scale, the mean scores of females were perceived to be higher in both scales. It has also been delineated that anxiety levels, depression, and stress rates are high in females and young people, and this difference may be by dint of the inability of males to express their fears due to gender roles (47). In another study with healthcare professionals, and using CPDI, it was articulated that females had relatively higher mild-to-moderate stress (42.7% vs 31.4%) compared to males, with a severe stress ratio of 10.4% vs. 4.3% (48). In this study, when the sub-dimensions of the CPDI scale were examined, negative mood, tiredness and hyperactivity, and somatization were determined commensurate with the total score of the scale. It was expressed that females had higher scores than males, and singles had a higher change in behavior and cognitive abilities scores than married ones. This may be in consequence of the fact that married people exhibit a more stable situation based on their family responsibilities.

In plentiful studies performed in the literature during the pandemic, it is clear that health workers experience fear of contamination and death when working under high risk, and thereupon they are inclined to develop psychological disorders, namely, depression, anxiety, and stress. As the fear of COVID-19 scale score increases, the stress scale value increases. It can be put forward that health workers experience similar psychological problems since, on top of all the other things, they feel that they are stigmatized and discriminated against in this process. This situation may also give rise to stress factors (49-51). All in all, our findings are mostly supported by the literature.

CONCLUSION

In the development of not only epidemics but also innumerable other diseases, especially chronic diseases, adequate and balanced nutrition is humans' most essential defense weapon. In truth, the most basic approach to

warrant adequate and balanced nutrition is the condition of accessing sufficient healthy food for those living in society. To be able to make sure the said desired to act, all stakeholders from decision makers to researchers need to take over vital duties. In order for such an understanding of nutrition to be fully embraced and internalized by society, trainings on the subject should be given to raise the awareness of the shareholders and healthy nutrition should be made a part of the lifestyle. Lastly, we hold the belief that it would be beneficial to support mental health within the scope of preventive measures, as the psychological distress experienced by individuals during the pandemic process may have longer-term side effects.

Ethics Committee Approval: The study was approved by the Non-Interventional Ethics Committee of Ankara Medipol University (14.01.2021, 53).

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The Change in the Susceptible Populations with the Shift in Hepatitis A Epidemiology

Hepatit A Epidemiyolojisi ve Duyarlı Nüfusta Değişim

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ABSTRACT

Aim: The incidence of hepatitis A (HepA) has decreased due to vaccination and improved hygiene conditions. However, the age of onset of the disease has shifted from childhood to adulthood. Children with HepA are mildly symptomatic, whereas the course of the disease in adults may be severe. The aim of this study was to examine the change in HepA seroprevalence and identify the population susceptible to HepA.

Material and Methods: A total of 10132 patients who were tested anti-Hepatitis A virus immunoglobulin G (anti-HAV IgG) between 2016 and 2019 were reviewed retrospectively, and included in this study. The patients were divided into five groups according to their age, and seropositivity rates were compared between age groups. The relevant data of the healthcare professionals were also evaluated separately.

Results: The overall seropositivity rate was 60.1% (n=6088). The seropositivity rate was found 29.0% (n=944) in the 18 to 24 years range, 49.7% (n=837) in the 25 to 29 years range, 60.6% (n=689) in the 30 to 34 years range, 76.6% (n=784) in the 35 to 39 years range, and 93.3% (n=2834) in the ≥40 years groups. The seropositivity rate was found 36.1% (n=1781) and 82.9% (n=4307) in patients <30 and ≥30 years groups, respectively (p<0.001).

Conclusion: In recent decades, there has been a significant change in HepA seroprevalence. This change has resulted in the emergence of a young adult population susceptible to possible HepA outbreaks. Thus, seronegative young adults may be considered at risk for HepA and routine vaccination may be considered.

Keywords: Hepatitis A; hepatitis A virus; hepatitis A vaccine.

ÖZ

Amaç: Aşılama ve hijyen koşullarının iyileşmesi sayesinde hepatit A (HepA) insidansı azalmıştır. Bununla birlikte, hastalığın başlangıç yaşı çocukluk çağından yetişkinlik çağına doğru kaymıştır. HepA olan çocuklar hafif semptomatik iken erişkinlerde ise hastalığın seyri şiddetli olabilir. Bu çalışmanın amacı, HepA seroprevalansındaki değişimi incelemek ve HepA'ya duyarlı olan popülasyonu belirlemektir.

Gereç ve Yöntemler: Bu çalışmada, 2016 ve 2019 yılları arasında anti-Hepatit A virüsü immünoglobulin G (anti-HAV IgG) testi çalışılmış olan toplam 10132 hastanın verileri geriye dönük olarak incelendi ve çalışmaya dahil edildi. Hastalar yaşlarına göre beş gruba ayrıldı ve bu yaş grupları arasında seropozitiflik oranları karşılaştırıldı. Sağlık çalışanlarının ilgili verileri de ayrıca değerlendirildi.

Bulgular: Genel seropozitiflik oranı %60,1 (n=6088) idi. Seropozitiflik oranı 18 ile 24 yaş aralığında %29,0 (n=944) olarak, 25 ile 29 yaş aralığında %49,6 (n=837) olarak, 30 ile 34 yaş aralığında %60,6 (n=689) olarak, 35 ile 39 yaş aralığında %76,6 (n=784) olarak ve ≥40 yaş grubunda %93,3 (n=2834) olarak bulundu. Seropozitiflik oranı, <30 ve ≥30 yaş gruplarında, sırasıyla, %36,1 (n=1781) ve %82,9 (n=4307) olarak bulundu (p<0,001).

Sonuç: Son yıllarda, HepA seroprevalansında önemli bir değişiklik olmuştur. Bu değişiklik, olası HepA salgınlarına duyarlı olan genç bir yetişkin popülasyonun ortaya çıkmasına neden olmuştur. Bu nedenle seronegatif genç yetişkinler HepA açısından risk altında kabul edilebilir ve rutin aşılama yapılması düşünülebilir.

Anahtar kelimeler: Hepatit A; hepatit A virüsü; hepatit A aşısı.

INTRODUCTION

Approximately 1.5 million people worldwide are infected with the hepatitis A virus (HAV) annually. However, the actual number is estimated to be ten times higher (1). HAV is transmitted through the consumption of contaminated water and food and contact with infected persons (2).

Hepatitis A (HepA), defined as liver parenchyma infection caused by HAV is the most common form of acute viral hepatitis. Children with HepA are mildly symptomatic, whereas adults with HepA may have a serious clinical course, which may require hospitalization. Acute hepatitis usually resolves within two months. However, in approximately 10% of the patients, it may persist for up to 6 months or relapse. Rarely, it may also result in fulminant hepatitis and death (3).

HepA is a preventable disease by vaccination. Including the HAV vaccine in the national vaccination programs (NVP) and vaccinating people who have risk factors is recommended for countries in highly and moderately endemic regions (4). In accordance with these recommendations, the Ministry of Health of the Republic of Turkey included the HAV vaccine in its childhood NVP in 2012.

In addition to NVP, improved infrastructure and sanitary conditions caused a shift in the onset of HepA from childhood to adulthood (5). Therefore, it has been speculated that a group of adolescents and young adults who are not vaccinated may become susceptible to HAV, and thus, in a possible epidemic, loss of workforce, increased treatment costs, and increase in HepA-related morbidity and mortality may occur (6).

Most of the epidemiological studies on HepA in Turkey were conducted during the period when the HepA vaccine was not included in NVP (7-9). Only a few epidemiological studies examined the changes in the prevalence of HepA in adolescents and young adults who were not included in NVP. However, these studies may not fully reflect the changes in age-related HepA seroprevalence since they were conducted before the inclusion of the HAV vaccine in NVP (10-12).

This study aimed to examine the changes in HepA seroprevalence and to identify the population susceptible to HepA.

MATERIAL AND METHODS

This study was designed as retrospectively in İstanbul Medeniyet University Göztepe Training and Research Hospital. A total of 12814 patients who were tested for anti-Hepatitis A virus immunoglobulin G (anti-HAV IgG) between January 2016 and January 2019 were included. Patients under 18 years of age and repetitive test results were excluded. The remaining 10132 patients were included. Considering people under the age of 40 as young adults, we formed four groups under the age of 40 and one group over the age of 40. In this direction, the patients were divided into five groups according to their age; 18 to 24 years, 25 to 29 years, 30 to 34 years, 35 to 39 years, and over the age of 40. Based on previous studies, we divided the entire population into two groups as those under 30 years of age and above, since we considered those under 30 as the susceptible population (7-10). The serum samples were analyzed by the chemiluminescence microparticle immunoassay (Architect i2000, Abbott, U.S.) method. Per the manufacturer's instructions, anti-HAV IgG signal to cut-off (S/Co) values of less than 1 were considered negative, and greater than or equal to 1 were considered positive. The HAV-IgG seropositivity was compared between the groups.

The present study was performed in accordance with the principles of the Declaration of Helsinki, and the relevant national laws. The study protocol was approved by the Ethics Committee of İstanbul Medeniyet University with the decision number 2019/142).

Statistical Analysis

Descriptive statistics were reported as median, interquartile range, and minimum-maximum for numerical variables that were determined not to conform to the normal distribution, and as numbers and percentages for categorical variables. The Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests were used to analyze the normality of numerical variables. The Mann-Whitney U test was used to compare two independent groups. In order to compare the categorical variables, Pearson's chi-squared test, and Fisher's exact test were used in 2x2 tables, and the Fisher-Freeman-Halton test was used in RxC tables. Jamovi project (Jamovi, version 2.2.5.0, 2022, retrieved from https://www.jamovi.org), Jeffreys' Amazing Statistics Program (JASP) software package (version 0.16.1, retrieved from https://jasp-stats.org), and R-project 4.1.3 for Windows (R Core Tea, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2022, retrieved from https://www.R-project.org) software package were used in the statistical analysis. A p value of ≤ 0.05 was deemed to indicate statistical significance.

RESULTS

A total of 10132 patients who were tested for anti-HAV IgG between January 2016 and January 2019 were included. The patient characteristics were presented in Table 1. The overall anti-HAV IgG seropositivity rate was 60.1% (n=6088). The patients with anti-HAV IgG positivity were significantly older ($p<0.001$). In the subgroup analysis considering the age of the five groups, the groups also differed significantly in terms of the distribution of the anti-HAV IgG-positivity ($p<0.001$). The frequency of anti-HAV IgG-positivity increased from 29.0% to 93.3% as the age groups got older (Figure 1). The seropositivity rate was found as 29.0% (n=944) in the 18 to 24 years range group, 49.7% (n=837) in the 25 to 29 years range group, 60.6% (n=689) in the 30 to 34 years range group, 76.6% (n=784) in the 35 to 39 years range group, and 93.3% (n=2834) in the age of ≥40 years group. When the age was considered in two groups, <30 and ≥30 years old, the anti-HAV IgG-positivity rate was found as 82.9% (n=4307) in patients ≥30 years old, while it was 36.1% (n=1781) in patients <30 years old. A significantly higher prevalence of HAV infection was noted in patients ≥30 years old (OR=8.570, 95% CI: 7.812-9.402, $p<0.001$). There was a significantly higher number of male patients than female patients among the patients with anti-HAV IgG-positivity ($p<0.001$). While the rate of anti-HAV IgG-positivity was 66.6% (n=3108) in male patients, it was 54.5% (n=2980) in female patients. A significantly higher anti-HAV IgG-positivity was noted in male patients (OR=1.661, 95% CI=1.532-1.801, $p<0.001$). In

Table 1. Demographic and clinical characteristics of the patients

	Anti-HAV IgG Positivity (n=6088)	Anti-HAV IgG Negativity (n=4044)	p
Age (year), median (IQR), [min-max]	44 (35-54) [18-98]	22 (20-26) [18-77]	**<0.001**
Age groups, n (%)			
18-24 years	944 (29.0)	2306 (71.1)	
25-29 years	837 (49.7)	848 (50.3)	
30-34 years	689 (60.6)	448 (39.4)	**<0.001**
35-39 years	784 (76.6)	240 (23.4)	
≥40 years	2834 (93.3)	202 (6.7)	
Age group, n (%)			
<30 years	1781 (36.1)	3154 (63.9)	**<0.001**
≥30 years	4307 (82.9)	890 (17.1)	
Gender, n (%)			
Male	3108 (66.6)	1560 (33.4)	**<0.001**
Female	2980 (54.5)	2484 (45.5)	
Occupation, n (%)			
Healthcare Professionals	2302 (62.6)	1373 (37.4)	**<0.001**
Others	3786 (58.6)	2671 (41.4)	

Anti-HAV IgG: anti-Hepatitis A virus immunoglobulin G, IQR: interquartile range (25th-75th percentile)

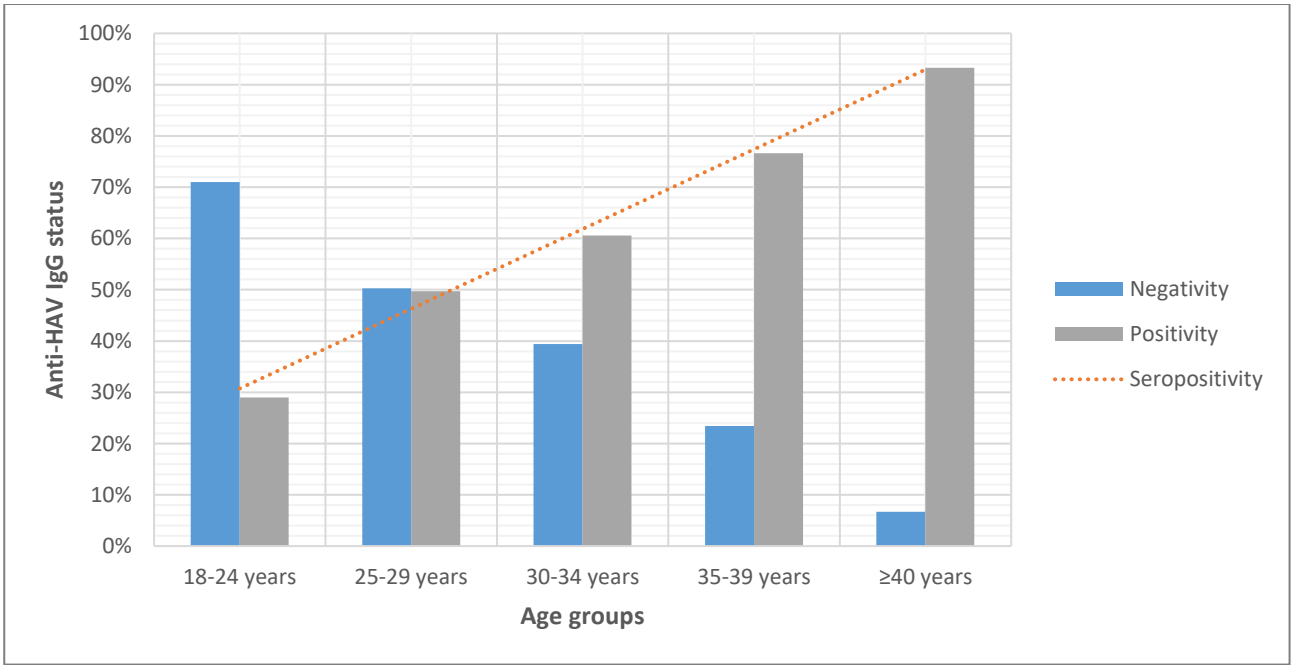

Figure 1. Distribution of patients' anti-HAV IgG characteristics by age groups

addition, there was a significantly higher number of healthcare professionals among patients with anti-HAV IgG-positivity (n=2302, 62.6% vs. n=3786, 58.6%, OR=1.183, 95% CI: 1.088-1.285, p<0.001).

The seropositivity rate among the healthcare professionals was 62.6% (n=2302/3675). The seropositivity rate of the healthcare professionals aged ≥30 years was 79.0% (1445/1829). The prevalence of HAV infection was also significantly higher among healthcare professionals aged ≥30 years (OR=4.343, 95% CI=3.756-5.020, p<0.001) than that among healthcare professionals aged <30 years (Table 2, Figure 2).

DISCUSSION

In the presented study, we investigated the anti-HAV IgG characteristics of a large cohort of patients in our tertiary care referral center which resides in the highly populated and cosmopolitan city of Istanbul. The anti-HAV IgG characteristics of the patients included in the study were analyzed to detect the HepA seroprevalence according to the age groups and determine the population susceptible to HepA. We demonstrated that the seropositivity in younger age groups was low and increased with age.

Globally, the incidence of HAV has decreased in the last two decades due to increased accessibility to clean water

Table 2. The distribution of anti-HAV IgG characteristics in healthcare professionals aged<30 and ≥30 years

	Anti-HAV IgG Positivity (n=2302)	Anti-HAV IgG Negativity (n=1373)	p
Age group, n (%)			
<30 years	857 (46.4)	989 (53.6)	**<0.001**
≥30 years	1445 (79.0)	384 (21.0)	

Anti-HAV IgG: anti-Hepatitis A virus immunoglobulin G

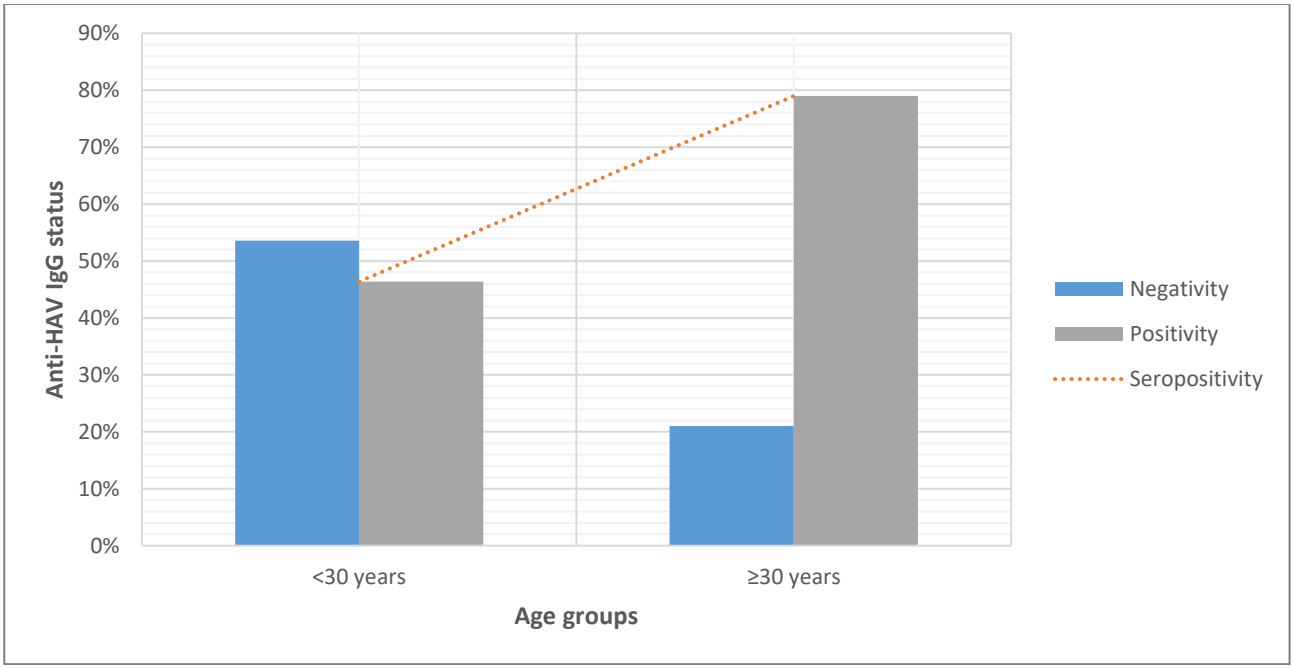

Figure 2. Distribution of healthcare professionals' anti-HAV IgG characteristics by age groups

resources and improved sanitary conditions (5). Similarly, the incidence of HepA tends to decrease in Turkey but still, the seropositivity rate in the general population was found to be between 74-91% (8-10). In comparison, the seropositivity rate was found as 60% in this study.

The frequency of HepA infection is closely related to the countries' development level and socioeconomic status. In this context, HepA seropositivity in young adults has gradually decreased in developed countries yet remains high in developing countries (13). HepA seropositivity rate in the 20-25-year-old age group was 13% in North America, 35% in Western Europe, 91% in South Asia, 83% in the Middle East and North Africa, and 100% in Sub-Saharan Africa (5).

In a study conducted in South Korea, seropositivity was found to be low in young adults (12.7%, 16.0%, and 26.7% in 20-24, 25-29, and 30-34-year-old age groups, respectively). On the other hand, it was observed that the incidence of acute HepA increased in individuals aged 20-39 years and that severe hepatitis clinic-related hospital admissions and treatment costs increased with age (14). The range of seropositivity rate in the below-30-year-old age group reported in the studies that investigated the seroprevalence by age in Turkey was 57.1-83.6% (8-10). In a prospective study with 630 individuals in Istanbul in 2012, the seropositivity rate in the 20-24-year-old age group was 69% (7). In comparison, the seropositivity rates of our cohort in the 18-24- and 25-29-year-old age groups were 29.0% and 49.7%, respectively. The seropositivity rate was found as 36.1% in the below-30-year-old age group in our study, indicating a significant difference between the below-30 and over-30-year-old age groups in that respect.

Given that there was a significantly lower number of patients with seropositivity among young adults can be attributed to the vaccination campaign intended for children, which plays an important role in contagiousness, and the improvement of sanitary conditions.

HepA incidence in the European Union Countries also decreased between 1997 and 2011, from 10.0 to 2.5 per 100,000 population. However, outbreaks have been reported in recent years due to travel to highly endemic areas, frozen imported foods, and risky sexual behavior (15). In 2017, 649 HepA cases were reported in the California State of U.S. as part of the epidemic originating from homeless people. Of these cases, 417 people were hospitalized, and 21 died (16). These data indicate that even in developed countries, the vaccination rate of people with risk factors remains low, and the disease may be disregarded.

The healthcare workers are considered to be in the risk group for HAV infection. In the study by Kutlu et al. (17) conducted with students in the faculty of dentistry, HAV IgG-positivity was found to be 24.9%, and the majority of the students were found to be susceptible to HepA. In comparison, the seropositivity rate in healthcare professionals in the below-30-year-old age group in this study was 46.4%. The difference between the seropositivity rates in our study may be attributed to the fact that the students are typically aged below 25. The serology of healthcare professionals in the high-risk group should be analyzed, and adequate efforts should be made, especially for the immunization of young healthcare professionals.

HepA vaccine is very effective and safe in preventing the disease. Healthcare professionals, sewer workers, travelers to countries located in highly endemic regions, chronic liver disease patients, and men who have sex with men are considered risky groups for HepA and are recommended to have the HepA vaccine (18,19). Other than these risk groups, the HepA vaccine was not found to be cost-effective in studies conducted for routine vaccination of adults with the HepA vaccine. However, these studies were carried out only in countries located in slightly endemic regions and did not cover the countries located in moderately endemic regions such as Turkey (20,21) and it

is a shortcoming that the riskier seronegative adults living in countries located in the moderately and highly endemic regions are not recommended to have the vaccine. Additionally, the refugee influx from the Middle East, which is a highly endemic region, to moderately to slightly endemic countries, including Turkey, increases the risk for HepA outbreaks even more.

Including young adults in the national vaccination programs will raise concerns about the increase in health expenditures. However, it may be possible to reduce the cost of vaccination by maintaining the effectiveness of immunization. As a matter of fact, Curran et al. (22) demonstrated the efficacy and safety of a single dose of the HAV vaccine using a mathematical modeling method in the Mexican Public Health System. In studies, the effectiveness of the vaccine was found to be >98% in the first 10 years with a single dose vaccination. However, it was observed that antibody titers decreased in the following years. Double-dose vaccination is preferred because there is insufficient data on single-dose vaccination. There is a need for studies evaluating the cost-effectiveness of a single dose of the HepA vaccine (23,24).

CONCLUSION

Including young adults susceptible to HepA in the groups recommended for being vaccinated with the HAV vaccine may be considered. Further studies on single-dose HAV vaccination are needed in the context of reducing the cost of vaccination. Additionally, prospective studies are needed to evaluate the epidemiology and prognosis of acute HepA in adults and the cost-effectiveness of vaccinating seronegative young adults.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of İstanbul Medeniyet University (27.03.2019, 142).

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Ellagic Acid Inhibits TGFβ1/Smad-Induced Renal Fibrosis in Diabetic Kidney Injury

Elajik Asit, Diyabetik Böbrek Hasarında TGFβ1/Smad Kaynaklı Böbrek Fibrozisini İnhibe Eder

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ABSTRACT

Aim: Free radical formation increases due to hyperglycemia occurring in the pathogenesis of diabetes mellitus (DM), and as a result, oxidative stress occurs. Hyperglycemia-mediated oxidative stress plays an important role in the pathogenesis of diabetic nephropathy. The antihyperglycemic, antioxidative, anti-apoptotic, and anti-inflammatory effects of ellagic acid (EA) have been demonstrated by many studies. In this study, it was aimed to demonstrate the antifibrotic effect of EA on TGFβ1/Smad signaling in rats with streptozotocin induced diabetic nephropathy.
Material and Methods: A total of 24 male Sprague Dawley rats, weighing 200-250 g, were used in this study. The animals were divided into four groups as control, EA, DM, and DM+EA. The kidney tissues were used for histological and immunohistochemical procedures. While the collagen density in kidney tissues was revealed by Masson's trichrome staining, the expression levels of fibrotic markers TGFβ1, p-Smad3, and αSMA were determined by the immunocytochemical method.
Results: It was shown that the collagen density in the renal tissue of the DM group increased significantly in the intertubular area, while the collagen density in the EA-treated DM group was statistically significantly decreased. When TGFβ1, p-Smad3, and αSMA immunopositivity in kidney tissue sections of all groups were evaluated, the highest staining intensity was in the DM group, while the intensity of staining was close to the control group in the treatment group. It was observed that αSMA, TGFβ1, and p-Smad3 protein expression were down-regulated with EA treatment.
Conclusion: EA reduced fibrosis in diabetic nephropathy by returning profibrotic parameters to normal levels.
Keywords: Diabetes mellitus; ellagic acid; fibrosis; kidney; TGFβ/smad.

ÖZ

Amaç: Diabetes mellitus (DM) patogenezinde meydana gelen hiperglisemi nedeniyle serbest radikal oluşumu artar ve bunun sonucunda da oksidatif stres meydana gelir. Hiperglisemi aracılı oksidatif stres, diyabetik nefropatinin patogenezinde önemli bir rol oynar. Elajik asit (EA)'in antihiperglisemik, antioksidatif, antiapoptotik ve antiinflamatuar etkileri birçok çalışma ile gösterilmiştir. Bu çalışmada, streptozotosin ile indüklenen diyabetik nefropatili ratlarda EA'nın TGFβ1/Smad sinyalizasyonu üzerine antifibrotik etkisinin gösterilmesi amaçlandı.
Gereç ve Yöntemler: Bu çalışmada, ağırlığı 200-250 g arasında olan toplam 24 adet erkek Sprague Dawley cinsi sıçan kullanıldı. Hayvanlar kontrol, EA, DM ve DM+EA grupları olmak üzere dört gruba ayrıldı. Böbrek dokuları histolojik ve immünohistokimyasal prosedürler için kullanıldı. Masson trikrom boyaması ile böbrek dokularındaki kollajen yoğunluğu ortaya koyulurken, fibrotik belirteçler olan TGFβ1, p-Smad3 ve αSMA'nın ekspresyon seviyeleri ise immünositokimyasal yöntem ile belirlendi.
Bulgular: DM grubunun böbrek dokusundaki kollajen yoğunluğunun intertübüler alanda önemli bir ölçüde arttığı gösterilirken, EA ile tedavi edilen DM grubunda ise kollajen yoğunluğunun istatistiksel olarak anlamlı bir derecede azaldığı gösterildi. Tüm grupların böbrek doku kesitlerinde TGFβ1, p-Smad3 ve αSMA immünopozitifliği değerlendirildiğinde ise en yüksek boyanma yoğunluğu DM grubunda olurken, tedavi grubunda boyanma yoğunluğu ise kontrol grubuna yakındı. αSMA, TGFβ1 ve p-Smad3 protein ekspresyonunun EA tedavisi ile aşağı regüle edildiği gözlendi.
Sonuç: Elajik asit, diyabetik nefropatide profibrotik parametreleri normal seviyelere döndürerek fibrozu azaltmıştır.
Anahtar kelimeler: Diabetes mellitus; elajik asit; fibrozis; böbrek; TGFβ/smad

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease associated with glucose intolerance and hyperglycemia. The most common complication of DM is diabetic nephropathy (1,2). Among the pathological changes of diabetic nephropathy, glomerulosclerosis, tubular inflammation, tubular atrophy, and inter-tubular fibrosis are common (3). Renal fibrosis seen in diabetic nephropathy may damage tissue and thus organ function and finally lead to kidney failure (4,5). Renal fibrosis is associated with transforming growth factor β (TGFβ) / suppressor of mothers against decapentaplegic (Smad) signaling. In diabetic conditions, elevated glucose and its metabolites induce TGFβ1 expression (6,7). The interaction of TGFβ1 with its receptor leads to the uptake of Smad2/3 (8,9). Subsequently, the phosphorylated Smad2/3 complex is translocated to the nucleus, enabling the expression of genes such as fibronectin and α-smooth muscle actin (αSMA) (10,11). Accumulation of fibronectin and αSMA proteins in the glomerular and inter-tubular parts cause interstitial fibrosis. Blocking TGFβ/Smad signaling has been reported to be effective in preventing the progression of diabetic kidney disease (12,13).

Although the etiology of complications of DM is variable, it has not been fully elucidated. However, it is widely accepted that diabetes is a source of oxidant stress (14,15). Ellagic acid (EA) is a polyphenolic compound found in many foods, including raspberries, strawberries, blackberries, pomegranates, cranberries, and walnuts. EA has been shown to have strong antioxidant, anti-aging, anti-proliferative, anti-atherosclerotic, anti-cancer, and anti-mutagenic properties in-vivo and in-vitro studies (16-19). In addition, some experimental studies have shown that EA has antidiabetic and antihyperglycemic effects that reduce glucose intolerance (20,21).

This study aimed to contribute to the elucidation of the effect of EA on the renal fibrosis pathway by investigating the effect of EA on diabetic kidney damage. For this purpose, the expressions of the proteins involved in the fibrosis pathway were revealed by histological and immunohistochemical methods.

MATERIAL AND METHODS

Animals and Experimental Groups

In this study, a total of 24 male Sprague Dawley rats, weighing 200-250 g, were used. This experimental study was approved by the Animal Experiments Local Ethics Committee of Gazi University (G.Ü.ET-22.083). The animals were fed at standard temperature (~24° C) on a 12 h light/dark cycle. Diabetes was induced in animals with a single dose (55 mg/kg) of streptozotocin (STZ) (CAS 18883-66-4, Santa Cruz, CA) injection. Blood glucose level was measured 48 hours after STZ administration. Animals with blood glucose levels of 250 mg/dl were included in the DM group. Diabetic status was confirmed for 8 weeks. The animals were divided into four groups of six animals each: Control, EA, DM, and DM+EA (treatment). EA (sc-202598A, Santa Cruz, CA) was prepared by dissolving in 0.2% dimethyl sulfoxide (DMSO). EA and DM+EA groups were given 100 mg/kg/day orally for 35 days (21,22). After the experiments, all rats were euthanized. The kidneys were used for histological and immunohistochemical procedures.

Histological Analysis

After routine histological procedures, kidney tissues were fixed in 10% formalin and embedded in paraffin. Then, 4 µm thick sections taken from the paraffin block were stained with Masson's trichrome (GBL, 5022, Turkey) for histological evidence of fibrosis. The fibrotic area ratios in the x200 magnification images obtained with the light microscope were calculated with the Image J program.

Immunohistochemistry

Deparaffinized sections were retrieved at high temperatures with citrate buffer (pH, 6.0). Sections were incubated first with 3% hydrogen peroxide (TA-125-HP, Thermo Scientific, USA) and then with Ultra V block (TP-125-HL, Thermo Scientific, USA). Then, the sections were incubated with TGFβ1 primer antibody (bs-0086R, rabbit polyclonal, Bioss Inc., USA), p-Smad3 primer antibody (bs-3425R, rabbit polyclonal, Bioss Inc., USA), and αSMA primer antibody (bsm-52392R, rabbit monoclonal, Bioss Inc., USA) at 1:200 dilution for overnight at 4°C. Sections were then processed according to the secondary antibody kit (TP-125-HL, Thermo Scientific, USA) protocol. The coloration results from the reaction with the aminoethyl carbazole (AEC) (TA-125-HA, Thermo Scientific, USA) chromogen became visible. Background staining was done with Mayer's hematoxylin and the sections were evaluated under a light microscope. In each section, 6 areas were randomly determined at x200 magnification. Immunopositivity values in the areas were determined as a percentage using the Image J program. Two methods were used for negative controls. The first was primary antibody exclusion, and the other was the use of normal rabbit IgG (bs-0295 P, Bioss Inc., USA) as the negative reagent control (NRC).

Statistical Analysis

Mean, standard deviation, median, minimum and maximum values were used to define the variables. Data distribution was evaluated by the Shapiro-Wilk test. The Kruskal-Wallis test was used for univariate analyzes of the variables in the study. Pairwise comparisons of groups with significant differences were made using the Mann-Whitney U test and evaluated by applying Bonferroni correction (0.05/group number). Statistical analyzes were performed using IBM SPSS Statistics 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Armonk, NY), and $p<0.05$ was considered statistically significant.

RESULTS

Histological Results

According to Masson's trichrome staining results, while dense collagen structure was remarkable in the DM group, this density decreased in the treatment group. Collagen density in the EA group was statistically significantly decreased compared to the control group ($p=0.003$). The DM group showed a statistically significant increase in areas of fibrosis compared to the control and EA groups (both $p<0.001$). In addition, the fibrotic area value in the treatment group was statistically significantly decreased compared to the DM group ($p<0.001$, Table 1, Figure 1, 3A).

Immunohistochemical Results

When TGFβ1, p-Smad3, and αSMA immunopositivity in kidney tissue sections of all groups were evaluated, it was observed that the highest staining was in the DM group, while the intensity of staining was close to the control group in the treatment group (Table 2, Figure 2).

Table 1. The descriptive values of the fibrotic area of all groups (%)

		Control	EA	DM	DM+EA	p
Fibrotic Area	mean±SD	4.04±1.18	3.11±1.52	14.08±4.11	8.33±2.43	**<0.001**
	median [min-max]	4.10 [1.62-6.56]	2.78 [0.46-6.51]	15.32 [6.25-20.64]	8.25 [5.09-14.66]	

EA: ellagic acid, DM: diabetes mellitus, SD: standard deviation

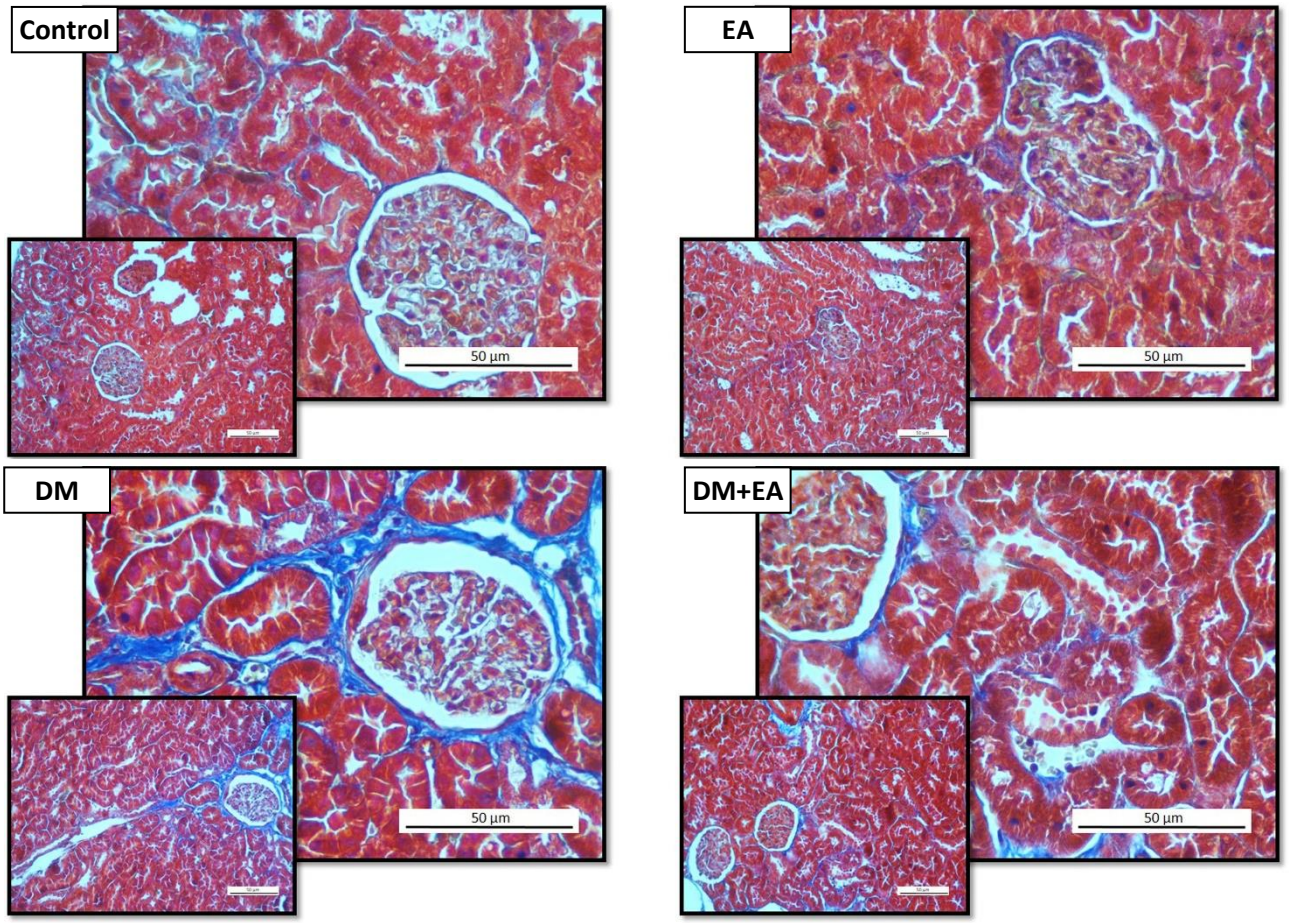

Figure 1. Representative microphotographs showing the kidney. Collagen deposition is highlighted in blue on kidney tissues of control, EA, DM, and DM+EA groups by Masson's trichrome staining (x400; thumbnail x200). EA: ellagic acid, DM: diabetes mellitus

When the TGFβ1 immunopositivity density was evaluated statistically, the values of the EA group showed a statistically significant decrease compared to the control group (p=0.006). The immunostaining intensity of the DM group increased significantly compared to the control and EA groups (both p<0.001). The decrease in staining intensity in the treatment group was statistically significant compared to the DM group (p<0.001) (Figure 2, 3B).

When the p-Smad3 immunopositivity density was evaluated statistically, there was no significant difference between the control and EA group (p=0.180), while the immunostaining density of the DM group increased statistically significantly compared to the control and EA groups (both p<0.001). The decrease in staining intensity in the treatment group was statistically significant compared to the DM group (p<0.001) (Figure 2, 3C).

When the αSMA immunopositivity density was evaluated statistically, there was no significant difference between the control and EA group (p=0.173), while the immunostaining density of the DM group increased significantly compared to the control and EA groups (both p<0.001). The decrease in staining intensity in the treatment group was statistically significant compared to the DM group (p<0.001). In addition, when the treatment group was compared with the control and EA groups, the difference was statistically insignificant (p=1.000, p=0.898, respectively) (Figure 2, 3D).

Table 2. Immunopositivity descriptive values of TGFβ1, p-Smad3, and αSMA of all groups (%)

		Control	EA	DM	DM+EA	p
TGFβ1	mean±SD	7.17±2.39	5.75±2.97	27.96±6.49	12.57±2.83	**<0.001**
	median [min-max]	6.66 [3.64-14.80]	4.97 [2.46-13.26]	28.52 [17.94-42.16]	12.13 [8.94-20.56]	
p-Smad3	mean±SD	4.42±2.05	5.21±2.40	21.42±6.98	10.99±2.26	**<0.001**
	median [min-max]	4.10 [1.51-8.69]	4.77 [1.98-10.25]	21.00 [10.25-38.16]	10.67 [7.49-16.25]	
αSMA	mean±SD	9.21±3.64	8.01±2.75	24.08±4.39	9.49±3.43	**<0.001**
	median [min-max]	8.66 [3.26-18.26]	7.63 [4.59-15.36]	25.08 [13.26-32.16]	8.76 [5.05-20.23]	

TGFβ1: transforming growth factor β1, p-Smad3: phosphorylated suppressor of mothers against decapentaplegic 3, and αSMA: α-smooth muscle actin, EA: ellagic acid, DM: diabetes mellitus

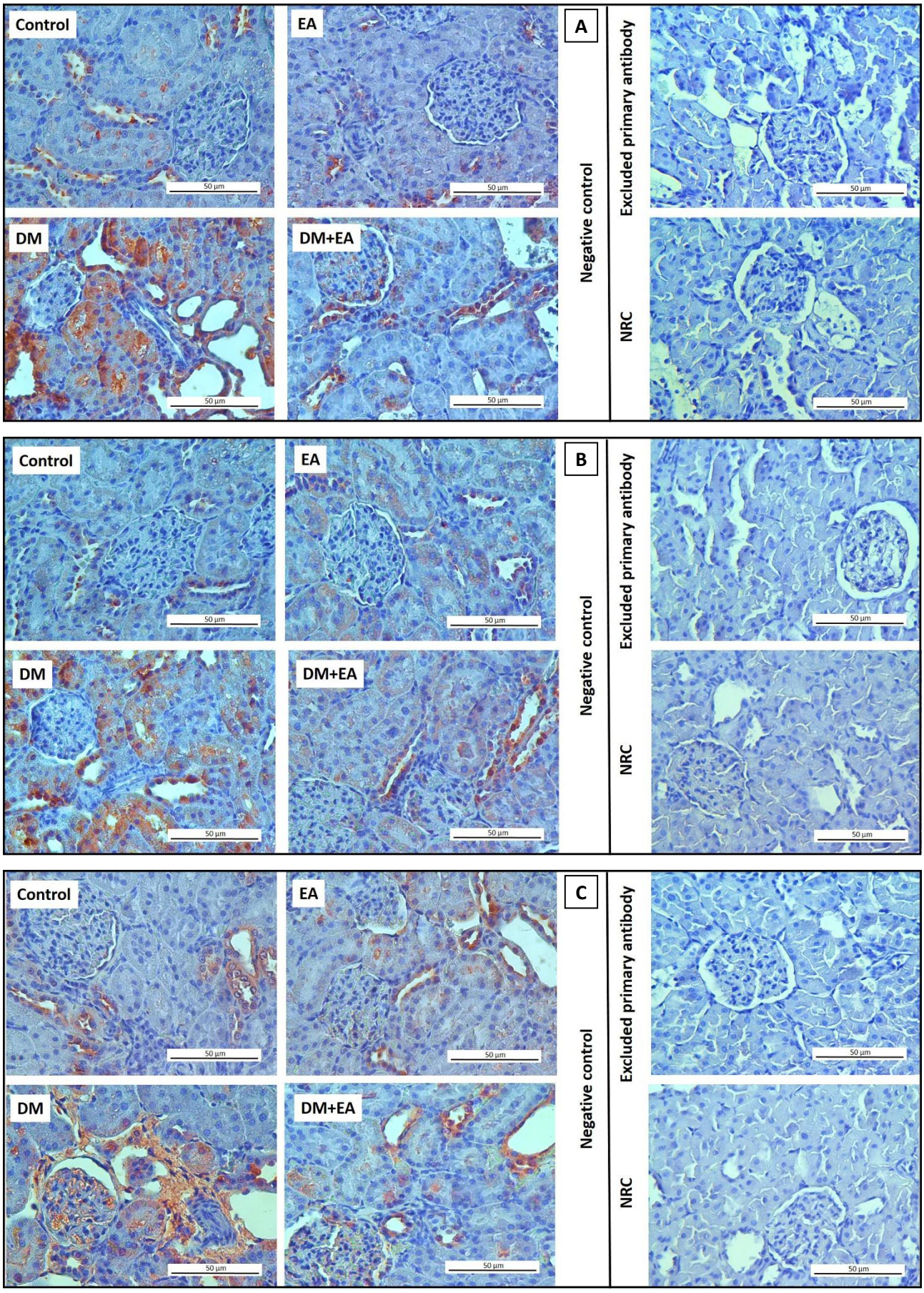

Figure 2. Representative microphotographs for the negative control and immunostaining with TGFβ1 (A), p-Smad3 (B), αSMA (C) primary antibodies in kidney tissues of all the groups. No staining occurred in controls with nonspecific IgG of the same isotype as primary antibodies (x400; AEC-hematoxylin). TGFβ1: transforming growth factor β1, p-Smad3: phosphorylated suppressor of mothers against decapentaplegic 3, and αSMA: α-smooth muscle actin, EA: ellagic acid, DM: diabetes mellitus, NRC: negative reagent control

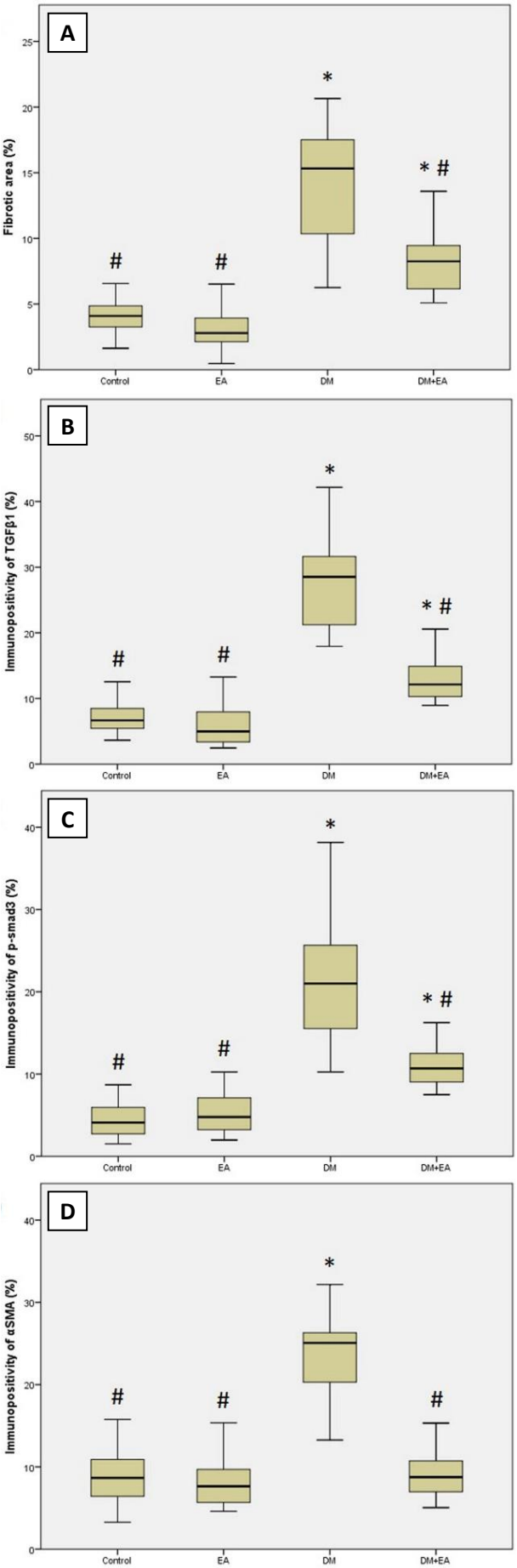

Figure 3. Quantitative summary of fibrotic area staining ratios (A), and immunopositive staining ratios (B-D).
*: p<0.05 vs. control, #: p<0.05 vs. DM (Bonferroni correction Mann-Whitney U tests)

Briefly, evidence was provided to show that elevated glucose increases TGFβ1 protein expression in the kidney. Increased TGFβ1 protein levels in diabetic kidneys triggered phosphorylated Smad3 translocated to the nucleus, activating TGFβ/Smad signaling. As a result of this activation, αSMA expression was induced in the intertubular area. In conclusion, renal fibrosis was induced in rats with diabetic kidney injury. TGFβ/Smad signal inhibition was observed with EA treatment. As a result, it was determined that fibrosis decreased with decreased collagen density and decreased αSMA expression.

DISCUSSION

DM is an endocrine metabolic disease with many side effects. Oxidative stress plays an important role in the onset and progression of diabetes and its complications. The most common complication of DM is diabetic nephropathy. Therefore, oxidative stress due to hyperglycemia plays an important role in the development of diabetic nephropathy (1,2). Among the pathological changes of diabetic nephropathy, glomerular and tubulointerstitial damage are the leading ones (3). Renal fibrosis seen in diabetic nephropathy is the accumulation of fibrotic matrix and scar formation in response to serious injury (4,5). Reducing oxidative stress remains an important goal in the treatment of diabetic nephropathy. Phytochemicals with antioxidant properties and free radical scavengers are frequently used in recent studies (23-26). In experimental diabetes studies, antioxidant applications have been shown to reduce hyperglycemia caused by STZ (23,27).

Gallic acid-derived EA has been shown to reduce myocardial infarction areas and suppress cardiac fibrosis in myocardial infarction and regulate the expression of antiapoptotic genes and mitochondrial respiratory enzyme activities (25,26). In a study investigating the effect of EA on testis by creating diabetes with STZ, it was found that EA reduced the expression of Nrf-2, which is a marker of oxidative stress, and the apoptotic index (22).

Smad proteins are important in the TGFβ signaling pathway. Different components of the Smad signaling pathway interact with regions on Smad proteins, inducing phosphorylation of Smad proteins and signal initiation. Smad2 and Smad3 are transported to the nucleus along with other transcription factors. Smad7 has a negative effect on TGFβ signaling, leading to polyubiquitination and degradation of TGFβ receptors. This shows that Smad factors are the main mediators of the fibrinogenic effects of TGFβ (28,29). In a study by Shu et al. (28) in which they investigated the hepatoprotective effects of limonin, a natural tetracyclic triterpenoid compound, it was shown that in a liver fibrosis model, limonin suppressed TGFβ-induced Smad2/3 phosphorylation and subsequent nuclear translocation, and increased Smad7 expression. In the diabetes study of Meng et al. (24), it was shown that silymarin application in myocardial fibrosis reduced collagen and fibrous structure, downregulated TGFβ1 and Smad2/3 levels, and upregulated Smad7 levels.

In our study, when we evaluated the effects of EA on TGFβ1/Smad signaling in diabetic kidney tissues, it was

observed that TGFβ1 protein, which plays an important role in the fibrotic pathway in diabetic kidney tissues, increased significantly and decreased with EA treatment. It was observed that high glucose up-regulated the expression of p-Smad3, the activated form of Smad3, which causes the activation of TGFβ1 expression and TGFβ1 transcription in the kidney, and down-regulated these levels with treatment.

In a myocardial infarction study with EA, it was reported that EA reduces cardiac fibrosis, suppresses HDACs expressions and expression of fibrous-related genes, decreases cardiac fibroblast proliferation, and is a phytochemical with anti-fibrotic properties (26). In the study of Li et al. (9), in which they created renal fibrosis in mice, it was shown that collagen accumulation and α-SMA expression increased. Collagen deposition was visualized by Masson's trichrome staining and a significant increase was detected. In the study, TGFBR1 induction by lentivirus injection method was regulated by MicroRNA-10a/b post-transcriptional mechanism; collagen deposition, fibrotic gene expressions, and α-SMA expression were down-regulated.

In our study, a significant increase in collagen density in the intertubular area of the kidney tissue of the diabetic injury group was demonstrated by Masson's trichrome staining. Collagen density was found to be statistically significantly decreased in the DM group treated with EA. While the expression level of the profibrotic marker αSMA increased in DM groups in line with fibrosis, its expression level was significantly decreased in the treatment groups. In particular, it was noteworthy that the αSMA expression levels of the control and treatment groups were similar.

CONCLUSION

In conclusion, TGFβ1 and p-Smad3 expression levels in the treatment group were statistically significantly decreased compared to the DM group. While the expression level of the profibrotic marker αSMA increased in DM groups in line with fibrosis, its expression level was significantly decreased in the treatment groups. It is thought that this study will shed light on studies investigating different signaling pathway mechanisms in the relationship between diabetes and EA.

Ethics Committee Approval: The study was approved by the Animal Experiments Local Ethics Committee of Gazi University (21.07.2022, G.Ü.ET-22.083).

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Evaluation of Anti-Mullerian Hormone in Predicting In Vitro Fertilization Cycle Outcomes

Anti-Mülleryan Hormonun İn Vitro Fertilizasyon Siklus Sonuçlarına Etkisinin Araştırılması

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ABSTRACT

Aim: This study was conducted to explore the effect of serum anti-Mullerian hormone (AMH) level on in vitro fertilization (IVF) cycle outcomes.
Material and Methods: A total of 142 patients included in this study, were divided into three groups according to their serum AMH levels as Group 1: AMH level 5-10 ng/ml (n=108), Group 2: AMH level 10-15 ng/ml (n=20), and Group 3: AMH level >15 ng/ml (n=14). Demographic characteristics were recorded. The duration of infertility and stimulation, the number of cycles, initial, final, and total doses of gonadotropins, and estradiol (E2) and progesterone levels on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the total number of oocytes retrieved, the number of mature oocytes, the number and quality of the embryo, and also endometrial thickness on the day of trigger, OPU and ET, the distance of embryo-fundus, the day of ET, and pregnancy outcomes were all recorded.
Results: While the IVF treatment indications and pregnancy outcomes were similar between the groups, body mass index (BMI) was significantly higher in Group 2 and Group 3 than in Group 1 ($p<0.001$). The total doses of gonadotropin were significantly higher in Group 2 than in Group 1 and Group 3, and the total oocyte count was also significantly higher in Group 3 than in Group 1 ($p=0.006$, and $p=0.015$, respectively)
Conclusion: AMH levels were associated with BMI and total oocyte count, but not with mature oocyte count, oocyte quality, and pregnancy outcomes.
Keywords: AMH levels; IVF cycle outcomes; BMI; oocyte count and quality; pregnancy outcomes.

ÖZ

Amaç: Bu çalışma, serum anti-Mülleryan hormon (AMH) seviyesinin in vitro fertilizasyon (IVF) siklus sonuçları üzerindeki etkisini araştırmak için yürütülmüştür.
Gereç ve Yöntemler: Bu çalışmaya dahil edilen toplam 142 hasta, serum AMH düzeylerine göre, Grup 1: AMH düzeyi 5-10 ng/mL (n=108), Grup 2: AMH düzeyi 10-15 ng/mL (n=20) ve Grup 3: AMH düzeyi >15 ng/mL (n=14) olmak üzere üç gruba ayrıldı. Demografik özellikler kaydedildi. İnfertilite ve stimülasyon süresi, siklus sayısı, gonadotropinlerin başlangıç, son ve toplam dozları ile tetikleme, oosit toplama (oocyte pick up, OPU) ve embriyo transferi (ET) gününde östradiol (E2) ve progesteron düzeyleri, toplanan toplam oosit sayısı, matur oosit sayısı, embriyo sayısı ve kalitesi ve ayrıca tetikleme, OPU ve ET gününde endometrial kalınlık, embriyo-fundus mesafesi, ET günü ve gebelik sonuçlarının tamamı kaydedildi.
Bulgular: IVF tedavi endikasyonları ve gebelik sonuçları gruplar arasında benzer iken, vücut kitle indeksi (VKİ) Grup 2 ve Grup 3'te Grup 1'e göre anlamlı olarak daha yüksekti ($p<0,001$). Toplam gonadotropin dozları Grup 2'de Grup 1 ve Grup 3'e göre anlamlı olarak daha yüksek ve toplam oosit sayısı da Grup 3'te Grup 1'e göre anlamlı olarak daha yüksek idi (sırasıyla, $p=0,006$ ve $p=0,015$).
Sonuç: AMH seviyesi VKİ ve toplam oosit sayısı ile ilişkilidir, fakat matur oosit sayısı, oosit kalitesi ve gebelik sonuçları ile ilişkili değildir.
Anahtar kelimeler: AMH seviyeleri; IVF siklus sonuçları; VKİ; oosit sayısı ve kalitesi; gebelik sonuçları.

INTRODUCTION

Anti-Mullerian hormone (AMH) is a homodimeric glycoprotein secreted by the granulosa cells of small antral follicles. It belongs to the transforming growth factor β (TGF-β) family and is located on chromosome 19p13.3 (1). In addition to being an important predictor of ovarian reserve, AMH is also significant in the detection of ovarian response to hormonal stimulation (2). One line of the literature showed that the number of retrieved oocytes was correlated with serum AMH levels in in vitro fertilization (IVF). We know that a high AMH level does not always mean that the number of fertilized oocytes will be high (3,4). Although there is a strong positive correlation between serum AMH level and oocyte quantity, the oocyte and embryo quality are controversial (5-7). In addition, previous studies have demonstrated that a high serum AMH level was correlated with follicular fluid AMH concentration that affects fertilization conditions negatively by changing the ratios of oestradiol-testosterone (8,9).

Therefore, the present study was conducted to explore the effect of serum AMH level on IVF cycle outcomes, especially on the conflicting results observed in the literature.

MATERIAL AND METHODS

This was a retrospective study conducted at the IVF clinic of Etlik Zübeyde Hanım Women's Health Training and Research Hospital of Ankara, Turkey, and was carried out with a total of 142 women. The study protocol was approved by the local ethics committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.07.2022, 2022/143). The women were divided into three groups according to the serum AMH levels, Group 1: AMH level 5-10 ng/mL (n=108), Group 2: AMH level 10-15 ng/mL (n=20), Group 3: AMH level >15 ng/mL (n=14), respectively.

The exclusion criteria included having a history of chronic disease, undergoing preimplantation genetic diagnosis, freeze-thaw, and mild or natural cycle protocols, multiple embryo transfer, severe male factor infertility defined as azoospermia or total progressive motile sperm count being less than 1 million, and the study also excluded the patients with moderate to severe ovarian hyperstimulation syndrome (OHSS).

Demographic characteristics viz. maternal age, gravidity, abortion, live birth, body mass index (BMI), IVF treatment indications (unexplained infertility, male factor, tubal factor), duration of infertility and stimulation, the number of cycles, initial, final, and total doses of gonadotropins (recombinant follicle-stimulating hormone (FSH), Gonal-F® Merck, Germany, human menopausal gonadotropin (hMG), Menopur®, Ferring Pharmaceuticals, Germany), estradiol (E2) and progesterone levels on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the total number of oocytes retrieved, the number of mature oocytes, the number and quality of embryo (10), endometrial thickness on the day of trigger, OPU and ET, the distance of embryo-fundus, the day of ET and pregnancy outcomes were all recorded for reference. The gonadotropin releasing hormone (GnRH) antagonist protocol (recombinant FSH, Gonal-F® Merck, Germany, hMG, Menopur®, Ferring Pharmaceuticals, Germany, GnRH antagonist (141 Cetrotide®, Merck, Germany) and agonist trigger (Gonapeptyl® Ferring Pharmaceuticals, Germany) were administrated in all groups (11). The same luteal phase support was provided for all three groups.

Serum AMH level was determined using the IMMULITE 2000 Immunoassay System (Siemens, Berlin, Germany). The detection limit was 0.02-24 ng/mL and the inter-assay and intra-assay coefficients of variation were 3.77%~3.99% for AMH.

Statistical Analysis

Statistical analysis was performed by IBM SPSS Statistics Version 26.0. Descriptive statistics and frequency tables were used to examine the obtained results. Parametric tests were performed for the data which was normally distributed and the analysis of variance (ANOVA) test was used in the analysis of three or more independent groups. Non-normally distributed data were analyzed by the Kruskal Wallis-H test as a non-parametric test. Bonferroni correction test was applied for pairwise comparisons. The relationships between the two qualitative variables were analyzed with the Pearson chi-square test. A p value of <0.05 was considered to be statistically significant.

RESULTS

A total of 142 women were recruited in the study. No significant difference was found in IVF treatment indications and pregnancy outcomes between the three groups according to the AMH value (Table 1).

The comparison of demographic and obstetric characteristics, laboratory data, and duration of infertility between the groups was shown in Table 2. The comparison of IVF cycle characteristics and embryo outcomes between the AMH groups was shown in Table 3.

Table 1. The comparison of IVF treatment indications and pregnancy outcomes according to the AMH value

	Group 1 (n=108) (AMH 5.0-10.0 ng/mL)	Group 2 (n=20) (AMH 10.1-15.0 ng/mL)	Group 3 (n=14) (AMH >15.0 ng/mL)	p
Tubal factor, n (%)	8 (7.4)	2 (10.0)	0 (0.0)	0.509
Male factor, n (%)	32 (29.6)	6 (30.0)	1 (7.1)	0.200
Unexplained infertility, n (%)	68 (63.0)	12 (60.0)	11 (87.6)	0.477
Pregnancy outcomes, n (%)				
No pregnancy	73(67.6)	10 (50.0)	10 (71.4)	
Biochemical	5 (4.6)	2 (10.0)	0 (0.0)	0.479
Clinical	30 (27.8)	8 (40.0)	4 (28.6)	

IVF: in vitro fertilization, AMH: anti-Mullerian hormone (AMH)

Table 2. The comparison of demographic and obstetric characteristics, laboratory data, and duration of infertility according to the AMH value

	Group 1: AMH 5.0-10.0 ng/mL (n=108)		Group 2: AMH 10.1-15.0 ng/mL (n=20)		Group 3: AMH >15.0 ng/mL (n=14)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Maternal age (years)	28.78±3.02	28 [23-35]	29.00±3.33	29.5 [24-35]	28.93±2.67	28 [26-33]	0.955
Gravidity	0.60±0.86	0 [0-4]	0.70±0.66	1 [0-2]	0.29±0.46	0 [0-1]	0.188
Abortion	0.36±0.73	0 [0-4]	0.55±0.61	0.5 [0-2]	0.14±0.36	0 [0-1]	0.061
Live birth	0.15±0.47	0 [0-3]	0.10±0.31	0 [0-1]	0.14±0.36	0 [0-1]	0.929
BMI (kg/m^2)	25.98±4.45^a	25.1 [17.0-40.0]	30.28±4.59^b	30.8 [19.0-37.6]	28.91±3.04^b	29.5 [23.0-33.2]	**<0.001**
E2 (pg/mL), day of trigger	3246.96±1981.83	2946 [437-9438]	4741.87±3179.94	3000 [1400-12115]	4795.81±3520.88	3308 [506-11041]	0.132
Progesterone (ng/mL), day of trigger	1.04±1.43	0.8 [0.1-13.1]	1.28±0.79	1.3 [0.3-2.9]	0.94±0.59	0.8 [0.1-2.1]	0.160
E2 (pg/mL), day of OPU	2241.69±1357.84^a	2061.4 [253.4-7061.7]	3443.42±1761.84^b	3000 [1048.8-7731.9]	3577.18±2125.50^b	3000 [514.0-8128.0]	**0.001**
Progesterone (ng/mL), day of OPU	8.16±4.96	7.3 [0.7-26.8]	9.54±5.27	9.6 [2.5-25.9]	6.01±3.64	5.6 [0.8-16.3]	0.063
E2 (pg/mL), day of ET	1649.45±1417.02	1346.4 [137-6480]	1678.09±1046.43	1677.0 [125-3756]	998.07±820.23	556.5 [325-2275.6]	0.305
Progesterone (ng/mL), day of ET	69.24±69.07	60.0 [1.0-326.6]	49.00±38.24	51.2 [3.1-127.8]	25.98±35.21	15.9 [4.5-112.0]	0.106
Number of cycle	1.71±1.07^a	1 [1-5]	2.45±1.05^b	3 [1-4]	2.00±0.88^{ab}	2 [1-3]	**0.004**
Duration of infertility (month)	68.76±37.49^a	60 [5-180]	95.40±47.80^b	96 [24-168]	89.14±47.47^{ab}	84 [24-216]	**0.022**

AMH: anti-Mullerian hormone, BMI: body mass index, E2: estradiol, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation, ^{a,b}: different superscripts denote the significant difference according to the post hoc test results

Table 3. The comparison of IVF cycle characteristics and embryo outcomes according to the AMH value

	Group 1: AMH 5.0-10.0 ng/mL (n=108)		Group 2: AMH 10.1-15.0 ng/mL (n=20)		Group 3: AMH >15.0 ng/mL (n=14)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Initial doses of gonadotropins (IU)	158.33±28.60	150 [100-225]	166.25±29.82	175 [100-200]	143.75±29.72	150 [87-200]	0.069
Final doses of gonadotropins (IU)	126.97±48.91^{ab}	150 [25-275]	148.75±46.40^a	150 [25-225]	104.46±34.53^b	100 [50-150]	**0.014**
Total doses of gonadotropins (IU)	1517.62±488.83^a	1400 [800-4350]	1753.75±405.43^b	1718.8 [1013-2575]	1339.29±267.42^a	1337.5 [825-1700]	**0.006**
Duration of stimulation (day)	10.38±1.60^a	10 [7-15]	11.30±1.53^b	11.5 [9-14]	11.00±1.10^{ab}	11 [9-13]	**0.019**
Follicle number (15-17 mm)	5.02±3.45	4.5 [0-22]	4.95±3.15	4 [0-11]	5.79±2.64	6 [2-11]	0.462
Follicle number (≥17 mm)	4.50±3.06	4 [0-13]	4.65±3.70	4 [0-11]	4.14±3.28	3.5 [0-10]	0.904
Total oocyte count	16.93±8.32^a	15 [2-38]	21.15±9.95^{ab}	22 [6-37]	22.71±7.63^b	25 [5-33]	**0.015**
Mature oocyte count	12.64±7.51	11 [0-32]	14.25±7.95	12.5 [4-35]	14.29±7.46	13 [0-26]	0.463
Grade 1 embryo	0.44±0.56	0 [0-2]	0.50±0.51	0.5 [0-1]	0.21±0.42	0 [0-1]	0.261
Grade 2 embryo	0.30±0.48	0 [0-2]	0.65±0.45	1 [0-2]	0.36±0.63	0 [0-2]	0.462
Grade 3 embryo	0.14±0.35	0 [0-1]	0.10±0.31	0 [0-1]	0.07±0.27	0 [0-1]	0.721
Endometrial thickness (mm), day of	10.14±2.14	9.9 [5.6-17.0]	10.18±1.51	10.0 [7.5-12.8]	10.49±2.61	9.8 [6.5-15.0]	0.860
Endometrial thickness (mm), day of OPU	9.53±2.14	9.1 [4.5-16.0]	9.24±1.74	8.8 [7.0-13.0]	9.32±2.79	8.6 [5.0-14.3]	0.773
Endometrial thickness (mm), day of ET	9.94±2.11	9.6 [7.0-17.2]	9.53±1.72	9.5 [7.2-13.3]	10.41±1.85	10.6 [7.4-12.9]	0.464
Distance embryo-fundus	11.11±3.51	11.0 [0.8-19.0]	10.24±4.61	10.4 [1.1-18.0]	12.99±4.31	13.5 [6.4-20.0]	0.238
Day of embryo transfer	3.74±0.97	3 [3-5]	3.94±1.03	3 [3-5]	4.13±0.99	4.5 [3-5]	0.425

AMH: anti-Mullerian hormone, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation, ^{a,b}: different superscripts denote the significant difference according to the post hoc test results

There was no significant difference in maternal age, gravidity, abortion, live birth, E2, and progesterone level on the trigger day, progesterone level on the OPU day, E2, and progesterone level on the ET day between the groups. As a result of the Bonferroni correction method, BMI was significantly higher in Group 2 and Group 3 than in Group 1, OPU E2 level was significantly higher in Group 2 and Group 3 than in Group 1, the number of cycle was significantly higher in Group 2 than in Group 1, and duration of infertility was significantly longer in Group 2 than in Group 1 ($p<0.001$, $p=0.001$, $p=0.004$, and $p=0.022$, respectively, Table 2).

There was no significant difference in the initial doses of gonadotropins, the number of follicles, mature oocyte counts, grade 1-2-3 embryo, and endometrial thickness on the day of trigger, OPU and ET, embryo-fundus distance, and the day of ET between the groups. As a result of the Bonferroni correction method, the final doses of gonadotropin were significantly higher in Group 2 than in Group 3, the total doses of gonadotropin were significantly higher in Group 2 than in Group 1 and Group 3, the duration of stimulation was significantly longer in Group 2 than in Group 1, and total oocyte count was significantly higher in Group 3 than in Group 1 ($p=0.014$, $p=0.006$, $p=0.019$, and $p=0.015$, respectively, Table 3).

DISCUSSION

In the present study, it was observed that as the AMH level increased BMI and the total oocyte count increased, the mature oocyte count, oocyte quality, and pregnancy outcome did not change. While it was expected that the increase in the total oocyte count would also be reflected on the number of mature oocyte and embryo, this did not take place for the present study. It is worth highlighting that the number of oocyte retrieved did not always mean an increased fertilized quality of oocytes.

In contrast with the current study, it was demonstrated in the literature that there occurred a negative correlation between AMH level and BMI (12). In fact, numerous theories have been put forward for the analysis of the association between BMI and AMH level (13). It has been argued that insulin resistance in obese women may disrupt the function of granulosa cells through lipotoxic effect and alter AMH production (14,15).

When the literature was reviewed, it was understood that the AMH level had a positive correlation with the number of retrieved oocytes and the OPU E2 level in IVF cycles was in agreement with the present study (6,16). The results were controversial regarding embryo quality. Garcia-Velasco et al. (17) reported that high AMH level blocks aromatase expression in granulosa cell that contributes to an intraovarian hyperandrogenic environment, which impairs oocyte development and causes poor quality embryo. In addition, Grossman et al. (18) revealed that the expression of cytochrome P450 aromatase (CYP19) via FSH in granulosa cells is inhibited by AMH that leads to a detrimental effect on the development of oocytes. In accordance with our study, Lie Fong et al. (19) demonstrated that there was no correlation between AMH level and embryo quality.

The mean goal of IVF treatment was to obtain the maximum number of high-quality embryos and increased the live birth rate as much as possible with minimum complications. Therefore, the optimization and individualization of IVF treatment have been accepted as the best practice, especially for patients with high AMH level. Because of the concerns regarding OHSS, cycle cancellation was more common in high AMH levels resulting in an increase in the number of failed IVF cycles as shown in the present study (20). In addition, in the current study, the initial gonadotropin doses were similar in all groups, but as the AMH level increased, we gradually reduced the doses through the end of the ovarian stimulation due to the risk of OHSS. In this way, even if we administered different doses of gonadotropin, the number of mature oocytes obtained was similar, so the controlled low-dose ovarian stimulation seemed more rational in those with high AMH levels.

The literature results are conflicting on the association between AMH level and pregnancy outcome, namely, a number of studies showed a positive relationship in this sense while some others demonstrated an inverse relationship. Wang et al. (21) indicated that endometrial cells contain AMH protein and when it binds its receptor, cellular viability is declined. This could explain the inverse relation between high AMH levels and decreased implantation rate. On the other hand, Kaya et al. (22) demonstrated that high AMH level had a positive correlation with pregnancy rate. In the current study, the pregnancy outcomes do not seem to be affected by AMH values.

CONCLUSION

In conclusion, AMH levels were associated with BMI and total oocyte count but not with mature oocyte count, oocyte quality, and pregnancy outcomes. Extensive studies are required to confirm the results of this study.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.07.2022, 143).

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Synchronous Bilateral Flexure Tumor Causing Ileus and Requiring Surgical Treatment

İleusa Neden Olan ve Cerrahi Tedavi Gerektiren Senkron Bilateral Köşe Tümörü

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ABSTRACT

This case report presented a simultaneous right colon tumor detected perioperatively in a patient who developed ileus due to a metastatic left colon tumor in the preoperative period. A seventy-six-year-old man was admitted with epigastric pain, nausea, and vomiting. There was tenderness and defense on deep palpation on the epigastrium. On computed tomography, there were multiple hypodense lesions on the liver, a mass at the level of the splenic flexure that obliterates the lumen. In addition, there were numerous air-fluid levels due to tumoral mass on splenic flexure. Emergency surgery was performed, and during surgery, there were tumoral masses at the hepatic flexure and splenic flexure. Palliative total abdominal colectomy with end ileostomy was performed due to megacolon. The patient died due to sudden cardiac arrest on the 1st postoperative day.
Keywords: Colorectal cancer; intestinal obstruction; synchronous neoplasms.

ÖZ

Bu olgu sunumunda, preoperatif dönemde metastatik sol kolon tümörü nedeniyle ileus gelişen bir hastada perioperatif dönemde saptanan eş zamanlı sağ kolon tümörü sunulmaktadır. Yetmiş altı yaşında erkek hasta epigastrik ağrı, bulantı ve kusma şikayetleriyle başvurdu. Derin palpasyonda epigastriumda hassasiyet ve defans mevcuttu. Bilgisayarlı tomografide karaciğerde çoklu hipodens lezyonlar, splenik fleksura seviyesinde lümeni oblitere eden bir kitle vardı. Ayrıca splenik fleksuradaki tümöral kitleye bağlı olarak çok sayıda hava-sıvı seviyesi mevcuttu. Acil cerrahi uygulandı ve ameliyat sırasında hepatik fleksura ve splenik fleksurada tümöral kitleler görüldü. Megakolon nedeniyle palyatif total abdominal kolektomi ile uç ileostomi yapıldı. Hasta ameliyat sonrası 1. günde ani kalp durması nedeniyle kaybedildi.
Anahtar kelimeler: Kolorektal kanser; bağırsak tıkanıklığı; senkron neoplazmlar.

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INTRODUCTION

According to Cancer Statistics 2022, in the USA, colorectal cancer (CRC) ranks 3rd for both sexes in terms of estimated new cases and estimated deaths (1). Historically, studies have defined CRCs within three compartments of the gut: distal colon, proximal colon, and rectum (2). Proximal colon cancers include cancers located proximal two-thirds of the transverse colon, ascending colon, and cecum. In contrast, distal colon cancers are located in one-third of the transverse, descending, and sigmoid colon. On the other hand, rectal cancers include cancers located from the proximal side of the rectum up to the dentate line. The most common location of CRC is the cancers located in the rectosigmoid region (3).

Almost all CRCs are seen in a single tumoral focus. Synchronous multiple CRCs have an incidence of 1.1-8.1% among all CRCs (4). Synchronous CRCs were defined as having more than one primary CRC at the same time, a distance of at least 5 cm between two primary tumors detected simultaneously, and a second primary tumor detected within six months of the detection of the first primary cancer (5). Detection of synchronous CRCs is essential, and if overlooked, the second tumor may appear as advanced metachronous cancer. If the second tumor is detected preoperatively, it affects the width and type of surgical resection (6).

This case report aimed to present a simultaneous right colon tumor detected perioperatively in a patient who developed ileus due to a metastatic left colon tumor in the preoperative period.

CASE REPORT

A seventy-six-year-old man was admitted to the emergency department of a tertiary health center with epigastric pain, nausea, and vomiting for about 2 days. He had lost weight (approximately 20 kilograms-from 106 kg to 86 kg) for about four months. He had hypertension and diabetes mellitus but did not have any history of surgery. In addition, there was no history of cancer in family members.

The patient's vital signs on admission were as follows: blood pressure: 158/88 mm Hg, pulse rate: 97 beats per minute, oxygen saturation on room air: 92%, and body temperature: 37.3° Celsius. On physical examination of the abdomen, there was tenderness and defense on deep palpation on the epigastrium and a palpable solid mass on the right upper quadrant. Other system examinations were unremarkable, including the digital rectal examination.

In the laboratory, total bilirubin (1.8 mg/dL), direct bilirubin (0.8 mg/dL), alanine transaminase (60 U/L), aspartate transaminase (245 U/L), gamma-glutamyl transferase (495 IU/L), alkaline phosphatase (291 U/L), lactate dehydrogenase (3025 U/L), creatinine kinase (329 U/L), C-reactive protein (97.2 mg/L), and platelet count ($610x10^9$/L) were higher than normal ranges, but hemoglobin (11.1 g/dL) level was lower. In addition, serum levels of carcinoembryonic antigen (43.83 ng/mL) and cancer antigen 19-9 (221.70 U/mL) were higher. Abdominal ultrasonography (USG) showed only multiple solid hypoechoic lesions on the liver.

The patient was admitted to internal medicine with a prediagnosis of malignancy. Endoscopy showed antral gastritis with esophagitis, and colonoscopy revealed a tumoral obstruction in the sigmoid colon that did not allow proximal passage of the colonoscope. On day 1 of the follow-up, the patient had severe abdominal pain and distension. Oral intake stopped, and nasogastric decompression was performed due to the risk of aspiration. Additionally, enema treatment was started twice a day. On the report of the computed tomography (CT), there were multiple hypodense lesions in the liver (Figure 1a) and obstructive masses at the level of the splenic flexure (Figure 1b) and the level of the hepatic flexure (Figure 1c). In addition, there were numerous air-fluid levels due to tumoral mass on splenic flexure (Figure 2). Despite laxative therapy and decompression therapy, the patient has increased abdominal distension, no gas stool, and continued vomiting; emergency surgery was planned. During exploration, there were tumoral masses at the hepatic and splenic flexure. In addition, multiple hepatic metastases were present. Palliative total abdominal colectomy with end ileostomy was performed due to a megacolon (Figure 3). The patient was followed up in the intensive care unit during the postoperative period. Oral feeding was stopped, and intravenous fluid replacement was started. The patient died due to sudden cardiac arrest on the 1st postoperative day.

Tumor pathology in the hepatic flexure was compatible with moderately differentiated adenocarcinoma; its dimensions were 60*45*8 mm (pT3, Figure 4a, 4b, and 4c). Tumor pathology in the splenic flexure was consistent with moderately differentiated adenocarcinoma; its dimensions were 70*45*20 mm (pT3, Figure 5a, 5b, and 5c). Both proximal and distal surgical margins were negative. MLH1, MSH2, MSH6, and PMS2 nuclear positive staining was observed in neoplastic cells, but no loss of expression was detected (proficient mismatch repair, pMMR).

DISCUSSION

Synchronous CRCs constitute 1.1-8.1% of all CRCs, and their preoperative detection directly affects surgical treatment and prognosis (7). Synchronous CRCs are more common in men and are seen in the older population than in patients with solitary CRC (8). Synchronous CRCs develop on a genetic or environmental common etiology. Studies of molecular colorectal carcinogenesis have shown that chromosomal instability, microsatellite instability (MSI), and gene methylation have been identified in many of the predisposing factors for synchronous cancers (9). It has been determined that the rate of MSI is higher in synchronous CRCs than in solitary tumors (10).

The diagnosis of CRC begins with a careful history and physical examination, including a rectal exam (11). It is also essential to question family history. It is a simple test, a screening test over 50 years that screens for occult blood in stool in cases with suspected colon cancer (12). Screening colonoscopy should be performed in cases with positive stool occult blood tests. Screening colonoscopy should also be planned in patients with a negative stool occult blood test and a family history with symptoms suggestive of colon cancer. In cases where a mass is seen in screening colonoscopy and the CRC, diagnosis is confirmed pathologically, abdominal CT, MRI, and PET-CT should evaluate the extent/stage of cancer (13). Appropriate treatment (surgery/neoadjuvant therapy) should be planned for the patient, considering the genetic pattern. This algorithm is the usual way of diagnosing CRC. However, detecting synchronous CRCs is essential in terms of prognosis and surgical treatment decisions. It is stated that synchronous tumors show a worse prognosis than solitary tumors (14). For synchronous CRCs, the most critical factor affecting the surgical technique, the width of the resection, and thus the quality of life after the operation is the localization of the tumors. Some studies stated that synchronous CRCs were mainly located in the right colon (15). However, studies also noted that left colon localization or localization in different colon segments is more frequent (16). In the present case, the patient with high carcinoembryonic antigen and cancer antigen 19-9 was admitted with epigastric

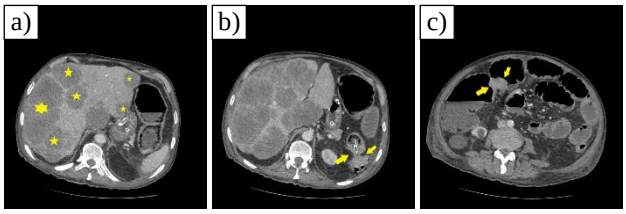

Figure 1. On the axial view of computed tomography, there were; **a)** multiple metastatic foci in the liver (yellow asterisks), **b)** a totally-obstructive tumoral mass at the level of the splenic flexure, and **c)** the hepatic flexure (yellow arrows)

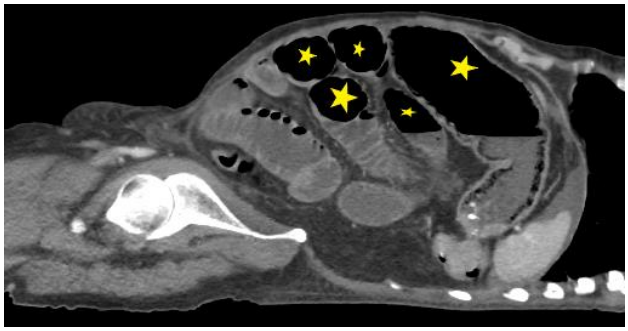

Figure 2. On the sagittal view of computed tomography, there were multiple air-fluid levels (yellow asterisks)

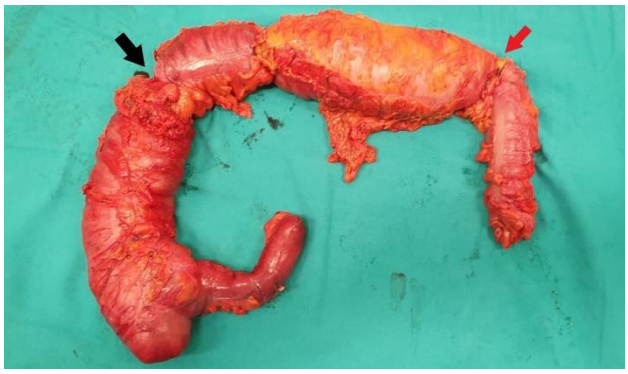

Figure 3. Resection material; the tumor on the hepatic flexure (black arrow), and the splenic flexure (red arrow)

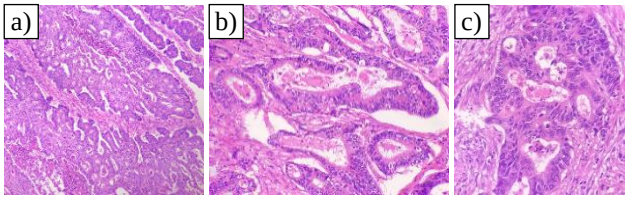

Figure 4. Pathological image of the tumor located at hepatic flexure; **a)** neoplastic glands displayed cribriform pattern (H&E, x100), **b)** neoplastic glands (H&E, x200), **c)** neoplastic glands (H&E, x400)

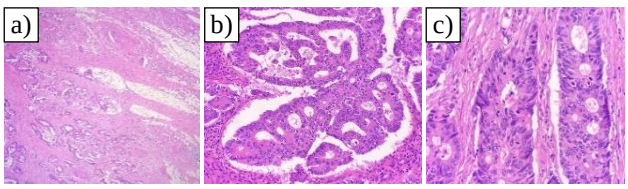

Figure 5. Pathological image of the tumor located at splenic flexure; **a)** neoplastic glands invading into subserosal adipose tissue (H&E, x40), **b)** neoplastic glands with a cribriform pattern (H&E, x200), **c)** neoplastic glands (H&E, x400)

pain, nausea, and vomiting. Colonoscopy and CT showed a tumoral mass at the level of the sigmoid colon. But different from preoperative evaluation, the patient had an obstructive tumor at the level of the splenic flexure. In addition, he had an obstructive tumor at the level of hepatic flexure, which was detected incidentally.

Surgical treatment of CRCs localized to the same colon segment is radical anatomical resection of the localized segment. However, studies have reported that different preferences from past to present are still accepted regarding the surgical technique for tumors located in different segments. Some authors have recommended wide resections for synchronous CRCs to avoid future recurrent surgeries due to metachronous tumors due to overlooked tumors (17). Recent studies have stated that multiple colon resections in solitary synchronous CRCs did not show a different result from wide resections regarding postoperative complications, length of hospital stay, and survival (18). We performed a total abdominal colectomy due to double-segment obstruction and ileus.

Mechanical bowel obstruction due to a distal tumor is an essential condition that complicates the detection of a synchronous CRC. It is challenging to diagnose proximal tumors in tumors that do not allow proximal passage of the colonoscope. In such cases, intraoperative colonoscopy or triple contrast computed tomography is recommended (19). Although tomography helps diagnose a synchronous tumor, palpation of all colon segments and tumor investigation are important during exploration. In this case, we detected a synchronous tumor at the hepatic flexure.

CONCLUSION

Colorectal cancer is a worldwide health problem. While most CRC cases present with a solitary tumor, it should be kept in mind that synchronous CRCs may be seen in very few cases. Synchronous CRCs are primarily seen in CRC cases with distal total obstruction that does not allow proximal passage of the colonoscope. In these cases, it is essential to evaluate all colon segments with tomography. Surgery type was chosen due to surgical urgency, patient's condition and performance status, and surgeon's experience. Total colectomy with a diverting stoma is a surgical option that can be applied in synchronous CRCs presenting with ileus in emergency conditions.

Informed Consent: Written informed consent was obtained from the patient for publication.

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Endoscopic Removal of Migrated Nissen Fundoplication Mesh

Migrasyon Olan Nissen Fundoplikasyon Meshinin Endoskopik Çıkarılması

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ABSTRACT

Laparoscopic Nissen fundoplication is known to be a safe and feasible method in the treatment of hiatal hernias. However, a recurrence rate of 42% has been reported. Using mesh repair in patients undergoing Nissen fundoplication has shown fewer recurrence rates than the primary suture, even though there have been few complications reported. Some complications that may occur with mesh placement are; severe fibrosis, esophageal strictures, esophageal perforations, mesh migration to the upper gastrointestinal tract, and mesh erosion in the intestinal wall, etc. In this report, the migration of the leathery esophagus developed after about 11 years was presented. In such cases, patients may have several surgical options. Among these options, the mesh was completely removed endoscopically. No further surgery was required. In conclusion, it is recommended to use a very selective mesh in the laparoscopic repair of the hiatal hernias, considering the surgeon's experience, hiatus anatomy, and patient's symptoms.
Keywords: Nissen fundoplication; mesh migration; endoscopic removal.

ÖZ

Laparoskopik Nissen fundoplikasyonunun hiatal hernilerin tedavisi için güvenli ve uygulanabilir bir yöntem olduğu bilinmektedir. Bununla birlikte, %42 gibi bir nüks oranı da bildirilmiştir. Nissen fundoplikasyonu yapılan hastalarda meş onarımının kullanılması, bildirilen birkaç komplikasyon olmuş olsa bile, primer sütüre göre daha az nüks göstermiştir. Meş konulmasıyla oluşabilecek bazı komplikasyonlar şunlardır; şiddetli fibrozis, özofagus darlıkları, özofagus perforasyonları, barsak duvarında meş erozyonu ve üst gastrointestinal sisteme meş migrasyonu, vb. Bu raporda, yaklaşık 11 yıl sonra gelişen meşin özofagusa migrasyonu sunulmuştur. Bu gibi durumlarda, hastalarda birkaç cerrahi seçenek bulunabilir. Bu seçeneklerin arasından, endoskopik olarak meş tamamen yerinden çıkarıldı. İleri cerrahiye ihtiyaç duyulmadı. Sonuç olarak, hiatal hernilerin laparoskopik onarımı için cerrahın deneyimi, hiatus anatomisi ve hastanın semptomları da dikkate alınarak çok seçici bir meş kullanılması önerilir.
Anahtar kelimeler: Nissen fundoplikasyonu; mesh migrasyonu; endoskopik çıkarma.

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INTRODUCTION

Open and laparoscopic Nissen fundoplication surgeries are commonly performed for hiatus hernias (1-3). However, high recurrence rates have been reported after these operations (2,4). Therefore, to reduce recurrence rates, recurrences were reduced by using mesh after cruroraphy. Although there are mesh-related complications, recurrences have decreased significantly with the use of mesh (1,4,5). Complications related to mesh include severe fibrosis, erosion of the esophageal wall and mesh migration, esophageal strictures and related swallowing difficulties, esophagus and stomach perforation, etc. (1-3). When complications develop in patients, the procedure is performed according to the complication (3,5).

This case report presented a patient with the complete transmural migration of the mesh and the surgical management of the case.

CASE REPORT

A 57-year-old female patient presented to the hospital with abdominal pain, dysphagia, and dyspeptic complaints, who had undergone laparoscopic Nissen fundoplication with polytetrafluoroethylene dual mesh due to gastroesophageal reflux disease 11 years ago. The patient's complaints have occurred in the last 1 year and have not had any complaints for 10 years. The patient had dysphagia and dyspeptic complaints due to partial gastroesophageal occlusion due to mesh compression. An upper gastrointestinal endoscopy was performed on the patient. In the endoscopy, it was observed that most of the mesh migrated in the cardia part of the stomach (Figure 1).

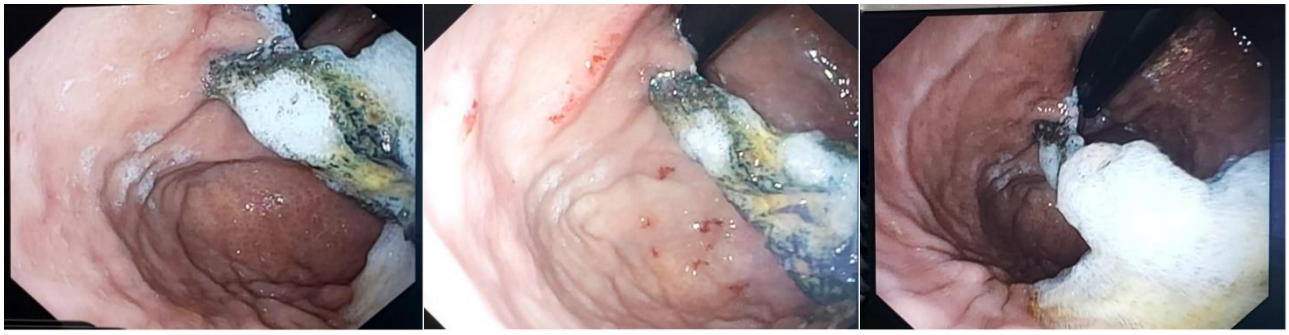

Figure 1. Endoscopic images of the surgical mesh migrated farther into the gastric lumen

The mesh was pulled into the stomach (Figure 2) and taken out endoscopically (Figure 3). Mesh removed an endoscopic grasping forceps (Olympus, FG-25C-1) using a standard adult upper endoscope (GIF-Q180; Olympus). The mesh out of the stomach was measured at about 3 cm (Figure 4).

The patient was hospitalized at the general surgery service. The esophageal wall was inspected again after the endoscopic extraction and we saw that there was no other pathology except some superficial ulcerated areas.

The patient was observed at the hospital for 24 hours in terms of the risk of developing an unexpected complication. Oral intake was stopped and parenteral treatment was started. No complications developed, and any further surgery was not required. The patients was discharged after 5 days.

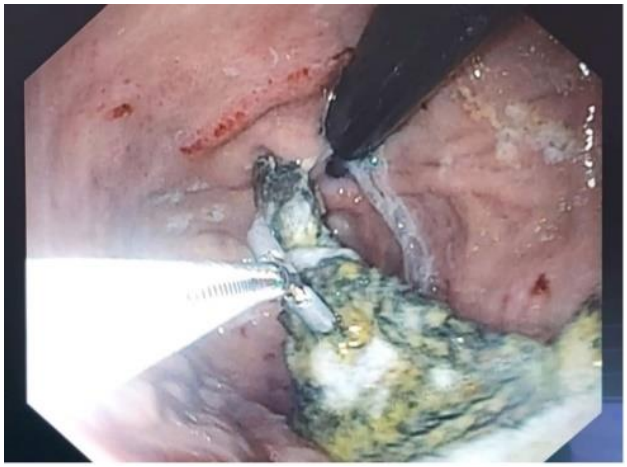

Figure 2. Endoscopic image of the surgical mesh grasping with stent removal forceps

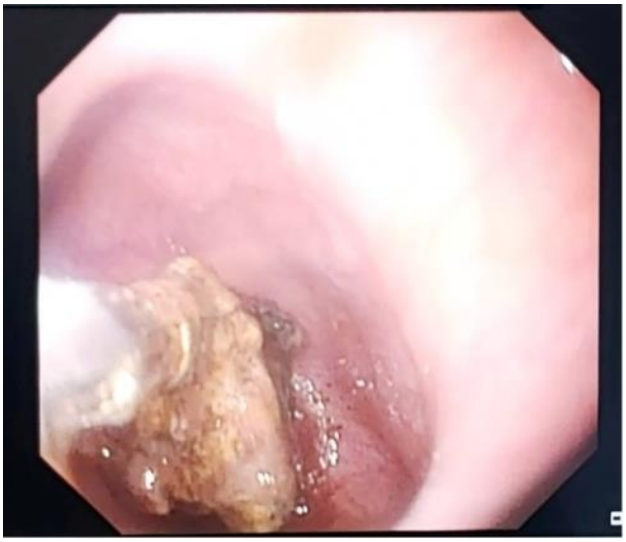

Figure 3. Endoscopic image revealing a mobile foreign body corresponding to the migrated surgical mesh (more distally) and the stent (more proximally)

Figure 4. The removed surgical mesh

DISCUSSION

The use of mesh reduces recurrences from 40% to 2% in patients who have undergone open or laparoscopic Nissen fundoplication and crurorаphy (2,4). Long-term complications are scarce (6). However, the use of mesh also brings some complications. Among these complications, stenosis, and difficulty in swallowing due to severe fibrosis, perforation, and mesh migration due to mesh erosion, abscess, and fistula formation are the most important (2,5,7). Goldschmiedt et al. (8) reported mesh-related esophageal perforation in 1992. Serious clinical conditions reported to occur in these patients.

Our patient had abdominal pain, dysphagia, and dyspeptic complaints before the mesh was removed, and no complications developed after the mesh was removed. In our patient, in whom the mesh completely migrated, endoscopic removal was achieved and any further surgery was not required. Transmural mesh migration to the posterior esophageal wall developed and was healed by fibrosis, and perforation did not occur. When the mesh was removed, it was observed that there was no injury to the serosal and mucosal wall of the esophagus, and it was completely free. In the endoscopic examination, no fundoplication was observed in the fundus. The clinic improved very quickly.

Full thickness migration of the mesh to the stomach wall enabled diagnosis and removal of the mesh by upper gastrointestinal endoscopy (4,9). It has been reported in the literature that very few mesh migrations exceed full thickness (3,10). In our case, full-thickness migration enabled the removal of the mesh with the help of upper gastrointestinal endoscopy. In order to reduce mesh reactions, some authors have suggested alternative methods such as the use of biological mesh (9).

Complete transmural gastric migrations of the mesh were rarely reported (2). Some authors recommend the biological mesh as an alternative (6), to decrease these kinds of complications.

The use of mesh in open or laparoscopic hiatal hernia repair significantly reduces recurrences. Patients should be informed about the development of complications after operations performed with mesh. In patients with mesh migration, in case of full-thickness migration of the mesh, the mesh can be removed by upper gastrointestinal endoscopy.

CONCLUSION

Some of the complications related to mesh use may be seen as in this case, like fibrosis, dysphagia, stenosis at the esophagogastric junction, or complete transmural gastric migration of the mesh. These complications sometimes can be life-threatening, can require complex surgical intervention, and may result in severe morbidity for the patient. For this reason, predicting possible complications is important. It is recommended to use a very selective mesh and to have an experienced surgeon for laparoscopic repair of hiatal hernias, considering the hiatus anatomy and the patient's symptoms.

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ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

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