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Investigation of the Factors Associated with Mortality in Catheter-Related Bloodstream Infections: Five-Year Observation

Kateter İlişkili Kan Dolaşımı Enfeksiyonlarında Mortalite ile İlişkili Faktörlerin Araştırılması: Beş Yıllık Gözlem

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ABSTRACT

Aim: Intravenous catheter use can cause various infections ranging from infection at the site of catheter entry to bacteremia and colonization. The purpose of this study was to identify the causative micro-organisms, and effects on morbidity-mortality of catheter-related bloodstream infections developing over the last five years.
Material and Methods: Data for 194 patients who underwent central intravenous catheter insertion in our hospital's intensive care unit and other departments between November 2014 and August 2019 were analyzed retrospectively. Blood samples taken from the catheter or the catheter tip, and blood samples collected simultaneously from the peripheral vein were included in the study, and culture results were recorded. Patients' demographic data and the effects of the factors identified on morbidity and mortality were subjected to statistical analysis.
Results: Ninety-two (47.4%) of the 194 patients included in the study were female and 102 (52.6%) were male, and mortality rate was 62.4% (n=121). The frequency of underlying medical conditions such as asthma, congestive heart failure, and cerebrovascular event, and receiving treatments such as immunosuppression, transfusion, tracheostomy, nasogastric tube, and mechanical ventilation were higher in mortal cases than non-mortal cases. A total of two hundred and forty microorganisms were detected in 194 patients, 121 (50.4%) of which were Gram negative bacteria, while 68 (28.3%) were Gram positive bacteria, and 51 (21.3%) were Candida species.
Conclusion: As a result, it was observed that the advanced age, underlying diseases and presence of resistant microorganisms were higher in mortal cases.
Keywords: Catheter-related bloodstream infections; mortality; advanced age.

ÖZ

Amaç: İntravenöz kateter kullanımı hastalarda kateter girişindeki enfeksiyondan bakteriyemi ve kolonizasyona kadar çeşitli enfeksiyonlara neden olabilir. Bu çalışmanın amacı, son beş yılda gelişen kateter ilişkili kan dolaşımı enfeksiyonlarının etken mikroorganizmalarını tanımlamak ve morbidite-mortalite üzerine etkisini ortaya koymaktır.
Gereç ve Yöntemler: Kasım 2014 ve Ağustos 2019 tarihleri arasında hastanemizin yoğun bakım ünitesinde ve hastanemizin diğer bölümlerinde santral intravenöz kateter yerleştirilen 194 hastanın verileri geriye dönük olarak incelendi. Kateterden veya kateter ucundan alınan kan örnekleri ve periferik venden aynı anda toplanan kan örnekleri çalışmaya dahil edildi ve alınan kan örneklerinin kültür sonuçları kaydedildi. Hastaların demografik verileri ile saptanan faktörlerin morbidite ve mortalite üzerine olan etkileri istatistiksel olarak değerlendirildi.
Bulgular: Çalışmaya dahil edilen 194 hastanın 92'si (%47,4) kadın, 102'si (%52,6) erkek cinsiyette idi ve mortalite oranı %62,4 (n=121) idi. Astım, konjestif kalp yetmezliği ve serebrovasküler olay gibi altta yatan tıbbi durumların sıklığı ve immünosupresyon, transfüzyon, trakeostomi, nazogastrik tüp ve mekanik ventilatör uygulanması gibi tedavilerin sıklığı mortal olgularda mortal olmayan olgulardan daha fazla idi. Yüz doksan dört hastada toplam 240 adet mikroorganizma üremesi saptanmış olup bunların 121 (%50,4)'i Gram negatif bakteri, 68 (%28,3)'i Gram pozitif bakteri ve 51 (%21,3)'i *Candida* spp idi.
Sonuç: Sonuç olarak ileri yaş, altta yatan hastalıklar ve dirençli mikroorganizma varlığının mortal olgularda daha fazla olduğu görülmüştür.
Anahtar kelimeler: Kateter ilişkili kan dolaşımı enfeksiyonu; mortalite; ileri yaş.

INTRODUCTION

In addition to raising costs, healthcare-related infections extend hospital stays and exacerbate poor prognosis and mortality (1). Catheter-related bloodstream infection (CRBSI) is the third most common nosocomial infection after ventilator-associated pneumonia and catheter-related urinary tract infection. The incidence of CRBSI decreases when protective bundles and infection control measures are applied, although the rate of infection may vary depending on the site of the catheter, the size of the hospital, and intensive care conditions (2). More than two billion intravenous devices are applied annually worldwide (3). In the USA, 250,000 patients are diagnosed with CRBSI every year, an average of 80,000 of whom are being treated in intensive care units (4). In addition to prolonging hospital stays and causing higher costs, CRBSI is one of the deadliest infections, with a mortality rate of 12-25% (5-7). Central venous catheters (CVCs) allow micro-organisms to enter and colonize the body. These pathogenic micro-organisms adhere to the surface of the catheter within the first 24 hours and form a biofilm layer that prepares the ground for infection by competing with the host cells (8,9). This allows micro-organisms to protect themselves against both antimicrobials and the immune system, and catheters need to be removed in most cases (10). Micro-organisms frequently implicated in CVC infections include *Acinetobacter baumannii*, *Staphylococcus epidermidis*, *Enterococcus faecium*, and *Candida albicans* (9,11).

The purpose of this study was identify the causative micro-organisms, and the effects on morbidity-mortality of CRBSI developing in our hospital over the last five years.

MATERIAL AND METHODS

Data for 194 patients in whom CVCs were inserted in our hospital's intensive care unit and other departments between November 2014 and August 2019 were analyzed retrospectively. Development of CRBSI was identified from data from the laboratory and clinic-based active surveillance system based on US Centers for Disease Control and Prevention criteria (2). The culture results of blood samples taken from the catheter or the catheter tip and blood samples taken from the peripheral vein were examined. Catheter samples were seeded using the semi-quantitative culture method. Culture plates were incubated at 37° C for 48 hours. Cultures from peripheral venous and catheter blood were incubated in a BACTEC 9120 (Becton Dickinson, USA) automated blood culture device. Conventional methods and/or the VITEK 2 automated system (bioMérieux, France) were used to identify the growing bacteria. Antibiotic susceptibility tests were performed according to "Clinical and Laboratory Standards Institute (CLSI)" standards (12) before 2016, and in line with "European Committee on Antimicrobial Susceptibility Testing (EUCAST)" standards (13) after 2016. Additional diseases such as asthma, hypertension, diabetes, and risk factors including mechanical ventilation, transfusion and immunosuppression were investigated. Infection Control Committee surveillance records were used to collect all data.

This study was approved by the Clinical Research Ethics Committee of Düzce University Medical Faculty on 15.06.2020 with decision number 130 and was conducted according to the Helsinki Declaration principles.

Statistical Analysis

SPSS v.22 software was used for data analysis. The data were expressed as numbers and percentages. Relationships between categorical variables were examined by Pearson chi-square, Fisher's exact and Fisher-Freeman-Halton tests. A p value of <0.05 was considered significant.

RESULTS

One hundred ninety-four patients, 92 (47.4%) female and 102 (52.6%) male, were included in the study. One hundred twenty-one (62.4%) patients with CRBSI died. One hundred eleven (57.2%) patients were aged 65 and over, and 83 (42.8%) were under 65. The mortality rate was significantly higher in patients aged 65 and over ($p<0.001$). Twenty-eight (14.4%) patients were treated on the wards and 166 (85.6%) in intensive care units. While the frequency of patients hospitalized in the internal intensive care unit (IICU) was higher in mortal cases than the non-mortal cases, the frequency of patients hospitalized in internal ward and pediatric intensive care unit (PICU) were lower in mortal cases than the non-mortal cases ($p<0.001$). Analysis of catheter sites revealed similar relationships between jugular, femoral, umbilical, or subclavian catheter applications and mortality rates ($p=0.903$). Analysis of comorbidities and risk factors revealed that the presence of asthma, congestive heart failure (CHF) and cerebrovascular event (CVE) were associated with mortality rates ($p=0.015$, $p=0.033$ and $p=0.039$, respectively). While the frequency of immunosuppression ($p=0.048$), transfusion ($p=0.046$), nasogastric tube ($p<0.001$), and mechanical ventilation ($p=0.005$) in mortal cases was higher than the non-mortal cases, the frequency of enteral nutrition ($p=0.011$) was lower in mortal cases than the non-mortal cases. While infection with single or multiple factors caused no statistically significant difference in mortality rates ($p=0.167$), the mortality rate in resistant microorganism growth was higher than that in susceptible agent growth ($p=0.004$). All these data are summarized in Table 1.

A total of 240 microorganisms were detected in 194 patients in the study. Infection occurred in 149 (76.8%) patients with a single agent and in 45 (23.2%) of patients with multiple agents. One hundred twenty-one (50.4%) of the 240 micro-organisms were identified as Gram negative bacteria, 68 (28.3%) as Gram positive bacteria, and 51 (21.3%) as Candida species. The distribution of agents is shown in Table 2. Extended spectrum beta-lactamase (ESBL) positivity rates were 62% in *Klebsiella pneumoniae* strains and 60% in *Escherichiae coli* strains, while the carbapenem resistance rate was 34% in *K. pneumoniae* strains. In addition, two carbapenem-resistant *Enterobacter aerogenes* and one colistin-resistant *Acetinobacter baumannii* strain were detected. Vancomycin resistance was detected in three out of 17 *E. faecium* strains among the enterococci.

DISCUSSION

The 2019 National Vascular Access Management Guide lists risk factors as prolonged hospitalization before catheter insertion, colonization of the inserted area and lumen, long-term catheter insertion, presence of internal jugular and femoral catheter in adults, prematurity, neutropenia, lack of intensive care nurses, catheter care

errors, total parenteral nutrition (TPN) support, and blood transfusion in children (10,11). The site of CVC is important for the risk of developing complications such as thrombophlebitis due to the local skin flora causing the infection (14-16). One randomized controlled study comparing the femoral and subclavian catheter insertion sites reported a higher colonization rate in the femoral region (17). Another study compared the subclavian and internal jugular veins and reported that catheters inserted into the internal jugular vein were exposed to higher colonization (18). A study conducted in 2017 reported infection rates of 36% for the internal jugular vein, 35.5% for the femoral vein, and 30% for the subclavian vein (19).

Table 1. Distribution of the features of patients and agents in mortal and non-mortal cases, n (%)

	Mortal (n=121)	Non-mortal (n=73)	p
Gender			
Female	62 (51.2)	30 (41.1)	0.170
Male	59 (48.8)	43 (58.9)	
Age			
≥65	82 (67.8)	29 (39.7)	**<0.001**
<65	39 (32.2)	44 (60.3)	
Clinics			
SICU	62 (51.2)[a]	35 (47.9)[a]	**<0.001**
IICU	48 (39.7)[a]	11 (15.1)[b]	
Internal ward	9 (7.4)[a]	17 (23.3)[b]	
PICU	1 (0.8)[a]	9 (12.3)[b]	
Surgical ward	1 (0.8)[a]	1 (1.4)[a]	
Catheter type			
Femoral	72 (59.5)	42 (57.5)	0.903
Juguler	35 (28.9)	21 (28.8)	
Subclavian	13 (10.7)	10 (13.7)	
Umbilical	1 (0.8)	0 (0.0)	
Risk factors*			
Asthma	9 (7.4)	0 (0.0)	**0.015**
CHF	26 (21.5)	7 (9.6)	**0.033**
CVE	16 (13.2)	3 (4.1)	**0.039**
Hypertension	60 (49.6)	36 (49.3)	0.971
Diabetes	39 (32.2)	17 (23.3)	0.183
CAD	22 (18.2)	8 (11.0)	0.178
CRF	19 (15.7)	14 (19.2)	0.533
COPD	13 (10.7)	3 (4.1)	0.104
Enteral feeding	109 (90.1)	56 (76.7)	**0.011**
TPN	53 (43.8)	29 (39.7)	0.578
Immunosuppression	52 (43.0)	21 (28.8)	**0.048**
Transfusion	91 (75.2)	45 (61.6)	**0.046**
Nasogastric tube	108 (89.3)	46 (63.0)	**<0.001**
Mechanical ventilation	93 (76.9)	42 (57.5)	**0.005**
Hemodialysis	43 (35.5)	21 (28.8)	0.331
Agent features			
Single agent	89 (73.6)	60 (82.2)	0.167
Multiple agent	32 (26.4)	13 (17.8)	
Resistancy state			
Group of resistant agent	95 (78.5)	43 (58.9)	**0.004**
Group of sensitive agent	26 (21.5)	30 (41.1)	

SICU: Surgical intensive care unit, IICU: Internal intensive care unit, PICU: Pediatric intensive care unit, *: more than one factors in a patient, CHF: Congestive heart failure, CVE: Cerebrovascular event, CAD: Coronary artery disease, CRF: Chronic renal failure, COPD: Chronic obstructive pulmonary disease, TPN: Total parenteral nutrition, Group of resistant agent: Carbapenem resistant Gram negative bacteria, methicillin resistant staphylococcus, vancomycin resistant enterococcus and Candida spp., Group of sensitive agent: Vancomycin sensitive enterococcus, methicillin sensitive staphylococcus, carbapenem sensitive Gram negative bacteria and others

In the present study, rates of use were 58.5% in the femoral vein, 29% in the jugular vein, 12% in the subclavian vein, and 0.5% in the umbilical vein. Although the highest rate of use was observed in the femoral vein, analysis of mortality rates revealed identical values for the femoral catheter and jugular catheter, at 63%, and a rate of 57% for the subclavian catheter. Although mortality rates for the subclavian catheter were relatively low, no significant difference in mortality rates was detected among the groups.

Age and the presence of risk factors such as immunosuppression and malignancy are important factors in the diagnosis of CRBSI (20). In a study by Hajjej et al. (21), the presence of diabetes mellitus or presence of sepsis at the time of catheter insertion, prolonged catheterization, and the use of antibiotics before insertion, even in a single dose, were identified as independent risk factors for the development of infection, with a reported mortality rate of 21.8%. In Wittekamp et al. (22), the incidence of infection was high in central venous and arterial catheters, but even higher in arterial catheters. In a study from Turkey, advanced age, being treated in intensive care, use of antibiotics during catheterization, and prolonged catheterization were associated with the development of infection (14). In the present study, 57.2% of patients were over 65, 85.6% were being treated in the intensive care unit, and 37.6% were immunosuppressive. When the departments in which patients were hospitalized were compared, mortality rates were higher in the IICU. We attributed this to patients receiving treatment in IICU being hospitalized for longer periods due to advanced age and

Table 2. Distribution of causative microorganism species

Microorganism (n=240)	n (%)
Gram negative bacteria species	**121 (50.4)**
Acinetobacter baumanni	44 (18.3)
Klebsiella pneumonia	29 (12.1)
Pseudomonas aeruginosa	18 (7.5)
Escherichiae coli	5 (2.1)
Serratia marcescens	5 (2.1)
Enterobacter cloacae	5 (2.1)
Enterobacter aerogenes	4 (1.7)
Klebsiella oxytoca	3 (1.3)
Pseudomonas putida	2 (0.8)
Stenotrophomonas maltophilia	2 (0.8)
Burkholderia cepaciae	2 (0.8)
Morganella morganii	1 (0.4)
Pantoae spp	1 (0.4)
Gram positive bacteria species	**68 (28.3)**
MRCNS	31 (12.9)
Enterococcus faecium	17 (7.1)
Enterococcus faecalis	8 (3.3)
MRSA	7 (2.9)
MSSA	4 (1.7)
Corynebacterium spp.	1 (0.4)
Fungal types	**51 (21.3)**
Candida albicans	26 (10.8)
Candida tropicalis	10 (4.2)
Candida parapsilosis	9 (3.8)
Candida glabrata	3 (1.3)
Candida famata	1 (0.4)
Candida lipolytica	1 (0.4)
Candida guilliermondi	1 (0.4)

MRCNS: Methicillin-resistant coagulase negative staphylococcus, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*

comorbid disease, and to infections that developed with more resistant agents.

The present study also examined the effects of comorbid conditions, enteral nutrition, TPN, and mechanical ventilation on mortality rates. Asthma, CHF, CVE, and other risk factors such as transfusion, immunosuppression, tracheostomy, nasogastric tube, mechanical ventilation, and pulmonary artery catheter (PAC) were associated with mortality. While enteral nutrition is associated with mortality, it has been reported that TPN is not (10). This may be due to enteral nutrition, tracheostomy, nasogastric tube, PAC and mechanical ventilation being the most frequently applied interventions or treatments, especially in long-term hospitalizations and intensive care patients, and to the majority of our patients being treated in the ICU. Pichitchaipitak et al. (23) reported that long-term TPN use increased the rate of CRBSI.

While Gram-negative bacilli or *S. aureus* may be an agent in catheters inserted for a short period, Coagulase-negative staphylococci are generally factors in extended catheter insertion (10,24). In their study of hematology, oncology, and intensive care patients, 78% of whom were immunosuppressive, Demirel et al. (25) observed that half of the factors consisted of Gram negative bacteria (50%) and that Gram positive bacteria consisted of only 24.5%. The rate of Candida species was 23.9%. In a study involving hemodialysis patients, catheter infection factors were reported to be 60% Gram positive bacteria, 38% Gram negative bacteria, and 2% *Candida* spp. The authors of that study reported that only 20% of these patients had temporary catheters, the remaining 80% having permanent catheters, and that the incidence of infection in the temporary catheter group was higher than that in the permanent catheter group (26). All patients in the present study had temporary catheters, and the fact that information of the numbers of days with catheters could not be provided is a limitation of our study.

The relationship between susceptibility and mortality has also been investigated in recent research. In one study conducted in the hemodialysis unit, the reported carbapenem susceptibility of Gram-negative bacteria was 87.5%, with an overall mortality rate of 10% (26). Analysis of the effects of micro-organisms on mortality in the present study revealed no significant difference between infections with single or multiple factors in terms of mortality rates. At least one resistant agent or *Candida* spp. was detected in the cultures of 95 patients who died (78.5%). When our patients were divided into two groups based on the resistance status of microorganisms, the mortality rate was significantly higher in the group with resistant agents compared to the other group.

CONCLUSION

In conclusion, central catheter insertion is an invasive procedure and may result in morbidity and mortality by preparing the ground for infection. The purpose of this study was to contribute to the literature for Turkey by evaluating our own hospital's catheter infection data in the preceding five years. Advanced age and hospitalization in the IICU were more common in the mortal cases than the non-mortal cases. In addition, the frequency of resistant microorganisms in CRBSI was higher in mortal cases than non-mortal cases.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (15.06.2020, 130).

Conflict of Interest: None declared by the authors.

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Evaluation of the Effect of Aortic Stenosis and Severity on Left Ventricular Function by Isovolumic Myocardial Acceleration

Aort Darlığı ve Ciddiyetinin Sol Ventrikül Fonksiyonuna Etkisinin İsovolumik Miyokardiyal Akselerasyon ile Değerlendirilmesi

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ABSTRACT

Aim: Tissue Doppler-derived isovolumic acceleration (IVA) is a parameter that evaluates the systolic function of both ventricles, without being affected by pre-load and post-load. We aimed to detect left ventricular systolic dysfunction at an early stage with IVA in patients with asymptomatic aortic stenosis (AS).
Material and Methods: A total of 105 patients were included in the study, 75 of which had isolated AS and 30 were free of any valve disease. Patients with AS were divided into three groups (mild, moderate and severe) according to their aortic valve area (AVA) and aortic peak velocities, as determined by means of a transthoracic echocardiography. Conventional echocardiography, systolic and diastolic Tissue Doppler parameters [peak myocardial velocity during isovolumic contraction (IVV), myocardial velocity during ejection phase (Sm), early diastolic myocardial velocity (e'), late diastolic myocardial velocity (a'), and acceleration time (AT)] were calculated in all patients. IVA was obtained by dividing the IVV flow rate by the AT time.
Results: The systolic parameters IVV ($p<0.001$), Sm ($p<0.001$), IVA ($p=0.002$) and diastolic parameters e' wave ($p<0.001$), a' wave ($p=0.001$) were found to be significantly lower in patients with AS compared to the control group. However, this relationship observed in IVA was not different in AS subgroups ($p=0.122$). Sm and e' waves were positively correlated with AVA ($p=0.001$, $p<0.001$, respectively) and negatively correlated with aortic peak gradient ($p=0.008$, $p<0.001$, respectively), but IVA was not correlated.
Conclusion: Left ventricular function is impaired in patients with AS and this is independent of the severity of AS.
Keywords: Aortic stenosis; isovolumic acceleration; tissue Doppler imaging.

ÖZ

Amaç: Doku Doppler kaynaklı isovolumik akselerasyon (İVA) her iki ventrikülün sistolik fonksiyonunu ön ve ard yükten etkilenmeden değerlendiren bir parametredir. Bu çalışmada asemptomatik aort darlığı (AD) olan hastalarda, sol ventrikül sistolik disfonksiyonun İVA ile erken aşamada tespiti amaçlanmıştır.
Gereç ve Yöntemler: Çalışmaya izole AD bulunan 75 hasta ve herhangi bir kapak hastalığı bulunmayan 30 hasta olmak üzere toplam 105 hasta dahil edildi. AD hastaları, transtorasik ekokardiyografi ile belirlenen aort kapak alanı (AKA) ve aortik pik velositeye göre üç gruba (hafif, orta ve ileri) ayrıldı. Konvansiyonel ekokardiyografi parametreleri, sistolik ve diyastolik doku Doppler parametreleri [isovolumik kontraksiyon esnasında oluşan pik miyokardiyal velosite (IVV), ejeksiyon fazında oluşan miyokardiyal velosite (Sm), erken diyastolik miyokardiyal velosite (e'), geç diyastolik miyokardiyal velosite (a'), akselarasyon zamanı (AT)] tüm hastalarda hesaplandı. İVA, IVV akım hızının AT süresine bölünmesiyle elde edildi.
Bulgular: Sistolik parametrelerden IVV ($p<0.001$), Sm ($p<0.001$), İVA ($p=0.002$) ve diyastolik parametrelerden e' dalgası ($p<0.001$), a' dalgası ($p=0.001$) AD bulunan hastalarda kontrol grubuna göre anlamlı şekilde düşük izlendi. Fakat İVA da izlenen bu ilişki AD alt gruplarında anlamlı değildi ($p=0.122$). Sm ve e' dalgasının, AKA ile pozitif yönde (sırasıyla $p=0.001$, $p<0.001$) aortik pik gradiyentle negatif yönde korelasyonu vardı (sırayla $p=0.008$, $p<0.001$), ancak İVA ise korele değildi.
Sonuç: AD olan kişilerde sol ventrikül fonksiyonu bozulmuştur ve bu AD ciddiyetinden bağımsızdır.
Anahtar kelimeler: Aort darlığı; isovolumik akselerasyon; doku Doppler görüntüleme.

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INTRODUCTION

Aortic stenosis (AS) is the most common acquired heart valve disease in the population (2-7%) over 65 years (1). With the onset of symptoms, it shows a very rapid progression (2,3). 50-60% of patients who are not treated surgically are lost in approximately 2 years (4,5). Decreased left ventricular (LV) ejection fraction (EF) and the development of symptoms in AS are associated with poor prognosis (6). In addition, the presence of subclinical systolic dysfunction has been associated with mortality in patients with asymptomatic severe AS with preserved EF (7).

Recently, tissue Doppler imaging (TDI) and/or strain/strain-rate imaging have proven to be effective in demonstrating both global and regional LV systolic functions. Although LV EF is preserved in patients with AS, subclinical systolic dysfunction in the left ventricle has been demonstrated by both the S' wave obtained by TDI and the strain measured using two and three-dimensional speckle-tracking (8-16). It is known that TDI measurements are angle-dependent, influenced by the pre-load and after-load, and strain/strain-rate imaging requires complex programs, requires good image quality and is time consuming. Isovolumic acceleration (IVA), which is calculated by tissue Doppler method, is an easily measurable parameter in showing right ventricular (RV) and LV systolic functions. It is unaffected by pre-load and after-load (17-20). Experimental and clinical studies with IVA have shown strong correlation with invasive and non-invasive measurements of LV function (18-21). Although it is so advantageous, it is not used enough in our daily practice.

Its easy application will allow early intervention before myocardial damage becomes apparent or symptoms begin. There are no previous data comparing the severity of AS with healthy people. In this study, we aimed to evaluate the effect of asymptomatic AS on LV systolic function using TDI-derived IVA.

MATERIAL AND METHODS

A total of 105 patients; 75 patients with isolated AS (36 male, 39 female, mean age 65.7±11.5) and 30 patients without any valve disease (15 male, 15 female, mean age 63.9±6.0) were included in the study. All were evaluated by transthoracic echocardiography (TTE) and TDI. Those with AS were divided into three groups (mild, moderate and severe) according to their aortic valve area (AVA) and aortic peak velocities (APV) determined by TTE. Mild AS (AVA>1.5 cm^2, APV<3 m/sec) was found in 24 patients, moderate AS (AVA=1-1.5 cm^2, APV=3-4 m/sec) in 20 patient and severe AS (AVA≤1.0 cm^2, APV≥4 m/sec) was found in 31 patients (22).

In addition, patients with mildly more severe valve disease other than AS, low ejection fraction (EF<50), congenital heart disease, subvalvular and supravalvular AS, left bundle branch block, atrial fibrillation, pacemaker rhythm, chronic renal failure, ischemic ECG changes, and those with angina and/or acute coronary syndrome were all excluded from the study. Approval was sought and obtained by the ethics committee of the Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital on December 16, 2013, decision number 16. Written informed consent was obtained from all patients prior to enrollment and the study was performed in the Helsinki Declaration.

Conventional Echocardiography

A GE-Vivid 6 instrument (Horten, Norway) 2-4 MHz transducer was used to perform the echocardiographic evaluation of the patients and all images were recorded on digital media. Patients were evaluated with the parasternal long axis, apical four cavities, two cavities and five cavities images according to the guidelines. Two-dimensional, M-mode, PW, CW-Doppler and color flow Doppler echocardiographic measurements were performed. All images were recorded in a single-lead ECG recording and were calculated by averaging 5 consecutive cycles.

In the parasternal long axis window, LV end diastolic (LVEDD) and LV end systolic diameters (LVESD), LV outflow tract (LVOT) diameter, left atrial (LA) diameter, LV septum thickness (IVS) and posterior wall thickness (PW) were measured. Left ventricular mass (LVM) was calculated using the Devereux equation (23):

$$LVM=0.8\{1.04[([LVEDD+IVST+PWT]^3 - LVEDD^3)]\}+0.6$$

The left ventricular mass index (LVMI) was calculated by dividing the LVM by the body surface area. LVOT cross sectional area (CSA) was calculated by taking the LVOT diameter. LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) were measured from the apical four-chamber and two-chamber images and the LV EF was calculated using the modified Simpson method (24). The CW-Doppler was placed on the aortic valve from the apical 5-chamber image and the aortic time velocity integral (TVI), maximal and mean aortic gradients were measured. LVOT TVI was measured by the PW-Doppler over the LVOT. AVA was calculated using the continuity equation (6,22):

$$LVOT\ CSA\ (cm^2) = 0.785 \times (LVOT\ Diameter)^2$$

$$AVA = (LVOT\ CSA \times LVOT\ TVI) / Aortic\ TVI$$

In addition, the PW-Doppler was used to measure the early (E) and late (A) diastolic flow velocities and deceleration time (DT).

Tissue Doppler Imaging (TDI)

TDI measurements were performed at a high frame rate (>150 fps), using minimal optimal gain, with transducer frequency between 3.5 and 4.0 MHz, with the Nyquist limit set to 15-20 cm/sec. The monitor sweep rate was set to 50 to 100 mm/sec in order to optimize the spectral analysis of the myocardial velocities. Apical window images were selected to quantify regional wall motions simultaneously with Doppler inflow and outflow currents and to minimize the angle between Doppler beam and wall motion. A 5 mm pulsed Doppler sample volume was placed in the basal portion of the LV medial and lateral wall, at the end of the expiration on apical 4-chamber images (25). Peak myocardial velocity during isovolumic contraction (IVV), myocardial velocity during ejection phase (Sm), early diastolic myocardial velocity (e'), late diastolic myocardial velocity (a') flow rates and acceleration time (AT), were calculated by TDI. All measurements were calculated by averaging 5 consecutive cycles. IVA was obtained by dividing the IVV flow rate by the AT time (Figure 1):

$$IVA = IVV / AT$$

Global LV tissue Doppler measurements were obtained by averaging the tissue Doppler parameters measured from the septal and lateral walls.

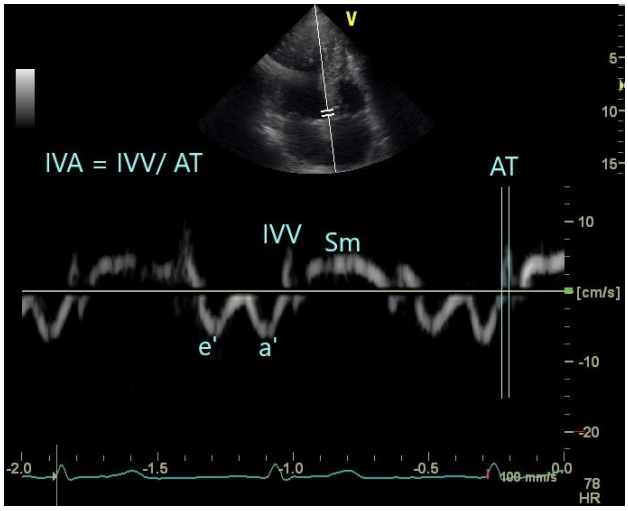

Figure 1. Tissue Doppler imaging-derived IVV and AT obtained from left ventricular septal basal wall of patients with aortic stenosis. IVV; peak myocardial velocity during isovolumic contraction; AT; isovolumic acceleration time, Sm; myocardial velocity during ejection phase, e'; early diastolic myocardial velocity, a'; late diastolic myocardial velocity, IVA; isovolumic acceleration

Statistics Analysis

In all statistical analysis, we used the SPSS v.21.0 statistical package. Distribution of the variables were tested with the Kolmogorov-Smirnov normality test. Descriptive statistics were expressed as mean±standard deviation (SD) or median, interquartile range (IQR) and minimum-maximum values depending on the distribution pattern. Categorical variables were expressed as frequency and percentages. If there was a normal distribution, the independent samples t test was used to compare two groups, one-way ANOVA was used when comparing more than two groups; if there was no normal distribution, the Mann-Whitney U test was used to compare two groups, the Kruskal-Wallis test was used to compare more than two groups. Tukey and Mann-Whitney U test with Bonferroni correction were used to determine which group caused the difference. The correlation analysis was performed using the Spearman correlation analysis and categorical variables were analyzed with Pearson chi-square test. The cases where the p value was less than 0.05 were evaluated as statistically significant.

RESULTS

Clinical Properties

There was no statistically significant difference in traditional risk factors and drug use between AS patients and the control group (Table 1). There was no statistically significant difference in these parameters between the subgroups of patients with AS (Table 1).

Two Dimensional and CW-Doppler Echocardiography Parameters

LVEDD, LVVESD, LV EF, LVEDV and LVESV parameters did not differ between the AS and the control group (Table 2). Similarly, no difference was found in these parameters between the AS subgroups (Table 2).

IVS, PW, LVMI and LA diameter were found to be significantly higher in the AS group compared to the control group (Table 2). LVMI and IVS thickness were found to be significantly lower in mild AS compared

Table 1. Demographic characteristics of the patients with aortic stenosis and control group

	AS Total (n=75)	Control (n=30)	p	Severity of AS			
				Mild (n=24)	Moderate (n=20)	Severe (n=31)	p
Age (year)	65.7±11.5	63.9±6.0	0.408	61.6±11.6	68.0±10.7	67.6±11.4	0.098
Gender, n (%)							
Male	36 (48.0)	15 (50.0)	0.853	11 (45.8)	9 (45.0)	16 (51.6)	0.870
Female	39 (52.0)	15 (50.0)		13 (54.2)	11 (55.0)	15 (48.4)	
Body mass index (kg/m²)	28.4±4.9	28.5±3.9	0.575	28.8±4.3	28.6±6.3	27.8±6.3	0.683
Body surface area (m²)	1.9±0.2	2.0±0.2	0.086	1.9±0.2	1.9±0.2	1.8±0.2	0.412
Heart rate (beats/min)	76.4±14.0	79.7±11.7	0.242	75.6±13.4	78.7±15.5	75.5±13.7	0.700
Systolic BP (mm Hg)	138 (29) [103-207]	130 (20) [110-159]	0.192	140 (45) [105-200]	140 (33) [103-183]	131 (22) [110-207]	0.332
Diastolic BP (mm Hg)	79 (20) [42-130]	70 (10) [60-90]	0.507	80 (16) [57-130]	79 (20) [57-110]	70 (23) [42-101]	**0.032**
Hypertension, n (%)	54 (72.0)	16 (53.3)	0.067	17 (70.8)	15 (75.0)	22 (71.0)	0.941
Diabetes mellitus, n (%)	23 (30.7)	8 (26.7)	0.685	7 (29.2)	7 (35.0)	9 (29.0)	0.886
Current smoking, n (%)	29 (38.7)	9 (30.0)	0.404	2 (8.3)	6 (30.0)	7 (22.6)	0.181
CAD, n (%)	15 (20.0)	3 (10.0)	0.219	7 (29.2)	9 (45.0)	13 (41.9)	0.499
Hyperlipidemia, n (%)	25 (33.3)	10 (33.3)	1.000	8 (33.3)	4 (20.0)	13 (41.9)	0.268
Drug use, n (%)							
ACE inhibitor	25 (33.3)	12 (40.0)	0.518	9 (37.5)	10 (50.0)	6 (19.4)	0.067
ARB	16 (21.3)	3 (10.0)	0.173	6 (25.0)	2 (10.0)	8 (25.8)	0.351
Statin	26 (34.7)	6 (20.0)	0.140	9 (37.5)	4 (20.0)	13 (41.9)	0.258
Beta blocker	30 (40.0)	7 (23.3)	0.106	8 (33.3)	7 (35.0)	15 (48.4)	0.458
CCB	16 (21.3)	5 (16.7)	0.589	2 (8.3)	5 (25.0)	9 (29.0)	0.159

AS: aortic stenosis, BP: blood pressure, CAD: coronary artery disease, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blockers, CCB: calcium channel blocker, descriptive statistics were given as mean±standard deviation for normally distributed variables, otherwise median (interquartile range) [minimum-maximum] were used

to severe AS (p<0.001, p=0.002, respectively), but there was no statistically significant difference between the other subgroups (p>0.017). PW thickness was significantly lower in mild and moderate AS subgroups than in the severe AS subgroup (p<0.001, p=0.004, respectively), but there was no statistically significant difference between the mild and moderate AS subgroups (p=0.230). LA diameters increased with the degree of AS, but this increase was not statistically significant (p=0.058).

PW-Doppler and Tissue Doppler Echocardiography Parameters

E wave, A wave, DT, E/e' ratio, e' wave, a' wave, IVV, Sm parameters were found to be statistically significantly different when the AS and the control groups were compared (Table 3). When subgroups of AS were compared to each other, E wave, A wave, DT, E/A ratio, and a' wave parameters were not observed to be different between the groups (Table 3).

E/e', e', IVV and Sm were found to be statistically different among subgroups of AS (p=0.005, p<0.001, p=0.016, p=0.029, respectively). When the groups were compared with each other, the E/e' and Sm were significantly different in the mild and severe AS subgroups, the e' wave in the mild to moderate AS and mild to severe AS, the IVV parameter was statistically different between the mild and moderate AS subgroups (p=0.001, p=0.011, p=0.016, p<0.001, p=0.003, respectively). There was no significant difference in other subgroup comparisons (p>0.017).

IVA was significantly lower in the AS group than in the control group (p=0.002). However, in the subgroup analysis, IVA was did not differ in severity of the stenosis (p=0.122).

Table 2. Comparison of two-dimensional, M-mode and CW-Doppler echocardiographic parameters of aortic stenosis patients and control group

	AS Total (n=75)	Control (n=30)	p	Severity of AS			
				Mild (n=24)	Moderate (n=20)	Severe (n=31)	p
LVEDD (mm)	48.4±4.5	47.8±3.5	0.532	48.3±4.8	50.0±4.6	40.7±4.0	0.183
LVESD (mm)	30.3±4.5	29.8±2.7	0.527	30.2±4.9	31.7±4.7	29.4±3.9	0.213
IVS (mm)	12.0 (3.0) [0.9-2.1]	10.0 (1.0) [0.9-1.3]	**<0.001**	11.5 (1.0) [0.9-1.5]	12.0 (3.0) [1.0-1.6]	13.5 (3.0) [1.2-2.1]*	**<0.001**
PW (mm)	12.0 (2.0) [0.9-1.7]	10.0 (0.5) [0.9-1.2]	**<0.001**	11.0 (1.8) [0.9-1.3]	11.0 (2.0) [1.0-1.4]	12.0 (2.7) [1.1-1.7]#	**<0.001**
LVEF (%)	61.0 (5.0) [50-70]	60.0 (3.7) [55-70]	0.980	62.0 (4.0) [52-69]	62.0 (7.0) [55-70]	60.5 (5.0) [50-70]	0.830
LV diastolic volume (ml)	112.1±24.6	104.9±24.2	0.179	109.8±26.5	112.1±22.6	114.0±25.1	0.822
LV systolic volume (ml)	40.0 (19.0) [23-85]	40.5 (12.5) [27-80]	0.511	37.5 (26.0) [23-68]	40.0 (13.0) [27-80]	43.0 (23.2) [25-85]	0.904
LVMI (gr/m^2)	123.4±33.7	91.8±17.6	**<0.001**	106.4±20.5	121.4±29.6	137.5±38.7&	**0.008**
LA diameter (cm)	3.9 (0.6) [2.8-5.0]	3.6 (0.3) [3.2-4.2]	**0.003**	3.8 (0.4) [2.8-4.5]	3.9 (0.9) [3.0-5.0]	4.0 (0.5) [3.4-4.9]	0.058
		Aortic valve area (cm^2)		1.7±0.1	1.3±0.2	0.8±0.2	**<0.001**^
		Aortic peak velocity (m/sn)		2.7±0.1	3.5±0.3	4.6±0.5	**<0.001**^
		Aortic peak gradient (mm Hg)					
		Maximum		29.3±2.4	50.1±7.7	87.4±19.1	**<0.001**^
		Mean		16.1±1.7	30.4±10.8	52.8±12.6	**<0.001**^

AS: aortic stenosis, LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, IVS: left ventricular septum thickness, PW: posterior wall thickness, LVEF: left ventricular ejection fraction, LV: left ventricle, LVMI: left ventricular mass index, LA: left atrium; *: p=0.002 vs mild, #: p<0.001 vs mild and p=0.004 vs moderate, &: p<0.001 vs mild, ^: p <0.001 between all subgroups, descriptive statistics were given as mean±standard deviation for normally distributed variables, otherwise median (interquartile range) [minimum-maximum] were used

Table 3. Comparison of PW and tissue Doppler echocardiographic parameters of aortic stenosis patients and control group

	AS Total (n=75)	Control (n=30)	p	Severity of AS			
				Mild (n=24)	Moderate (n=20)	Severe (n=31)	p
E wave (cm/sn)	80.0 (40.0) [40-150]	78.5 (23.0) [33-100]	**0.013**	78.0 (29.0) [46-135]	84.0 (40.0) [50-120]	77.5 (33.0) [40-150]	0.711
A wave (cm/sn)	102.2±29.1	78.8±17.5	**<0.001**	95.2±27.6	107.5±25.0	104.3±32.3	0.334
E/A rate	0.78 (0.5) [0.4-2.1]	0.96 (0.4) [0.4-1.7]	0.147	0.78 (0.5) [0.5-1.5]	0.75 (0.5) [0.5-1.3]	0.79 (0.4) [0.4-1.5]	0.580
DT (msn)	306.2±73.5	255.5±60.7	**0.001**	279.9±51.1	324.3±88.4	314.9±74.3	0.094
E/e' rate	11.7 (8.3) [4.5-37.5]	7.1 (4.7) [2.7-12.1]	**<0.001**	9.5 (6.4) [4.5-16.2]	13.8 (9.7) [6.1-25.0]	12.8 (10.7) [8-37.5]*	**0.005**
e' (cm/sn)	6.5 (1.2) [2.0-14]	9.7 (0.6) [6.0-15.0]	**<0.001**	7.5 (2.0) [4.0-14.0]#	6.0 (2.0) [2.0-9.0]	5.5 (2.0) [4.0-11.0]	**<0.001**
a' (cm/sn)	10.4±2.4	12.1±2.0	**0.001**	11.2±0.2	10.3±0.2	9.7±0.3	0.078
IVV (cm/sn)	7.0 (4.0) [4.0-19.0]	8.7 (5.0) [4.0-19.0]	**<0.001**	7.5 (3.0) [4.0-16.0]&	5.0 (2.0) [4.0-9.0]	7.5 (4.0) [2.0-12.0]	**0.016**
Sm (cm/sn)	7.0 (2.0) [3.0-12.0]	8.2 (2.0) [7.0-12.0]	**<0.001**	8.2 (3.0) [5.0-12.0]	6.5 (2.0) [3.0-10.0]	6.5 (2.0) [3.0-12.0]^	**0.029**
IVA (m/sn^2)	2.2 (1.4) [0.6-5.7]	2.9 (1.9) [1.1-5.5]	**0.002**	2.3 (1.1) [1.2-4.2]	1.9 (0.7) [1.0-3.7]	2.7 (1.7) [0.6-5.7]	0.122

AS: aortic stenosis, E: early diastolic flow velocities, A: late diastolic flow velocities, DT: deceleration time, e': peak myocardial velocity during early diastole, a': peak myocardial velocity during atrial contraction, IVV: peak myocardial velocity during isovolumic contraction, Sm: peak myocardial velocity during systole, IVA: myocardial acceleration during isovolumic contraction, *: p=0.001 vs mild, #: p=0.016 vs moderate and p<0.001 vs severe, &: p=0.003 vs moderate, ^: p=0.011 vs mild, descriptive statistics were given as mean±standard deviation for normally distributed variables, otherwise median (interquartile range) [minimum-maximum] were used

Correlation between TDI Velocities and Conventional Echocardiographic Parameters

In the Spearman correlation analysis, E/e' ratio, IVS and PW thickness were negatively correlated with AVA, whereas LVMI, e' wave, a' wave and Sm parameters were positively correlated with AVA. When the correlation of echocardiographic parameters with aortic peak gradient (APG) was examined, Sm and e' wave were negatively correlated, whereas E/e', LVMI, IVS, PW thickness were positively correlated. However, there were no correlations between IVA (Table 4).

Table 4. Correlation between tissue Doppler imaging velocities and conventional echocardiographic parameters

	Aortic valve area		Aortic peak gradient	
	r	**p**	**r**	**p**
E/e' rate	-0.380	**0.001**	0.363	**0.001**
LVMI	0.413	**0.001**	0.360	**0.002**
Global IVA	0.039	0.744	0.090	0.444
e'	0.531	**<0.001**	-0.526	**<0.001**
a'	0.340	**0.007**	-0.201	0.087
Sm	0.388	**0.001**	-0.305	**0.008**
IVV	0.163	0.165	-0.017	0.889
IVS	-0.495	**<0.001**	0.533	**<0.001**
PW	-0.487	**<0.001**	0.518	**<0.001**

E: early diastolic flow velocities, e': peak myocardial velocity during early diastole, LVMI: left ventricular mass index, IVA: myocardial acceleration during isovolumic contraction, a': peak myocardial velocity during atrial contraction, Sm: peak myocardial velocity during systole, IVV: peak myocardial velocity during isovolumic contraction, IVS: left ventricular septum thickness, PW: posterior wall thickness

DISCUSSION

In our study, TDI-derived LV systolic and diastolic velocities were found to be reduced in patients with AS compared to the control group. While we determined the relationship between the severity of stenosis, systolic and diastolic parameters, we were unable to determine the relationship of IVA in the subgroups of AS. This is the first study to compare the severity of AS with LV systolic parameters, with the inclusion of a control group. Thus, we understood that subclinical systolic dysfunction occurred independently of AS severity.

LV systolic function is normal in most patients with severe AS. Impaired myocardial contraction can be detected using the tissue Doppler and/or the speckle tracking strain method without EF decline (8-16,26). Detection of subclinical systolic dysfunction often results in a poor prognosis and has been shown to improve after aortic valve replacement (15,27,28). Similarly, Nieh et al. (29) found that echocardiographic parameters of patients operated for severe AS did not change LV diameter, mass and EF, but improvements in systolic and diastolic parameters measured by TDI were noted at a mean follow-up of 120 days. Subclinical systolic dysfunction detected by TDI has been shown in other valve pathologies and systemic diseases (30-34).

In our study, LVMI, IVS and PW thickness were higher in the AS group compared to the control group, as expected, and this increase correlated with the severity of AS. In the subgroup analysis, likewise Galema et al. (12), LWMI and IVS thickness differed from mild to severe AS, and as with Rajani et al. (35), there was no difference between moderate to severe AS in terms of LWMI. In our study, PW thickness was different between both mild to severe and moderate to severe AS. It is known that LV hypertrophy occurs to compensate for the pressure burden caused by AS (36). Post-operative mortality and morbidity were found to be high in patients with LV hypertrophy and undergoing aortic valve surgery (37). While only 20% of patients with AS have impaired EF, many patients with preserved EF have experienced increased LVMI heart failure rates and subclinical systolic dysfunction (38).

Although LV EF is not reduced in patients with AS, emerging symptoms such as dyspnea and fatigue have been associated with disturbances in diastolic parameters, measured by non-invasive methods (8,12,14). In our study, diastolic parameters (mitral flow E, A, E/A, DT) measured by conventional methods were different from the control group, regardless of the severity of AS. Galema et al. (12), in their study comparing healthy controls with patients with symptomatic severe AS, as well as Jassal et al. (8) in their own study, divided mild-moderate AS patients into three groups, and similarly to our study, did not find a significant relationship between the degree of stenosis and the traditional diastolic function parameters. When diastolic functions were measured by the more sensitive TDI method, we found that AS was impaired compared to controls and this impairment was associated with the degree of AS, similar to what was reported in other studies (8,10-13,39). Diastolic dysfunction in AS may be associated with increased myocardial stiffness, decreased LV compliance, increased LVMI, increased end-diastolic pressure and impaired LA function. Truong et al. (40) showed that high LV diastolic pressures before transcatheter aortic valve replacement were associated with mortality.

In patients with severe AS, a positive correlation was shown between end-diastolic LV pressure and E/e' ratio, measured by invasive method (10). In addition, E/e' >15 was associated with an elevated mean LA pressure; in our study, the LA diameter, LVMI and E/e' ratio were found to support LV diastolic dysfunction. Polito et al. (41), while comparing moderate and severe AS with the control group, found that the LVMI and E/e' ratio was similarly high. In their study where they used LA volume instead of LA diameter, this value was also significantly higher. In previous studies, increased E/e' was associated with symptoms, surgical necessity and mortality in AS (42).

In many studies, AS patients with preserved systolic function were evaluated for LV subclinical systolic functions by TDI method and a significant decrease was found compared to the control groups (10,11,13-15,39). Systolic dysfunction cannot be detected by conventional echocardiography, but can be detected by the TDI method, LVH and subendocardial ischemia due to increased pressure load, and thus longitudinal contraction is affected (43). The systolic parameters measured by the TDI method are evaluated in the longitudinal axis functions of the left ventricle and show loss of function at the subclinical stage, without obvious LV systolic dysfunction. In our study, Sm, IVV and IVA, which showed LV subclinical systolic dysfunction, were decreased in the AS group, and only the decrease in Sm wave was moderately correlated with AVA and APG. Similar to our study, Poh et al. (39) found 53 patients with AS in their study correlated Sm and AVA index. Barthelemy et al. (34) compared RV function in critically ill patients with invasive

methods and non-invasive methods, while they could not find the correlation of IVA, but they observed that Sm wave correlated with RV EF.

In our study, although LV IVA decreased in patients with AS, this decrease did not correlate with the severity of stenosis like Sm; this may be due to the Sm wave being affected more by preload and afterload. In support of our study, Ertürk et al. (33) found that LV IVA decreased in patients with mitral stenosis, but could not correlate this decrease with the severity of mitral stenosis. If we consider IVA as the time to overcome the resistance against the stenosis in front of it, in patients with mild AS, the IVA extends for a certain period and remains constant regardless of resistance. This stability suggests that a solid left ventricle is sufficient to overcome the resistance in front of it, regardless of the valve area and gradient. Further clinical studies suggest that IVA may become significant between groups or in the severe AS group, which may be explained by this difference with patients with low EF.

Study Limitations

Our study had some limitations; the number of patients was low, the systolic function parameters were not compared with parameters measured by invasive methods. In addition, patients were not evaluated with strain/strain rate imaging and the value of systolic function parameters in predicting clinical deterioration and surgery in long-term follow-up was not investigated. Although asymptomatic patients were included in our study, the existing coronary lesions and myocardial ischemia were not known. Patients with systolic dysfunction were not included in our study.

CONCLUSION

We found that asymptomatic AS with normal EF, systolic and diastolic function parameters measured by the TDI method were impaired and this deterioration was associated with degree of stenosis. We could not determine the relationship with the degree of stenosis by IVA during LV contraction.

Ethics Committee Approval: The study was approved by the Ethics Committee of Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital (16.12.2013, 16).

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Inferior Gluteal Nerve Injury Due to Intramuscular Injection

İntramüsküler Enjeksiyona Bağlı İnferior Gluteal Sinir Yaralanması

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ABSTRACT

Aim: The purpose of this study was to determine the clinical features of the inferior gluteal nerve (IGN) injury due to intramuscular (IM) injection.
Material and Methods: Patients with clinical and electrodiagnostic features of the sciatic nerve (SN) and possible IGN injuries due to IM injection were included in this retrospective study. The presence of an IGN injury was considered in patients with weakness in the gluteus maximus (GM) muscle or in those who demonstrated needle electromyography (EMG) abnormality in the GM muscle.
Results: There were 44 (95.6%) patients with an SN injury only, 1 (2.2%) patient with both an SN and an IGN injury, and 1 (2.2%) patient with an IGN injury only. The complaints of the patient with an IGN injury only occurred within hours to days after the IM injection; this patient had no muscle weakness. The complaints of the patient with both IGN and SN injuries occurred minutes to hours after IM injection; this patient had mild weakness in the plantar flexion of the foot. In 40 of the patients with only an SN injury, complaints occurred immediately after or within a few seconds following the IM injection, while complaints occurred within minutes to hours in the remaining 4 patients.
Conclusion: Although rare when compared to SN injury, the IGN can be injured by IM injection. Therefore, the GM muscle should be examined with needle EMG in patients with complaints associated with IM injection. Muscle weakness may not occur in nerve injuries due to IM injections.
Keywords: Electromyography; inferior gluteal nerve; intramuscular injection; sciatic nerve.

ÖZ

Amaç: Bu çalışmanın amacı intramüsküler (İM) enjeksiyona bağlı gelişen inferior gluteal sinir (İGS) yaralanmasının klinik özelliklerinin belirlenmesidir.
Gereç ve Yöntemler: Klinik ve elektrodiagnostik özellikleri İM enjeksiyona bağlı gelişen siyatik sinir (SS) ve olası İGS yaralanmaları ile uyumlu olan hastalar bu geriye dönük çalışmaya dahil edildi. Gluteus maksimus (GM) kasında güçsüzlük ya da GM kasında iğne elektromiyografi (EMG) anormalliği olan hastalarda İGS yaralanması olduğu kabul edildi.
Bulgular: Sadece SS yaralanması olan 44 (%95,6) hasta, hem SS hem İGS yaralanması olan 1 (%2,2) hasta ve sadece İGS yaralanması olan 1 (%2,2) hasta mevcuttu. Sadece İGS yaralanması olan hastanın şikayetleri İM enjeksiyondan sonra saatler ile günler içinde oluşmuştu ve bu hastanın kas güçsüzlüğü yoktu. Hem İGN hem SS yaralanması olan hastanın şikayetleri İM enjeksiyondan sonra dakikalar ile saatler içinde oluşmuştu ve bu hastanın ayak plantar fleksiyonunda hafif derecede güçsüzlük mevcuttu. Sadece SS yaralanması olan hastaların 40'ında şikayetler İM enjeksiyonu takiben hemen ya da saniyeler içinde ortaya çıkarken geri kalan 4 hastada ise şikayetler IM enjeksiyonu takiben dakikalar ya da saatler içinde oluşmuştu.
Sonuç: SS yaralanması ile karşılaştırıldığında nadir olsa da, İM enjeksiyon ile İGS yaralanabilir. Bu nedenle İM enjeksiyon ile ilişkili şikayetleri olan hastalarda GM kası iğne EMG ile değerlendirilmelidir. İM enjeksiyonlara bağlı gelişen sinir yaralanmalarında kas güçsüzlüğü oluşmayabilir.
Anahtar kelimeler: Elektromiyografi; inferior gluteal sinir; intramüsküler enjeksiyon; siyatik sinir.

INTRODUCTION

The sciatic nerve (SN) injury can occur as a result of hip surgery, intramuscular (IM) injection, or trauma (1-4). The degree of injury can range from mild to severe and can even result in disability (3). The SN is formed from the ventral rami of the L4, L5, S1, S2, and S3 spinal nerves. The SN leaves the pelvis through the greater sciatic foramen inferior to the piriformis muscle (5,6). The inferior gluteal nerve (IGN) originates from the ventral rami of the spinal nerves of L5, S1, and S2 and exits the pelvis along a similar course to that of the SN. Along this course, the IGN runs medial and very close to the SN (6). Because the superior gluteal, posterior femoral cutaneous, and pudendal nerves also pass through the greater sciatic foramen, they can be injured, along with the IGN and SN, whether by IM injection or other conditions (2,7). There are also reports that the IGN specifically can be injured by conditions such as schwannoma or colorectal carcinoma (8,9). The aim of the study was to find the clinical and electrodiagnostic features of an SN and/or possible IGN injury due to an IM injection.

MATERIAL AND METHODS

Subjects

Patients who applied to our neurology outpatient clinic and laboratory of clinical neurophysiology between July 2018 and January 2020 were analyzed retrospectively. Patients with clinical and electrodiagnostic findings of an SN and/or an IGN injury due to IM injection were included in the study, provided that their complaints started after an IM injection. An SN injury due to an IM injection (SNIII) was considered in patients with sensory abnormalities or muscle weakness, or, alternately, in those who exhibited electrodiagnostic findings consistent with an SN injury. The inclusion criteria for an IGN injury were weakness in the gluteus maximus (GM) muscle; abnormal needle electromyography (EMG) findings in the GM muscle; or sensory abnormality over the lower outer quadrant (the greater trochanter) as the IGN may have sensory branches (10). The patients with an IGN injury had to have normal needle EMG findings in the gluteus medius, as well as in the L3, L4, L5, and S1 paraspinal muscles. Lumbosacral magnetic resonance imaging (MRI) findings of these patients should not be compatible with radiculopathy. Individuals with polyneuropathy (or a disease that may cause polyneuropathy such as diabetes mellitus), neurodegenerative disease, lumbosacral radiculopathy, and low back pain were excluded from the study. Muscle strength was analyzed using the Medical Research Council (MRC) scale (11). The Turkish version of the Leeds assessment of neuropathic symptoms and signs (LANSS) was used to evaluate neuropathic complaints (12).

This study was approved by the Ethics Committee of Adana City Training and Research Hospital (number: 45/620, date. December 4, 2019).

Electrodiagnostic Tests

The Cadwell Sierra Summit EMG unit (Cadwell laboratories, Kennewick, Washington, USA) was used for nerve conduction studies and needle EMG. Electrodiagnostic tests were performed if the temperature of the limb was ≥32 ºC, otherwise, cold limbs were heated. Low-high band filters for sensory and motor nerve conduction were set at 20Hz-2kHz and 20Hz-10kHZ, respectively. Stimulation and recordings were performed with surface electrodes. Nerve stimulation was performed supramaximally. The sweep speeds for sensory and motor nerve conduction studies were set as 1 ms/division and 5 ms/division, respectively. Sensitivity levels for sensory and motor nerve conduction studies were 10 μV/division and 2 mV/division, respectively. Reference values for nerve conduction studies were obtained from previous studies (13-15). Peroneal, tibial, superficial peroneal, peroneal, and sural nerve conduction studies were performed bilaterally (1). Compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) amplitudes were measured from peak to peak. To exclude peroneal neuropathy, peroneal nerve points were stimulated at the ankle, fibula head, and popliteal fossa. The peroneal nerve CMAP was recorded from both the extensor digitorum brevis (EDB) and the tibialis anterior (TA) muscles. The recording electrode was placed over the abductor hallucis muscle to obtain the tibial nerve CMAP. Superficial peroneal and sural sensory nerve conduction studies were performed antidromically. Sensory nerve conduction velocity was calculated using onset latency. The reference lower limits for the CMAP amplitudes of the tibial nerve and the peroneal nerve recorded from the EDB/TA muscle were 4.4 mV, 2.6 mV / 1.7 mV, respectively. The reference lower limit for amplitudes of both the superficial peroneal and sural nerves was 5 μV (13-15). The amplitude of CMAP or SNAP was considered abnormal if the CMAP or SNAP amplitude was lower than the reference lower limit, or lower than 50% of the CMAP or SNAP amplitude of the intact extremity nerve. Needle EMG was performed using a concentric needle electrode (length=50mm, diameter=0.46mm, Bionen Medical Devices, Florence, Italy). Low-high band filters for needle EMG were 10Hz-10kHz. The sweep speed for active denervation and motor unit action potential (MUAP) analysis was 10 ms/division. Sensitivity levels for active denervation and MUAP analysis were 50-100 μV and 500-1000 μV, respectively. Needle EMG was performed visually. Positive sharp wave (PSW) and fibrillation potentials were carefully analyzed. The MUAP was analyzed during mild muscle contraction. If the MUAP amplitude was >4 mV and duration was >15 ms, the MUAP was considered neurogenic. Needle EMG was applied to the medial gastrocnemius, TA, peroneus longus, biceps femoris short head, vastus lateralis and GM muscles of the patients. To exclude lumbosacral plexopathy or lumbosacral radiculopathy, needle EMG was also performed on the gluteus medius and L3, L4, L5 and S1 paraspinal muscles of some patients. Further, hip and lumbosacral MRIs were analyzed in some patients to exclude lumbosacral radiculopathy and masses in the gluteal region.

Statistical Analysis

The Shapiro-Wilk test was used to determine the distribution of the data. Mean±standard deviation was calculated for descriptive statistics. Categorical variables were summarized as percentage and frequency. Statistical Package for the Social Sciences (SPSS IBM Corp; Armonk, NY, USA) v.22.0 was used to perform the statistical analysis.

RESULTS

Forty-six patients (34 male, 12 female) were included in the study. Apart from these forty-six patients, three patients had clinical findings compatible with SNIII. However, these three patients had needle EMG abnormality in the L4, L5, and S1 paraspinal muscles in addition to the GM muscle, and also had lumbosacral MRI findings compatible with lumbosacral radiculopathy. The mean age of the patients was 39.9±14.7 (range, 19-69) years. The mean body mass index (BMI) of the patients was 21.8±3.4 (range, 15.6-31.9) kg/m^2, respectively. Twelve (26.1%) patients had a BMI <18.5 kg/m^2 while 4 (8.7%) had a BMI >25 kg/m^2. The mean interval between the time of IM injection and the time of electrodiagnostic test was 9.7±9.8 (range, 0.7-36.1) months. The IM gluteal injection was applied by a nurse or a paramedic to the upper outer quadrant of the gluteal region of all patients. The reasons for IM injections were upper respiratory tract infection in 14 (30.4%) patients, muscle or joint pain in 8 (17.4%), generalized pain in 7 (15.2%), abdominal pain in 5 (10.9%), headache in 4 (8.7%), urinary tract infection in 4 (8.7%), toothache in 3 (6.5%), and allergy in 1 (2.2%). IM agents were analgesics in 35 (76.1%) patients, antibiotics in 5 (10.9%) patients, antibiotics+analgesics in 3 (6.5%) patients, and an allergy drug in 1 (2.2%) patient. IM agents were unknown in two patients.

The mean LANSS score of the patients was 14.1±5.8 (range, 3-24). The LANSS score was ≥12 in 30 (65.2%) of the patients. The right lower extremity was affected in 16 (34.8%) patients. The neurological examination findings of the patients are shown in Table 1. Sensory abnormality over the sole of the foot only was observed in 3 (6.5%) patients, while sensory abnormality in the skin area supplied by the peroneal nerve only was observed in 14 (30.4%) patients. Five patients had weakness in only peroneal nerve innervated muscles. None of the patients exhibited weakness in only tibial nerve innervated muscles. Table 2 shows abnormalities of nerve conduction studies in patients. Needle EMG abnormalities of patients are shown in Table 3. SN injury only was present in 44 (95.6%) patients (33 male, 11 female), both SN and IGN injuries in 1 (2.2%) patient (male), and only an IGN injury in 1 (2.2%) patient (female). The complaints of 40 (87.0%) patients with SN injuries occurred immediately following IM injection, while 5 (10.9%) patients occurred within minutes to hours following IM injection.

The patient suffering exclusively with an IGN injury -a thirty-year old woman- applied to our EMG laboratory four weeks after IM injection. Diclofenac was applied to the patient intramuscularly due to generalized pain. The complaint of this patient started within hours to days following IM injection, and progressed over the following days. Within two to three weeks, the pain and paresthesia severity of the patient increased and reached its peak. The patient had hip pain, paresthesia (pins and needles), and sensory loss over the lateral gluteal region, but had no weakness. Bilateral tibial, peroneal, superficial peroneal, and sural nerve conduction studies were normal. The patient had PSW and fibrillation potentials in the GM muscle, but there were no needle EMG abnormalities in other muscles including the MG, TA, PL, biceps femoris short head, vastus lateralis, gluteus medius, L3, L4, L5, and S1 paraspinal muscles. The complaints of this patient improved within two months. She had normal hip and lumbosacral MRIs.

The patient with both SN and IGN injuries -a fifty-eight year old man- expressed complaints within minutes following the IM injection. Diclofenac was given intramuscularly due to an upper respiratory infection. This patient was referred to our EMG laboratory thirty days after IM injection. The MRC score of his foot plantar flexion was four. There was sensory loss in the dorsal region and sole of the foot. The tibial nerve CMAP and sural SNAP amplitudes were reduced. There were PSW and fibrillation potentials in GM and MG muscles, but no neurogenic MUAPs were present in these muscles. Needle EMG was normal in the TA, PL, biceps femoris short head, gluteus medius, vastus lateralis, and L3, L4, L5 and S1 paraspinal muscles. He had normal hip and lumbosacral MRIs. After two months, sensory complaints of the patient decreased, but muscle weakness persisted.

Needle EMG was applied to the L3, L4, L5 and S1 paraspinal muscles in 37 (80.4%) patients with an SN injury only; there was no active denervation in the paraspinal muscles of these patients. Thirty-nine (84.8%) of the patients with an SN injury only had lumbosacral MRIs and their findings were not compatible with lumbosacral radiculopathy.

Table 1. Clinical features of the patients

Neurologic examination	n (%)
Sensory abnormality	
Dorsum of foot or lateral of foot (peroneal)	40 (87.0%)
Sole of foot (tibial)	29 (63.0%)
Posterolateral leg (sural)	28 (60.9%)
Lateral aspect of the GM muscle	1 (2.2%)
None	2 (4.3%)
Weakness	
Peroneal nerve innervated muscles	38 (82.6%)
Tibial nerve innervated muscles	24 (52.2%)
Knee flexors	31 (67.4%)
GM muscle	0 (0.0%)
None	3 (6.5%)

GM: Gluteus maximus

Table 2. Nerve conduction studies of the patients

CMAP and SNAP amplitude abnormality	n (%)
Peroneal nerve EDB	26 (56.5%)
Peroneal nerve TA	16 (34.8%)
Peroneal nerve EDB or TA	34 (73.9%)
Tibial nerve	28 (60.9%)
Superficial peroneal nerve	30 (65.2%)
Sural nerve	35 (76.1%)
None	2 (4.3%)

CMAP: Compound muscle action potential, SNAP: Sensory nerve action potential, EDB: Extensor digitorum brevis, TA: Tibialis anterior

Table 3. Needle electromyography abnormalities of the patients, n (%)

Muscle	Active Denervation	Neurogenic MUAPs	Active Denervation or Neurogenic MUAPs
TA	24 (52.2%)	18 (39.1%)	30 (65.2%)
PL* (n=43)	16 (37.2%)	18 (41.9%)	24 (55.8%)
TA or PL	25 (54.3%)	23 (50.0%)	31 (67.4%)
MG	23 (50%)	19 (41.3%)	31 (67.4%)
Biceps femoris - short head* (n=44)	22 (50%)	19 (43.2%)	31 (70.5%)
GM	2 (4.3%)	0 (0.0%)	2 (4.3%)
None	7 (15.2%)	21 (45.7%)	1 (2.2%)

MUAP: Motor unit action potential, TA: Tibialis anterior, PL: Peroneus longus, MG: Medial gastrocnemius, GM: Gluteus maximus, *: Note that the short head of the biceps femoris and the peroneus muscles cannot be examined by needle electromyography in 3 and 2 patients, respectively

DISCUSSION

SNIII is associated with factors such as the angle of the injector, the amount of gluteal protective tissue, and the position of the patient (3,16-18). Thin individuals are more likely to have less gluteal protective tissue, so these individuals may be at risk for SNIII (3,4). The fact that 12 patients in this study had a BMI <18.5 kg/m^2 may support this observation. In addition, there were only 4 (8.7%) patients with BMI >25 kg/m^2. In this study, it was found that analgesics were among the IM agents associated with SNIII. This may be related to frequent use of analgesics. Many drugs that are administered intramuscularly, such as vitamins and antibiotics, can cause SNIII (3). Peripheral nerve injury is associated with the neurotoxicity of the drug. Some neurotoxic drugs such as benzylpenicillin, chlorpromazine, and diazepam can cause peripheral nerve injury even if they are injected extra-fascicularly (19).

It is known that the peroneal part of the SN is more affected than the tibial part in SNIII (1,3,4). This can be explained by the fact that the peroneal part is more lateral and has less connective tissue than the tibial part (20,21). In this study, weaknesses were more prominent in peroneal nerve innervated muscles. Also, sensory abnormalities were higher in the skin area innervated by the peroneal nerve. Yuen et al. (1) found that only the peroneal part was affected in approximately 10.0% of patients with an SN injury, and there were no patients with only the tibial part affected. In our study, five patients had weakness in peroneal innervated muscles only and no patients had weakness in tibial innervated muscles exclusively. Therefore, it should be noted that while more than one branch of the SN is affected in SNIII, the peroneal part is more severely affected (1,3,4). Although patients often complain of muscle weakness and pain, sensory abnormalities can be found in most patients with a careful neurological examination. In this study, the sensory examination of 44 (95.6%) patients was abnormal. Neuropathic pain is an important symptom in patients with nerve injury due to IM injection. The high number of patients with LANSS scores ≥12 in our study supports this situation.

Three (6.5%) patients had no weakness and 2 (4.3%) patients had normal nerve conduction studies, while all patients except one had needle EMG abnormalities. These findings indicate that electrodiagnostic tests are important in patients with SN and/or IGN injuries due to IM injection, and that needle EMG should be applied to these patients. Needle EMG also plays an important role in differential diagnosis. Three patients who were excluded from the study had needle EMG abnormalities compatible with lumbosacral radiculopathy. The patient with an IGN injury only had normal nerve conduction study results and needle EMG of the muscles innervated by the SN and its branches. Therefore, even if the nerve conduction studies are normal in patients with complaints associated with IM injection, needle EMG should be applied to the GM muscle to exclude an IGN injury. It should be noted that nerve conduction studies, including those of the sural sensory nerve, may have normal results in an SN injury. The sural SNAP amplitude abnormality found in this study (76.1%) was close to that found in another study (1).

IGN paresis is a rare condition. It can be damaged alone or along with other nerves. Inferior gluteal neuropathy has been reported as a result of colorectal carcinoma, schwannoma, IM injection or inadequate stabilization of the back due to lumbar lordosis (2,7-9). Obach et al. (2) reported 137 cases with nerve injuries due to gluteal IM injection. In 2 of these patients, the IGN was injured along with other nerves. The IGN is located very close and medial to the SN during part of its course. This indicates that the SN and the IGN can be injured together (2,6). In this study, the SN and IGN were injured together in 1 patient. IM injection can damage the nerve directly or damage the nerve through diffusion as a result of IM injection very close to the nerve or to the epineurium. While complaints begin immediately following IM injection directly to the nerve, complaints begin within minutes-hours following IM injection very close to the nerve (3,18,20). In this study, in most patients, complaints began immediately following IM injection, similar to previous studies (3,18). However, the complaints of the patient with an IGN injury only started within hours and the complaints intensified within days. While the IGN is more superficial in the medial part of GM muscle, it is slightly deeper in the lateral part (5). Since the nerve is located deeper in the region where the IM injection was performed, the possibility of intraneural injection appears to be difficult. However, there may still be a possibility of an IGN injury due to IM injection. If the IM injection is made into the fatty tissue around the nerve, complaints may start later due to toxic swelling, vascular lesions, necrosis, or fibrosis (2). This may be one of the reasons for the late onset of the complaints of the patient with the IGN injury only. To protect from the nerve injury, IM injection should not be administered deep into the GM muscle, especially when applied to the medial part of this muscle.

There is a study stating that most of the IGN (75.0%) has a sensory branch (10). It was found that these sensory branches originate from the terminal motor branches of the IGN, and are located mostly in the lower outer quadrant of the GM muscle and rarely in its upper outer quadrant. In addition to the superior and inferior cluneal nerves, the sensory branch or branches of the IGN was thought to supply the skin area over the posterior of the greater trochanter (10). Paresthesia in the lateral of the hip in the patient with the IGN injury can only be explained by the injury of the sensory branch of the IGN. In addition, severe hip pain in some patients may be due to the injury of the IGN and its sensory branches.

There were some limitations of this study. It could be noted that the only cause in patients with needle EMG abnormality in the GM muscle is not an IGN injury. However the hip and the lumbosacral MRIs of the patients with IGN injuries were normal. In addition, needle EMG was normal in the gluteus medius and paraspinal muscles of these two patients. Electrodiagnostic tests were not performed for the posterior femoral cutaneous and pudendal nerves. This was another limitation. It has been considered that further studies including electrodiagnostic tests for these nerves in patients with symptoms after IM injection could be important and interesting.

CONCLUSION

The IGN can be injured by IM injection. Therefore, the GM muscle should be analyzed for an IGN injury by needle EMG in patients with complaints following IM injection. If there are abnormal needle EMG findings in the GM muscle, it is useful to examine the lumbosacral paraspinal and the gluteus medius muscles (and other additional muscles) with needle EMG to exclude lumbosacral radiculopathy or plexopathy, as well as nerve injuries such as to the superior gluteal nerve. It should also be noted that muscle weakness may not occur in nerve injuries due to IM injections.

Ethics Committee Approval: The study was approved by the Ethics Committee of Adana City Training and Research Hospital (04.12.2019, 45/620).

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The Ultrasonographic Evaluation of Vena Cava Inferior Diameter as an Intraabdominal Pressure Indicator

İntraabdominal Basınç Göstergesi Olarak Vena Kava İnferior Çapının Ultrasonografi ile Değerlendirilmesi

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ABSTRACT

Aim: Intraabdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are accepted as a significant cause of morbidity and mortality. The aim of this study is to investigate the utility of ultrasonography (US) as a non-invasive technique for evaluation of IAH, ACS and abdominal perfusion pressure (APP).
Material and Methods: Ninety-five patients with intensive care unit requirement, who applied to emergency department and also received a urine catheter were included in the study. During first evaluation intraabdominal pressure (IAP) calculated via measuring intravesical pressure. Inferior vena cava (VCI) diameter, pulsed wave (PW) and central venous pressure (CVP) were recorded by using US. Patients were divided into three groups according to their IAP (IAP <12 mm Hg, IAP =12-20 mm Hg, IAP >20 mm Hg). Each group were evaluated separately in terms of VCI inspirium (i) and expirium (e) diameters.
Results: Mean age of the patients was 68.6±14.5 (range, 24-91) years. Median IAP was 9.55 mm Hg and mean APP was 70.41±17.67 mm Hg. VCIi and VCIe diameters were significantly different in Group 1 with normal (<12 mm Hg) IAP (p<0.001). Correlation between VCI diameter and CVP among all patients were significant (p<0.001).
Conclusion: A significant correlation between both VCIi and VCIe diameters, and CVP values in case of IAH presence was found in this study. We think that, recognition of IAP with non-invasive methods via evaluating high values of VCI diameter and CVP is effective for reducing morbidity and mortality providing early diagnosis and treatment.
Keywords: Vena cava inferior; intraabdominal pressure; abdominal compartment syndrome; intraabdominal hypertension.

ÖZ

Amaç: İntraabdominal hipertansiyon (IAH) ve abdominal kompartıman sendromu (ACS) anlamlı morbidite ve mortalite nedenleri arasında kabul edilmektedir. Bu çalışmanın amacı IAH, ACS ve abdominal perfüzyon basıncı (APP) değerlendirilmesinin non-invaziv bir teknik olarak ultrasonografi (US) ile yapılabilirliğinin araştırılmasıdır.
Gereç ve Yöntemler: Çalışmaya acil servise başvuran ve yoğun bakım ihtiyacı olan mesane sondası takılan 95 hasta dahil edildi. İlk muayenede mesane içi basınç ölçümü yöntemi ile intraabdominal basınçları (IAP) ölçüldü. Vena kava inferior (VCI) çapı, pulsed wave (PW) ve santral venöz basınç (CVP) değerleri US ile kaydedildi. Hastalar IAP değerlerine göre 3 gruba (İAB <12 mm Hg, İAB =12-20 mm Hg, İAB >20 mm Hg) ayrıldı. Her grup kendi içinde VCI inspiryum (i) çapı ve VCI ekspiryum (e) çapı ile ayrı ayrı değerlendirildi.
Bulgular: Hastaların yaş ortalaması 68,6±14,5 (aralık, 24-91) yıl idi. Hastaların ortanca IAP değerleri 9,55 mm Hg olarak saptanırken APP ortalaması 70,41±17,67 mm Hg idi. IAP'si normal (<12 mm Hg) olan Grup 1 ile diğer gruplar arasında VCIi ve VCIe çapları açısından istatistiksel anlamlı fark saptandı (p<0,001). Tüm hastalarda, VCI çapı ile CVP arasındaki korelasyon anlamlı bulundu (p<0,001).
Sonuç: Bu çalışmada, IAP varlığı ile hem VCIi ve VCIe çapları arasında, hem de CVP değerleri arasında anlamlı bir korelasyon saptanmıştır. Yüksek VCI çap ve CVP değerlerinin değerlendirilerek IAP'ın non-invaziv yöntemlerle tanınmasının erken tanı ve tedavi imkanı sağlayarak morbidite ve mortaliteyi azaltmada etkili olabileceğini düşünmekteyiz.
Anahtar kelimeler: Vena kava inferior; intraabdominal basınç; abdominal kompartıman sendromu; intraabdominal hipertansiyon.

INTRODUCTION

Intraabdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are accepted as a significant cause of morbidity and mortality in both surgical and non-surgical patients. Increased intraabdominal pressure (IAP) can lead multiorgan dysfunction. Consequently in high risk patients, adding IAP measurement to other vital parameters has a significant prognostic value (1). Multiorgan dysfunction can be a preventable entity, if IAH and ACS are recognized in the early time period of treatment (1). Thus, decreasing IAP whether pharmacologically or surgically for maintaining peripheral organ perfusion becomes a requirement (2). Including IAH, early diagnosis and intervention reduces morbidity and mortality in surgical patients (2-4). When urinary catheterization is the preferred invasive method for measuring IAP, in this study we aimed to reveal the utilization of ultrasonography (US) as a non-invasive technique for evaluating IAP, IAH and abdominal perfusion pressure (APP).

MATERIAL AND METHODS

Patients with urinary catheterization and intensive care unit (ICU) need that checked into emergency department between October 2012 and October 2013 were included in the study. Exclusion criteria were as followed; pregnancy, present nephrostomy, bladder surgery history, right ventricle deficiency, right ventricle hypertrophy, tricuspid valve dysfunction and pericardial tamponade. Same medical professionals measured blood pressure by using a sphygmomanometer, arterial pulse and central venous pressure (CVP). Ninety-five patients with surgical, non-surgical and/or traumatic etiologies were included. IAP values were recorded for the first evaluation in emergency room or ICU. Vena cava inferior (VCI) diameters were measured with US. Demographic features of the patients, their mechanic ventilator requirements and first laboratory findings were recorded. IAP values were taken daily and simultaneous VCI diameters were measured during inspirium and expirium (VCIi, VCIe). US evaluation was made in supine position while convex probe was in epigastric region aiming for right shoulder. VCI diameter were measured at the site of right before it enters right atrium. Philips M2540A EnVisor C model© US was used. Highest and lowest pulsed wave (PW) Doppler values were recorded. Bladder pressure was measured for IAP. After inserting urinary catheter, 25 mL serum were injected into bladder and a CVP manometer was connected to the catheter. In supine position during expirium IAP was measured accepting the symphysis pubis as zero-reference point. Patients were divided into 3 groups according to IAP values in line with diagnostic options and general acceptance (5). Group 1; IAP <12 mm Hg, Group 2; IAP =12-20 mm Hg, Group 3; IAP >20 mm Hg. Ethics committee approval was received for this study from Ethical Comittee of Necmettin Erbakan University Meram Medical Faculty. The registration identification number is 2012/60. All included patients and/or their relatives signed an informed consent form.

Statistical Analysis

Descriptive statistics were presented as frequency and percentages for categorical variables. Distribution of the numerical data were examined by Kolmogorov-Simirnov test. Mean and standard deviation were given for variables distributed normally, while median, 1st and 3rd quartile and minimum-maximum values were given for variables not distributed normally. Normally distributed variables were compared between groups by ANOVA and post hoc analysis was done with Tukey method. Kruskal-Wallis test was used for comparison of groups in terms of variables not distributed normally, and post hoc analyses were performed with the Mann Whitney U test with Bonferroni correction. Spearman correlation coefficient was used for the correlation analysis. SPSS v.16 was used for statistical analyses, and a p value of 0.05 was accepted as significant.

RESULTS

During the 12 months of study period, a total of 95 patients that were admitted to emergency department were included in the study. There were 54 (56.8%) males and 41 (43.2%) females, with a mean age of 68.6±14.5 (range, 24-91) years. Mean systolic and diastolic pressure were 111.7±22.5 (range, 50-176) mm Hg and 65.6±16.1 (range, 40-140) mm Hg respectively.

Fifty-eight (61.1%) patients had abdominal distention, 14 (14.7%) patients had abdominal defense and rebound findings on physical examination. Seventeen (17.9%) patients had abdominal defense without rebound. Of these 58 patients with abdominal distention, IAH was identified in 29 (50.0%) patients. Five (5.3%) patients had IAH without abdominal distention. Distribution of patients' complaints and symptoms are revealed in Table 1.

In Table 2, IAP, mean arterial pressures (MAP), PW, CVP, APP, VCIi and VCIe values are shown.

Table 1. Distribution of complaints and symptoms during first emergency department evaluation, n (%)

Deterioration of general condition	48 (50.5)
Abdominal pain	16 (16.8)
Shortness of breath	15 (15.8)
Gastrointestinal bleeding	5 (5.3)
Syncope	4 (4.2)
Fever	3 (3.2)
Low urinary output	2 (2.1)
Toxic exposure	2 (2.1)

Table 2. IAP, MAP, PW, CVP, APP, VCIi and VCIe values of the patients

	Median	**Q_1 - Q_3**	**Min-Max**
IAP (mm Hg)	9.55	5.1 - 13.9	2.2 - 35.2
MAP (mm Hg)	80	70 - 90	43.3 - 150
PW (highest)	45	37 - 48	25 - 80
PW (lowest)	29	25 - 35	11 - 48
CVP (cm)	9	7 - 11	0 - 28
	Mean±SD		**Min-Max**
APP* (mm Hg)	70.41±17.67		18.0 - 141.9
VCIi* (mm)	1.74±0.44		0.90 - 3.00
VCIe* (mm)	1.89±0.46		0.98 - 3.14

IAP: Intraabdominal pressure, MAP: Mean arterial pressure, PW: Pulsed wave, CVP: Central venous pressure, APP: Abdominal perfusion pressure, VCIi: Vena cava inferior during inspirium, VCIe: Vena cava inferior during expirium, Q_1: 1st quartile, Q_3: 3rd quartile, Min: Minimum, Max: Maximum, SD: Standard deviation

Mean VCIi diameter and mean VCIe were 1.74±0.44 (range, 0.90-3.00) mm and 1.89±0.46 (range, 0.98-3.14) mm, respectively. Median highest PW value was 45 (range, 25-80) cm/sec and median lowest PW value was 29 (range, 11-48) cm/sec. Median CVP value was 9 (range, 0-28) cm H_2O. There is a positive correlation between CVP and VCIi (r=0.499, p<0.001), and CVP and VCIe (r=0.444, p<0.001) values. Correlation between IAP and VCIi (r=0.596, p<0.001), and VCIe (r=0.581, p<0.001) diameters were found significant. IAP and PW (both high and low) values did not correlate statistically (p=0.318 and p=0.669, respectively).

APP (mean arterial pressure-intraabdominal pressure) upper limit is accepted as 60 mm Hg. In our study 30% of patients had less than 60 mm Hg of APP value.

There were 59 (62.1%), 25 (26.3%) and 11 (11.6%) patients in Group 1, Group 2, and Group 3 respectively (Table 3). Among all groups there were a significant difference between mean values of VCIi and VCIe (p<0.001). Patients in Group 1 with normal (<12 mm Hg) IAP values had statistically different VCIi and VCIe diameters when compared to other groups (p<0.001).

CVP values differ between all groups (p=0.003). When CVP values were compared, there was significant difference between Group 1 and Group 3 (p=0.006), but no significance was found between Group 1 and Group 2 (p=0.093), and Group 2 and Group 3 (p=0.496).

DISCUSSION

Intraabdominal hypertension is a sustained or repeated IAP more than 12 mm Hg. Increased IAH may lead splanchnic hypoperfusion and/or multiorgan dysfunction if left untreated. After abdominal surgery IAP varies approximately from 3 to 15 mm Hg. IAH can occur during endotracheal entubation for short term. Also patients with burns, pancreatitis, traumatic injury or shock status may develop IAH. Excessive intravenous fluid administration can cause IAH. Prevalence of IAH in ICU patients is 18-58.8% (6). In our study we calculated the incidence of IAH as 37.9%. Another study conducted by Malbrain et al. (7) revealed that the incidence is 59% in critically ill patients. In ICU patients, evaluating IAH should be considered because of its high incidence rates. Simonson et al. (8) reported that higher survival rates may be possible with early interventions via education of health professionals in related departments about measuring and evaluation of IAP. Arabadzhiev et al. (9) reported in their study with ICU patients that early decompression decreases mortality rates. Therefore, routine IAP evaluation in ICU population can reduce both mortality rates and length of hospital stay. Ravishankar et al. (10) reported that physicians usually evaluate IAH of their patients only if necessary under certain clinical conditions and in this study, only 27% of clinicians took measurement of IAH 4 to 8 hours intervals. Although it is the most accurate method to measure IAH directly with a catheter placed inside the abdomen, it is not practicable to be an invasive method and because of the risk of infection. Indirect measurements can be accomplished by other several methods (11). The most commonly used method of indirect measurement is the transvesical measurement method described by Kron et al (12). This method is an impractical method because it requires an invasive procedure such as bladder catheterization. The World Society of the Abdominal Compartment Syndrome (WSACS), an international multidisciplinary consensus to study the causes and consequences of abdominal hypertension, has not yet found a consensus on the amount of fluid to be delivered to the bladder in its studies (13). In addition, various manometers have been developed for continuous measurement and monitoring, but have not found sufficient use. Urinary infection which may occur in intensive care patients with infection tendency with a minor intervention may adversely affect the prognosis of these patients. Another indirect method of measurement was the measurement of catheter insertion into the VCI and was not favored by the presence of continuous catheter in the groin and complications related to catheter. Transgastric measurement method is difficult to use in practice due to the insertion of an intragastric balloon. Especially in ICU patients, continuous monitoring is not preferred because of complications caused by reflux and aspiration. In our study, technical difficulties can be eliminated by indirect measurement of IAP with US, and the presence of IAH can be determined with a standard approach.

In our study, IAP measurement was performed with the help of a manometer after giving 25 ml of saline into the bladder via bladder catheter. The measurement of VCIi and VCIe diameters of the patients was non-invasive and provided faster results. A significant relationship was found between the patients' IAP and VCIi and VCIe diameters. In Group 1, an increase in the VCI diameter was observed in parallel with the increase in IAP; however, this increase did not change as the IAP stages increased and no difference was found. The reason for this is that the increase in advanced intraabdominal pressure has not been able to extend VCI more further. IAH benefits from medical treatment and early decompression surgeries when early recognition. Therefore, it is thought that the VCI measurements to be applied in patients will be useful in the diagnosis of IAP and increase in early period and this will give the chance of an early intervention in patients with IAP.

Table 3. Comparison of VCIi, VCIe and CVP values

	Group 1 (n=59) (IAP <12 mm Hg)	**Group 2 (n=25) (IAP =12-20 mm Hg)**	**Group 3 (n=11) (IAP >20 mm Hg)**	**p**
VCIi (mm)	1.58±0.40	1.90±0.39	2.19±0.33	**<0.001**
VCIe (mm)	1.75±0.44	2.02±0.39	2.35±0.36	**<0.001**
CVP (cm)	8 (5) [0 - 20]	10 (3) [4 - 22]	12 (4) [9 - 28]	**0.003**

VCIi: Vena cava inferior during inspirium, VCIe: Vena cava inferior during expirium, CVP: central venous pressure, IAP: Intraabdominal pressure, descriptive statistics were given as mean±standard deviation or median (interquartile range) [minimum-maximum]

When an acute or chronic increase in IAP occurs, the diaphragm becomes elevated, leading to a progressive reduction in lung and chest wall compliance by increasing intrathoracic and pleural pressures. As a result, increased ventilation/perfusion mismatch leads to hypoxia, hypercapnia and mechanical ventilator requirement (14). The VCI diameter can alter by changes in respiration movements and total body fluid. During inspiration, intrapleural pressure becomes negative and causes increased venous return to the right side of the heart, leading to a reduction in intraluminal pressure. VCI was first shown as enlarged by Weil in patients with right heart failure. To date, VCI has been visualized to assess volume status in patients with heart failure and dialysis requirement (15). In a study conducted by Tetsuka et al. (16), it has been reported that VCIe is a marker of circulating blood volume. In particular, the correlation between VCI end-expiratory diameter and circulating blood volume is noteworthy. In our study, we found a significant relationship between VCIi and VCIe diameter and CVP.

Lyon et al. (15) demonstrated that the collapse of VCI diameter correlates with CVP in the supine position of a lying patient. Marcelino et al. (17) investigated whether the VCI was correlated with CVP in patients who were followed up in surgical or non-surgical ICUs and ultimately found that the VCI index (VCIe-VCIi / VCIe) correlated with CVP.

Wachsberg et al. (18) reported in their study of seven patients with IAH, intrahepatic VCI diameter was examined with computed tomography (CT) and US and the diameter of intrahepatic VCI was found increased in these patients. In our study, both inspiratory and expiratory diameters were measured where extrahepatic VCI enters to the right atrium of the heart. Also, CVP values of patients were measured and a significant correlation was found between CVP values and VCI diameters for each group. These results show us that both CVI diameter and CVP value are related and possible abnormal CVP increment may be warning for IAP elevation.

CONCLUSION

The significance of the correlation between the early stages of the elevated IAP and the increase in VCI diameters will allow early diagnosis and treatment of IAH. It should be kept in mind that high CVP measurements, like VCI diameter measurement, are also a warning to demonstrate IAP increase. Especially in the follow-up of ICU patients, the fact that IAP measurements should not be neglected, because IAP increase can be detected non-invasively in the early period. Thus, it can be concluded that the decrease in morbidity and mortality resulting from IAH can be achieved.

Ethics Committee Approval: The study was approved by the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine (27.11.2012, 2012/60).

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Serum Uric Acid Level and Cardiovascular Disease Development Risk in Stage 3-5 Chronic Kidney Disease Patients

Evre 3-5 Kronik Böbrek Hastalarında Serum Ürik Asit Seviyesi ve Kardiyovasküler Hastalık Gelişim Riski

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ABSTRACT

Aim: The patients with chronic kidney disease (CKD) have higher risk of cardiovascular disease (CVD) than the general population and this risk increases in advanced CKD stages. The data about the association between CVD and uric acid level in stage 3-5 CKD patients are limited in the literature. The aim of this study is to investigate whether uric acid levels are associated with cardiovascular events and mortality in the CKD patients.

Material and Methods: Patients who were followed up with the diagnosis of stage 3-5 CKD between June 2014 and December 2019 were evaluated retrospectively. A hundred stage 3-5 CKD cases above the age of eighteen were included in the study. The patients were divided into two groups according to average serum uric acid levels; <7 mg/dL and ≥7 mg/dL. Any confirmed diagnosis of stroke, myocardial infarction, coronary heart disease or heart failure was accepted positive for the history of CVD.

Results: Cardiovascular event development was observed in 15 patients and mortality in 1 patient. CVD were seen in 10 (21.3%) patients in group 1 while 5 (9.4%) patients in group 2. Although the cardiovascular events were seen more in the group 1, this difference was not statistically significant (p=0.098).

Conclusion: Although CVD is seen higher in the low uric acid (<7 mg/dL) group, no significant association was found between serum uric acid level and development of CVD in stage 3-5 CKD patients, in this study. Prospective studies with larger sample sizes may provide better evidence regarding possible relationship.

Keywords: Hyperuricemia; chronic kidney disease; cardiovascular disease.

ÖZ

Amaç: Kronik böbrek hastalığı (KBH) olanlarda kardiyovasküler hastalık (KVH) riski genel popülasyondan daha yüksektir ve bu risk ileri KBH evrelerinde artmaktadır. Evre 3-5 KBH hastalarında KVH ve ürik asit seviyesi arasındaki ilişki hakkındaki veriler literatürde sınırlıdır. Bu çalışmanın amacı, KBH hastalarında ürik asit düzeyinin kardiyovasküler olaylar ve mortalite ile ilişkili olup olmadığının incelenmesidir.

Gereç ve Yöntemler: Haziran 2014 ve Aralık 2019 tarihleri arasında evre 3-5 KBH tanısı ile takip edilen hastalar geriye dönük olarak değerlendirildi. On sekiz yaş üstü 100 evre 3-5 KBH olgusu çalışmaya dahil edildi. Hastalar ortalama serum ürik asit düzeylerine göre <7 mg/dL ve ≥7 mg/dL olmak üzere iki gruba ayrıldı. Doğrulanmış inme, miyokard enfarktüsü, koroner kalp hastalığı veya kalp yetmezliği tanısı olması KVH öyküsü için pozitif kabul edildi.

Bulgular: On beş hastada kardiyovasküler olay gelişimi, 1 hastada mortalite izlendi. Grup 1'deki 10 (%21,3) hastada ve grup 2'deki 5 (%9,4) hastada KVH görüldü. Grup 1'de kardiyovasküler olaylar daha fazla görülmesine rağmen, bu fark istatistiksel olarak anlamlı değildi (p=0.098).

Sonuç: Bu çalışmada, düşük ürik asit (<7 mg/dL) grubunda KVH daha yüksek görülmekle birlikte, evre 3-5 KBH hastalarında serum ürik asit düzeyi ile KVH gelişimi arasında anlamlı bir ilişki bulunamamıştır. Daha fazla örneklem büyüklükleri ile ileriye dönük çalışmalar, olası ilişki hakkında daha iyi kanıt sağlayabilir.

Anahtar kelimeler: Hiperürisemi; kronik böbrek hastalığı; kardiyovasküler hastalık.

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INTRODUCTION

Chronic kidney disease (CKD) is an important health problem all over the world (1). The patients with CKD have higher risk of cardiovascular disease (CVD) than the general population and this risk increases in advanced CKD stages (2). Hypertension, smoking, diabetes, hyperlipidemia and high serum uric acid levels are the examples of traditional risk factors for CVD. The end product of purine metabolism is uric acid. Uric acid have some roles in formation of free radicals (3), platelet adhesiveness (4,5), and oxidative stress (6). A relationship between uric acid and CVD in the general population have been reported by a few studies (7-10) but not by other studies (11-14). And also there are studies reporting that lower uric acid levels were associated with mortality (15,16). Therefore, there are conflicting results about the association between serum uric acid levels and cardiovascular disease or all-cause mortality.

Urinary excretion of uric acid is decreased in chronic renal disease and as a result of this, serum uric acid levels are generally higher in CKD patients than the general population (17). The data about the association between CVD and uric acid level in stage 3-5 CKD patients are limited in the literature. Weiner et al. (18) reported that uric acid was not an independent predictor of a composite outcome of myocardial infarction, stroke, and all-cause mortality in stage 3-5 CKD patients. On the other hand, a relationship between uric acid and cardiovascular mortality has been shown by two studies (19,20). There are conflicting data in the literature about hyperuricemia and cardiovascular events in CKD patients. We therefore aimed to investigate whether uric acid levels are associated with adverse CVD and mortality in the CKD patients.

MATERIAL AND METHODS

The files of patients who were followed up with the diagnosis of stage 3-5 CKD between June 2014 and December 2019 were evaluated retrospectively. The ethics committee of Necmettin Erbakan University, Faculty of Medicine approved the study protocol (2020/2788, 21.08.2020). A hundred stage 3-5 CKD cases above the age of eighteen were included in the study. The patients who needed renal replacement treatment were excluded from the study. Patients with hyperuricemia-related disease such as gout, malignancy and at the beginning using a uric acid lowering therapy were excluded from the study. The Modification of Diet in Renal Disease (MDRD) formula, eGFR = 175 × creatinine$^{-1.154}$ × age$^{-0.203}$ × 1.212 (if black) × 0.742 (if female), was used to calculate the eGFR (21). Early morning spot urine protein-creatinine ratio was used to detect the daily protein loss. Any confirmed diagnosis of stroke, myocardial infarction, coronary heart disease or heart failure was accepted positive for the history of CVD. The average of three separate serum uric acid value (at least 6 months between the samples) of patients during the follow-up period was taken. The patients were divided into two groups according to the average serum uric acid levels; first group consists of the patients who had average serum uric acid below 7 mg/dl and second group consists of the patients who had higher than 7 mg/dl average serum uric acid (22). We investigated whether there was any difference in terms of cardiovascular event and mortality according to uric acid level during the follow-up period.

Statistical Analysis

Statistical analysis was performed with the SPSS v.22 statistical software. The normality of the variables was determined with Kolmogorov-Smirnov test. Descriptive statistics for were given as mean±standard deviation, and median, minimum-maximum with interquartile range according to the distribution of the variable. The groups were compared with the independent samples t test or Mann-Whitney U test, as appropriate. Categorical variables were analyzed by Pearson chi-square test or Fisher's exact test, and given as frequency and percentage. A p value of <0.05 was considered statistically significant.

RESULTS

The study included 100 stage 3-5 CKD patients (53 females, 47 males) with a mean age of 59.7±14.3 years. The mean follow-up was 33.1±8.4 (median=31.5, range, 18-49) months. Baseline characteristics of patients are summarized in Table 1. The number of patients with a history of hypertension was 92, diabetes mellitus was 38, glomerulonephritis was 15, polycystic kidney disease was 5 and amyloidosis was 3. Twenty six patients had previous cardiovascular disease history. CVD development was observed in 15 patients and mortality in 1 patient in follow up period. Out of 100 patients, 83 were using ACE inhibitors or ARBs and 55 were using diuretics.

Patients were divided into two groups according to uric acid levels (group 1: uric acid <7 mg/dL and group 2: uric acid ≥7 mg/dL). The mean age of group 1 was 59.9±15.3, the mean age of group 2 was 59.9±13.5 years. Gender distribution of groups were as follows; in group 1 there were 23 (48.9%) male and 24 (51.1%) female, and in group 2 there were 24 (45.3%) male and 29 (54.7%) female. The mean follow-up time of group 1 was 33.9±8.2 months, and group 2 was 32.4±8.6 months. There were no difference between the two groups in terms of gender, age, and follow up time (p=0.715, p=0.916, p=0.381, respectively). Demographic and laboratory parameters by groups according to the uric acid levels were shown in Table 2.

There were no difference between groups in terms of comorbidities and frequency of use of ACE inhibitors, ARBs and diuretics (Table 3). CVD were seen in 10 (21.3%) patients in group 1 while in 5 (9.4%) patients in group 2. Although the cardiovascular events were seen more in the group with low uric acid, this difference was not statistically significant (p=0.098).

Table 1. Baseline characteristics of patients (n=100)

Age (years)	59.7±14.3
BMI (kg/m^2)	28.1±3.4
Uric acid (mg/dL)	7.1±1.4
Creatinine (mg/dL)	2.1±0.9
eGFR (mL/min/1.73 m2)	33.3±14.2
Albumin (g/dL)	4.2±0.5
Hemoglobin (g/dL)	12.9±1.6
Calcium (mg/dL)	9.3±0.7
Phosphorus (mg/dL)	4.6±0.6
PTH (pg/mL)	132.4±89.1
Spot urine protein/creatinine*	594 (1496) [1.49-15078]

BMI: body mass index, eGFR: estimated glomerular filtration rate, PTH: parathyroid hormone, *: median (interquartile range) [minimum-maximum]

Table 2. Demographic and laboratory parameters of patients by uric acid groups

	Group 1 (n=47)	Group 2 (n=53)	p
Age (years)	59.9±15.3	59.9±13.5	0.916
BMI (kg/m^2)	27.9±3.4	28.1±3.5	0.753
Follow-up time (months)	33.9±8.2	32.4±8.6	0.381
Uric acid, (mg/dL)	6.0±0.8	8.1±0.9	**<0.001**
Creatinine (mg/dL)	2.0±0.8	2.1±0.9	0.448
eGFR (mL/min/1.73 m2)	35.5±14.8	31.7±13.4	0.157
Albumin (g/dL)	4.2±0.5	4.1±0.5	0.373
Hemoglobin (g/dL)	13.1±1.5	12.8±1.7	0.298
Calcium (mg/dL)	9.2±0.8	9.4±0.7	0.292
Phosphorus (mg/dL)	4.6±0.6	4.7±0.6	0.282
PTH (pg/mL)	86 (90) [21-385]	123 (100) [39-457]	**0.003**
Spot urine protein/creatinine	524 (1251) [1.49-6836]	620 (1657) [22-15078]	0.236

BMI: body mass index, eGFR: estimated glomerular filtration rate, PTH: parathyroid hormone, descriptive statistics were given as mean±standard deviation or median (interquartile range) [minimum-maximum]

Table 3. Characteristics of patients by uric acid groups

	Group 1 (n=47)	Group 2 (n=53)	p
Comorbidities			
Hypertension	45 (95.7)	47 (88.7)	0.276
Diabetes mellitus	20 (42.6)	18 (34.0)	0.377
Glomerulonephritis	5 (10.6)	10 (18.9)	0.250
Polycystic kidney disease	0 (0.0)	5 (9.4)	0.058
Amyloidosis	0 (0.0)	3(5.7)	0.245
Medications			
ACE inhibitors and ARBs	38 (80.9)	45 (84.9)	0.590
Diuretics	29 (61.7)	26 (49.1)	0.205
Cardiovascular Event	10 (21.3)	5 (9.4)	0.098

ACE: angiotensin-converting enzyme, ARBs: angiotensin II receptor blockers

DISCUSSION

Based on our study results there is no meaningful relationship between serum uric acid levels and cardiovascular event in CKD patients. There are prospective studies reporting that there is a relationship between basal hyperuricemia and the development of cardiovascular event and mortality (16,23). Although there are data reported in this direction in the literature but uric acid has not been identified as a causal risk factor for CVD. On the other hand it's reported that there are important links between uric acid and diseases which are known to be associated with the development of cardiovascular events, such as hypertension, impaired glucose metabolism and dyslipidemia (13,15). Therefore, it may be a more correct approach to evaluate uric acid as a risk marker for the development of CVD.

Although it is not statistically significant level, we found that the cardiovascular events development risk was higher in those with low uric acid levels. Gerber et al. (15) reported that the development of cardiovascular events was higher in those with low uric acid levels. Li et al. (24) and Dong et al. (25) reported in their study that hemodialysis patients with low uric acid levels were associated with high risk for cardiovascular mortality and all-cause mortality. Aker Karagöz et al. (26) reported that there was no relationship between uric acid level and the development of CVD in hypertensive patients. In another study conducted by Shao et al. (27), there was no significant relationship between uric acid level and coronary heart disease in type 2 diabetes patients.

Some researchers reported the combined results for female and male patients after correction for sex while others preferred sex specific investigation in uric acid studies (13,16). Since serum uric acid level in males is higher than females, we think that gender-specific analyzes are required in studies related to uric acid. In our study, there were 23 (48.9%) males and 24 (51.1%) females in the low uric acid group whereas 24 (45.3%) males and 29 (54.7%) females in the high uric acid group. There was no difference in terms of gender distribution between the two groups. When we included all cases (53 females, 47 males) the uric acid averages of females and males were found similar (7.1±1.4 vs 7.1±1.3). Cardiovascular event development rate was similar in males and females.

The fact that studies have very different results might depend on differences in patient populations, different follow-up times and statistical regulations. While other studies examined the development of cardiovascular events over a single basal uric acid value, but we evaluated the cardiovascular event development rate with the average of three uric acid samples taken at different times during the follow-up period. In this way, we think that we made a more accurate assessment for the patients with a low serum uric acid level at the beginning and raised later, and also for the patients that had a high level of uric acid at the beginning and fall down later. We think that examinations based on a single value could be misleading. In conclusion, although CVD is seen higher in stage 3-5 CKD patients with low uric acid levels, no significant association was found between serum uric acid level and development of CVD. Detailed prospective studies with huge number of patients and longer follow up time are required to comment the possible relationship.

Ethics Committee Approval: The study was approved by the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine (21.08.2020, 2020/2788).

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The Effect of Fingolimod (FTY720) Treatment on Liver Enzyme Levels in Relapsing-Remitting Multiple Sclerosis Patients

Fingolimod (FTY720) Tedavisinin Relapsing-Remitting Multipl Skleroz Hastalarında Karaciğer Enzim Düzeylerine Etkisi

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ABSTRACT

Aim: Multiple sclerosis (MS) is a chronic inflammatory pathology affecting the central nervous system. Many therapeutic options have been approved against MS until today. In this study, it was aimed to investigate the effect of fingolimod treatment (FT) on the liver enzyme levels of relapsing-remitting multiple sclerosis (RRMS) patients.

Material and Methods: Body mass index, FT (0.5 mg/day) duration, and liver enzyme (alanine aminotransferase, ALT; gamma glutamyl transferase, GGT) levels of 102 RRMS patients (66 female, 36 male, mean age was 40.9±10.9 years) were gathered from polyclinic records retrospectively.

Results: The FT duration of MS patients was between 0.5 and 6 years. Increased ALT and GGT levels were detected in RRMS patients after >3 month-long FT. After FT, ALT and GGT levels elevated in males almost 2 times higher than in females. It was observed that ALT and GGT levels increased by 1.3 and 1.5 times in females, while 1.6 and 1.9 times in males, respectively. Of the MS patients with increased transaminases post-FT, 7 (23.3%) males and 8 (17.4%) females were at upper limit of normal for ALT whereas 9 (34.6%) males and 14 (32.6%) females as for GGT. Age and FT duration did not affect ALT and GGT levels.

Conclusion: Overall, FT elevated ALT and GGT levels of RRMS patients. Thus, it is of high importance to monitor MS patients throughout FT. So that, we suggest tracking ALT and GGT levels during and after FT to prevent possible liver damage or the occurrence of other systemic diseases.

Keywords: Relapsing-remitting multiple sclerosis; fingolimod; liver enzyme; ALT; GGT.

ÖZ

Amaç: Multipl skleroz (MS) merkezi sinir sistemini etkileyen kronik inflamatuvar bir patolojidir. Günümüze kadar MS'e karşı birçok tedavi seçeneği onaylanmıştır. Bu çalışmada, fingolimod tedavisinin (FT) relapsing-remitting multipl skleroz (RRMS) hastalarının karaciğer enzim düzeylerine etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Yüz iki RRMS hastasının (66 kadın, 36 erkek, ortalama yaş 40,9±10,9 yıl) vücut kitle indeksi, FT (0,5 mg/gün) süreleri ve karaciğer enzim (alanin aminotransferaz, ALT; gama glutamil transferaz, GGT) seviyeleri poliklinik kayıtlarından geriye dönük olarak incelendi.

Bulgular: MS hastalarının FT süresi 0,5 ile 6 yıl arasındaydı. Üç ay ve daha fazla FT süresi olan RRMS hastalarında ALT ve GGT seviyelerinin arttığı tespit edildi. FT sonrası, ALT ve GGT seviyeleri erkeklerde kadınlara kıyasla yaklaşık olarak 2 kat daha yüksekti. Kadınlarda ALT ve GGT seviyelerinin sırasıyla 1,3 ve 1,5 kat, erkeklerde ise bu seviyelerin 1,6 ve 1,9 kat arttığı görüldü. FT sonrası transaminaz seviyesi artan MS hastalarından 9 (%34,6) erkek ve 14 (%32,6) kadın GGT için normalin üst seviyesindeyken, 7 (%23,3) erkek ve 8 (%17,4) kadın ALT için normalin üst seviyesindeydi. Yaş ve FT süresi, ALT ve GGT seviyelerini etkilemiyordu.

Sonuç: Sonuç olarak, FT RRMS hastalarının ALT ve GGT seviyelerini yükseltmiştir. Bundan dolayı, MS hastalarının FT süresince takibi oldukça önemlidir. Bu yüzden, MS hastalarında olası karaciğer hasarı ya da diğer sistemik hastalıkların önlenmesi için FT sırasında ve sonrasında ALT ve GGT seviyelerinin takip edilmesini öneriyoruz.

Anahtar kelimeler: Relapsing-remitting multipl skleroz; fingolimod, karaciğer enzimi; ALT; GGT.

INTRODUCTION

Multiple sclerosis (MS) is a central nervous system (CNS) disease characterized by inflammation, demyelination, and neurodegeneration (1). Several medications that change the course of MS have been identified since the discovery of the disease. But today, despite great advances in MS therapy, we are still not at the desired point regarding a long-lasting cure. Fingolimod is the first oral medication approved by the United States Food and Drug Administration to attenuate seizures and disability rates in relapsing-remitting multiple sclerosis (RRMS) type (2). Fingolimod acts as a modulator of sphingosine-1-phosphate (S1P) that is found in immune system cells (3). S1P is involved in several biological processes via interacting with G-protein-coupled S1P receptor. In the immune system, S1P activates lymphocytes by binding to their S1P receptors and also blocks their release from lymph nodes (4-6). Eventually, autoreactive B and T lymphocytes are blocked to reach to CNS and cause inflammation (7).

Although the effectiveness and reliability of fingolimod treatment (FT) against MS have been shown previously (8,9), there are also side effects of this immunomodulatory drug such as altered liver enzyme levels (10). In previous studies comparing the effect of fingolimod with placebo, 0.5 mg/day FT caused asymptomatic increases in liver transaminase levels of the patients with 7-8% of MS patients having 3-fold and 2-4% having 5-fold higher levels than the upper limits of normal (ULN). These increments in liver enzyme levels were observed ~3-4 months after the start of FT and reduced to within normal limits (WNL) ~2-3 months after the cessation of FT (11-13).

It is of pivotal importance to know and monitor the side effects of fingolimod as an immunosuppressive drug in MS therapy. It has been previously well documented by "Central and East European (CEE) MS Expert Group" that 3 to 4 months of FT caused asymptomatic elevations in liver enzyme levels (14). On the other hand, the asymptomatic elevations in liver transaminases can highly vary depending on the studied population (15,16). In this context, the aim of this retrospective study was to analyze alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) liver enzyme levels before and after FT in RRMS patients.

MATERIAL AND METHODS

Patients

Permission for this study was obtained from Non-invasive Clinical Studies Ethical Committee in Hatay Mustafa Kemal University (02/18, 17/01/2019). 102 patients, who were 18-65 year-old, diagnosed with RRMS based on the McDonald 2017 criteria (17), and started their first FT in Hatay Mustafa Kemal University Medical Faculty Research Hospital Neurology Polyclinic between 2013-2019 were included in this retrospective study. MS patients medicated with anti-inflammatory drugs or who had any disease or disease history affecting liver enzyme levels such as chronic liver disease, chronic inflammatory disease, and cancer were excluded from the study (Figure 1).

Data Collection

We collected the medical records of RRMS patients including age, sex, FT (0.5 mg/day) duration, body mass index (BMI), and ALT and GGT levels before and at least 3 months after FT (the first measurements post-FT) by retrospective inquiry of the clinical patient database of the Neurology Polyclinic in the Research Hospital of Hatay Mustafa Kemal University. The following reference ranges of Hatay Mustafa Kemal University Research Hospital were used for liver enzyme parameters in this study: For ALT levels, 10-49 U/L WNL and >49 U/L ULN in males and females; for GGT levels, 9-36 U/L WNL and >36 U/L ULN in females and 12-64 U/L WNL and >64 U/L ULN in males.

Statistical Analysis

The normal distribution of the variables was assessed using Kolmogorov-Smirnov test. We used non-parametric tests since our data did not exhibit normal distribution. The data were expressed as mean±standard deviation (SD) and median (with interquartile range and minimum-maximum values) for continuous variables, and as percentage for categorical variables. Wilcoxon signed-rank test was used to compare the liver enzyme levels before and after FT. Spearman's correlation test was used to analyze any association of liver enzyme levels with age and FT duration. A p value of less than 0.05 was accepted as statistically significant. All statistical analyses were performed using SPSS v.22.0 statistical package.

RESULTS

The baseline characteristics of the RRMS patients are shown in Table 1. Of the 102 RRMS patients included in the present study, 66 (64.7%) were female and 36 (35.3%) were male. The mean age of MS patients, who were between 18 and 63 years old, was 40.9±10.9, 42.4±10.8 for female and 38.1±10.8 for male patients. There was no significant difference between the mean ages of males and females (p=0.054). The FT duration of MS patients in our study was between 0.5 and 6 years, and the mean FT duration was 1.5±1.2 years.

We observed that the ALT and GGT levels of MS patients increased significantly after FT compared to before FT (p<0.001, Figure 2, Table 2).

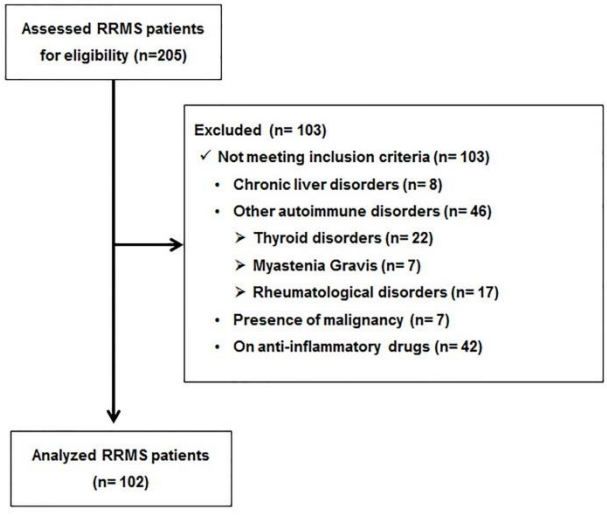

Figure 1. CONSORT flow diagram indicating the inclusion/exclusion criteria of RRMS patients

Table 1. Baseline characteristics of RRMS patients

Gender, n (%)	
Female	66 (64.7)
Male	36 (35.3)
Age (year), mean±SD	
General	40.9±10.9
Female	42.4±10.8
Male	38.1±10.8
BMI (kg/m^2), mean±SD	
General	26.8±3.3
Female	27.4±2.6
Male	23.1±1.8
FT period (year), mean±SD	1.5±1.2

RRMS: relapsing-remitting multiple sclerosis, SD: standard deviation, BMI: body mass index, FT: fingolimod treatment

Of the MS patients with increased ALT levels, 38 (82.6%) of females were WNL and 8 (17.4%) of them exceeded ULN, while 23 (76.7%) of males were WNL and 7 (23.3%) of them exceeded ULN. From the MS patients with increased GGT levels, 29 (67.4%) of females were WNL and 14 (32.6%) of them exceeded ULN while 17 (65.4%) of males were WNL and 9 (34.6%) of them exceeded ULN.

There was no significant correlation of increased liver ALT and GGT levels with the age (r=-0.068, p=0.559 for ALT, and r=-0.020, p=0.869 for GGT) or with the FT duration (r=-0.129, p=0.268 for ALT, and r=0.122, p=0.316 for GGT).

Table 2. ALT/GGT levels in general and in female and male RRMS patients pre- and post-FT

		Pre-FT		Post-FT		p
		Mean±SD	**Median (IQR) [Min-Max]**	**Mean±SD**	**Median (IQR) [Min-Max]**	
ALT (U/L)	**General (n=102)**	23.02±12.13	20 (12) [7-73]	32.75±21.36	25.5 (22) [10-276]	**<0.001**
	Female (n=66)	21.61±11.10	19 (12) [9-73]	28.18±17.77	23.5 (16) [10-90]	**<0.001**
	Male (n=36)	25.61±13.62	20 (13) [7-66]	41.11±24.87	34 (27) [13-127]	**<0.001**
GGT (U/L)	**General (n=102)**	23.46±22.39	16.5 (17) [4-150]	39.48±45.10	23 (32) [3-235]	**<0.001**
	Female (n=66)	20.98±23.31	14 (10) [4-150]	31.48±38.15	18 (23) [3-227]	**<0.001**
	Male (n=36)	28.00±20.12	23.5 (21) [8-93]	54.14±53.15	30.5 (46) [9-235]	**<0.001**

ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, RRMS: relapsing-remitting multiple sclerosis, FT: fingolimod treatment, SD: standard deviation, IQR: interquartile range

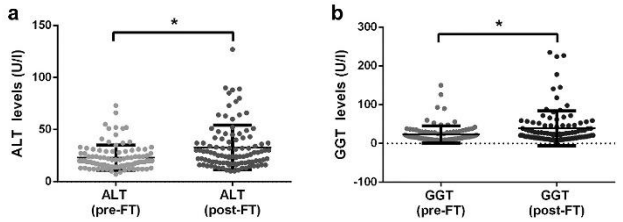

Figure 2. Liver enzyme levels before and after FT in RRMS patients, **a)** ALT, **b)** GGT, *: p<0.001, FT: fingolimod treatment, RRMS: relapsing-remitting multiple sclerosis, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase

DISCUSSION

FT, despite well-tolerated in MS patients, has several side effects including macular edema, lymphopenia, hypertension and more commonly increased liver enzymes (6,18,19). In the present study, we retrospectively evaluated the effect of FT on liver enzyme levels (ALT and GGT) in RRMS patients and observed that both ALT and GGT were increased in the patients.

The effects of FT on liver enzyme levels of MS patients have been shown in previous studies. In a study conducted with Japanese RRMS patients, the increase in enzyme levels was the most common side effect of 0.5 mg/day FT for 12 months (20). In the same study, it was also observed that ALT and AST levels of MS patients reached peak levels at 3 months of FT while GGT levels peaked after 6.5 months of FT and in the patients with persistently high enzyme levels FT was withdrawn. In another study with 122 RRMS patients that evaluated the effectiveness and reliability of FT, ALT and GGT levels in ~25% of MS patients increased significantly (21). In the present study, we also compared the liver enzyme levels of RRMS patients before and after FT and observed, as consistent with the previous studies, that ALT and GGT levels elevated significantly with FT compared to the baseline.

It has been reported that ALT and AST levels can change significantly among different populations (15,16). Despite limited studies concerning confounder effects of demographic features on liver enzyme levels after FT, in general, increased ALT levels were observed 15% more frequently in male patients than in females (22,23). Besides, other studies investigating the effect of fingolimod on MS patients reported that the patients with increased liver enzymes mostly consisted of males (24,25). Similarly, we also observed that ALT levels after FT increased significantly in 80.5% of males while this ratio was 68.1% in females. As to GGT levels, FT led to elevations in ~64% of both males and females. We also detected that ALT and GGT levels after FT in males were ~1.4 and 1.7 times higher respectively than in females. Among female MS patients, the mean ALT and GGT levels after FT were ~1.3 and 1.5 times higher respectively than before FT, while among male MS patients, the mean ALT and GGT levels after FT were ~1.6 and 1.9 times higher respectively than before FT. In a controlled study conducted in 358 RRMS patients, ALT levels showed a dose-dependent elevation after FT and increased in 29 patients, exceeded ULN in 25 patients by 3-fold and 5-fold in 8 patients (12). In a similar study conducted in Latin American RRMS patients, ALT levels after FT reached 3-, 5-, and 10-times of ULN (26). In parallel with previous studies, we noticed that 23.4% and 34.6% of male MS

patients exceeded ULN of ALT and GGT levels respectively, whereas 17.9% and 32.6% of female MS patients exceeded ULN of ALT and GGT levels respectively. A previous report underlined that monitoring liver enzymes during/after FT is important to sustain a functional liver. Based on this report by the CEE MS Expert Group, liver transaminases should be screened periodically (i.e. at moth 1, 3, 6 post-FT) and FT should be discontinued if the liver enzyme levels exceed five times ULN, which should normally return to baseline levels within two months, to prevent hepatic failure (14). Furthermore, owing to its prominent anti-inflammatory and neuroprotective effects, FT was also utilized as a treatment option in other neurodegenerative and/or neuroinflammatory disease models such as schizophrenia, optic neuritis, Alzheimer's disease, Parkinson's disease, epilepsy, etc. (6,27). In a phase-II trial study testing the safety and efficacy of FT on amyotrophic lateral sclerosis (ALS) patients, fingolimod was reported to be well-tolerated in ALS patients and caused no similar side effects like in MS patients (28).

As to our knowledge, there is no study in the literature analyzing the correlation of increased levels of liver enzymes with the age of MS patients and FT duration. In the present study, there was no significant correlation of increased ALT and GGT levels with the age of RRMS patients or FT duration.

CONCLUSION

The present study confirmed that FT caused significant elevations in ALT and GGT levels of RRMS patients. For this reason, we suggest that ALT and GGT levels should be followed routinely during and after FT to prevent probable liver failures.

Study Limitations

In the present study, we included routinely analyzed liver enzyme levels in our polyclinic, i.e. ALT and GGT but not AST.

Ethics Committee Approval: The study was approved by the Ethics Committee of Hatay Mustafa Kemal University Faculty of Medicine (17.01.2019, 02/18).

Conflict of Interest: This manuscript was produced based on the master thesis of Duygu Tap, as the first author here, and all authors declare that there is no conflict of interest.

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BK Virus Infections in Pediatric Patients with Hematopoietic Stem Cell Transplantation

Hematopoetik Kök Hücre Transplantasyonu olan Pediatrik Hastalarda BK Virüs Enfeksiyonları

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ABSTRACT

Aim: BK virus (BKV)-associated hemorrhagic cystitis (HC) is a common complication in patients after hematopoietic stem cell transplantation (HSCT). The aim of this study was to investigate the incidence of BKV infection in pediatric patients receiving HSCT.
Material and Methods: Total of 51 patients aged between 16 months and 16 years old and followed up between October 2015 and September 2017 were included in the study. The patients were monitored by quantitative real-time polymerase chain reaction (Anatolia Geneworks, Turkey) test for the detection of BKV DNA in urine and blood.
Results: Of patients, 46 received allogeneic HSCT and 5 autologous HSCT. BKV DNA positivity was detected in urine and/or blood of total 27 (52.9%) patients in whom 26 (56.5%) of 46 patients with allogeneic transplantation, and 1 (20.0%) of 5 patients with autologous transplantation. BKV viral load in urine $>10^7$ copies/ml required for preemptive treatment was detected in 12 (26.1%) of 46 patients received allogeneic HSCT. The development of HC was prevented in 9 (75.0%) of the 12 patients given preemptive treatment, while 3 (25.0%) cases developed HC and cured by treatment. BKV viruria was detected $>10^9$ copies/ml in two weeks before the onset of HC and was accepted as a prognostic indicator for predictive diagnosis of HC. BKV viremia was found $>10^4$ copies/ml in 1 patient within two weeks before the onset of cystitis.
Conclusion: Screening for BKV infection, especially BKV viruria in HSCT patients, is recommended for the predictive diagnosis of HC in patients at high risk.
Keywords: BK virus; hematopoietic stem cell transplantation; hemorrhagic cystitis; polymerase chain reaction.

ÖZ

Amaç: BK virus (BKV) ile ilişkili hemorajik sistit (HS), hematopoetik kök hücre transplantasyonu (HKHT) yapılan hastalarda yaygın görülen bir komplikasyondur. Bu çalışmanın amacı, HKHT yapılan çocuk hastalarda BKV enfeksiyonu insidansının araştırılmasıdır.
Gereç ve Yöntemler: Çalışmaya Ekim 2015 ile Eylül 2017 tarihleri arasında izlenen, yaşları 16 ay ile 16 yıl arasında olan toplam 51 hasta dahil edilmiştir. Hastalar BKV DNA'nın idrar ve kanda tespiti için kantitatif gerçek-zamanlı polimeraz zincir reaksiyonu (Anaolia Geneworks, Türkiye) testiyle monitörize edilmiştir.
Bulgular: Hastaların 46'sına allojenik HKHT ve 5'ine otolog HKHT yapılmıştır. Allojenik nakil yapılan 46 hastanın 26'sında (%56,5) ve otolog nakil yapılan 5 hastanın 1'inde (%20,0) olmak üzere toplam 27 (%52,9) hastanın idrar ve/veya kanında BKV DNA pozitifliği saptanmıştır. Allojenik HKHT yapılan 46 hastanın 12 (%26,1)'sinde preemptif tedavi için gereken idrarda $>10^7$ kopya/ml BKV viral yük düzeyi tespit edilmiştir. Preemptif tedavi uygulanan 12 hastanın 9'unda (%75,0) HS gelişmesi önlenirken 3'ünde (%25,0) HS gelişmiş ve tedaviyle iyileşmiştir. HS gelişmeden önceki iki hafta içinde BKV virurisi $>10^9$ kopya/ml olarak tespit edilmiş ve HS prediktif tanısı için prognostik bir gösterge olarak kabul edilmiştir. BKV viremisi 1 hastada sistit gelişmesinden önceki iki hafta içinde $>10^4$ kopya/ml olarak tespit edilmiştir.
Sonuç: Yüksek riskli hastalarda HS prediktif tanısı için BKV enfeksiyonu, özellikle HKHT hastalarında BKV virüri taraması önerilir.
Anahtar kelimeler: BK virüs; hematopoietik kök hücre transplantasyonu; hemorajik sistit; polimeraz zincir reaksiyonu.

INTRODUCTION

Hemorrhagic cystitis (HC) associated with BK virus (BKV) is a common complication in patients with hematopoietic stem cell transplantation (HSCT). BKV is a small (40-45 nm), non-enveloped DNA virus with icosahedral capsid and circular double-stranded genome, belonging to the Polyomaviridae family. BKV was first isolated by Gardner in 1971 from the urine sample of a renal transplant patient who developed ureteral stenosis and acute renal failure and it was named BKV according to the patient's initials. BKV infections are very common worldwide and more than 90% of adults are seropositive. BKV primer infection typically occurs during early childhood, before 10 years, often at age 4-5 years. Primary BKV infections are usually asymptomatic or mild upper respiratory tract infections. BKV is transmitted mainly by the respiratory route. After primary infection, viremia develops and BKV spreads to (infects) many different organs and enters latent phase. BKV remains latent especially in the uroepithelial cells of the kidney and urinary tract. BKV does not cause disease in immunocompetent healthy individuals in the latent phase but occasionally reactivates and manifests itself as asymptomatic viruria. However, the disease will develop in the case of immunodeficiency or in transplant recipients who undergo immunosuppressive treatment, primarily in kidney or bone marrow transplant patients. Serious complications of BKV reactivation are HC in allogeneic HSCT recipients and nephropathy that develops most commonly in renal transplant recipients. BKV may also lead to asymptomatic hematuria, ureteral stenosis and nephropathy in patients with HSCT. BKV infection is common in patients after allogeneic HSCT, but rarely seen in autologous HSCT patients. HC is an important complication after HSCT. HC is divided into two types based on the onset time of cystitis in patients with allogeneic HSCT. Early onset HC develops in the pre-engraftment period during the conditioning regimen, especially within 48-72 hours after the initiation of conditioning regimen or within 1 week of transplantation. It is caused by the direct toxicity of chemotherapeutic drugs such as cyclophosphamide and busulfan used in the conditioning regimen or of the pelvic radiation to the urothelial mucosa. In the post-engraftment period, factors such as viral infections and acute graft versus host disease (GVHD) are responsible for late-onset HC occurring. BKV is the major cause of late-onset HC after allogeneic transplantation. BKV-associated HC occurs between 2 and 8 weeks (1 week to 6 months) after transplantation. The incidence of BKV-associated HC after allogeneic HSCT is 13% on average. The rate of BKV-associated HC is 18% (8-25%) in children with allogeneic HSCT, and 16% (7-54%) in adults (1). The aim of this study was to investigate the incidence of BKV infection in children with HSCT.

MATERIAL AND METHODS

A total of 51 pediatric patients aged between 16 months and 16 years, who underwent HSCT (46 allogeneic and 5 autologous,) between October 2015 and September 2017, were prospectively studied. Patients were informed for consent. The study protocol was approved by the Institutional Ethics Committee of Cukurova University (dated 13.05.2016 and numbered 53/7). Thirty-one male and 20 female patients, aged between 16 months and 16 years, were included in the study. The patients were randomly divided into 5 groups according to their age (Table 1). Other demographic and clinical characteristics of the patients are also shown in Table 1 and, information about donor gender and type, transplant type, stem cell source and complications are shown in Table 2. Conditioning regimen was applied to patients for approximately 10 days before HSCT. Of the 51 patients, 36 (70.6%) received myeloablative treatment (29 cyclophosphamide and busulfan, 4 cyclophosphamide, ATG, fludarabine and busulfan, and 3 cyclophosphamide, fludarabine and busulfan), and 15 (29.4%) received low-density treatment (13 of the patients received cyclophosphamide, ATG and fludarabine, and 2 cyclophosphamide). Patients were followed up one week prior to transplantation, one per week for first 3 months after transplantation, and one every month up to 1 year after transplantation for BKV viruria and viremia. The extraction of viral DNA from urine and plasma samples was performed with Magnesia Viral Nucleic Acid Extraction Kit EP (Geneworks Anatolia, Turkey). The Bosphorus BKV quantification kit v1 (Anatolia Geneworks, Turkey) was used for detection of BKV DNA in urine and blood samples. For each patient sample, 10 µl of the sample DNA extract was added to the mixture consisting of 14.9 µl BKV master mix and 0.1 µl internal control (IC). Positive and negative controls were included in each study. Amplification was performed in a Qiagen Montania 4896 real-time polymerase chain reaction (PCR) instrument according to the manufacturer's protocol as 1 cycle of 14:30 min at 95 °C (first denaturation), and 50 cycles of 30 seconds at 97 °C (denaturation) and 90 seconds at 53 °C (annealing and synthesis), following the manufacturer's protocol.

All patients with allogeneic HSCT received cyclosporin and methotrexate for GVHD prophylaxis. Prophylactic acyclovir treatment was given to all transplant patients for 90 days. In addition Prophylactic intravenous immunoglobulin (IVIG) was used (0.5 gr/kg) on the day before transplantation and on day 5 after transplantation in 23 of 46 patients with allogeneic HSCT. Preemptive reduction of immunosuppression was started in patients with high-level BKV viruria (viral load $>10^7$ copies/ml) HC was treated with cidofovir, oral levofloxacin, platelet transfusion and bladder irrigation according to the clinical condition of the patient.

Statistical Analysis

Descriptive statistics were given as mean±standard deviation for numerical variables. Categorical variables were summarized with frequencies and percentages.

RESULTS

The ages of 51 patients included in the study were between 16 months and 16 years. The mean age of the patients was 6.9±4.4 years. 31 (60.8%) of the patients were male and 20 (39.2%) were female. Twenty-seven (52.9%) of 51 patients had BKV DNA positivity in their urine and/or blood. Twelve patients (23.5%), with urine BKV DNA levels above $>10^7$ copies/ml who required for preemptive treatment, were in allogeneic HSCT group (26.1%, 12/46).

In addition, BKV viremia was detected in 6 (13.0%, 6/46) of these 12 patients, and 4 of these 6 patients (8.7%) has a viral load >10^4 copies/ml. In 27 patients with BKV DNA positivity, the mean initiation time of viruria was 9.4±9.7 weeks (1st week to 12th months) while in 12 patients with a viral load of more than 10^7 copies/ml, the mean time to onset of high-level BKV viruria was 6.1±8.4 weeks (1st week to 7th months).

Three of 51 patients had HC median 56 (55-61) days after transplantation and all were in the allogeneic HSCT group (6.5%, 3/46). HC developed in 1 (7.1%, 1/14) of 5 patients who received a low-density treatment regimen and had a viral load >10^7 copies/ml. HC developed in 2 (6.2%) of 7 patients with high-level viruria who underwent myeloablative regimen. Data about the treatment regimens are given in Table 3.

The presence of BKV DNA in urine and/or blood was investigated before transplantation. It was found positive in 4 (7.8%) patients and only in urine (<10^7 copies/ml). In 2 (50.0%) of these patients, the viral load of urine BKV DNA was >10^7 copies/ml and preemptive treatment was applied to these patients. During the follow-up, viremia developed once in these 2 patients and the blood viral load of BKV DNA was determined as $2.8x10^3$ copies/ml and $6.0x10^2$ copies/ml, but HC was not developed. On the other hand, in 10 (21.3%) of 47 patients with BKV DNA negativity in urine and/or blood before transplantation, had BKV DNA viral load >10^7 copies/ml after transplantation.

Twelve (26.1%) of 46 patients who receipt allogeneic transplantation developed GVHD, 8 (17.4%) of them were acute and 4 (8.7%) were chronic GVHD. In 5 (62.5%) of 8 patients who developed acute GVHD, the viral load of urine BKV DNA was >10^7 copies/ml. High-level viremia was detected in only 7 (18.4%) of 38 allogeneic HSCT patients who did not develop acute GVHD, and acute GVHD is seen as a risk factor for BKV infection.

Prophylactic IVIG was given to 23 patients who underwent allogeneic stem cell transplantation. Five (21.7%) of these patients had high-level viruri and only 1 (4.3%, 1/23) had HC. In 7 (30.4%) of 23 patients who were not given IVIG, the viral load in urine was >10^7 copies/ml and HC was observed in 2 (8.7%) patients.

HC was seen in 3 (25.0%) of 12 patients who received preemptive therapy and all of these patients had urinary BKV DNA viral load >10^9-10^{11} copies/ml in 2 weeks prior to the development of HC, whereas only 1 patient had >10^4 copies/ml BKV viremia. With preemptive therapy, the development of HC was prevented in 9 (75.0%) of 12 patients.

Eight patients died in the study group, 1 of them died by GVHD and pneumonia, 3 of them by GVHD and 4 of them had died by other reasons. As a result, none of the patients who was underwent HSCT died due to BKV infection.

DISCUSSION

After primary infection, viremia develops and BKV passes into latent phase by spreading into many organs. BKV remains latent in the kidney and uroepithelial cells in particular. In healthy individuals, asymptomatic BKV viruria can be seen in the latent phase. In the other hand, BKV causes severe complications such as nephropathy in patients with bone marrow and kidney transplantation and receiving immunosuppressive therapy. Approximately 90% of the general population is infected with BKV. BKV infections are usually seen in early (<10 years) childhood (2). BKV infections in transplant recipients commonly are

Table 1. Demographic and clinical features of patients, n (%)

Gender, n (%)	
Male	31 (60.8)
Female	20 (39.2)
Age, n (%)	
0-2 years	9 (17.6)
3-5 years	15 (29.4)
6-10 years	15 (29.4)
11-15 years	9 (17.6)
16-18 years	3 (5.9)
Preparation regimen, n (%)	
Myeloablative regimen	36 (70.6)
Low-density regimen	15 (29.4)
Diagnosis, n (%)	
Fanconi aplastic anemia	9 (17.6)
Thalassemia major	18 (35.3)
Acute myeloid leukemia	2 (3.9)
Acute lymphoblastic leukemia	3 (5.9)
Hodgkin lymphoma	1 (2.0)
Chronic granulomatous disease	1 (2.0)
Severe combined immunodeficiency	3 (5.9)
Non-Hodgkin's lymphoma	2 (3.9)
Neuroblastoma	4 (7.8)
Diamond Blackfan anemia	1 (2.0)
T-cell lymphoma	1 (2.0)
Sickle cell anemia	2 (3.9)
Ewing sarcoma	1 (2.0)
Myelodysplastic syndrome	1 (2.0)
Aplastic anemia	1 (2.0)
Hemophagocytic lymphohistiocytosis	1 (2.0)

Table 2. Donor gender and type, transplant type, stem cell source and complications, n (%)

Transplant type	
Allogeneic	46 (90.2)
Autologous	5 (9.8)
Donor gender (n=46)	
Male	24 (52.2)
Female	22 (47.8)
Donor type (n=46)	
HLA compatible relative	44 (95.7)
HLA compatible unrelated	2 (4.3)
GVHD (n=46)	
Absent	34 (73.9)
Acute GVHD	11 (23.9)
Chronic GVHD	1 (2.2)
Stem cell source	
Bone marrow	39 (76.5)
Peripheral blood stem cells	11 (21.6)
Cord blood and bone marrow	1 (2.0)
Complications	
Fever	30 (58.8)
Gastroenteritis	20 (39.2)
Skin rash	16 (31.4)
Oral mucositis	14 (27.5)
Renal dysfunction	1 (2.0)
Hemorrhagic diathesis	3 (5.9)
Cystitis	3 (5.9)
Liver dysfunction	6 (11.8)
Pneumonia	1 (2.0)
Hematuria	3 (5.9)
Organomegaly	8 (15.7)

GVHD: Graft versus host disease

Table 3. Transplant type, treatment regimen and viral load distribution of urine BKV DNA of patients, n (%)

	Myeloablative Regimen (n=36)			**Low-Density Regimen (n=15)**		
	BKV DNA $<10^7$ copy/ml	**BKV DNA $>10^7$ copy/ml**	**BKV DNA (+)**	**BKV DNA $<10^7$ copy/ml**	**BKV DNA $>10^7$copy/ml**	**BKV DNA (+)**
Allogeneic	10 (27.7)	7 (19.4)	17 (47.2)	4 (26.7)	5 (33.3)	9 (60.0)
Autologous	-	-	-	1 (6.7)	0 (0.0)	1 (6.7)
Total	10 (27.7)	7 (19.4)	17 (47.2)	5 (33.3)	5 (33.3)	10 (66.7)

seen in genitourinary tract in consequence of BKV's genitourinary epithelium tropism. In bone marrow transplant patients, BKV viruria is associated with various clinical manifestations such as asymptomatic hematuria, HC, ureteral stenosis and interstitial nephritis. In the case of immunosuppression, BKV reactivation is observed and according to the studies, BKV viruria is 40-87% and its viremia is 17-67% (3,4). In patients undergoing chemotherapy treatment after HSCT and solid organ transplantation, the immune system is suppressed and as a result, the virus reactivates and may cause HC (5,6). HC is characterized by hematuria due to inflammation of the bladder mucosa. Accompanying symptoms are dysuria, frequent urination and suprapubic pain (2). HC has been reported as 6.5-25% in patients who underwent HSCT and it is associated with high morbidity and mortality. BKV viruri is usually seen in 2 to 8 weeks after HSCT and continues for 1 week to 2 months. In many studies, it has been reported that high viral load in urine is a sign of HC. In particular, the viral load $>9.0x10^6$ copies/ml in the urine and $>10^4$ copies/ml in the blood are risk indicators for the development of HC. Deaths also have been reported because of BKV associated HC.

Other important risk factors associated with the development of HC except BKV infection; incompatible donor, high intensity preparation regimen (anti-thymocyte globulin, cyclophosphamide, busulfan), acute GVHD, age and radiotherapy (7,8).

In our study; high-level viruri ($>10^7$ copies/ml) were detected in 12 (26.1%) of 46 patients who underwent allogeneic transplantation. BKV DNA positivity in urine before transplantation urinary and acute GVHD were seen as risk factors for the development of high-level BKV viruri. BKV viruria with $>10^7$ copies/ml levels in patients with and without acute GVHD were 62.5% and 18.4%, respectively. The presence of acute GVHD was identified as a risk factor for the increase of BKV DNA viral load to the level requiring preemptive therapy.

BKV DNA was positive ($<10^7$ copies/ml) before transplantation in 4 of 51 patients who underwent HSCT. Viruria (BKV DNA viral load $>10^7$ copies/ml) and viremia ($<10^4$ copies/ml) were detected in 2 (50.0%) of 4 patients. Of 47 patients with BKV DNA negativity before transplantation, 10 (21.3%) patients had BKV DNA viral load $>10^7$ copies/ml after transplantation, with a lower rate. Pre-transplant BKV viruri positivity is considered to be a risk factor for increased levels of viral load requiring preemptive treatment and for the development of high-level BKV viruri. The rate of viruri in 51 patients who underwent HSCT was 52.9% and is close to 50% rate of Bogdanovic et al. (9). The BKV viruri rate in allogeneic HSCT patients was 56.5% and was close to other studies (8,10-13).

Urinary viral load was $>10^7$ copies/ml and preemptive treatment was applied in 12 (26.1%) of allogeneic HSCT patients. HC developed in 3 of the patients who had preemptive therapy. Two weeks before the occurrence of HC, $>10^9$ copies/ml of the BKV virus was a risk indicator. Hayden et al. (7) similarly found the BKV viruri as $>10^9$ copies/ml at the time of 13 days before HC. Other studies have also detected BKV viruri at $>10^6$-10^{10} copies/ml in the 2-13 days before HC (9,11,14-16).

In our study, only 1 of 3 HC cases was found to be BKV viremia $>10^4$ copies/ml in 2 weeks prior to the development of cystitis and it is same with the results of Oshrine et al. (11) and Lee et al. (17) On the other hand, other studies reported levels of $>10^{2\text{-}6}$ copies/ml (13,14,16).

The rates of BKV viruri ($>10^7$ copies/ml) according to our study were lower in the myeloablative group (19.4%) than low-density regimen group (33.3%), while Giraud et al. (13) found it higher in the myeloablative group (30%) than low-density regimen group (19%). In addition, in our study, HC in patients with myeloablative and low-density treatment regimen was similar with 5.5% and 6.7%, respectively, while Giraud et al. (16) detected the incidence of HC as higher in the myeloablative group (78%) than the low-density regimen group (22%).

In this study, 62.5% of patients with acute GVHD had a viral load $>10^7$ copies/ml and this rate was 18.4% in patients without acute GVHD. This rate was 67% and 50% in the studies of Bogdanovic et al. (9) and Hayden et al. (7), respectively. They declared acute GVHD as a risk factor for BKV infection.

The rate of HC was 6.5% in allogeneic HSCT patients and it is close to 9% rate of Kwon et al. (15) and 8.9% rate of Lee et al. (17), and similar with 6% rate of Mori et al. (18), but lower than the other studies (7,10,11,19,20).

According to our results, HC occurred median 56 days after HSCT and it is close to result of Lee et al. (18, 69 days). But the many other studies reported that HC developed in a shorter time (12,14,15,19-21).

CONCLUSION

In conclusion, in all 3 cases with HC, the viral load in the urine was 10^9 copies/ml in the 2 weeks prior to the development of HC, and this finding was a prognostic marker for the development of HC. Early detection of viral infections will be effective in preventing the progression of the disease by providing timely initiation of preemptive therapy with the monitoring of BKV viral load in patients with HSCT. More prospective studies in the future for predictive diagnosis and preemptive therapy of BKV-associated HC may help to reduce the complications and mortality associated with transplantation in HSCT recipients.

Ethics Committee Approval: The study was approved by the Ethics Committee of Çukurova University Faculty of Medicine (13.05.2016, 53/7).

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Evaluation of the Relationship between Serum 25-Hydroxyvitamin D Levels and Pulmonary Functions in Adult Asthma

Serum 25-Hidroksivitamin D Düzeylerinin Yetişkin Astım Hastalarında Pulmoner Fonksiyonlar ile İlişkisinin Değerlendirilmesi

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ABSTRACT

Aim: This study was aimed to determine the relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and the pulmonary functions in adult asthma patients.
Material and Methods: A total of 131 asthmatic patients' general characteristics, asthma symptoms, pulmonary function test, asthma control test (ACT) scores, serum 25(OH)D levels, body weight, and height were recorded. Body mass indexes (BMIs) of the patients were calculated and World Health Organization criteria were used for the classification. Patients with serum 25(OH)D levels below 20 ng/mL were classified as having a deficiency, those with 21-29 ng/mL as having an insufficiency, and those with 30 ng/mL and above as having normal serum vitamin D levels.
Results: The serum 25(OH)D levels of patients in the uncontrolled asthma group, according to the ACT score, were found to be lower than those of patients in the controlled group (p=0.002). It was determined that as the serum 25(OH)D levels of the patients increased, the ACT scores also increased significantly (r=0.280, p=0.001). Additionally it was found that each 1 ng/mL increase in 25(OH)D level was associated with an increase of 0.176 L in forced vital capacity (FVC). In addition it was determined that as the serum 25(OH)D levels increased, the patients' FVCs also increased (OR=1.056, 95% CI=1.003-1.113, p=0.038). Although the change was not statistically significant (p=0.081), as serum 25(OH)D levels increased, the ACT scores also increased.
Conclusion: Vitamin D insufficiency and deficiency were frequently found in adults with asthma and there was a relationship between vitamin D deficiency and pulmonary function.
Keywords: Asthma; pulmonary function; vitamin D deficiency.

ÖZ

Amaç: Bu çalışmanın amacı, yetişkin astım hastalarında serum 25-hidroksivitamin D [25(OH)D] düzeyleri ile pulmoner fonksiyonlar arasındaki ilişkiyi saptamaktır.
Gereç ve Yöntemler: Toplam 131 astım hastasının genel özellikleri, astım semptomları, solunum fonksiyon testi, astım kontrol testi (AKT) skorları, serum 25(OH)D seviyeleri, vücut ağırlığı ve boy uzunluğu verileri kayıt altına alınmıştır. Hastaların beden kütle indeksleri (BKİ) hesaplanmış ve sınıflandırma için Dünya Sağlık Örgütü kriterleri kullanılmıştır. Serum 25(OH)D seviyesi 20 ng/mL'nin altında olan bireyler D vitamini eksikliği, 21-29 ng/mL olanlar D vitamini yetersizliği ve 30 ng/mL ve üstü olanlar ise normal serum D vitamini düzeyi olarak sınıflandırılmıştır.
Bulgular: AKT skoruna göre kontrolsüz astım grubundaki bireylerin serum 25(OH)D düzeyleri, kontrollü gruptaki bireylerden daha düşük bulunmuştur (p=0,002). Bireylerin serum 25(OH)D düzeyleri arttıkça AKT skorlarının da anlamlı şekilde arttığı saptanmıştır (r=0,280; p=0,001). Buna ek olarak serum 25(OH)D seviyesindeki her 1 ng/mL'lik artışın zorlu vital kapasite (forced vital capacity, FVC)'de 0.176 L'lik bir artış ile ilişkili olduğu bulunmuştur. Ayrıca serum 25(OH)D seviyeleri arttıkça, bireylerin FVC düzeylerinin de arttığı tespit edilmiştir (OR=1,056; %95 GA=1,003-1,113; p=0,038). İstatistiksel olarak önemli olmamakla birlikte (p=0,081), serum 25(OH)D seviyeleri arttıkça AKT skorlarının da arttığı belirlenmiştir.
Sonuç: Astım hastası olan yetişkinlerde D vitamini yetersizliği ve eksikliğinin sık görüldüğü ve D vitamini eksikliği ile solunum fonksiyonu arasında bir ilişki olduğu belirlenmiştir.
Anahtar kelimeler: Astım; pulmoner fonksiyon; D vitamini eksikliği.

INTRODUCTION

Asthma is a heterogeneous disease characterized by oversensitivity of the airway and chronic airway inflammation, which develops in response direct or indirect stimuli (1). The World Health Organization (WHO) has reported that 339 million people have asthma worldwide (2). According to WHO estimates, there were 4484.8 deaths (95% CI: 2414.1-7593.1) due to asthma at the global level in the year 2016 (3). Although the presence of atopy, exposure to environmental allergens and smoking are well known to be important risk factors associated with the onset of asthma, in recent years vitamin D insufficiency has been noticed to be a risk factor for asthma (4).

Vitamin D is indicated to be much more than a micronutrient. Vitamin D 1,25-dihydroxy, the active metabolite of vitamin D [$1,25(OH)_2D$] is considered to be a hormone, as it is structurally and functionally similar to steroid hormones (4). Studies have shown that vitamin D reduces proinflammatory markers (interleukin-6 and tumour necrosis factor-α) levels, reduces the antigen delivery of monocyte-macrophage to T cells, prevents the maturation of dendritic cells and shows immunomodulatory effect in addition to the endocrine role as a result of the anti-proliferative effect on lymphocytes (4,5). The effect of vitamin D on the pathogenesis of asthma can also be described as its inhibitory effect on the antigen-providing cells that are essential for the onset and substitution of the cellular immune response. Vitamin D has been shown to inhibit dose-dependent Th1 cells and inflammatory cytokines (such as interleukin-1 alpha-beta, interleukin-12, interferon-gamma), increasing the expression of Th2-dependent cytokines. Vitamin D has also been reported to have anti-proliferative effects on T cells and to suppress antibody production directly or indirectly in B cells; it also reduces the risk of asthma by activating Treg cells and suppressing peripheral autoreactive T cells. Due to these properties, vitamin D is believed to have a role in the reduction of asthma-related morbidity as well as prevention of asthma (6,7). Studies have shown a positive correlation between vitamin D deficiency and severity of asthma in asthma patients, longer hospitalizations and an increased usage of emergency services due to shortness of breath, and a deficiency of vitamin D in asthma patients (8-11). However, these studies have generally been conducted in the pediatric population, and the studies examining the effect of vitamin D insufficiency on asthma are limited. Therefore, this study was aimed to determine the relationship between serum vitamin D levels and the pulmonary functions in adult asthma patients.

MATERIAL AND METHODS

This retrospective study included 131 patients over the age of 18 who had received an asthma diagnosis between 1 March 2019 and 1 June 2019 at the Chest Diseases Polyclinic of the Training and Research Hospital at the University of Health Sciences Antalya. Since serum vitamin D levels may be affected by the time of year, all the patients were selected from patients admitted in the same season (spring). Pregnant, diabetic or cancer patients, vitamin D supplement users and smokers who smoked more than 10 packs/year were not included in the study.

The patient examination information filled in by the chest disease specialist was accessed from the hospital information system: age, gender, underlying chronic diseases, smoking status, asthma symptoms, asthma control test (ACT) scores, serum vitamin D and serum IgE levels and body mass indexes (BMIs) [body weight (kg)/height $(m)^2$] were calculated. The WHO criteria were used for the classification of BMI (12). Patients with serum 25-hydroxyvitamin D [25(OH)D] levels below 20 ng/mL were classified as having a deficiency, those with 21-29 ng/mL as having an insufficiency, and those with 30 ng/mL and above as having normal serum vitamin D levels (13). A Spiro Zan respiratory function test device was used for spirometry evaluations. Forced expiratory volume (FEV1), forced vital capacity (FVC), peak expiratory flow (PEF), forced expiratory volume/forced vital capacity (FEV1/FVC) and forced medium expiratory flow (MEF2575) values were used in the study.

ACT results recorded during the examination were utilized to evaluate the control of asthma-related symptoms. The ACT is a questionnaire that evaluates how well asthma has been controlled, and its Turkish version has been tested for validity and reliability. A score of 20 or more points indicates that the asthma is "controlled" while 19 or fewer points indicates that it is "uncontrolled" (14).

Ethical Committee (Training and Research Hospital, University of Health Sciences Antalya) approval was obtained on 7 November 2019 (Decision no. 24/4).

Statistical Analysis

The descriptive statistics are presented as frequencies, percentage, mean, standard deviation, median, interquartile range, and minimum-maximum values. The relationships between serum vitamin D levels and ACT scores, respiratory function parameters, serum IgE and eosinophil levels were evaluated using the Pearson or Spearman correlation test. The patients were divided into tertile according to serum vitamin D levels. The Shapiro-Wilk test was used in the normality test. The Kruskal-Wallis test was used for non-parametric comparison of numerical variables according to the tertiles, and the Dunn Bonferroni post-hoc test was used for significant cases. The ANOVA test was used to compare the tertiles where there was an assumption of normal distribution. The independent effects of vitamin D on different respiratory function parameters were evaluated using the multiple linear regression model. The effect of insufficient/deficiency vitamin D level on respiratory function parameters and ACT score was examined by logistic regression analysis. The Hosmer-Lemeshow test was used to test the model's goodness of fit. The SPSS v.22.0 package program was used in all statistical analyses and $p<0.05$ was considered statistically significant.

RESULTS

The general characteristics of patients are shown in Table 1. It was determined that the majority of the patients participating in the study (n=79, 60.3%) were female and approximately half of all patients (n=56, 42.7%) were between the ages of 18 and 33. 92 (70.2%) of the patients had had asthma for 1-5 years and more than half of them did not have any other chronic disease. In patients with chronic diseases, hypertension (n=24, 18.3%), psychiatric diseases (n=19, 14.5%) and gastritis/ulcer (n=10, 7.6%) were the most common diseases. More than half of the

patients were found to be overweight (n=49, 37.4%) or obese (n=31, 23.7%). According to the ACT score, the majority of the patients were in the uncontrolled asthma group (n=83, 63.4%) and serum vitamin D levels of 73.3% (n=96) were deficient (≤20 ng/mL). It was determined that as the serum 25(OH)D levels of the patients increased, the ACT scores (p=0.008), FVC (p=0.004) and PEF (p=0.051) levels also increased. Although the BMI values of the patients in the first tertile were the highest (28.1±5.38 kg/m^2) and those of the patients in the third tertile were the lowest (26.6±4.81 kg/m^2), this difference was not statistically significant (p=0.291, Table 2).

Table 3 shows a positive, significant correlation between serum vitamin D level and ACT scores (r=0.295, p=0.001), FVC (r=0.294, p=0.001), FEV1 (r=0.217, p=0.022) and PEF (r=0.180, p=0.040). In the multiple linear regression analysis, it was found that each 1 ng/mL increase in vitamin D level was associated with an increase of 0.176 units in FVC (p=0.005), 0.158 L in FEV1 (p=0.028) and 0.544 point in ACT score (p=0.024, Table 4). Table 5 shows the association of the vitamin D insufficiency with the respiratory function parameters and the ACT score. Accordingly, it was determined that as the serum vitamin D levels increased, the patients' FVCs also increased (OR=1.056, 95% CI=1.003-1.113, p=0.038). Although the change was not statistically significant (p=0.081), as serum vitamin D levels increased, the ACT scores also increased.

Table 1. General characteristics of patients, n (%)

Sex	
Male	52 (39.7)
Female	79 (60.3)
Age (year)	
18-33	56 (42.7)
34-49	41 (31.3)
50-65	34 (26.0)
Asthma Year	
1-5 years	92 (70.2)
≥ 6 years	39 (29.8)
Asthma treatment	
Receiving ICS treatment	107 (81.7)
Not receiving ICS treatment	24 (18.3)
Chronic disease status	
None	78 (59.5)
Hypertension	24 (18.3)
Psychiatric	19 (14.5)
Gastritis/ulcer	10 (7.6)
Body mass index (kg/m^2)	
Normal	51 (38.9)
Overweight	49 (37.4)
Obese	31 (23.7)
Asthma severity	
Controlled (ACT ≥20)	48 (36.6)
Uncontrolled (ACT ≤19)	83 (63.4)
Serum 25(OH)D (ng/mL)	
Deficient (≤20 ng/mL)	96 (73.3)
Insufficient (20.1-29.9 ng/mL)	35 (26.7)

ICS: inhaled corticosteroids, ACT: asthma control test, 25(OH)D: 25-hydroxyvitamin D

Table 2. The asthma control test score, pulmonary function parameters, IgE, eosinophils and body mass index of patients according to serum vitamin D levels

	Tertile 1 (n=43)	Tertile 2 (n=43)	Tertile 3 (n=45)	p
ACT score	19.5±2.8	20.9±3.1	21.5±2.8	0.008
FVC	85 (19) [57-107]	89 (26) [59-125]	94 (13) [56-138]	0.004
FEV1	82.6±14.3	87.5±17.0	89.5±11.9	0.104
FEV1/FVC	103.4±12.9	103.4±11.1	101.2±9.3	0.553
PEF	66.8±17.9	73.3±19.9	75.6±16.4	0.051
MEF2575	71.0±25.5	78.3±26.5	75.4±22.6	0.397
IgE	99 (113) [17-694]	110 (152) [17-603]	141 (130) [17-1660]	0.573
Eosinophils	200 (200) [0-600]	100 (200) [0-1000]	200 (300) [0-1000]	0.285
BMI (kg/m^2)	28.1±5.4	26.7±4.9	26.6±4.8	0.291

Tertile ranges are as follows: Tertile 1: ≤13.89 ng/mL, Tertile 2: 13.9-19.3 ng/mL, Tertile 3: 19.31-29.9 ng/mL, normally distributed variables are presented as mean±standard deviation while not-normally distributed variables are presented as median, interquartile range, and minimum-maximum, ACT: asthma control test, FVC: forced vital capacity, FEV1: forced expiratory volume, PEF: peak expiratory flow, MEF2575: forced medium expiratory flow, BMI: body mass index

Table 3. Correlation between serum vitamin D level and asthma control test score, respiratory function parameters, serum IgE, eosinophils and body mass index

	r	p
ACT score	0.295	0.001
FVC	0.294	0.001
FEV1	0.217	0.022
FEV1/FVC	-0.143	0.140
PEF	0.180	0.040
MEF2575	0.090	0.309
IgE	0.068	0.440
Eosinophils	0.080	0.364
BMI (kg/m^2)	-0.109	0.217

FVC: forced vital capacity, FEV1: forced expiratory volume, PEF: peak expiratory flow, MEF2575: forced medium expiratory flow, BMI: body mass index

Table 4. Multiple linear regression analysis of the relationship between serum vitamin D levels and pulmonary function parameters

	B	SE	Beta	t	p	95% CI	
Constant	6.680	3.110		2.148	0.034	0.525	12.835
ACT score	0.544	0.239	0.275	2.278	0.024	0.071	1.016
FVC	0.176	0.062	0.432	2.844	0.005	0.053	0.298
FEV1	0.158	0.071	0.417	2.222	0.028	0.300	0.017
PEF	0.029	0.034	0.090	0.840	0.403	-0.039	0.097

F=2.403, p=0.010, β: coefficient of regression, SE: standard error, CI: confidence interval, ACT: asthma control test, FVC: forced vital capacity, FEV1: forced expiratory volume, PEF: peak expiratory flow

Table 5. Logistic regression analysis of the relationship between vitamin D insufficiency and deficiency between pulmonary function parameters and asthma control test score

	OR	95% CI		p
FVC	1.056	1.003	1.113	0.038
FEV1	0.955	0.899	1.015	0.138
PEF	1.001	0.973	1.030	0.960
ACT score	1.202	0.977	1.477	0.081

OR: odds ratio, FVC: forced vital capacity, FEV1: forced expiratory volume, PEF: peak expiratory flow, ACT: asthma control test

DISCUSSION

This study found that the prevalence of vitamin D insufficiency in the adult asthmatic population in Turkey (Antalya) was quite high. At the same time, there was a significant relationship between serum vitamin D level and FVC, FEV1 and PEF.

The prevalence of asthma and the rates of vitamin D insufficiency are increasing worldwide. Vitamin D is thought to have a role in asthma pathogenesis because it has immunomodulatory effects such as improving immune system tolerance and maintaining epithelial barrier integrity (15). In Turkey, vitamin D deficiency and insufficiency were found in 76.4% in children with asthma between the ages of 1-4, and in 90.6% of children in another similar study (16,17). Although the number of studies of asthmatic adults is quite limited, the prevalence of vitamin D deficiency found in asthma patients in other research is similar to that observed in our study (18-21). In a study conducted on 435 adult asthma patients in China to determine the relationship between serum vitamin D level and pulmonary function, a significant relationship was found between vitamin D deficiency (50 nmol/L) and FEV1/FVC ratio and FEV1 (18). Similarly, Beyhan-Sagmen et al. (19) suggested that the FEV1 levels of asthmatic patients with serum vitamin D insufficiency in Turkey were low and that there was a significant linear relationship between vitamin D and FEV1. In one study, every 22.7 mL increase in FEV1 was found to cause a 1 ng/mL increase in serum vitamin D (20). Despite this, 91% of adult asthmatic patients in Costa Rica had serum vitamin D levels below 30 nmol/L, and although there was a linear relationship between serum vitamin D levels and FEV1, this relationship was not statistically significant (21). In a study involving 760 asthmatic patients in Norway, a significant correlation between serum vitamin D levels and FEV1 was observed only in males because the males participating had a lower level of lung function than the females (22). In this study, it was determined that as the serum vitamin D levels of the asthma patients increased, the FVC and PEF levels increased, and in multiple linear regression analysis, each 1 ng/mL increase in vitamin D level was associated with an increase of 0.176 L in FVC, 0.158 L in FEV1 and 0.544 point in ACT score. Obesity causes the emergence of asthma as a result of its mechanical and inflammatory effects and increases the severity of asthma over time. Patients with a BMI of 35 or more are reported to be approximately twice as much at risk of asthma (23). There is evidence that the active form of vitamin D modulates intracellular ionized calcium signaling in adipocytes, inhibits uncoupling protein-2 (UCP-2), decreases lipolysis, and increases lipogenesis. Accordingly, vitamin D deficiency is thought to play an important role in the development of obesity (24). In our study, more than half of the patients with asthma were found to be overweight (n=49, 37.4%) or obese (n=31, 23.7%). In addition, although it was determined that patients' BMIs increased as their serum vitamin D levels decreased, this difference was not found to be statistically significant. Sutherland et al. (20) stated that each unit increase in BMI in adult asthmatic patients caused a decrease in serum vitamin D level of 0.71 ng/ml and that there was a strong inverse correlation between serum vitamin D levels and BMI, especially in asthmatic patients not receiving inhaled corticosteroid (ICS) therapy. This was associated with higher obesity rates in asthmatic patients not receiving ICS treatment compared to other groups. Despite this, similarly to our study, Li et al. (18) observed lower serum vitamin D levels in asthmatic patients who were obese, but reported that this relationship was not significant. The fact that the sample of our study was small and that more than one-third of the patients had a normal BMI may explain why this relationship was not significant.

The ACT is the most commonly used test today, is easily understood by patients and their families and shows the severity of the asthma (25). It has been stated that there is a relationship between serum vitamin D levels and clinical parameters such as asthma severity, exacerbation, admission to an emergency department and number of hospitalizations (16,26,27). Studies in children have shown that serum vitamin D deficiency exacerbates asthma by 2.6 times (26) and hospital or emergency department admission by 1.5 times (9) compared to the previous year. Few studies have determined the relationship between asthma control and serum vitamin D levels in adults. While there was no relationship between serum vitamin D level and ACT scores in one study, when classified according to serum 25(OH)D levels, ACT scores were reported to be lower in the group with severe vitamin D deficiency (19). In one study conducted with the elderly, the serum vitamin D levels of patients in the uncontrolled asthmatic group were shown to be lower than those of patients in the controlled group (28). In this study, it was determined that as the serum 25(OH)D levels of the patients increased, the ACT scores also increased, and that there was a low-level, positive and significant relationship between the ACT scores and serum vitamin D level.

In randomized controlled studies, vitamin D supplements at different doses were found to increase serum 25(OH)D levels (29,30), decrease exacerbation rates of asthma and increase ACT scores (28,30,31), and positively affect FEV1 (31,32).

However, some limitations should be noted. First, since the study is retrospective, there was no record of the nutrient consumption of patients and the amount of vitamin D taken in orally could not be calculated. In addition, the patients were not asked about the amount of time they had spent in the sun. Nevertheless, the fact that patients constituting the sample of the study were admitted in the same season (spring) is one of the strengths of the study. Another limitation of the study was the low number of samples and the fact that it was a cross-sectional retrospective study.

CONCLUSION

This study tried to explain the role of vitamin D in adult asthma patients. It was determined that vitamin D deficiency was frequently found in adults with asthma and there was a relationship between vitamin D deficiency and pulmonary function. In addition, it was determined that patients with high serum vitamin D levels have a better asthma course. Since the results of this study are thought to have been affected by factors such as diet, exposure to sun, etc., further studies are recommended that consider multiple factors that may affect the relationship between asthma and vitamin D.

Ethics Committee Approval: The study was approved by the Ethics Committee of Antalya Training and Research Hospital (07.11.2019, 24/4).

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Evaluation of the Relationship between Silent Cerebral Lesions and Triglyceride/HDL-Cholesterol in Patients with First Stroke Attack

İlk İnme Atağı Olan Olgularda Sessiz Serebral Lezyonların Trigliserid/HDL-Kolesterol Oranı ile İlişkisinin Değerlendirilmesi

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ABSTRACT

Aim: Triglyceride/high-density lipoprotein cholesterol (TG/HDL-C) ratio is defined as the serum atherogenicity index. High TG/HDL-C ratio is related with vascular diseases, insulin resistance and metabolic syndrome. The TG/HDL-C ratio in cerebrovascular diseases, especially in silent cerebral lesions hasn't been well studied. The aim of this study was to evaluate the frequency of silent cerebral ischemia (SCI) and leukoaraiosis (LA), and its relationship with TG/HDL-C ratio in patients admitted with the first ischemic stroke attack.
Material and Methods: We retrospectively evaluated 200 patients who admitted to Bulent Ecevit University Faculty of Medicine, Department of Neurology with the diagnosis of acute first ischemic stroke. Silent cerebral lesions were defined as LA and SCI with magnetic resonance imaging. TG/HDL-C ratio was calculated by dividing TG levels by HDL-C levels.
Results: Silent cerebral lesions were detected 124 (83.2%) of 149 patients. LA severity was evaluated according to Fazekas score, 22 (14.8%) of patients were grade 0, 49 (32.9%) of them were grade 1, and 78 (52.3%) of them were advanced periventricular white matter hyperintensity (adv-PWMH) group. TG/HDL-C ratio in SCI group was higher than the group without SCI, but it wasn't statistically significant (p=0.091). A significant increase was observed in the TG/HDL-C ratio, as LA severity increased. TG/HDL-C ratio was significantly higher in adv-PWMH group (p=0.050).
Conclusion: High serum atherogenicity index is associated with atherosclerosis and vascular endothelial dysfunction. With this simple, inexpensive and effective test method, high-risk group of LA and SCI could be identified.
Keywords: Silent cerebral ischemia; leukoaraiosis; TG/HDL-C ratio; serum atherogenic index.

ÖZ

Amaç: Trigliserid/yüksek yoğunluklu lipoprotein kolesterol (TG/HDL-K) oranı serum aterojenite indeksi olarak tanımlanmıştır. Yüksek TG/HDL-K oranı vasküler hastalıklar, insülin direnci ve metabolik sendrom ile ilişkilidir. Serebrovasküler olaylarda özellikle sessiz serebral lezyonlarda TG/HDL-K oranı ile ilgili çalışmalar azdır. Bu çalışmanın amacı, ilk iskemik inme atağı ile başvuran hastalarda sessiz serebral iskemi (SSİ) ve lökoariozis (LA) sıklığını ve TG/HDL-K oranı ile ilişkisini değerlendirmektir.
Gereç ve Yöntemler: Bülent Ecevit Üniversitesi Tıp Fakültesi Nöroloji bölümüne akut ilk iskemik inme tanısı ile başvuran 200 hasta geriye dönük olarak değerlendirildi. Manyetik rezonans görüntüleme ile sessiz serebral lezyonlar SSİ ve LA olarak tanımlandı. TG/HDL-K oranı TG değerinin HDL-K değerine bölünmesi ile hesaplandı.
Bulgular: Yüz kırk dokuz hastanın 124 (%83,2)'ünde sessiz serebral lezyon saptandı. LA şiddeti Fazekas skorlamasına göre değerlendirildi, hastaların 22 (%14,8)'si grade 0, 49 (%32,9)'u grade 1 ve 78 (%52,3)'i ileri düzey periventriküler beyaz cevher lezyonu (advanced periventricular white matter hyperintensity, adv-PWMH) grubundaydı. SSİ grubunda TG/HDL-K oranı SSİ olmayan gruba göre daha yüksek bulundu, ancak istatistiksel olarak anlamlı değildi (p=0,091). LA şiddeti arttıkça TG/HDL-K oranında anlamlı bir artış olduğu izlendi. TG/HDL-K oranı, adv-PWMH grubunda anlamlı derecede yüksek idi (p=0,050).
Sonuç: Yüksek serum aterojenite indeksi ateroskleroz ve vasküler endotelyal disfonksiyon ile ilişkilidir. Bu basit, ucuz ve etkili test yöntemi ile LA ve SSİ yüksek risk grubu belirlenebilir.
Anahtar kelimeler: Sessiz serebral iskemi; lökoariozis; TG/HDL-K oranı; serum aterojenite indeksi.

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INTRODUCTION

Stroke is a common, serious, and disabling health-care problem. Stroke is the second leading cause of death and a major cause of disability worldwide. World Health Organization has defined stroke as a clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than of vascular origin (1).

Stroke is associated with high mortality, morbidity, hospitalization rate and serious economic burden in developing countries. Knowing and preventing risk factors for stroke is important for a new direction to treatment approaches and take early measures (1,2).

Silent cerebral lesions have been defined as leukoaraiosis (LA) and silent cerebral ischemia (SCI). SCI has been defined as ischemic lesions in patients without neurological symptoms and a history of stroke (3,4). LA is cerebral white matter abnormalities which is characterized by demyelination and ischemic injury (5,6).

Studies have shown that SCI and LA increase the risk of stroke, mortality and morbidity after stroke (3,4,7). Thus, detecting and preventing risk factors of SCI and LA have gained prominence. It has been shown that various risk factors and pathophysiological mechanisms for SCI and LA. Lipohyalinosis, atherosclerosis and endothelial dysfunction are among these (8,9). The well-known risk factor is serum cholesterol level. According to international guidelines, it is recommended to control lipid profile, especially low density lipoprotein (LDL) levels (10). Recent studies have shown that LDL particle types are more important than their total amount for vascular complications (9,11). It has been determined that small dense low density lipoprotein (LDL) and small high density lipoprotein (HDL) particles are more effective for the development of atherosclerosis. According to studies, there is a strong relationship between high triglyceride/high density lipoprotein cholesterol (TG/HDL-C) ratio and plasma small dense LDL ratio (12). Atherogenic index of plasma (AIP) is calculated as the ratio between the TG value and HDL-C value (mg/dL). (TG/HDL-C) AIP is a major risk factor for metabolic syndrome, cardiovascular diseases and insulin resistance (9,13-15). Total cholesterol/triglyceride (TC/TG) ratio is another index which reflects LDL particle size (9). There are few studies about the role of TG/HDL-C ratio in silent cerebral lesions in cerebrovascular diseases (16).

In this study, we aimed to evaluate the relationship between TG/HDL-C ratio and silent cerebral lesions in patients with first ischemic stroke.

MATERIAL AND THE METHODS

Participants

Two hundred patients, who admitted to Zonguldak Bulent Ecevit University Faculty of Medicine, Department of Neurology, with first-ever ischemic stroke between 2016 and 2019 were recorded retrospectively. Patients with cerebral or subarachnoid hemorrhage, symptomatic stroke history and under 35 years of age were excluded. Demographic data of the patients (age, gender, body mass index, diabetes mellitus, hypertension, ischemic heart disease, hyperlipidemia, smoking, alcohol and drug use) was recorded retrospectively. Fifty-one patients with diabetes mellitus, hypertension, ischemic heart disease, hyperlipidemia, atrial fibrillation, smoking, alcohol use, use of antiplatelet, anticoagulant, antihypertensive or antilipidemic medication and subjects with missing covariate data were excluded the study. Lipid profiles included total cholesterol (TC), LDL cholesterol, HDL-C and TG were recorded in 149 patients. The TG/HDL-C ratio was calculated after dividing absolute TG levels by absolute HDL-C levels in peripheral blood. TG/HDL-C ratio (AIP) and TC/TG ratios were calculated. Our study was approved by Bulent Ecevit University Clinical Research Ethics Committee (29.04.2020, 09/6).

Magnetic Resonance Imaging Protocol

Magnetic resonance imaging (MRI) findings of the patients were recorded. 1.5 Tesla MRI (PHILIPS, INTERA) was used to identify silent cerebral lesions. It is scanned in 22 sections and 5 millimeters thickness. T1 (TR/TE: 582/15), T2 (TR/TE: 5835/110), T2 FLAIR (TR/TE: 6000/120), Diffusion (TR/TEd: 4832/81) and ADC (TR/TEd: 4832/81) sections were analyzed.

SCI was defined as focal hyperintensity on FLAIR and T2-weighted images, hypointensity on T1-weighted image, lesions of 3-20 mmin size. Under 3mm lesions seen hyperintensity on T2-weighted image, hypointesity on T1-weighted and FLAIR, were described as Virchow-Robin Space (3,4).

LA severity was evaluated by Fazekas scale (17,18):

Grade 0: No white matter lesions.

Grade 1: Small-sized white matter lesion adjacent to the ventricle (punctate)

Grade 2: White matter lesion that holds 1/3 of ventricular cerebral cortex distance

Grade 3: White matter lesion that holds 2/3 of ventricular cerebral cortex distance

Grade 2-3 lesions were considered as advanced periventricular white matter hyperintensity (adv-PWMH). MRI results of the patients were examined by two different neurologists and a common decision was reached.

Statistical Analysis

Statistical analysis was performed using the SPSS v.18.0 program. The suitability of the normal distribution was assessed by Kolmogorov-Smirnov test. When the parametric test assumptions were provided Independent Samples t test was used to compare two groups in terms of numerical variables, and the Mann-Whitney U test was used when parametric test assumptions were not provided. One-way analysis of variance (ANOVA) was used to compare three groups when the parametric test assumptions were provided, and the Kruskal-Wallis test was used when not provided. Categorical variables were analyzed by Pearson Chi-square test. Descriptive statistics were given as mean±standard deviation or median with interquartile range and minimum maximum depending on the distribution of the continuous variables, while categorical variables were summarized as numbers and percentages. A p value of 0.05 was considered significant.

RESULTS

One hundred forty nine patients included in our study. Seventy nine (53.0%) patients were female, seventy (47.0%) patients were male, and the mean age was 66.5±12.6 years. SCI was detected in 83.2% (n=124) of the patients. 12.9% of the lesions were single and 87.1% of the

lesions were multiple. According to the Fazekas scoring, 22 (14.8%) of the patients were in the grade 0, 49 (32.9%) of the patients were in the grade 1, and 78 (52.3%) of the patients in adv-PWMH group. The prevalence of LA and SCI was high in patients with the first ischemic stroke attack. The demographic features of the groups with and without SCI were summarized in Table 1. When the groups with SCI (n=124) and without SCI (n=25) were compared, no statistically significant difference was observed with TC, TG, LDL-C, HDL-C. Although TG/HDL-C ratio was high in patients with SCI, it wasn't statistically significant (p=0.091). TC/TG ratio was low in patients with SCI but it wasn't statistically significant (p=0.084, Table 2).

The comparison of the patients' demographic features according to Fazekas scores were summarized in Table 3. There was an increase in LA severity with age (p<0,001), but no relation was found between gender and LA severity (p=0.458).

No statistically significant difference was observed in LA severity with HDL-C, LDL-C, TC, TG values. However, LA severity was significantly associated with high TG/HDL-C ratio (p=0.050). TG/HDL-C ratio was significantly higher in the adv-PWMH group (Table 4). In the adv-PWMH group, TC/TG ratio was lower, but it was not statistically significant (p=0.089).

DISCUSSION

Leukoaraiosis is associated with dementia, cognitive impairment, stroke risk, and small vessel diseases that increase infarct progression (19). Studies have shown that; a history of stroke, hypertension, diabetes mellitus, age, cerebral vessel atherosclerosis are known as risk factors for LA (7,19,20). The cause of LA is not known exactly, but the precise mechanisms and prognostic significance in LA are still actively investigated.

Although many studies have shown that hyperlipidemia is associated with the severity of LA (7,19,21), this relationship has not been shown in others (22). The relationship between TG and LA severity is conflicting in recent studies. While some studies have shown that low TG level was associated with LA (19,21), some studies have shown that high TG level was associated with LA (23). The mechanisms of the relationship between LA and TG level are not clearly known.

Table 1. Comparison of demographic characteristics of the patients with and without SCI

	With SCI (n=124)	Without SCI (n=25)	p
Age (year), mean±SD	67.4±13.8	64.8±11.2	0.282
Gender, n (%)			
Female	65 (52.5)	14 (56.0)	0.553
Male	59 (47.5)	11 (44.0)	

SCI: silent cerebral ischemia, SD: standard deviation

Table 3. Comparison of demographic characteristics of the patients according to Fazekas scoring

	Grade 0 (n=22)	Grade 1 (n=49)	adv-PWMH (n=78)	p
Age (year), mean±SD	56.1±11.2	63.3±11.7	71.5±8.9	0.001
Gender, n (%)				
Female	11 (50.0)	28 (57.1)	31 (39.7)	0.458
Male	11 (50.0)	21 (42.9)	47 (60.3)	

adv-PWMH: advanced periventricular white matter hyperintensity, SD: standard deviation

Table 2. Comparison of serum lipid values of the patients with and without SCI

	With SCI (n=124)			Without SCI (n=25)			p
	Median	Q_1-Q_3	Min-Max	Median	Q_1-Q_3	Min-Max	
TC (mg/dl)	181	151-204	84-309	174	149-205	70-304	0.755
LDL-C (mg/dl)	111.5	89-130	28-229	104	93-137	23-232	0.729
HDL-C (mg/dl)	37.9	30.6-42.1	15.2-76.2	37	32.7-47.5	25-91	0.593
TG (mg/dl)	139.5	96-190	55-617	120	87-163	37-407	0.152
TG/HDL-C	3.7	2.4-6.2	0.8-22.9	3.1	2.1-4.7	0.9-16.3	0.091
TC/TG	1.3	0.9-1.7	0.3-3.3	1.4	1.2-1.9	0.5-3.4	0.084

SCI: silent cerebral ischemia, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, Q_1-Q_3: 1st quartile - 3rd quartile, Min-Max: minimum - maximum

Table 4. Comparison of serum lipid values of the patients according to Fazekas scoring

	Grade 0 (n=22)			Grade 1 (n=49)			adv-PWMH (n=78)			p
	Median	Q_1-Q_3	Min-Max	Median	Q_1-Q_3	Min-Max	Median	Q_1-Q_3	Min-Max	
TC (mg/dl)	160	150-201	89-271	178	134-196	84-249	188	149-211	70-309	0.670
LDL-C (mg/dl)	100	88-120	43-168	114	88-136	23-232	117	102-137	52-186	0.490
HDL-C (mg/dl)	39.8	28.4-39.8	19.2-59	36.1	29.3-41	17-76.2	34.3	29.2-40	15.2-50.1	0.280
TG (mg/dl)	119	87-141	68-421	145	87-203	37-479	143.5	106-186	50-617	0.317
TG/HDL-C	3.3	2.0-4.8	1.3-15.1	4.2	1.9-6.5	0.8-22.9	4.5	3.2-6.6	1.2-16.3	0.050
TC/TG	1.4	1.0-1.9	0.6-2.6	1.2	0.9-1.9	0.3-3.3	1.1	0.8-1.5	0.3-2.4	0.089

adv-PWMH: advanced periventricular white matter hyperintensity, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, Q_1-Q_3: 1st quartile - 3rd quartile, Min-Max: minimum - maximum

More than half of the white matter is made up of myelin, and the myelin contains about 70% lipids. The decrease in TG level and the increase in severity of LA can be explained in this way. Genetic reasons should also be kept in mind (19). Studies are needed to reveal new clues in this area.

Recent studies have shown that LDL particle types are more important than their total amount for vascular complications (11). Small-dense LDL and small HDL particles have been found to be particularly effective for the development of atherosclerosis. Small and dense LDL particles are more sensitive to oxidation. It is more atherogenic than large and cholesterol-rich LDL particles by increasing vascular permeability and reducing interest in LDL receptors. A relationship was observed between increased TG/HDL-C ratio, that is, hypertriglyceridemia and low HDL levels, and increased small and dense LDL rates (12). The TG/HDL-C ratio was shown for the first time by Gaziano et al. (24) as the AIP. It is considered to be important risk factor especially for coroner artery disease. TG/HDL-C ratio is accepted as an indicator of harmful LDL particles. A significant relationship was observed between high TG/HDL-C ratio and recurrent stroke in a study conducted by Park et al (25).

The size of the LDL particles and circulating very low density lipoprotein (VLDL) levels and TG levels are inversely proportional (9,11). Therefore, it defined that LDL phenotypes based on TG/HDL-C ratios. Dense and small LDL particles, high TG/HDL-C ratio have been associated with coroner artery disease, cerebrovascular disease, metabolic syndrome and insulin resistance (9,13-15). Vascular endothelial dysfunction is associated with an increase in TG/HDL-C ratio and vascular endothelial dysfunction has been demonstrated in LA formation (22). The changes in brain microvascular endothelial activity causing cerebral small vessel disease, which is primarily responsible for the pathogenesis of LA. Dysfunction of vascular endothelial cells causes increased permeability of the blood brain barrier (26). Blood brain barrier disorder, disruption of cerebrovascular autoregulation, and chronic hypoperfusion are important mechanisms for the development of LA. Vascular endothelial dysfunction is a potential risk factor for the development of white matter lesions (27). In this study, we observed that the relationship between high TG/HDL-C ratio with severity of LA, especially in the advanced LA group. This strengthens the relationship between LA and vascular endothelial dysfunction. This is the first study to show the relationship between serum atherogenicity index and LA.

SCI is a common subclinical pathophysiology before ischemic stroke (3,4,7,9). Many risk factors and pathophysiological mechanisms have been identified for SCI development. Lipohyalinosis, atherosclerosis and endothelial dysfunction are among these (8,9). It has been shown in many studies that hyperlipidemia is a risk factor for SCI (3,4,7). Patients with multiple SCI had higher LDL and TC levels than the patients with single SCI (7). In the study investigating the relationship between the high TG/HDL-C ratio and the presence of SCI in healthy individuals with silent cerebral ischemia, a significant relationship was found between the high TG/HDL-C ratio and the presence of SCI. It was concluded that the high TG/HDL-C ratio is responsible for atherogenicity, small and dense LDL is more sensitive to oxidation and accelerates atherosclerosis (9). Atherosclerosis also causes extravasation of toxic substances into neuronal tissues, blockage of small arterioles and hypoperfusion (28).

The relationship between high TG/HDL-C ratio and SCI prevalence can be thought to be related to atherosclerosis. In our study, when the groups with and without SCI were compared, the TG/HDL-C ratio was high in the groups with SCI, but it was not statistically significant. TC/TG ratio was found to be low in the patients with SCI but it was not statistically significant.

CONCLUSION

Leukoaraiosis and SCI increase the risk of stroke and affect mortality and morbidity after stroke. Therefore, determination and prevention of risk factors of SCI and LA has gained prominence. High serum atherogenicity index is associated with atherosclerosis and vascular endothelial dysfunction. With this simple, inexpensive and effective test method could identify high-risk group of LA and SCI. Development of LA and SCI can be prevented by this method. There is a need for new prospective studies on this subject.

Ethics Committee Approval: The study was approved by the Ethics Committee of Bulent Ecevit University Faculty of Medicine (29.04.2020, 09/6).

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Serum Zonulin Levels and Social Cognition in Children with Attention Deficit Hyperactivity Disorder

Dikkat Eksikliği Hiperaktivite Bozukluğu olan Çocuklarda Serum Zonulin Düzeyleri ve Sosyal Biliş

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ABSTRACT

Aim: Alterations in gut-brain axis of patients with attention deficit hyperactivity disorder (ADHD) have been indicated in recent studies. The aim of this study was to determine whether serum level of zonulin, considered to be a regulator of permeability in both gut-blood and blood-brain barriers, was associated with ADHD symptoms, and to evaluate the relationship between zonulin and social cognition in children with ADHD.

Material and Methods: Serum level of zonulin was analyzed by enzyme-linked immunosorbent assay (ELISA) in 40 treatment-naive children with ADHD, and age and gender matched 40 healthy children as control group. DuPaul ADHD Rating Scale was used for ADHD symptoms and reading the mind in the eyes test (RMET) was administered by the clinician to examine the social cognitive abilities.

Results: We found that serum zonulin levels were significantly higher in ADHD group compared to control group (p=0.010). Also, children with ADHD have significantly lower RMET scores (p=0.007). Furthermore, we found statistically significant positive correlations between serum zonulin levels and ADHD symptoms (p<0.001) and a negative correlation between serum zonulin levels and RMET scores (p=0.001) in ADHD group.

Conclusion: The present study is the first to evaluate whether there is a relationship between serum zonulin levels and social cognition in children with ADHD. The results of our study indicate that zonulin may be associated with ADHD and social cognition. Further studies with larger samples are required to determine the role of zonulin in ADHD.

Keywords: Attention deficit hyperactivity disorder; zonulin; social cognition; gut-brain axis.

ÖZ

Amaç: Son çalışmalarda dikkat eksikliği hiperaktivite bozukluğu (DEHB) tanılı hastaların bağırsak-beyin eksenindeki değişiklikler belirtilmiştir. Bu çalışmanın amacı, hem bağırsak-kan hem de kan-beyin bariyerlerinde geçirgenliği düzenlemek için bir modülatör olarak kabul edilen zonulinin serum düzeyinin DEHB tanılı çocuklarda DEHB belirtileriyle ilişkili olup olmadığını belirlemek ve zonulin ile sosyal biliş arasında bir ilişki olup olmadığını değerlendirmektir.

Gereç ve Yöntemler: Zonulinin serum düzeyi, tedavi görmemiş 40 DEHB tanılı çocukta ve kontrol grubu olarak yaş ve cinsiyet yönünden eşleştirilmiş 40 sağlıklı çocukta enzime bağlı immünosorbent testi (enzyme-linked immunosorbent assay, ELISA) kullanılarak analiz edilmiştir. DEHB belirtileri, DuPaul DEHB Derecelendirme Ölçeği ile puanlanmış ve sosyal bilişsel becerileri incelemek için ise klinisyen tarafından gözlerden zihin okuma testi (reading the mind in the eyes test, RMET) uygulanmıştır.

Bulgular: DEHB grubunda serum zonulin düzeyleri kontrol grubuna göre anlamlı olarak yüksek olarak bulunmuştur (p=0.010). Ayrıca, DEHB tanılı çocukların RMET puanları önemli ölçüde düşük bulunmuştur (p=0.007). Ek olarak, DEHB grubunda serum zonulin düzeyleri ile DEHB semptomları arasında istatistiksel olarak anlamlı pozitif korelasyon (p<0.001) ve serum zonulin düzeyleri ile RMET skorları arasında ise negatif korelasyon (p=0.001) bulunmuştur.

Sonuç: Bu çalışma, DEHB tanılı çocuklarda serum zonulin düzeyleri ile sosyal biliş arasında bir ilişki olup olmadığını değerlendiren ilk çalışmadır. Çalışmamızın sonuçları, zonulinin DEHB ve sosyal biliş ile ilişkili olabileceğini göstermektedir. Zonulinin DEHB'deki rolünü belirlemek için daha büyük örneklemlerle yapılacak ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Dikkat eksikliği hiperaktivite bozukluğu; zonulin; sosyal biliş; bağırsak-beyin ekseni.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is the most prevalent neurodevelopmental disorder (1-3) and characterized by developmentally inappropriate symptoms of inattention, hyperactivity and impulsivity (4).

Social cognition is considered to be essential for successful social interaction and defined as the ability to understand other's mind and feelings (5,6). In addition to core symptoms of ADHD, it has been indicated that children with ADHD have difficulty in social cognitive abilities which is supposed to be mostly interpreted in the contex of fronto-striatal dysfunction (6-9). In a recent meta-analysis, performance of individuals with ADHD on social cognition was described to lie intermediate between autistic spectrum disorders (ASD) and healthy controls (7). Interestingly, indicating another overlapping aspect in ASD and ADHD, the gut microbiota and gut-brain axis has also been identified to be altered in patients with both ASD (10,11) and ADHD (12,13). Gut-brain axis is considered to involve the bi-directional communication between the gastrointestinal and central nervous systems (14). Zonulin (pre-haptoglobin 2) is known to be a modulator for regulator of permeability in gut-blood and blood-brain barriers via intercellular tight-junctions (15,16). Zonulin is considered to increase permeability of the small intestine and to contribute to the intestinal immunity (17). Furthermore, zonulin has been described to be associated with inflammation and autoimmunity (18,19), ASD (20) and ADHD (21). In addition, serum zonulin has been found to be related to social responsiveness (21).

We aimed to examine serum levels of zonulin and to explore whether there is an association between serum levels of zonulin and social cognition in treatment-naive children with ADHD. We have encountered no study investigating serum levels of zonulin and social cognition in children with ADHD.

MATERIAL AND METHODS

Participants

This research was carried out at the child and adolescent psychiatry outpatient clinic of Düzce University Medical Faculty. Based on zonulin levels found in a similar study in literature (20), the minimum sample size was calculated as 32, with 95% confidence level and 80% power. Simple random sampling method was used in sample selection. The research group consisted of 40 treatment-naive children aged between 8-12 years old diagnosed with ADHD, and age-gender matched 40 healthy children admitted to the child psychiatry outpatient clinic without any psychopathology. Diagnoses and comorbidities were determined through clinical examination based on "Diagnostic and Statistical Manual of Mental Disorders (DSM 5)" and the Interview Schedule for "Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (Kiddie-SADS-PL)", and children with exclusion criteria were excluded from the study. Exclusion criteria for the case group were clinically mental retardation, autism, medical comorbidities, comorbid psychopathologies except oppositional defiant disorder (ODD), taking medication, having an infection history for the last month. Exclusion criteria for the control group were psychiatric diseases, chronic physical diseases and having an infection history for the last month. BMI of all subjects, calculated according to BMI percentiles of World Health Organization, were found to be within normal range. All of the children gave verbal assent and parents provided informed consent. The study was performed in accordance with the ethical standards established in the Declaration of Helsinki. Medical Ethics Committee of Düzce University approved the study (approval date: 04.03.2019, number: 2019/38).

Measures

1. Kiddie Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version (K-SADS-PL) Turkish: This interview was developed (22) to evaluate present and lifetime psychopathology in children and adolescents and Turkish translation was also conducted (23,24).

2. Attention Deficit/Hyperactivity Disorder-Rating Scale-DuPaul (ADHD-RS): This scale evaluates ADHD symptoms according to DSM-IV criteria and contains 4-point Likert type 18-item (25). The scale had been used in Turkish children with ADHD (26-28).

3. Reading the Mind in the Eyes Test (RMET): RMET is designed to test comprehension of other's mental states by looking at eye expression (29-32). It was created by Baron-Cohen et al. (29) in 1997, and a revised form was published in 2001 (30). Turkish translation for children was performed by Girli (31).

Blood Sampling and Analysis

Blood samples of all participants were drawn from antecubital vein between 8:00 and 10:00 a.m. after an overnight fasting. Blood samples were centrifuged at 4,000 rpm for 5 min and the serum was stored in -80 °C until analysis time. Serum levels of zonulin were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the protocols of the manufacturers (Elabscience, USA, cat. No: E-EL- H5560). Analysis results were expressed in ng/mL.

Statistical Analyses

SPSS v.21 was used for the analyses. Relationships between dichotomous variables were assessed with Pearson chi-square test. The distribution of numerical variables was examined by Shapiro-Wilk normality test. Differences of continuous variables between groups, were analyzed using Mann-Whitney U test. The correlation between serum zonulin levels and the psychometric test scores was evaluated by Spearman's correlation analyses. Descriptive statistics were given as frequency, percentage, and median, interquartile range and minimum-maximum. A p value of 0.05 was accepted as significance level.

RESULTS

The mean age was 115.28±15.47 and 120.38±15.49 months, for ADHD group and the control group, respectively. Majority of the subjects were male, 80% (n=32) in ADHD and 75% (n=30) in control groups. Because we matched ADHD and control groups according to gender and age, ADHD and control groups did not differ significantly according to gender (p=0.592) and age (p=0.124). Children in ADHD group had significantly higher scores in terms of Du Paul ADHD-RS (p<0.001) and lower scores in terms of RMET (p=0.007) compared to children in the control group. Zonulin levels were found to be significantly higher in ADHD group (p=0.010, Table 1).

Table 1. Comparison of clinical parameters in ADHD and control groups

	ADHD (n=40)	Control (n=40)	p
DuPaul ADHD-RS Inattentive score	16 (2) [13-19]	3 (2) [1-4]	**<0.001**
DuPaul ADHD-RS Hyperactivity score	13.5 (4) [12-18]	2 (2) [0-3]	**<0.001**
DuPaul ADHD-RS Total score	29 (6) [26-35]	5 (2) [2-7]	**<0.001**
RMET	15 (2) [13-18]	16 (2) [13-20]	**0.007**
Zonulin	97.62 (58.92) [1.71-340.71]	68.27 (56.41) [1.81-199.88]	**0.010**

ADHD: attention deficit hyperactivity disorder, DuPaul ADHD-RS: attention deficit/hyperactivity disorder-rating scale, RMET: reading the mind in the eyes test, descriptive statistics were given as median (interquartile range) [minimum-maximum]

In ADHD group, the correlations between zonulin levels and age, RMET and DuPaul ADHD-RS (inattentive, hyperactivity, total) scores were examined by Spearman's correlation analyses. Although there was no correlation between zonulin level and age (p=0.937), zonulin level was found to be significantly negatively correlated with RMET score (r=-0.498, p=0.001) and positively correlated with inattentive (r=0.558, p<0.001), hyperactivity (r=0.704, p<0.001), and total (r=0.697, p<0.001) scores of DuPaul ADHD-RS (Table 2).

Table 2. Correlations between clinical parameters and serum zonulin levels in ADHD group

	Zonulin		RMET	
	r	**p**	**r**	**p**
Zonulin			-0.498	**0.001**
RMET	-0.498	**0.001**		
DuPaul-IA	0.558	**<0.001**	-0.467	**0.002**
DuPaul-HA	0.704	**<0.001**	-0.643	**<0.001**
DuPaul-Total	0.697	**<0.001**	-0.611	**<0.001**

ADHD: attention deficit hyperactivity disorder, DuPaul ADHD-RS: attention deficit/hyperactivity disorder-rating scale, RMET: reading the mind in the eyes test, IA: inattentive, HA: hyperactivity

DISCUSSION

We evaluated serum level of zonulin in treatment-naive children with ADHD with respect to ADHD symptomatology and social cognition. We found increased serum zonulin levels in treatment-naive children with ADHD compared to healthy controls. Furthermore, we found positive correlations between serum zonulin levels and ADHD symptoms and negative correlations between zonulin levels and RMET scores.

In literature, there are two studies examining zonulin levels in ADHD (21,32). In one of those studies, conducted in children with ADHD, no significant difference was found between serum zonulin levels of ADHD and control groups (32). However, consistent with our findings, Özyurt et al. (21) reported that zonulin was associated with hyperactivity and social responsiveness in children with ADHD. Our results are also in the same line with the study reporting an association between social communication problems and serum zonulin levels in children with ASD (20). In detail, zonulin expression is considered to be regulated by systemic inflammation in addition to local enteric inflammation (33). Interestingly, biomarkers of subclinical inflammation, especially interleukin-6 (IL-6), have been proposed to regulate the gene expression of haptoglobulin-2 encoding zonulin protein (34,35). IL-6 was reported to increase in individuals with both ASD (36) and ADHD (37). Considering all the results of the studies reporting zonulin increase in ASD (20) and ADHD (21), reported association between serum zonulin levels and social responsiveness (21) and mentioned findings in biomarker level together, we suggest that coinciding inflammatory processes might explain our finding of the association between serum zonulin levels and social cognition deficits in ADHD.

On the other hand, Özyurt et al. (21) posited that the relationship between zonulin and social problems in children with ADHD might be moderated by oxytocin functioning. This hypothesis was developed by authors based on preclinical studies showing changes in intestinal permeability with oxytocin (38) which was found to be correlated with social communication skills in ASD (39), and a clinical study reporting alterations in oxytocin functioning in children with ADHD and social deficits (40). Given the fact that social cognition is the fundamental step for social communication, altered oxytocin functioning might also be another mechanism explaining our finding of the relationship between serum zonulin levels and social cognition deficits in ADHD.

Alterations in zonulin levels have also been reported in autoimmune diseases (41), obesity (42), insulin resistance (34), type 1 and 2 diabetes (43-45), central nervous system diseases, chronic inflammatory diseases, cancers (46,47) and psychiatric conditions such as anxiety, depression, acute stress provocation (48,49). Furthermore, zonulin was also reported to be related to gut microbiota and dietary factors (50-52). We did not examine gut microbiota or dietary habits, however we excluded patients with medical comorbidities such as chronic inflammatory, immunological, endocrinological, allergic diseases and psychiatric comorbidities other than ODD, matched the groups according to age, gender and BMI and included only treatment- naive patients. We intended to increase the reliability of our results with this method.

Including only treatment-naive patients, excluding comorbidities with the K-SADS-PL, matching the groups in terms of age, gender and BMI are the strengths of this study. However, there are some limitations of this study. Firstly, the sample is consisted of relatively small number of patients. Secondly, we did not examine other factors such as gut microbiota and dietary habits. To evaluate social cognition in our study, we utilized one of the most frequently used tasks in social cognition research, known as RMET. RMET is known as an improved mind reading test that measures mentalization skills (29). However, an important limitation of this study is the lack of different measures reflecting social cognition skills in ADHD.

CONCLUSION

In conclusion, the results of our study indicate that zonulin may be associated with ADHD and social cognition deficits in children with ADHD. Further comprehensive studies with larger samples are required to determine the role of zonulin in ADHD.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (04.03.2019, 38).

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Cranial Magnetic Resonance Imaging as a Screening Tool for Evaluation of Silent Brain Ischemia in Severe Coronary Artery Disease: A Clinical Based Study

Ciddi Koroner Arter Hastalığında Sessiz Beyin İskemisini Değerlendirmede Tarama Aracı Olarak Kranial Manyetik Rezonans Görüntüleme: Klinik Tabanlı Bir Çalışma

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ABSTRACT

Aim: Silent brain ischemia (SBI), defined as ischemic changes and infarcts without neurologic signs, is an established marker of poor survival. Magnetic resonance imaging (MRI) is useful to define SBI and white matter hyperintensities that correspond to microangipathic ischemic disease. This study aimed to investigate the relationship among SBI, white matter lesions and the extent of coronary artery disease (CAD), and to determine possible predictors of SBI.
Material and Methods: A total 10640 patients who underwent coronary angiography were retrospectively screened to reveal 312 patients who had been evaluated with a subsequent cranial MRI within 6 months. CAD severity was established with Gensini score and MRIs were evaluated to determine presence of SBI and white matter hyperintensities scored by Fazekas. Finally, 58 patients with SBI and 254 without SBI consisted SBI and non-SBI groups.
Results: Patients with SBI were significantly older with higher prevalence of male gender than the non-SBI patients. Both Gensini and Fazekas scores were higher in SBI-group ($p<0.001$). Fazekas score was positively correlated with Gensini score ($r=0.219$, $p<0.001$) and age ($r=0.465$, $p<0.001$). In the logistic regression analysis; age, male gender and Gensini score were identified as the independent predictors of SBI.
Conclusion: Although SBIs don't present neurological symptoms they are associated with poor survival and future stroke. Our data suggest that cranial MRI may be a screening tool in risk stratification, particularly in elderly male patients with multivessel CAD. Our study also depicted that age, male gender and high Gensini scores are the independent predictors of SBI.
Keywords: Brain ischemia; coronary angiography; coronary artery disease; magnetic resonance imaging; stroke.

ÖZ

Amaç: Nörolojik bulgu göstermeyen iskemik değişiklikler ve enfarktlar olarak tanımlanan sessiz beyin iskemisi (SBI), kötü sağ kalımın bilinen bir göstergesidir. Manyetik rezonans görüntüleme (MRG), SBI ve mikroanjiopatik iskemik hastalığa karşılık gelen beyaz madde hiperintensitelerini göstermede yararlı bir yöntemdir. Bu çalışmada SBI, beyaz madde lezyonları ile koroner arter hastalığı (KAH) arasındaki ilişkiyi araştırmak ve SBI'nın olası belirleyicilerini belirlemek amaçlanmıştır.
Gereç ve Yöntemler: Koroner anjiyografi yapılan toplam 10640 hasta retrospektif olarak taranarak 6 ay içerisinde müteakip olarak kranial MRG ile değerlendirilmiş 312 hasta belirlendi. KAH ciddiyeti Gensini skoru ile tespit edildi ve MRG'ler SBI varlığı ile Fazekas skoru ile ölçülen beyaz madde hiperintensiteleri açısından değerlendirildi. Bunun sonucunda SBI olan 58 ve SBI olmayan 254 hasta, SBI ve SBI olmayan hasta gruplarını oluşturdu.
Bulgular: SBI olan hastalar, SBI olmayan hastalardan anlamlı şekilde daha yaşlı ve daha yüksek prevalansta erkek cinsiyette idi. SBI grubunda hem Gensini hem de Fazekas skorları daha yüksekti ($p<0.001$). Fazekas skoru, Gensini skoru ($r=0,219$; $p<0,001$) ve yaş ($r=0,465$; $p<0,001$) ile pozitif korelasyonlu idi. Lojistik regresyon analizinde; yaş, erkek cinsiyet ve Gensini skoru, SBI'nın bağımsız belirteçleri olarak belirlendi.
Sonuç: SBI nörolojik semptom göstermese de, kötü sağ kalım ve ileride yaşanacak inme ile ilişkilidir. Bulgularımız, kranial MRG'nin özellikle çoklu damar KAH olan yaşlı erkeklerde risk değerlendirmesi için bir tarama aracı olabileceğini göstermektedir. Çalışmamız ayrıca yaş, erkek cinsiyet ve yüksek Gensini skorunun SBI'nın bağımsız belirteçleri olduğunu göstermiştir.
Anahtar kelimeler: Beyin iskemisi; koroner anjiyografi; koroner arter hastalığı; manyetik rezonans görüntüleme; inme.

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INTRODUCTION

Silent brain ischemia (SBI) refers to brain disorders that are incidentally found in imaging modalities but do not cause significant neurological deficits (1,2). It is one of the manifestations of silent cerebrovascular disease which is a common finding in brain scans and calls attention to future stroke or dementia. Among other manifestations of silent cerebrovascular disease in neuroimaging is white matter hyperintensities that correspond to microangiopathic ischemic disease. SBI, on the other hand, is described as "a cerebral infarction that is evident by brain imaging but that is without a clinical syndrome characterized by rapidly developing clinical symptoms and signs of focal and at times a global loss of brain function" (3). It is not a rare entity with an estimated prevalence of 10-20% (4).

Although SBIs do not cause clinical symptoms, they are known to be closely associated with impending stroke (5). In addition, while SBIs do not result in focal neurological deficits, they have been shown to be related with neurocognitive and neuropsychiatric impairment and increase mortality (6,7). Nevertheless, the pathophysiology of SBIs remains unclear and various causes, such as small vessel disease, disorders of blood brain barrier, embolism or hypo perfusion have been suggested. Associated predisposing factors such as carotid atherosclerosis, heart failure or coronary artery disease (CAD) could have been identified.

SBIs present as hyperintense lesions on T2-weighted Magnetic resonance imaging (MRI), hyperintense gliotic rims on the fluid-attenuated inversion recovery (FLAIR) sequence, and diffusion-restricted acute lesions on diffusion-weighted imaging (DWI) (8). Although some studies have used computed tomography (CT) in their analyses (9), MRI has been shown to be more specific and sensitive in its ability to distinguish SBIs from enlarged perivascular spaces (6).

While prior studies have assessed the relationship between CAD and symptomatic cerebrovascular disease, the relationship of SBI with presence and extent of coronary atherosclerosis scored by Gensini scoring or brain deep white matter microangiopatic disease graded using Fazekas classification has not been evaluated yet. As such, the current study aimed to evaluate the possible associations of silent cerebrovascular disease in the form of SBI and brain white matter microangiopathic lesions with severity and extent of CAD. It also sought to investigate the distribution of localizations of such lesions within brain as well as to define the predictors of SBI.

MATERIAL AND METHODS

This study was approved by ethics committee of Namık Kemal University Faculty of Medicine in 30/11/2017 with 2017/94/10/09 approval number. Following ethics approval, coronary angiographic examinations that had been performed at the cardiology department between November 2015 and November 2018 were retrospectively evaluated. Of 10640 coronary artery examinations found in the hospital database, patients who had undergone subsequent brain MRI for evaluation of neurological disorders such as headache, migraine (without neurological deficit), vertigo, tinnitus, memory difficulties, paresthesias and syncope performed within 6 months following coronary angiography were identified. Patients with any neurological deficit, patients under 40 years of age or those already with the diagnosis of cerebrovascular disease, neurodegenerative diseases, demyelinating diseases, neoplastic diseases and vasculitis were excluded. At the end of selection procedure, 335 patients who had undergone both coronary angiography for differential diagnosis of CAD and brain MRI were identified. Of 335 patients, 8 patients who were under 40, 13 patients with diagnosis of stroke, 1 patient with lacunar infarct and 1 patient with motion artifact at the imaging were excluded from the study.

Finally, this study examined 312 patients with lesions that were detected with MRI, and the study group was categorized into two groups with regard to the presence of SBI; patients with SBI and patients without SBI (non-SBI) groups (Figure 1).

Coronary Angiography Protocol

Coronary angiography was performed as described earlier. Coronary arteries were identified as left anterior descending (LAD), circumflex (Cx), and right coronary artery (RCA), and coronary artery lesions were evaluated quantitatively by Gensini score. CAD was also classified as 0-VD for no vessel involvement, 1-VD for the involvement of 1 vessel, 2-VD for the involvement of 2 vessels, and 3-VD for the involvement of 3 vessels, respectively. Gensini scoring was calculated by a cardiologist who was blinded to the study groups as described previously (10).

Magnetic Resonance Imaging Protocol

The cranial MRI examination was performed with the GE Healthcare ™ Optima ™ MR360 1.5T (Marlborough, USA). Diffusion Weigthed Imaging (DWI), T2 Weighted (T2W) images, and Fluid Attenuated Inversion Recovery (FLAIR) sequences were evaluated during the cranial MRI examination. The parameters for the T2W images were Time of Repetition (TR): 4561 msec, Time of Echo (TE): 101.9 msec, Field of View (FOV): 24 cm x 19.2 cm, Matrix: 352 x 224, Bandwidht (BW): 1.25 KHz, thickness: 5.8 mm, gap: 1.5 mm, and Number of Excitation (NEX): 2. The parameters for the FLAIR sequences were TI: 2070

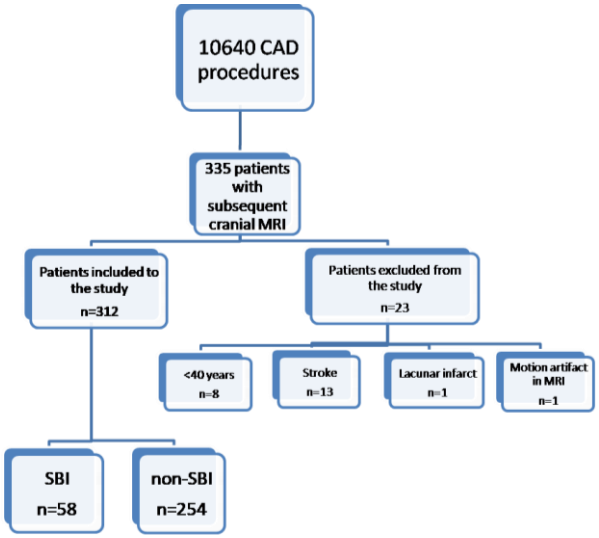

Figure 1. Flow chart of the study design. CAD: coronary artery disease, MRI: magnetic resonance imaging, SBI: silent brain ischemia

msec, TR: 8800 msec, TE: 86.3 msec, FOV: 24 cm x 24 cm, Matrix: 352 x 224, BW: 1.25 KHz , thickness: 5.8 mm, gap: 1.5 mm, and NEX: 2. Finally, the parameters for DWI were TR: 5200 msec, TE: 106.8 msec, BW: 1.25 KHz, FOV: 24 cm x 24 cm, Matrix: 128 x 128, and NEX: 2.

The cranial MRI examination was evaluated by an experienced neuroradiologist in a blind fashion to determine the presence of SBI and microangiopathic ischemic gliosis in the deep white matter. MRI examinations of all patients were performed during their initial evaluation. Upon the evaluation of microangiopathic ischemic gliosis, Fazekas scoring of lesions were used. In patients without any stroke symptoms, lesions larger than 3 mm in diameter were found to be compatible with SBI lesions, which are hyperintense on T2W images (Figure 2a) and suppressed with gliotic rim in the white matter on FLAIR sequences (Figure 2b). Lesions smaller than 3 mm in diameter were not identified as SBI lesions. As such, they were not included in the SBI group (11). Lesions larger than 3 mm in diameter but without peripheral gliotic rim were also excluded from the SBI group, receiving differential diagnoses as enlarged perivascular spaces or cystic lesions caused by brain parenchyma. In addition, the restricted diffusion of these lesions was evaluated with DWI (Figure 2c) and Apparent Diffusion Coefficient (ADC) map (Figure2d). Lesions with restricted diffusion were defined as acute lesions and were excluded from the study. Evidence of leukoaraiosis (microangiopatic ischemic gliosis) was noted during some of the cranial MRI examinations and staged according to the Fazekas scale. Lesions were evaluated according to the presence of periventricular and deep white matter lesions in T2W images and FLAIR sequences (12, Table 1).

Statistical Analysis

Statistical analyses were performed by SPSS v.17.0. The distribution of the data was determined using the Kolmogorov-Smirnov test. Normally distributed variables were presented as mean±standard deviation, non-normally distributed variables were presented as median, interquartile range, minimum-maximum, and categorical variables as number and percentage. The categorical variables were analyzed using the Chi-square test. The difference between numerical variables and SBI groups were assessed with Independent Samples t test or Mann-Whitney U test. Spearman rank correlation analysis was used to determine the relationship between Gensini score, Fazekas score and the age. Receiver operating characteristics (ROC) curve analysis was performed to find cut-off values of Gensini score for prediction of the presence of SBI lesions. Logistic regression analyses were performed to identify the predictors of SBI. By using enter method for logistic regression analysis, age, gender, Gensini score were added to the hypothesis to reveal the determinants that independently associate to SBI. A p value of <0.05 was accepted to be statistically significant.

RESULTS

This study provided assessments on the cranial MRIs of 312 patients. The demographic, angiographic and MRI characteristics of the study population are presented in Table 2. The mean age of the patients was 62.95±9.67 years (range, 41-94 years). Classification of the lesions as periventricular, deep white matter or basal ganglion lesions and the dimensions of lesions are also provided in Table 3. In the SBI group, the mean size of the SBI lesions evaluated by MRI was 6.23±2.52 mm and these lesions were mostly localized in the frontal lobes.

There was no significant difference between the SBI and non-SBI groups in terms of cardiovascular risk factors such as hypertension, diabetes mellitus, hyperlipidemia or smoking. Nevertheless, patients with SBI were significantly older than the non-SBI patients (mean age was 69.17±10.11 vs 61.54±9.00, p<0.001). It was also revealed that the male gender was more common in the SBI group (77.6% vs 54.3%, p=0.001).

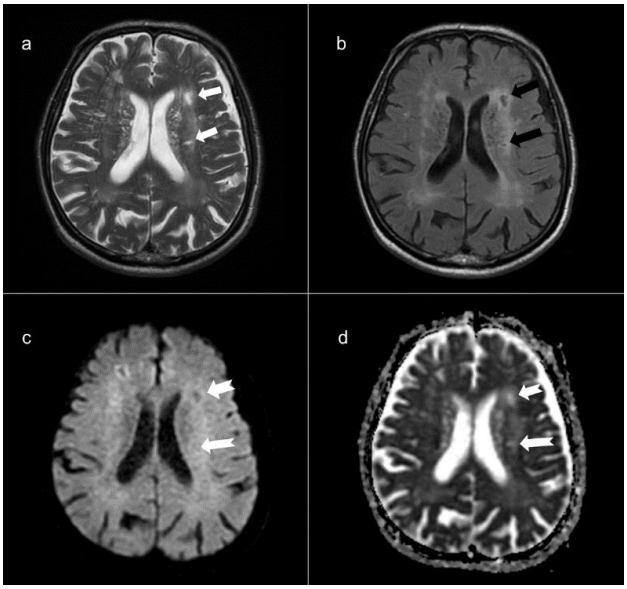

Figure 2. Axial T2W image (a) shows the hyperintense lesions with in the deep white matter (white arrows). T2W image also shows the enlarged perivascular spaces in the basal ganglia and Fazekas grade III lesions in white matter. On axial FLAIR image (b), these lesions suppressed and had gliotic rim (black arrows). Enlarged perivascular spaces have not periferic gliotic rim compared to the SBI. DWI (c) and ADC map (d) depicts that the lesions (white tailed arrows) do not show restriction of diffusion. ADC: apparent diffusion coefficient, DWI: diffusion weighted imaging, FLAIR: fluid attenuated inversion recovery, SBI: silent brain ischemia

Table 1. Fazekas classification of white matter hyperintensities on T2W and FLAIR images

Deep White Matter Lesions
0 Absent
1 Punctate foci
2 Beginning confluence
3 Large confluent areas
Periventricular White Matter Lesions
0 Absent
1 “caps” or pencil thin lining
2 Smooth “halo”
3 Irregular periventicular signal extending into the deep white matter

FLAIR: fluid attenuated inversion recovery

A significant relation was observed between the SBI and the number of VD (1 vs 0, p<0.001). While normal coronaries were more prevalent in non-SBI group (0 VD, p<0.001), 3 VD was more prevalent in SBI patients (p<0.001). The median Gensini scores of the SBI and non-SBI groups were 17 (0-128) and 5 (0-160), respectively. There was a statistically difference between the Gensini scores for the SBI and non-SBI groups, which were higher in the SBI group (p=0.001). The SBI group also showed a significant statistical relationship in terms of Fazekas score, with higher grade 2 and 3 Fazekas scores (p<0.001). Spearman correlation analysis demonstrated a weak positive correlation between age and both Fazekas and Gensini scores (r=0.465, p<0.001; r=0.213, p<0.001, respectively). A weak positive correlation was also observed between Gensini score and Fazekas score (r=0.219, p<0.001). With ROC analysis, the area under curve (AUC) for performance of Gensini score to diagnose SBI lesions was measured as 0.640 (95% CI= 0.560-0.719, p=0.001, Figure 3). Cut off value for Gensini score was 6.25. In the logistic regression analysis conducted to determine the predictors of SBI, age, male gender and Gensini score were identified as the independent predictors of SBI (p<0.001, Table 4).

Table 2. Comparison of SBI and non-SBI groups

	SBI (n=58)	non-SBI (n=254)	p
Demographic characteristics			
Age (years)	69.17±10.11	61.54±9.00	**<0.001**†
Male Gender, n (%)	45 (77.6)	138 (54.3)	**0.001**§
Hypertension, n (%)	41 (70.7)	189 (74.4)	0.561§
Diabetes mellitus, n (%)	30 (51.7)	126 (49.6)	0.771§
Hyperlipidemia, n (%)	32 (55.2)	138 (54.3)	0.908§
Smoking, n (%)	17 (29.3)	75 (29.5)	0.974§
Angiographic characteristics			
Gensini Score	17 (57) [0-128]	5 (26) [0-160]	**0.001**#
Number of vessels	1 (2) [0-3]	0 (1) [0-3]	**<0.001**#
Number of vessels, n (%)			
0	18 (31.0)	141 (55.5)*	**<0.001**§
1	18 (31.0)	52 (20.5)	
2	8 (13.8)	41 (16.1)	
3	14 (24.1)*	20 (7.9)	
MRI characteristics			
Fazekas Score	2 (1) [1-3]	1 (1) [0-3]	**<0.001**#
Fazekas Score, n (%)			
0	0 (0.0)	84 (33.1)*	**<0.001**§
1	11 (19.0)	134 (52.8)*	
2	22 (37.9)*	28 (11.0)	
3	25 (43.1)*	8 (3.1)	

SBI: silent brain ischemia, MRI: magnetic resonance imaging, †: Student t test, #: Mann-Whitney U test, §: Chi-square test, *: statistical difference in subgroups, values are presented as n (%) for categorical variables, and mean±standard deviation or median (interquartile range) [minimum-maximum] for numerical variables

Table 4. Possible predictors of SBI

Predictor	OR	95% CI	p
Age	0.922	0.88 - 0.95	**<0.001**
Male gender	0.436	0.21 - 0.89	**0.023**
Gensini score	0.989	0.98 - 1.00	**0.014**

SBI: silent brain ischemia, OR: odds ratio, CI: confidence interval, data in the table shows the logistic regression analysis in which possible predictors are presented

DISCUSSION

In this study we examined the relationship between atherosclerotic coronary artery involvement and asymptomatic cerebrovascular processes, namely SBI and ischemic microangiopathic changes in white matter. It was demonstrated that extend of CAD expressed by Gensini score was associated both with SBI and microangiopathic involvement in brain expressed by Fazekas scores. It was also revealed that coronary atherosclerosis was closely associated with SBI that was located mainly in frontal area. In clinical terms, clinicians need to take into account that the brain parenchyma has higher risks for development of silent white matter vascular injury and SBI in CAD patients with high Gensini scores.

This study clearly depicted the clinical importance of detecting the SBI lesions with cranial MRI in the multivessel CAD even if they have no symptoms of stroke. The association between brain parenchyma and ischemic diseases in the coronary arteries is indisputable. In patients with both symptomatic cardiac disease and CAD, the brain parenchyma is at risk of embolic or occlusive processes. CAD is an important predictive factor of SBI and stroke. Like in the current study, existing research has determined that with age, CAD is an important risk factor in the

Table 3. Localizations and incidence of SBI lesions in MRI

Localization	n (%)	Dimension (mm)
Deep White Matter		
Frontal	23 (39.7)	6.24±2.01
Parietal	6 (10.3)	4.36±1.07
Occipital	3 (5.2)	6.86±2.83
Periventricular		
Frontal	15 (25.9)	6.82±3.02
Occipital	5 (8.6)	6.80±4.57
Basal Ganglia		
Putamen	3 (5.2)	5.40±1.32
External capsule	2 (3.4)	3.36±2.88
Internal capsule	1 (1.7)	6.80

SBI: silent brain ischemia, MRI: magnetic resonance imaging, values are presented as n (%) for categorical variables, and mean±standard deviation for numerical variables

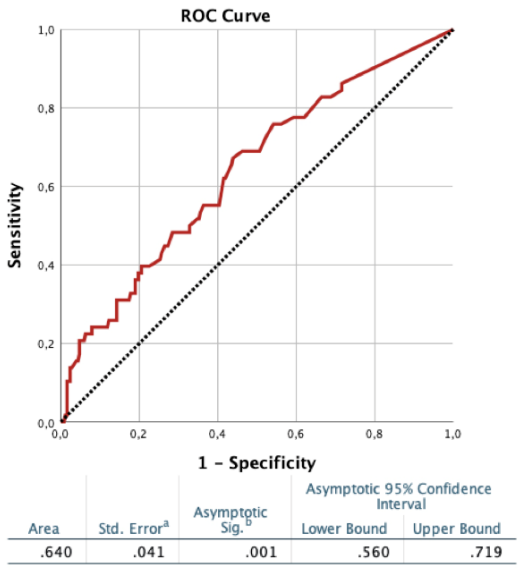

Figure 3. ROC analysis shows the diagnostic performance of Gensini score for predicting SBI lesions. ROC: receiver operating characteristic, SBI: silent brain ischemia

formation of SBI in the brain (13,14). Coronary atherosclerotic calcifications are also known to be an independent variable in the etiopathogenesis of stroke (15). It was also shown that there is a close relationship between impaired glucose intolerance and CAD (16).

In the literature epidemiological studies have shown that the prevalence of SBI is approximately 10-20% and that it has an annual incidence of approximately 3% (17). No study has determined the exact reason explaining why the SBIs develop or why they remain. It is also an argument whether they are silent or not, some of the authors have suggested that the disorder's silence may be due to its chronic impact or appearance in non-strategic areas (18). While evaluating the localization of lesions, it was shown here in that the deep white matter was the most affected area in the brain. The most frequent localizations of SBI lesions were presented in the deep white matter and periventricular white matter of frontal lobes. This supports the reported findings that deep white matter and frontal lobes are more prone to develop SBI lesions. It also might be explained by the locations of lesions why the neurocognitive and neurophysciatric symptoms are more frequent in patients with SBI lesions (19).

In addition to the studies in the literature, the current study revealed the obvious relationship between the SBI lesions and high grade Fazekas white matter lesions. Previous studies have defined leukoaraiosis (or microangiopathic ischemic gliosis) as incomplete ischemia that is caused by small vessel stenosis and led to microangiopathic ischemic disease. This is thought to be due to age-related chronic arteriolosclerosis resulting from long-term hypertension and ischemia caused by arterial stenosis (20,21). The relationship between microvascular disease in brain and deep white matter lesions is also expressed in the Fazekas classification. As an example, the present study demonstrated the increased risk of SBI in deep white matter lesions, especially in those that received ratings of 2 or 3 on the Fazekas scale. This suggests that microvascular disease of brain tend to coexist with SBI lesions. Thus, in their studies Zhang et al. (22) showed that white matter lesions in the brain with high grade Fazekas scale is an independent factor for SBI lesions. As such, intracranial arteriolosclerosis, which can be defined as small vascular disease, can indirectly reveal the presence of CAD (23).

The current study also indicated that CAD should be considered to be a strong predictor of SBI, especially in patients with 3-VD or with high Gensini scores. In addition, Hermann et al.'s (15) work determined that CAD was an independent variable for stroke. Lee et al. (24) also showed that hypertension is an independent predictor of SBI. As in the current study, Hoshide et al. (25) also noted that the likelihood of SBI in CAD was significantly higher in patients with 3-VD. However, unlike Hoshide et al.'s work, the present study also used Gensini scoring to assess CAD. Gensini scoring is a method that demonstrates CAD, stenosis occlusion, and the burden of CAD. By applying this method, the current study revealed a significant statistical relationship between SBI and Gensini scoring. As a result, it would be accurate to suggest that high Gensini score can be predictive of SBI lesions in asymptomatic patients.

MRI is the essential modality for diagnosis of the SBI's. FLAIR is the main sequence of the MRI which can differentiate SBI from the enlarged perivascular spaces which constitute the primary differential diagnosis. SBI's have peripheral hyperintense gliotic rim conversely to perivascular spaces on the FLAIR images. Although SBIs do not have any predictable anatomic localization, enlarged perivascular spaces have well defined anatomic locations and these lesions can be divided into three main types due to their localizations (26).

The current study determined that age, male gender and Gensini score were independent predictors of SBI. Existing literature has also shown that the development of SBI is powerfully linked to age (13,17). In accordance with previous studies, the current study determined a strong association between SBIs and age. As with a previous work (27), the present study revealed a statistically higher incidence of high-risk cases in males. In their meta-analysis, Fanning et al. (4) identified several strong predictors of SBI, such as age, hypertension, carotid artery disease, and metabolic syndrome. While this study found CAD to be a likely predictor of SBI, it identified gender, smoking, and alcohol as poor predictors of SBI.

The current study found no statistical significant differences in hypertension between the SBI and the non-SBI groups. While it is clear that there is a significant statistical difference in hypertension between SBI patients when compared to the normal population, the current study group consisted of patients with worse cardiovascular risk profile. For this possible reason, there was no statistically significant difference in hypertension between the present study's SBI group and normal non-SBI groups. Retrospective study design was the main limitation of the study.

CONCLUSION

In conclusion, although SBI lesions are asymptomatic, they potentially may led to brain deterioration and future loss in neurological function. For these reasons, diagnosis is vital. In patients with CAD, it is necessary to evaluate the presence of SBI with cranial MRI examinations, especially in elderly male patients with multivessel (3-VD) involvement and high Gensini scores, regardless of presence of any stroke symptoms.

Ethics Committee Approval: The study was approved by the Ethics Committee of Namık Kemal University Faculty of Medicine (30.11.2017, 2017/94/10/09).

Conflict of Interest: None declared by the authors.

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Comparison of Single Incision Laparoscopic and Classic Stamm Gastrostomy Methods in Children

Çocuklarda Tek Kesi Laparoskopik ve Açık Yaklaşım Gastrostomi Yöntemlerinin Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to compare single incision laparoscopic gastrostomy (SILG) with open surgery in children.
Material and Methods: Children who underwent laparoscopic and open gastrostomy surgeries between January 2016 and March 2020 were retrospectively evaluated. The data were arranged according to the patient's demographics, duration of surgery and anesthesia, time to start enteral feeding, and complications. For Stamm procedure, the abdomen was explored with an upper midline incision and a gastric tube was passed through a second incision. In the SILG method, only one incision was made. After inserting a camera was placed into a trocar, then a grasper was placed just next to the trocar. A part of stomach was removed through the incision. A foley catheter was placed visually into the lumen.
Results: There was no statistically significant difference between operation times for both surgical groups (p=0.844); the same was true for anesthesia times (p=0.919). The time taken to initiate and reach full enteral nutrition differed significantly between two groups (p=0.005). In general, when all complications were evaluated, more complications were found in the open surgery group (p=0.077). A remarkable number of maintenance problems (65.6%) were observed in both groups.
Conclusion: SILG is a technique that is not technically demanding and can be performed by any pediatric surgeon with experience in laparoscopy. Primary laparoscopic gastrostomy at the trocar insertion site can be performed easily and safely in all children with malnutrition due to low postoperative complication rates and applicable for all ages of children.
Keywords: Single incision laparoscopic gastrostomy; Stamm gastrostomy; children.

ÖZ

Amaç: Bu çalışmanın amacı çocuklarda tek insizyonlu laparoskopik gastrostomi (single incision laparoscopic gastrostomy, SILG) ile açık cerrahinin karşılaştırılmasıdır.
Gereç ve Yöntemler: Ocak 2016 ve Mart 2020 tarihleri arasında laparoskopik ve açık gastrostomi ameliyatı geçiren çocuklar geriye dönük olarak değerlendirildi. Veriler hastanın demografik özelliklerine, ameliyat ve anestezi süresine, enteral beslenmeye başlama zamanına ve komplikasyonlara göre düzenlendi. Stamm prosedürü için, üst orta hat kesiği ile karın eksplore edildi ve ikinci bir kesiden mide tüpü geçirildi. SILG yönteminde sadece bir kesi yapıldı. Bir trokar içine bir kamera yerleştirildikten sonra, trokarın hemen yanına bir yakalayıcı yerleştirildi. Kesiden midenin bir kısmı çıkarıldı. Lümen içerisine görsel olarak bir foley kateter yerleştirildi.
Bulgular: Her iki cerrahi grup için ameliyat süreleri arasında istatistiksel olarak anlamlı fark yoktu (p=0,844); aynı durum anestezi süreleri için de geçerliydi (p=0,919). Tam enteral beslenmeye başlama ve ulaşma süresi iki grup arasında önemli ölçüde farklılık gösterdi (p=0,005). Genel olarak tüm komplikasyonlar değerlendirildiğinde açık cerrahi grubunda daha fazla komplikasyon saptandı (p=0,077) Her iki grupta da dikkat çekici sayıda (%65,6) idame problemi görüldü.
Sonuç: SILG, teknik olarak zorlayıcı olmayan ve laparoskopi deneyimi olan herhangi bir çocuk cerrahı tarafından uygulanabilen bir tekniktir. Trokar giriş yerinde primer laparoskopik gastrostomi, düşük postoperatif komplikasyon oranları nedeniyle malnütrisyonlu tüm çocuklarda kolay ve güvenli bir şekilde yapılabilir ve her yaştaki çocuklara uygulanabilir.
Anahtar kelimeler: Tek kesi laparoskopik gastrostomi; Stamm gastrostomi; çocuk.

INTRODUCTION

If adequate nutritional intake within 2-3 weeks is not sufficient in children whose energy and nutritional needs cannot be fully met with only oral nutrition, enteral feeding via a gastrostomy tube (GT) should be considered in children (1). Nowadays, gastrostomy methods are one of the most commonly performed surgical processes in the pediatric patients, and the diseases associated with gastrostomy indications are extensive with the inclusion of neurological, metabolic, cardiopulmonary and urology disorders (2). Since it was first described in 1894, the main approach to gastrostomy has been open gastrostomy, usually using the Stamm technique (3). Percutaneous endoscopic gastrostomy (PEG) tube placement, which was introduced for the first time in 1980 and is a less invasive technique, is still frequently performed in pediatric and adult patients (4). On the other hand, the reporting of serious complications associated with the PEG technique in children led to new searches in pediatric patients and laparoscopic gastrostomy (LG) application gained popularity in the 1990s with the increasing usage of laparoscopy and other video-assisted techniques (5). Since the first time it was applied, LG has undergone different modifications in order to reduce complication rates and facilitate the operation technique (6). Minimally invasive surgery (MIS) has developed rapidly in recent years and has become the gold standard for the correction of many surgical pathologies. Less size of scars, minimum pain (preferentially in a small location), shorter healing time and the desire to seek perfection for an earlier retrieval of daily life quality have led to laparoscopic procedures being performed through a single little incision (7). Various methods such as classical PEG application, fluoroscopy-guided gastrostomy opening, and laparoscopic assisted PEG application are used today (8). In our research, we aimed to compare single incision laparoscopic gastrostomy (SILG) with open surgery in children.

MATERIAL AND METHODS

With the approval of the institutional review board numbered 2020/208, babies and children who had laparoscopic and open surgical gastrostomy surgeries performed in Düzce University Pediatric Surgery Clinic between January 2016 and March 2020 were retrospectively evaluated. The data were arranged according to the patient's age, gender, weight, duration of surgery, duration of anesthesia, time to start enteral feeding, and complications. Complications were considered as intraoperative, major postoperative, and tube care problems. Patients who underwent Nissen fundoplication during the first tube insertion were excluded from the study, and only plain gastrostomy cases were included in the study. Patients who initially had sufficient GT alone but later needed fundoplication were also included in the analysis. Within these criteria, a total of 32 GT placements were evaluated in the analysis. For a long time, an informed consent form, including information that the patient's data can be used, has been routinely obtained from the parents of the patients in all operations.

Operative Technique

For the open surgery of Stamm procedure, the abdomen was explored with a midline incision above the umbilicus and then the stomach was identified. A second much smaller cut was performed 2 cm under left costal angle at the anterior abdominal wall, and a gastric tube was passed through it. Gastrostomy was performed by placing a foley catheter in the lumen of the stomach at a suitable place on the anterior surface of the stomach, and closed with a 3-0 polyglactin purse string suture.

In the SILG method, only the second small incision in the classical gastrostomy method was used to reach the peritoneum. The incision was lowered into the peritoneum, opening directly under visualization. After inserting a 5 mm trocar, abdominal pressures were inflated with carbon dioxide to 6-12 mm Hg according to the patient's dimensions and comorbidities. With 5 mm 30 degree camera placed through the port, laparoscopic confirmation of the stomach position and the location of the gastrostomy, a 3-mm grasper was placed just next to the trocar (Figure 1A). A veinless part of greater curvature of stomach was retained (Figure 1B) and, after the trocar pulled out, removed through the incision (Figure 1C). The part of the stomach that was taken out was fixed to the fascia margins of the incision with polyglactin sutures. After the gastrostomy had been opened with cautery, a foley catheter was placed visually into the lumen and the stomach was closed with cerclage suture (Figure 1D). The proper position of the GT was confirmed by intraoperative administration of methylene blue to the stomach and the visualization of the dye from the nasogastric tube.

Statistical Analysis

Shapiro-Wilk test was used to examine normality assumption, and Independent samples t test was used to analyze numerical variables with normal distribution while Mann-Whitney U test was used for numerical variables not showing normal distribution. Categorical variables were analyzed with Pearson chi-square or Fisher's exact test. Descriptive statistics were given as mean±standard deviation or median, interquartile range, minimum, maximum, and categorical variables were summarized with frequency and percentage. Statistical analyses were done by SPSS v.22 statistical package and 0.05 level was considered as statistical significance level.

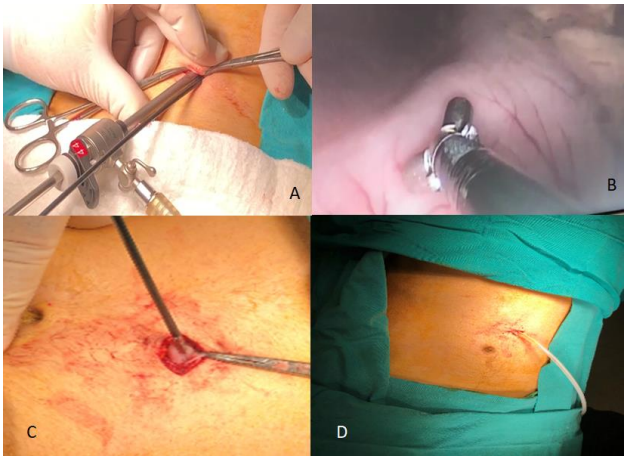

Figure 1. A) A grasper inserted from the side of the trocar in the same incision, B) Easy handling of the stomach from the appropriate non-vascular area with the grasper and, C) after the trocar pulled out, removed through the incision, D) Final state of gastrostomy

RESULTS

Study Group

A total of 32 GTs were placed between January 2016 and March 2020 using the open surgical approach or the SILG placement method. Of these, 17 were open Stamm gastrostomy (53.1%) and 15 were SILG (46.9%). The mean age for all patients was 6.4±5.5 years and the mean weight was 18.3±14.8 kg. The age (p=0.018) and weight (p=0.044) of the open Stamm gastrostomy patients were found to be higher than the SILG technique. The demographic properties of cases are summarized in Table 1.

Gastrostomy indications were mostly related to growth retardation (n=26, 81.3%) and nutritional difficulties (n=22, 68.8%) associated with neurological deficits. Other indications include mental disturbances due to traffic accidents and malignancies. No meaningful differences were found between the surgery groups in terms of procedure indication, gender, and the ratio of patients with neurological disorders or related diagnoses.

Surgical Outcome

There was no statistically significant difference between operation times for both surgical groups (p=0.844); the same was true for anesthesia times (p=0.919). It took an average of 53.87±3.52 hours for the patients in the SILG group, and 59.59±6.72 hours for the patients in the open surgery group to reach full enteral nutrition. The time taken to initiate and reach full enteral nutrition differed significantly between these two groups (p=0.005, Table 2). The launch time of enteral feeding was also different between the groups in favor of SILG (p=0.002, Table 2).

The GT of a total of 32 patients was replaced with a "low profile" button gastrostomy, and the time elapsed between the operation date and the conversion date did not differ for the two groups. The mean GT replacement time to a button gastrostomy for both groups was 86.4±6.9 days. In almost all children, this change was performed in the outpatient clinic with only topical anesthesia or no pain relief. About 76% of the patients were examined in the follow-up and mean follow-up time was 24.13±9.68 (range, 10-38) months for SILG group, and 37.94±16.39 (range, 1-60) months for open surgery group.

Complications

There was no postoperative death due to the operations in either groups. In general, when all complications were evaluated, more complications were found in the open surgery group (p=0.077, Table 3). Complications were divided into two as intraoperative complications and postoperative complications according to the literature (9). Intraoperative complications were not seen in both the SILG group and the open surgery group. Postoperative complications were also divided into care problems and other complications leading to returning to the operating room. Postoperative complications include intraperitoneal leakage, bleeding, gastric detachment, early separation of the tube, exit from the tube canal, pyloric occlusion and gastrocolic fistula (10). There were exactly 16 postoperative complications were encountered among all gastrostomy patients, 5 (33.3%) of which were in the SILG group and 11 (64.7%) in the other group (p=0.077). The most important complications in the open surgery group: leakage and pneumoperitoneum were detected in one patient and re-operated in the early period due to gastric separation. In another patient in the same group, the GT was dislodged in the canal, which could not be replaced and required surgery for repair. Just one tube associated with the SILG procedure had this complication and was changed without surgery.

A remarkable figure of maintenance issues (n=21, 65.6%) was observed in both surgical groups such as tube leakages, impairment in the function of the tube, local infections, skin granulomas, and late dislocations. When operative techniques were compared, there was no difference between the groups in terms of the frequency of these care issues (Table 3). All such complications and care problems were treated with nonsurgical procedures and conservative maintenance.

DISCUSSION

Nasogastric (NG) tube can be used in a short period in children who cannot be fed orally. Although the NG catheter provides complete enteral nutrition for nutritional purposes, it also has some disadvantages. These disadvantages may arise due to complications experienced during the NG probe insertion phase or long-term use. Complications such as pharyngeal discomfort, nasogastric syndrome, sinusitis, nasotracheal intubation, esophagitis, gastritis, and gastric bleeding may be encountered during the NG catheter insertion phase and in the short period after insertion. More serious complications in children fed

Table 1. Comparison of the demographic properties

	SILG (n=15)	Open Surgery (n=17)	p
Gender, n (%)			
Male	11 (73.3)	9 (52.9)	0.234
Female	4 (26.7)	8 (47.1)	
Age (month)	12 (104) [3-180]	108 (120) [6-180]	**0.018**
Weight (kg)	9.5 (16.1) [3.5-66]	21 (16.8) [5.3-45]	**0.044**

SILG: single incision laparoscopic gastrostomy, descriptive statistics were given as median (interquartile range) [minimum-maximum]

Table 2. Comparison of surgical outcomes

	SILG (n=15)	Open Surgery (n=17)	p
Operation time (min)	32.60±8.23	33.12±6.56	0.844
Anesthesia time (min)	47.00±8.43	47.29±7.88	0.919
Starting time of Enteral Nutrition (h)	21.33±2.09	29.06±8.25	**0.002**
Starting time of Total Enteral Nutrition (h)	53.87±3.52	59.59±6.72	**0.005**

SILG: single incision laparoscopic gastrostomy, descriptive statistics were given as mean±standard deviation

Table 3. Comparison of complications, n (%)

	SILG (n=15)	Open Surgery (n=17)	p
Intraoperative complications	-	-	-
Postoperative complications	5 (33.3)	11 (64.7)	0.077
Return to operating room	0 (0.0)	2 (11.8)	0.486
Maintenance issues	10 (66.6)	11 (64.7)	0.907

SILG: single incision laparoscopic gastrostomy

with NG catheter for a long time; erosion of the nasal wings, knotting of the catheter in the esophagus or stomach, GER, pneumothorax, tracheoesophageal fistula may develop (11). For these reasons, gastrostomy techniques have been developed and started to be used as the gold standard in patients with chronic diseases where NG cannot be used for a long time. Open Stamm gastrostomy was the most widely used method until the invention of the PEG technique which has been found after long years usage of open surgery. However, with the reporting of major problems associated with the PEG procedure in children, it has increased anxiety about the reliability and usefulness of this approach in the pediatric population. Because it requires the PEG tube to be blindly inserted from the peritoneal cavity into the stomach, creating a harmful potential to the surrounding viscera. After the discovery of laparoscopy and with the improvement of smaller devices, a large variety of surgical disorders in children, including gastrostomy, have been corrected with this surgical technique (12).

In the last 20 years, studies including laparoscopy-assisted gastrostomy experiences have been published. After these methods involving 2-3 ports, as technology and innovation continue to advance the field of MIS, single incision laparoscopic surgery (SILS) for a variety of general, bariatric, urological and pediatric surgical procedures is gaining popularity as a method to achieve a less "scar-free" abdomen. Compared to the traditional laparoscopic procedure, the single-site technique can be considered less invasive as it uses only one incision instead of 2 or 3. A number of reports have been published in the literature regarding the feasibility of SILS in various techniques in adults. However, there are very few studies on the usage of this new technique in children (13).

Before the procedure was decided, abdominal USG was absolutely performed in patients, and if anomalies such as hepatomegaly or splenomegaly were present, open surgery was preferred to avoid laparoscopy complications. Open surgery was also preferred in patients who had previously undergone intra-abdominal surgery or in the presence of conditions that prevent gastric mobilization, such as previous TEF operation, and in patients with lung problems that limit laparoscopy. In such patients with suspected adhesion, single port surgery is risky and 2, 3 or even 4 ports may be required (14), which prolongs the operation time and decreases the feasibility compared to open surgery. That's why we preferred the open approach in such cases.

Previously published studies comparing laparoscopic and open GT implantation but involving mostly adult patients could not show a statistically important difference in the rate of postoperative complications or tube revising (15). In our study, we did not encounter intraoperative complications in either group. However, the rate of postoperative complications was surprisingly higher in the open surgery group (33.3% and 64.7%, respectively). The two patients who were re-operated were in the open surgery group. On the other hand, this has been attributed to other patient-related situations rather than the methods employed. The patient, who was re-operated due to gastric detachment, had severe scoliosis. In the other patient whose catheter could not be inserted under polyclinic conditions, her parents applied to the hospital too late and the tract was closed. In any case, the laparoscopic procedure may have prevented such complications as it provides better visualization of the whole intraabdominal space and reduces the risk of wound dehiscence.

One more advantage of working under direct visualization is the opportunity to select a sensitive site for the appropriate gastric hole for tensionless connection to the abdominal wall. Thus, one of the major advantages of the SILG technique is the avoidance of critical complications such as unwanted injury to abdominal organs, like the insertion of the gastrostomy into the colon or liver and unforeseeable hemorrhages. So, the technique can replace other gastrostomy techniques, especially in young babies and toddlers. Compared to PEG, the simplicity and practicability of this single-site laparoscopic technique could potentially become the first choice technic for GT installment in young children. In our patient series, the SILG method was applied to patients between 3 months and 15 years old patients, there is no difficulty in choosing the age, and it can be easily applied to children of all ages. When compared in terms of tube maintenance problems, there was no difference between the two surgical groups. Similar problems were encountered in both groups and similar maintenance intervals were required. However, we experienced that tube changes can be done more easily in the SILG group. We think that this is because the stomach is fixed to the fascia in the SILG method. Likewise, the inability to reattach in case of involuntary tube dislocation was not observed in the SILG group, while one patient had to be re-operated because of this reason in the open surgery group.

On the other hand, the SILG technique is not applicable to every child and the risks associated with each surgical procedure vary depending on the characteristics of the patients and accompanying health situations. For instance, the pressure of the gas given during laparoscopy can be dangerous in any children with lung diseases. Furthermore, records of previous intraabdominal surgery especially the upper region could challenge the surgeon during laparoscopic surgery (16).

There were no statistically significant differences between the two surgical groups according to operation times and anesthesia durations. In the comparisons in the literature, generally laparoscopic methods were found to be longer in both times. We believe that the use of a single port in the SILG method, unlike other laparoscopic methods, has an effect on finding these times shorter. In addition, there was no difference between the open surgery group and the SILG group in terms of feasibility due to the use of a single trocar, single telescope and single grasper during the procedure, all of which are reusable.

Pain-related discomfort has been the subject of discussion when discussing laparoscopic techniques with a single incision. The hypothesis is that the larger the facial incision, the more pain the patient will experience. However, when we scan the patient files regarding the use of painkillers, we found no significant distinction between the two gastrostomy groups in terms of narcotic drugs or anti-inflammatory medication.

CONCLUSION

SILG is a technique that is not technically demanding and can be performed by any pediatric surgeon with experience

in laparoscopy. Primary LG at the trocar insertion site can be performed easily and safely in all children with malnutrition due to low postoperative complication rates and applicable for all ages of children.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (21.09.2020, 208).

Conflict of Interest: None declared by the authors.

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The Post-Transplant Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Relation to Graft Function among Renal Transplant Recipients

Renal Transplant Alıcılarında Transplant Sonrası Nötrofil-Lenfosit Oranı ve Trombosit-Lenfosit Oranı ile Graft Fonksiyonu Arasındaki İlişki

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ABSTRACT

Aim: The aim of this study was to evaluate the association of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) with acute rejection after kidney transplantation.

Material and Methods: A total of 36 adult renal transplant recipients (33 males, 3 females) with a median age of 41 (range, 19-64) years were included in this retrospective study conducted between January 2016 and January 2019. Data on patient demographics and laboratory findings (neutrophil, lymphocyte, platelet, creatinine, eGFR, serum uric acid and C-reactive protein) were recorded. Acute rejection was defined via renal biopsy in accordance with Banff criteria.

Results: Acute rejection occurred in 16 (44.4%) patients. NLR (median 3.75 vs. 1.99, p=0.001) and PLR (median 125.59 vs. 99.23, p=0.008) values were significantly higher in the acute rejection group than in the control group. Area under the curve was calculated to be 0.822 for NLR and to be 0.759 for PLR. Cut-off values were determined to be >2.5 (75% sensitivity and 75% specificity) for NLR and to be >108 (81% sensitivity and 65% specificity) for PLR. Univariate analysis revealed a strong correlation of acute rejection both with NLR >2.5 (Odds Ratio (OR)=0.267, 95% Confidence Interval (CI)=0.089-0.803, p=0.019) and PLR >108 (OR=0.231, 95% CI=0.066-0.810, p=0.022).

Conclusion: In kidney transplant patients, there is a strong relationship between high NLR and PLR values and the development of acute rejection. As simple, easy-to-access, inexpensive and non-invasive methods, PLR, and particularly NLR, may be potential tests to diagnose post-transplant acute rejection.

Keywords: Kidney transplantation; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; acute rejection.

ÖZ

Amaç: Bu çalışmanın amacı nötrofil-lenfosit oranı (NLO) ve trombosit-lenfosit oranı (TLO) ile böbrek nakli sonrası akut rejeksiyon arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntemler: Ocak 2016 ile Ocak 2019 tarihleri arasında yürütülen bu geriye dönük çalışmaya ortanca yaşı 41 (aralık, 19-64) yıl olan toplam 36 yetişkin (33 erkek, 3 kadın) böbrek nakli alıcısı dahil edildi. Hastaların demografik özellikleri ve laboratuvar bulguları (nötrofil, lenfosit, trombosit, kreatinin, eGFR, serum ürik asit, C-reaktif protein) kaydedildi. Akut rejeksiyon Banff kriterlerine göre renal biyopsi ile tanımlandı.

Bulgular: On altı (%44,4) hastada akut rejeksiyon gelişti. NLO (ortanca 3,75'e karşı 1,99; p=0,001) ve TLO (ortanca 125,59'a karşı 99,23; p=0,008) değerleri akut rejeksiyon grubunda kontrol grubuna göre anlamlı olarak daha yüksekti. Eğri altındaki alan, NLO için 0,822 ve TLO için 0,759 olarak hesaplandı. Cut-off değer NLO için >2,5 (%75 duyarlılık ve %75 özgüllük) ve TLO için >108 (%81 duyarlılık ve %65 özgüllük) olarak belirlendi. Tek değişkenli analiz, NLO >2,5 (Odds Ratio (OR)=0,267; %95 Güven Aralığı (GA)=0,089-0,803; p=0,019) ve TLO >108 (OR=0,231; %95 GA=0,066-0,810; p=0,022) ile akut rejeksiyon arasında güçlü bir korelasyon olduğunu ortaya koydu.

Sonuç: Böbrek nakli hastalarında, yüksek NLO ve TLO değerleri ile akut rejeksiyon gelişimi arasında güçlü bir ilişki vardır. Basit, erişimi kolay, ucuz ve invaziv olmayan yöntemler olarak TLO ve özellikle NLO, transplant sonrası akut rejeksiyonu teşhis etmek için potansiyel testler olabilirler.

Anahtar kelimeler: Böbrek nakli; nötrofil-lenfosit oranı; trombosit-lenfosit oranı; akut rejeksiyon.

INTRODUCTION

Although kidney transplantation is the most effective treatment option in the management of end-stage renal disease, the graft function loss and acute rejection (AR) are the major complications in the post-transplant period (1). Donor type, tissue adaptation, age, gender, primary diagnosis, delayed graft function, infections and vascular complications are amongst the several factors considered to be related to AR. Despite the improved success of immunosuppressive therapy in recent years, graft loss becomes inevitable in some patients (2,3).

While the needle biopsy is considered the standard approach in diagnosis of acute allograft rejection, it is an invasive method with potential complications such as hemorrhage, infection and graft loss.

Non-invasive methods in diagnosing AR have become increasingly used such as serum and urine biomarkers along with cytometric and PCR analyses. However, due to limitations of the currently available methods in terms of sensitivity, specificity, predictive value, accessibility and cost issues, the search for ideal test and methods continues (2). The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are considered strong predictors of inflammation and to be associated with worse prognosis in a variety of conditions that include chronic kidney disease (3), cancer (4), coronary artery disease (5), rheumatic diseases (6), heart transplantation (7) and kidney transplantation (8-10). Given the inflammatory nature of the rejection process, we have hypothesized that NLR and PLR may alter during the rejection process and in this way; they may serve as easily accessed, inexpensive and non-invasive methods of detecting AR.

Therefore, the objective of this study was to investigate post-transplant NLR and PLR in relation to acute graft rejection in renal transplants recipients.

MATERIAL AND METHODS

This retrospective single-center study enrolled 54 consecutive patients over 18 years of age who underwent kidney transplantation between January 2016 and January 2019 and were followed up in the transplantation clinic. Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Ufuk University Faculty of Medicine Ethics Committee (25.02.2020, 4). Data on patient demographics, clinical and laboratory findings and follow-up records were retrieved from the hospital database. Patients receiving ABO-compatible kidney transplantation for the first time were included in the study. Age of <18 years, prior history of transplantation, presence of postoperative vascular complications, administration of a steroid-free immunosuppressive treatment protocol (steroids increase neutrophils and decrease lymphocytes, thus modifying both NLR and PLR), any proven history of acute coronary syndrome, cancer, primary bone marrow disorder, thrombocytopenia, thrombocytosis, active autoimmune disease, active chronic inflammation due to untreated chronic infections, any diagnosis of BK nephropathy or CMV positivity, and systemic, urinary or local documented infection proven by a culture at any evaluation period were the exclusion criteria of the study.

Routine laboratory tests included complete blood count (neutrophil, lymphocyte, and platelet), creatinine, serum uric acid, C-reactive protein (CRP) and tacrolimus levels as well as estimated glomerular filtration rate (eGFR) measurement. The patients were not evaluated in the early post-transplant period to rule out the potential impact of surgery or high dose immunosuppression on white blood cells.

When indicated, anti-thymocyte globulin (ATG, Grafalon Neovii) was administered at 100 mg/g dose for 3 days. Following total 1500 mg intravenous methyl-prednisolone, all patients received oral prednisolone (0.8 mg/kg/day). Prednisolone dose was tapered to 30 mg/day at 1 month, to 20 mg/day at 2 months and to 5 mg/day after 3 months. In the maintenance treatment phase, calcineurin inhibitor (tacrolimus (Tac); 0.1 mg/kg/day, 2 doses per day) and antiproliferative agent (mycophenolate mofetil; maximum 2 g/day or mycophenolate sodium; maximum 1440 mg/g) were used along with the prednisolone. Tac doses were titrated as needed to achieve target blood levels. AR was defined as the increase in creatinine levels by 30% above the baseline values that was not attributable to any other causes, and in case of AR, renal biopsy was performed and treatment (pulse methyl-prednisolone, ATG, plasmapheresis, and intravenous immunoglobulin treatments alone or in combination) were administered according to Banff criteria (11).

Age- and median follow-up time-matched kidney transplant patients who had no rejection episode served as the control group. The blood samples collected on the day of the admission to the clinic were used for the analyses in the AR group, while blood samples collected on the last outpatient clinic visit day were used for the analyses in the control group.

NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. Varying levels of NLR have been considered to be prognostic in different disease settings, including NLR >5 in cancer patients and NLR >3.5 in cardiovascular and acute surgical patients (10). In general, a normal NLR is considered to be ≤3. A NLR value of 2.5 or greater was therefore considered as elevated in this study.

Statistical Analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY) package. We could not say that probability distributions of the all variables are normally distributed with Kolmogorov-Smirnov test, therefore Mann-Whitney U test was used for those variables. To estimate the predictive value of NLR and PLR for the AR, receiver operating characteristic (ROC) curve was plotted, and the area under the curve (AUC) was calculated to compare the discriminatory performance of NLR or PLR in prediction of the AR, with 95% confidence interval (CI). After determining the appropriate cut-off points, with univariate binary logistic regression we further examine the relationship from a categorical perspective. Data were expressed as median, 1st and 3rd quartiles, minimum-maximum, and percent where appropriate. A p value of <0.05 was considered statistically significant.

RESULTS

The final study group subjected to analysis included 36 patients since 18 patients were excluded due to active infection. Majority of patients (n=33, 91.7%) were males, the median age was 41 (range, 19-64) years and the follow-up period was 34.4 (range, 21.3-53.4) months. The primary etiology of end-stage renal disease included chronic glomerulonephritis in 14 (38.9%) patients, hypertension in 7 (19.4%) patients, secondary amyloidosis in 6 (16.7%) patients, type 2 diabetes mellitus in 3 (8.3%) patients, nephrolithiasis in 2 (5.6%) patients and polycystic kidney disease in 2 (5.6%) patients, while no detectable cause was evident in 2 (5.6%) patients.

Data on type of dialysis, tissue adaptation, type of transplantation and type of induction treatments of patients are provided in Table 1.

The median values for neutrophil, lymphocyte and platelets were 5130 (range, 1490-9940), 1890 (range, 1100-3500) and 227450 (range, 124000-308400) cell/mm^3, respectively. The median NLR was 2.40 (range, 1.05-8.02) and PLR was 113.99 (range, 65.26-237.90).

Acute allograft rejection occurred in 16 (44.4%) patients. In microscopic examination of kidney biopsies of patients with AR; tubulitis in 9 patients, peritubular capillaritis in 7 patients, endarteritis in 8 patients, with different densities, were observed. Thrombotic microangiopathy was observed in 2 patients. Amyloid was negative in all patients. In immunohistochemical examination; T cell infiltrates of different densities were observed with CD3 in 12 patients. While linear staining was observed in peritubular capillaries diffuse with CD4 in 10 patients, no specific staining was observed in peritubular capillaries with CD4 in 6 patients. SV40 was negative in all patients. In terms of histopathological diagnosis, borderline changes in terms of cellular rejection in 5 patients, acute T-cell mediated rejection in 9 patients (4 patients Banff grade IA, 3 patients Banff Grade IB, 2 patients Banff grade IIA), acute antibody-mediated and acute T-cell mediated rejection (Banff grade IIA) was observed in 2 patients. In addition, findings consistent with calcineurin inhibitor toxicity were observed in 4 patients.

Comparisons of the demographics and clinical features between the AR and control groups are summarized in Table 2. The median duration of follow up was 39.26 (range, 21.47-53.10) months in the AR group and 33.30 (range, 21.33-53.40) months in the control group. In the AR group, the median time from renal transplantation to AR was 1.72 (range, 0.03-26.30) months. The patients in the AR and control groups were similar in terms of duration of follow-up (p=0.924), age (p=0.975) and uric acid levels (p=0.823). When compared to control group, patients in the AR group had significantly higher baseline serum creatinine (p<0.001) and CRP levels (p<0.001) whereas significantly lower eGFR (p<0.001).

Table 1. Demographic and clinical characteristics in the overall study group (n=36)

	n (%)
Gender	
Male	33 (91.7)
Female	3 (8.3)
Dialysis type	
Preemptive	22 (61.1)
Hemodialysis	13 (36.1)
Peritoneal dialysis	1 (2.8)
Transplantation type	
Live	36 (100)
Cadaveric	0 (0.0)
Mismatch	
0 MM	3 (8.3)
1 MM	1 (2.8)
2 MM	8 (22.2)
3 MM	14 (38.9)
4 MM	4 (11.1)
5 MM	5 (13.9)
6 MM	1 (2.8)
Induction treatment	
ATG	10 (27.8)
Non-inducted	26 (72.2)
	Median (Q_1-Q_3) [Min-Max]
Age (year)	41 (30-53) [19-64]
Transplant time (month)	34.4 (28.6-47.2) [21.33-53.40]
Creatinine (mg/dL)	1.37 (1.25-1.78) [0.82-6.49]
eGFR	58 (45-68.5) [8.00-108.00]
Uric acid (mg/dL)	5.65 (5.15-6.45) [3.60-8.00]
CRP (mg/dL)	1.05 (0.25-4) [0.11-90.06]
Neutrophil (cell/mm^3)	5130 (3930-5980) [1490-9940]
Lymphocyte (cell/mm^3)	1890 (1710-2340) [1100-3500]
Platelet (cell/mm^3)	227450 (201600-254450) [124000-308400]
NLR	2.40 (1.82-3.29) [1.05-8.02]
PLR	113.99 (88.44-142.75) [65.26-237.90]

ATG: anti-thymocyte globulin, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, Q1-Q3: 1st quartile - 3rd quartile, Min-Max: minimum-maximum

Table 2. Comparison of demographical characteristic and biochemical parameters in study groups

	Acute Rejection (n=16)			**Control (n=20)**			**p**
	Median	**Q_1-Q_3**	**Min-Max**	**Median**	**Q_1-Q_3**	**Min-Max**	
Duration follow-up (month)	39.26	27.86-48.80	21.47-53.10	33.30	29.64-44.99	21.33-53.40	0.924
Age (year)	44.00	26.75-54.25	19-61	38.50	30.00-49.75	24-64	0.975
Creatinine (mg/dL)	1.76	1.53-3.15	1.34-6.49	1.27	1.12-1.37	0.82-1.70	**<0.001**
eGFR	44.00	23.75-53.02	8.0-66.00	66.71	62.25-77.00	43.00-108.00	**<0.001**
Uric acid (mg/dL)	5.55	5.20-6.52	3.70-8.00	5.70	5.05-6.40	3.60-7.70	0.823
CRP (mg/L)	1.70	1.10-7.97	0.50-9.06	0.35	0.08-1.62	0.01-8.50	**<0.001**
NLR	3.75	2.49-5.07	1.31-8.02	1.99	1.60-2.47	1.05-3.08	**<0.001**
PLR	125.59	112.97-162.79	71.88-237.90	99.23	83.09-120.31	65.26-146.93	**0.008**

eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, Q1-Q3: 1st quartile - 3rd quartile, Min-Max: minimum-maximum

Both NLR (median 3.75 vs. 1.99, p<0.001) and PLR (median 125.59 vs. 99.23, p=0.008) values were significantly higher in the AR group compared to control group (Table 2, Figure 1, Figure 2).

ROC analysis revealed NLR cut-off value >2.5 (AUC=0.822, 95% CI=0.677-0.967, standard error (SE)=0.074, p=0.019) to be a potential marker of AR with a sensitivity of 75% and specificity of 75%, while PLR cut-off value >108 (AUC=0.759, 95% CI=0.597-0.922, SE=0.083, p=0.022) was also a potential marker of AR with a sensitivity of 81% and specificity of 65%. In this regard, values of NLR>2.5 and PLR>108 were considered as elevated (Figure 3).

Univariate binary logistic regression analysis was performed to analyze the association of both NLR and PLR with AR. The NLR >2.5 (Odds Ratio (OR)=0.267, 95%CI=0.089-0.803, p=0.019) and PLR >108 (OR=0.231, 95% CI=0.066-0.810, p=0.022) were determined to be significantly associated with AR.

DISCUSSION

Our findings revealed significantly higher NLR and PLR values in the AR group when compared to control group, as well as the association of higher NLR and PLR values with an increased risk of AR in renal transplant recipients. NLR and PLR are inflammatory markers that are inexpensive and readily available in routine clinical practice. Growing evidence exists regarding the predictive value of these markers in several conditions such as cancer, cardiovascular disease, rheumatic diseases, infections and transplantations (7,12-17). Additionally there are studies showing that NLR and PLR levels are positively correlated with inflammatory cytokines in patients with end-stage renal disease (15). Turkmen et al. (14) reported that kidney transplant patients had a higher NLR than healthy subjects and concluded that the higher values were due to the ongoing inflammation in these patients (14).

AR is the major cause of graft dysfunction in kidney transplantation. Given that most of the patients who developed AR are initially asymptomatic, the diagnosis can often be delayed. Therefore, availability of an easily applicable marker for both prediction and early detection of AR is important for kidney transplant patients. The most frequently used parameter for AR prediction is the increase in serum creatinine, but the increase in creatinine is a reflection of the histological damage in the kidney in the late period of the rejection episode.

The mechanisms underlying the association of elevated NLR and PLR with the development of AR are considered complex and unclear. Inflammation-related disruptions in hematologic cell lines including neutrophilia and thrombocytosis lead to elevations of NLR and PLR. However, most of immunosuppressive treatments selectively inhibit the activation and proliferation of lymphocytes. In this regard, the combination of either neutrophilia or thrombocytosis with lymphopenia may contribute to development of AR. Several experimental studies in mice have indicated the possible role of neutrophils after transplantation, mainly via neutrophil activated β2-integrin, while the association of neutrophil penetration and gathering in the allograft tissue with AR has been reported to occur through β2-integrin in rodents. Neutrophils are known to induce the progression of AR of allografts and thus the inhibition of neutrophil infiltration into the allograft has been associated with lower likelihood of rejection and an improved survival of the allograft. In this regard, NLR is considered amongst the independent predictors of the risk of AR (18-20).

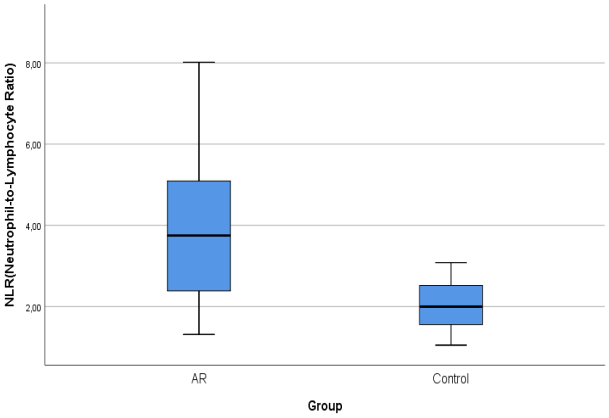

Figure 1. Comparison of NLR values between acute kidney rejection and control groups: the median NLR level was significantly higher in AR group (3.75 vs 1.99, p<0.001).

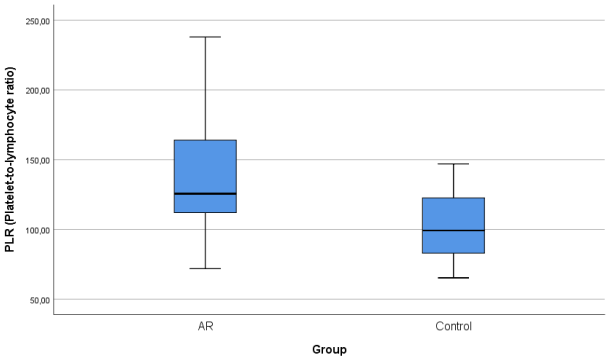

Figure 2. Comparison of PLR values between acute kidney rejection and control groups: the median PLR level was significantly higher in AR group (125.59 vs 99.23, p=0.008).

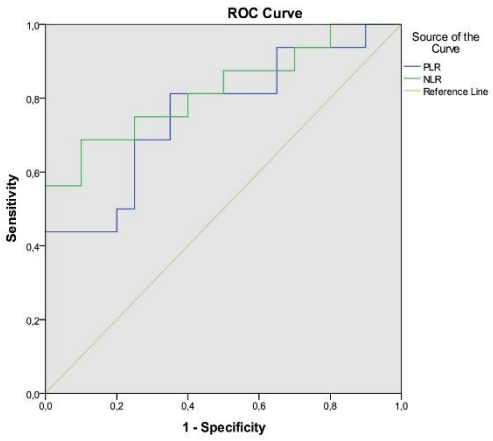

Figure 3. ROC curve analysis of the role of NLR and PLR in prediction of acute rejection. ROC: receiver operating characteristic, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio

Ergin et al. (16) reported that NLR values >2.5 were associated with AR (median NLR 4.06 vs. 1.24) in kidney transplant patients. In a study investigating early allograft rejection and NLR rate in 1531 liver transplant patients, the NLR rate was found to be significantly higher in patients with rejection (median 3.49 vs. 2.07) and NLR ≥2,85 (OR=1.89) was an independent predictor of early allograft rejection (21). Similar results were obtained in our study (median NLR 3.75 vs 1.99). In addition, we believe that the increase in NLR in AR patients was unrelated to corticosteroid dosage, because all the patients were using the same maintenance dosage.

In our study, the risk of developing AR in patients with an NLR value below 2.5 was 0.267 times lower than in patients with an NLR value above 2.5. Interestingly, Naranjo et al. (1) indicated 7-fold higher NLR in patients without evidence of acute cellular rejection than in patients with findings of acute cellular rejection, and similar trend (5.5-fold higher levels in the absence of rejection) was also reported for PLR and they explained this different result with the relative increase of lymphocyte count in patients who developed rejection (1).

Halazun et al. (10) reported the association of elevated preoperative NLR levels with a higher risk of developing graft function along with role of NLR over 3.5 in predicting (AUC=0.751) the delayed graft function. However in our study, patients with NLR over 2.5 were more likely to develop AR (AUC=0.822).

Turkmen et al. (22) reported that PLR can be used to predict inflammation in patients with end stage renal disease and it is superior to NLR when used as a marker of inflammation. Seropian et al. (7) reported that in heart transplant patients, NLR was a more useful marker than PLR at ROC analysis (AUC, 0.644 vs. 0.599) in prediction of 1-year mortality after transplantation. In the current head-to-head comparison of NLR and PLR, our findings revealed that NLR was more useful marker at ROC curve for predicting AR.

The main limitation of this study is that it was a retrospective single center study. In addition, the sample size was relatively small, and a single measurement of NLR and PLR may not accurately reflect the changes over time. Future studies that obtain serial changes of NLR and PLR would be useful to clarify to role of these ratios in the follow up of AR.

CONCLUSION

In conclusion, our findings indicate that PLR and specially NLR are non-invasive, useful, low-cost, widely available markers for AR in renal transplant patients. Further validation from prospective larger-scale controlled and multicenter cohorts seems to be helpful to determine the predictability of these tests in the early diagnosis of AR.

Ethics Committee Approval: The study was approved by the Ethics Committee of Ufuk University Faculty of Medicine (25.02.2020, 4).

Conflict of Interest: None declared by the authors.

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Ocular Pathologies in Children with Mental Retardation: A Prospective Study

Zihinsel Engelli Çocuklarda Oküler Patolojiler: Prospektif Bir Çalışma

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ABSTRACT

Aim: The aim of this study is to investigate the ocular pathologies in mentally retarded pediatric patients without Down syndrome or any certain chromosomal-genetic anomaly.
Material and Methods: A total of 189 patients, including 79 mental retarded and 110 healthy children, were included in this study between 2010 and 2011. Demographic factors (age, gender) of the patients, and affected side, visual acuity, esotropia, exotropia, anterior segment pathologies and posterior segment pathologies were evaluated. All pediatric patients in study group were divided as IQ level <34, 35-49, 50-69, and >70 according to Wechsler Intelligence Scale for Children-Revised Form.
Results: The mean age of the children with mental retardation was 11.85±6.19 (3-17) years, while the mean age of healthy children was 10.73±3.35 (3-15) years. While visual impairment was present in 8 of the 74 patients whose vision was evaluated, there was no impairment in the healthy group (p=0.001 and p=0.004 for right and left, respectively). Anterior segment pathology was detected in 2 (2.5%) cases, and posterior segment pathology in 3 (3.8%) cases in children with low IQ. While strabismus was detected in 7 (8.9%) patients with low IQ, no strabismus was found in healthy children. Five (6.3%) of the cases with mental retardation had exotropia and 2 (2.5%) had esotropia. Exotropia was found significantly higher in children with mental retardation compared to the control group (p=0.012).
Conclusion: Visual impairment, anterior-posterior segment pathology, exotropia had a high prevalence in children with mental retardation, and all ocular pathologies were related to low intellectual disability.
Keywords: Children; mentally retarded; vision disorders.

ÖZ

Amaç: Bu çalışmanın amacı Down sendromu veya herhangi bir kromozomal-genetik anomalisi olmayan zihinsel engelli pediatrik hastalarda oküler patolojilerin araştırılmasıdır.
Gereç ve Yöntemler: Bu çalışmaya 2010 ve 2011 yılları arasında 79 zeka geriliği olan ve 110 sağlıklı çocuk olmak üzere toplam 189 hasta dahil edildi. Hastaların demografik faktörleri (yaş, cinsiyet) ile etkilenen taraf, görme keskinliği, ezotropya, ekzotropya, ön segment patolojileri ve arka segment patolojileri değerlendirildi. Çalışma grubundaki tüm pediatrik hastalar Wechsler Çocuklar için Zeka Ölçeği-Yenilenmiş Formu'na göre IQ düzeyi <34, 35-49, 50-69 ve >70 olarak ayrıldı.
Bulgular: Zeka geriliği olan çocukların yaş ortalaması 11,85±6,19 (3-17) yıl iken sağlıklı çocukların yaş ortalaması 10,73±3,35 (3-15) yıl idi. Görmesi değerlendirilen 74 hastanın 8'inde görme bozukluğu varken, sağlıklı grupta ise hiç görme bozukluğu yoktu (sağ ve sol için sırasıyla p=0,001 ve p=0,004). Düşük IQ'lu çocuklarda, 2 (%2,5) olguda ön segment patolojisi ve 3 (%3,8) olguda ise arka segment patolojisi saptandı. Düşük IQ'lu hastaların 7 (%8,9)'sinde şaşılık saptanırken sağlıklı çocuklarda ise hiç şaşılık saptanmadı. Zeka geriliği olan olguların 5 (%6,3)'inde ekzotropya, 2 (%2,5)'sinde ise ezotropya vardı. Ekzotropya zeka geriliği olan çocuklarda kontrol grubuna göre anlamlı derecede daha yüksek olarak bulundu (p=0,012).
Sonuç: Zihinsel engelli çocuklarda görme bozukluğu, ön-arka segment patolojisi, ekzotropya yüksek prevalansa sahipti ve tüm oküler patolojiler düşük zihinsel engelle ilişkiliydi.
Anahtar kelimeler: Çocuk; zihinsel özürlü; görme bozuklukları.

INTRODUCTION

Mental retardation is defined by low intellectual (intelligence quotient, IQ <75) functioning and is accompanied by many ocular findings, including pathologies of the anterior and posterior ocular segments, as strabismus, refractive errors, visual acuity (1-4). The incidence of each ocular abnormality varies in different studies (5-7). Sauer et al. (7) examined the refractive errors and ocular pathologies between newborn and children with severe behavioral pathologies and developmental disabilities. The authors studied a retrospective review of ocular examinations that 222 children between the ages of 0 and 4 (mean 2.2±0.9) years were performed and 100 (45.0%) had an ocular abnormality. They resulted that the frequency of ocular abnormality was 33.3%, strabismus was 10.9%, and nystagmus was 12.2%. In addition, they demonstrated that ophthalmic pathologies are most frequent even at a young age in children with behavioral pathologies and developmental disorders (8). And also, most visual pathologies in children with retarded intellectual development can be treated; early diagnosis and intervention can have a lifelong positive effect on neurodevelopment (9,10). Adults with mental retardation have a significantly higher rate of pathological ocular findings compared to the healthy population (11,12). Similarly, children with mental retardation are twice more likely to have abnormal eye findings than healthy children (13). Causes of mental retardation can be examined in two main groups; first, chromosomal disorders, genetic syndromes and second ones whose causes are not fully determined (4). In Turkey, the mentally retarded in 60% of patients in a study conducted in patients with a genetic-chromosomal disorder was detected, in 40%, no specific cause could be determined (2,3). The aim of this study was to investigate the ocular findings in mentally retarded patients without Down syndrome or any certain chromosomal-genetic anomaly. In this current study, we studied ocular findings in children with mentally retardation and compared the results with healthy children.

MATERIAL AND METHODS

Between October 2010 and December 2011, 189 patients who met the inclusion criteria out of 200 patients (90 mentally retarded, 110 healthy) aged 0-18 years who applied to Duzce University Faculty of Medicine ophthalmology clinic with the diagnosis of motor or mental developmental retardation or for special education were included in the study. A prospective comparative evaluation was made in terms of routine ocular examination and strabismus.

This study was approved by the Duzce University Faculty of Medicine non-invasive health research ethics committee (2010/66), and all patients were informed about the study, informed consent form and ethical approval were obtained. Patients with IQ level of 75 and below were included in the study group, while patients with Down syndrome (n=4) and diagnosed chromosomal genetic anomaly (n=7) were excluded from the study. Patients with normal intelligence (IQ >80) who applied to a healthy pediatric outpatient clinic were included in the control group. In this study, a total of 189 patients, including 79 mental retarded and 110 healthy children were included.

The results of a complete ocular examination by a strabismus specialist including pattern and size of eye deviation, visual acuity, refractive errors and presence or absence of amblyopia was evaluated. Age and gender as the demographic factors of the patients, and affected side, visual acuity, esotropia, exotropia, anterior segment pathologies and posterior segment pathologies were evaluated. An autorefractor (Nikon Retinomax K-plus; Nikon, Melville, NY, USA) was used for refractive errors with in cycloplegia (30 mins after cyclopentolate 1%), in addition esotrophia and exotropia was evaluated using Hirschberg's test. All pediatric patients in study group were divided as IQ <34 (n=4), IQ 35-49 (n=20), IQ 50-69 (n=54), IQ >70 (n=1) according to Wechsler Intelligence Scale for Children-Revised Form (WISC-R).

Statistical Analysis

Statistical analyzes were performed using the number cruncher statistical system (NCSS) 2007 statistical software. In the evaluation of the data, in addition to descriptive statistical methods (mean, standard deviation), the distribution of variables was examined with the Kolmogorov-Smirnov normality test. Independent samples t test for comparison of groups in terms of normally distributed variables and also Chi-square and Fisher's exact tests were used for comparisons of qualitative data. The results were evaluated at the significance level of <0.05.

RESULTS

Seventy nine children (44 male, 35 female) with mental retardation (IQ <75) and 110 healthy children (62 males, 48 female) were included in this study. The mean age of children with mental retardation was 11.85±6.19 (3-17) years, while the mean age of healthy children was 10.73±3.35 (3-15) years. No statistically significant difference was observed between the mean age and gender distribution of both groups (p=0.111, p=0.927). When mental retardation was examined by IQ level, 1 patient had mild (IQ >70), 54 patients had moderate (IQ =50-69), 20 patients had severe (IQ =35-49), and 4 patients had very severe (IQ <34) IQ level.

In 5 patients with low IQ, the visual level could not be determined because they could not be cooperated. While visual impairment was present in 8 of the 74 patients whose vision was evaluated, there was no impairment in the healthy group (p=0.001 and p=0.004 for right and left, respectively). While anterior segment pathology was detected in 2 (2.5%) cases in children with low IQ, cataracts and other anterior segment pathologies were not found in the healthy group (p=0.173). In cases with low IQ, 1 patient had advanced myopia, 1 patient had optic atrophy and 1 patient had chorioretinal degeneration, while no posterior segment pathology was found in the healthy group (p=0.071 both right and left). While strabismus was detected in 7 (8.9%) patients with low IQ, no strabismus was found in healthy children. Five (6.3%) of the cases with mental retardation had exotropia and 2 (2.5%) had esotropia. Exotropia was found significantly higher in children with mental retardation compared to the control group (p=0.012). In terms of esotropia, no significant difference was found between children with mental retardation and the control group (p=0.173, Table 1, 2).

Table 1. Comparison of MR and control groups

	Control (n=110)	MR (n=79)	p
Age (years), mean±SD	10.73±3.35	11.85±6.19	0.111*
Gender, n (%)			
Male	62 (56.4)	44 (55.7)	0.927#
Female	48 (43.6)	35 (44.3)	
Exotropia	0 (0.0)	5 (6.3)	**0.012**ǂ
Esotropia	0 (0.0)	2 (2.5)	0.173ǂ
Visual Acuity (R)	0 (0.0)	8 (10.1)	**0.001**ǂ
Visual Acuity (L)	0 (0.0)	6 (7.6)	**0.004**ǂ
Anterior segment (R)	0 (0.0)	2 (2.5)	0.173ǂ
Anterior segment (L)	0 (0.0)	2 (2.5)	0.173ǂ
Posterior segment (R)	0 (0.0)	3 (3.8)	0.071ǂ
Posterior segment (L)	0 (0.0)	3 (3.8)	0.071ǂ

MR: mental retardation, SD: standard deviation, R: right, L: left, *: Independent samples t test, #: Chi square test, ǂ: Fisher's exact test

Table 2. Ocular pathologies by IQ level in children with mental retardation

	<34 (n=4)	35-49 (n=20)	50-69 (n=54)	>70 (n=1)
Gender, n (%)				
Male	4 (100)	11 (55.0)	29 (53.7)	0 (0.0)
Female	0 (0.0)	9 (45.0)	25 (46.3)	1 (100)
Exotropia	0 (0.0)	1 (5.0)	4 (7.4)	0 (0.0)
Esotropia	1 (25.0)	0 (0.0)	1 (1.9)	0 (0.0)
Visual Acuity (R)	1 (25.0)	1 (5.0)	5 (9.3)	1 (100)
Visual Acuity (L)	1 (25.0)	1 (5.0)	3 (5.6)	1 (100)
Anterior segment (R)	1 (25.0)	1 (5.0)	0 (0.0)	0 (0.0)
Anterior segment (L)	1 (25.0)	1 (5.0)	0 (0.0)	0 (0.0)
Posterior segment (R)	1 (25.0)	1 (5.0)	1 (1.9)	0 (0.0)
Posterior segment (L)	1 (25.0)	1 (5.0)	1 (1.9)	0 (0.0)

IQ: intelligence quotient, MR: mental retardation, R: right, L: left

DISCUSSION

We present ocular pathology in children with mentally retardation and compared the results with healthy children in this current prospective study. We resulted that refractive errors, anterior-posterior segment pathology and strabismus (especially exotropia) were most frequently seen in mentally retarded children than healthy.

In mental retardation, there is a slow growing development of intelligence, affecting all cognitive abilities (14). Accordingly, children with mental retardation experience deficiencies in social activities such as communication, self-management, social skills, self-care, using social resources and ensuring their personal safety (15). Behavioral research has shown that the impact of visual impairment on early development is profound, and early in life, visually impaired children begin to lag behind children who see developmentally similar (2). Adults with mental retardation have a higher rate of refractive errors and ocular pathologies compared to the general population (13,16). Similarly, children with mental retardation are twice as likely to have ocular pathology than healthy children (17,18). Akinci et al. (17) evaluated the ophthalmological findings in children with intellectual disability and in controls of mean intellectual development. The authors compared 724 cases with intellectual disability and 151 control children in their study. They demonstrated that increasing severity of intellectual disability was related to higher prevalence of nystagmus, strabismus, astigmatism, hypermetropia, and anisometropia. In addition they resulted that in children with moderate, severe and syndromic mental retardation, evaluation and treatment of eye findings are important in reducing future health and social care costs and improving their productive lives. In another study, Aslan et al. (18) examined 215 children with mental disabilities (90 Down syndrome, 125 nonprofound ID) and 116 healthy cases for causeless preventable visual impairment in mentally retarded children. The authors resulted that the children with intellectual disability has a high prevalence of preventable visual impairments, refractive errors, strabismus, and cataracts.

In cases of mental retardation not related to a prominent chromosomal genetic disorder such as Down syndrome in etiology, the incidence of strabismus was found to be significantly higher than in healthy children (19). In terms of the direction of strabismus, exotropia is more common than esotropia. Our study and some other series show that strabismus can be seen more in mental retardation than in the normal population (20,21). Sandfeld Nielsen et al. (20) studied that prevalence of refractive errors and strabismus in children with developmental delay. The authors examined 923 children with IQ ≤80, aged between 4-15 years in their study. They showed that strabismus was found in 26.8% of subjects (esotropia in 14.9%, exotropia in 10.3%, and other forms, including mixed types, in 1.6%). As a result, strabismus was significantly correlated with low IQ. In addition, there are case series reporting the incidence of strabismus of 40% or more in mentally retarded cases (15,16).

Exotropia was significantly more common in cases with mental retardation in our study. Similarly, there are studies emphasizing that exotropia is more common in mentally disabled people (21), as well as publications reporting that esotropia is more common (20,22). Gogate et al. (21) examined that results of horizontal strabismus surgery. The authors studied 529 children 9 years in their study. They showed that exotropia was most often than esotropia in children. On the other hand, Donnelly et al. (22) studied that the prevalence of childhood visual disorders: amblyopia (strabismus, refractive errors) and organic disease. The authors studied 1582 children aged 8-9 years in their study. They resulted that 198 children (12.5%) had a significant visual disorder: strabismus (3.98%), eso:exo rate 5:1, anisometropia (2.34%), ametropia (5.82%), organic defects (0.38%). It is noteworthy that esotropia is more common in Down syndrome cases (19,23). Exclusion of Down syndrome and cases with other chromosomal anomalies in our study may play a role in the detection of exotropia more frequently.

There are a few limitations in this current study. First, the number of patients was relatively small. Children with syndromic mental retardation could be included in our study and compared with non-syndromic and healthy children. Further prospective-multicenter studies with large number of patients are needed.

CONCLUSION

There is already a difficulty in communicative and social skills in children with mental retardation. Strabismus can complicate the social adaptation of the child, as it can increase negative social pressure on the child. Removing cosmetic or visual ocular problems can facilitate social adaptation by reducing social pressure in children with mental retardation.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (30.09.2010, 66).

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The Surgical Treatment of Tissue Necrosis due to Diclofenac Sodium Injection (Nicolau Syndrome)

Diklofenak Sodyum Ekjeksiyonuna Bağlı Doku Nekrozunun (Nicolau Sendromu) Cerrahi Tedavisi

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ABSTRACT

Aim: Nicolau syndrome (NS) is the necrosis of skin and subcuticular tissue, following injection of many drugs, which covers nearly a perforasome. This study aims to unveil our clinical approach and treatment alternatives against this rare pathology subsequent to diclofenac sodium.

Material and Methods: In this retrospective study, our approach to 16 patients who developed NS at the injection site after diclofenac sodium injection was evaluated. Patients' demographic data, concomitant diseases, location and size of necrosis, and surgical techniques were collected. Post-operative complications, hospitalization period and results were evaluated.

Results: There were total of 16 patients, 2 of which were male and 14 were female. The mean age was 60±14.4 years, and the mean body mass index (BMI) was 33±1.4 kg/m^2. The major concomitant comorbidity was diabetes mellitus, followed by primary hypertension. The mean defect size was 8x8x5 cm. Surgical debridement was applied to all 16 necrosis. Following the surgical debridement of necrosis, 8 patients were reconstructed with primary closure, 6 patients with fasciocutaneous flaps and 2 patients with delayed primary closure after a week. All patients heal completely without complications.

Conclusion: Utmost care must be given when performing diclofenac sodium injections to the patients with comorbidities, such as high BMI, diabetes mellitus, and primary hypertension. The caregiver must be certain that the needle is in the muscular plane and no perforator vessel to be harmed during this procedure. In case of a post-injection necrosis, an early intervention with surgical debridement and reconstruction is an efficient treatment.

Keywords: Diclofenac sodium; injection; intramuscular; Nicolau syndrome.

ÖZ

Amaç: Nicolau sendromu (NS), bazı ilaçların enjeksiyonlarını takiben gelişen ve yaklaşık bir perforazomu kaplayan boyutlara ulaşan yumuşak doku ve ciltte nekroz gelişmesi durumudur. Bu çalışmada klinikte sık karşılaşılmayan diklofenak sodyum enjeksiyonu sonrası gelişmiş bu nadir patolojiye yönelik klinik yaklaşımımız ve tedavi alternatiflerinin sunulması amaçlanmıştır.

Gereç ve Yöntemler: Bu geriye dönük çalışmada diklofenak sodyum enjeksiyonu sonrası enjeksiyon yerinde NS gelişen 16 hastaya yaklaşımımız değerlendirildi. Hastaların demografik verileri, eşlik eden hastalıkları, nekrozun yerleşimi ve boyutları ve cerrahi teknikler tarandı. Postoperatif komplikasyonlar, hastanede kalış süresi ve sonuçlar değerlendirildi.

Bulgular: Toplam 16 hasta mevcut olup bunların 14'ü kadın 2'si erkek idi. Ortalama yaş 60±14,4 yıl ve ortalama vücut kitle indeksi (VKİ) 33±1,4 kg/m^2 idi. En sık eşlik eden hastalık diyabetes mellitus, daha sonra primer hipertansiyon olarak gözlendi. Ortalama nekroz boyutu 8x8x5 cm olarak saptandı. Tüm 16 nekroza da cerrahi debridman uygulandı. Nekrozun cerrahi debridmanı sonrası 8 hastanın defekti primer kapatılırken 6 hastada fasyokutan flep yapıldı ve 2 hasta ise bir hafta sonra gecikmiş primer olarak onarıldı. Hastaların tümü sorunsuz olarak tamamen iyileşti.

Sonuç: Yüksek VKİ, diyabetes mellitus ve primer hipertansiyon gibi komorbid özellikler taşıyan hastalara diklofenak sodyum enjeksiyonu yapılırken çok dikkatli olunmalıdır. İşlem sırasında, iğnenin kas tabakasına kadar ulaştığından ve perforatör bir damara denk gelmediğinden emin olunmalıdır. Enjeksiyon sonrası nekroz gelişmesi durumunda erken dönemde cerrahi debridmanı takiben rekonstrüksiyon etkili bir tedavidir.

Anahtar kelimeler: Diklofenak sodyum; enjeksiyon; intramüsküler; Nicolau sendromu.

INTRODUCTION

Although it usually occurs after intramuscular injections, the Nicolau syndrome (NS) is a rare reaction, which may end up with the formation of a livedoid dermatitis-like necrotic defect rarely emerging after subcutaneous or intravenous injections (1). This pathologic state which was initially recorded upon injection of bismuth salts, ends up with a necrosis of the skin, the subcutaneous tissues, and even sometimes within the musculature. It is often attributed to the injection of NSAIDs, but it may also happen with local anesthesia, penicillin, and vitamin K (2). Although the mechanism behind this pathology is not clear, fat necrosis and local cyclooxygenase (COX) inhibition are believed to be the reason behind it (3). According to the literature, the common surgical approach towards NS cases comprises reconstruction of the harmed tissue following an adequate debridement. In this study we aimed to present our plastic surgery approach towards NS, based on 16 cases who treated in our clinic.

MATERIAL AND METHODS

Upon approval of the Istanbul Medeniyet University Clinical Research Ethics Committee (2020/0406), 16 NS cases had been detected which have been treated at our institution and retrospectively analyzed between the years of 2016 and 2020. All cases were patients who received intramuscular diclofenac sodium injections at different parts of the body. The demographics, body mass indexes (BMIs), pre-injection medical conditions, comorbidities, defect areas, preoperative-intraoperative and postoperative photographs of patients and their follow-up data were examined.

Surgical Technique

All patients except one patient (general anesthesia) was operated under spinal anesthesia and 1 gr of cephazoline sodium (intravenous) was prophylactically given to each one, an hour prior to the operation. Necrotic tissues were debrided above muscular fascia until yellow colored healthy fat tissue was observed. Deep tissue biopsy samples were also taken. Upon completion of debridement and irrigation, the depth and the surface area of the defect was measured and photographed. After debridement, if the defect is suitable for primary closure, the defect was sutured primarily (Figure 1). Defects that cannot be closed primarily were reconstructed with fasciocutaneous flaps (Figure 2). Two patients, who had an infected wound area that was not suitable for an immediate surgical closure, were treated with negative pressure wound therapy (NPWT). No limitations were applied for patient mobilization after surgery. All patients were monitored with hemovac drains and changed dressing every two days in the post-operative period. The drains were held until the drainage amount less than 25 cc/day. The patients were discharged from the hospital after drainage tubes had been expelled. The patients received 3x1 gr intravenous cephazoline sodium within the day of operation, followed by oral amoxicillin-clavulanic acid 2x1 gr for a week.

RESULTS

Sixteen patients with NS were treated at our clinic between 2016 and 2020, 14 of which were female and 2 were male. The mean patient age was 60±14.4 (range, 35-78) years. All of the patients, except the one (who was 35 years old), had comorbidities. Diabetes mellitus was one of the most common (n=10) comorbidities in the group, and primary hypertension (n=6) was the second. The average BMI was 33±1.4 (range, 31.4-35.2) kg/m^2 (Table 1).

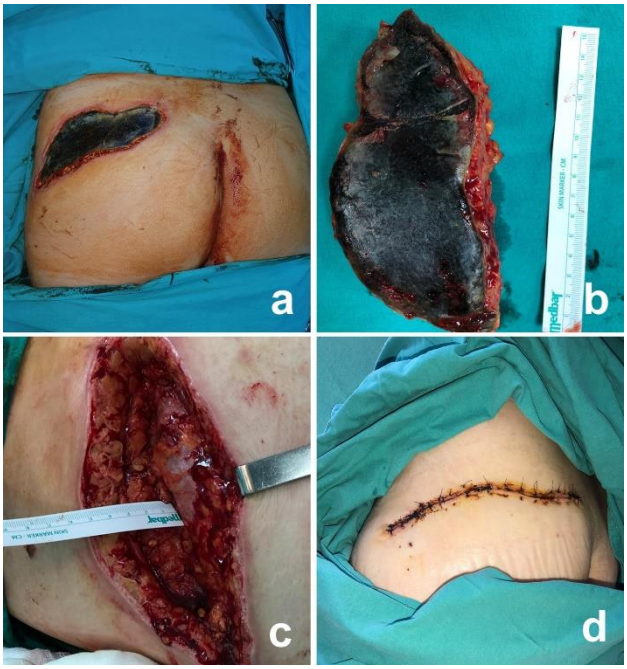

Figure 1. The necrosis on the left gluteal region 2 weeks after diclofenac sodium injection (a). The necrosis was debrided (b). The fat thickness was evaluated 5 cm after debridement (c). Postoperative 1st week of the gluteal region after primary closure (d).

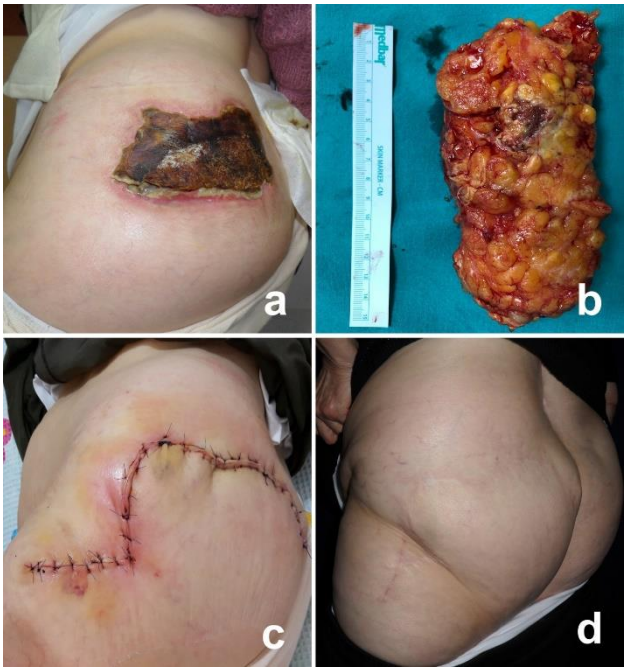

Figure 2. The necrosis on the left gluteal region 3 weeks after diclofenac sodium injection (a). The necrosis was debrided (b). The defect was reconstructed with a fasciocutaneous rotation flap (c). Patient at postoperative 6th month (d).

Intramuscular diclofenac sodium injection was the sole reason for the necrosis in our patient group. The location of necrosis varied by patient; 5 patient at the right gluteal, 6 patient at the left gluteal, 4 patient at the left thigh lateral border and 1 patient, who ignorantly self-injected herself, at the periumbilical region. The patients were operated at an average of 5 (2-9) weeks after the injection. As the necrosis depths were examined intraoperatively, it was observed that necrosis had reached to the deep fascia but the musculature was not harmed. The largest area of necrosis was 10x20x4 cm, and the smallest was 2x2x4 cm (average 8x8x5 cm). The defects of 8 patients were treated with primary closure. Six patients were reconstructed with rotational fasciocutaneous flaps. Two patients, who had an infected defect base and also had a pouche larger than the necrosis area, was treated with a NPWT device for two sessions (3 days for each session). After a week, an effective contraction had occurred at the defect, thus it was reconstructed with delayed primary closure. The average operation time was 64 (range, 30-95) minutes. No postoperative complications observed. The patients were hospitalized at an average of 5.1 (range, 2-10) days. The mean follow up period was 11.7 (range, 6-14) months. According to the wound cultures that were sampled in the operation, 2 patients had *E. coli*, 4 patients had normal skin flora, and the rest showed negative results. The culture positive cases were all amoxicillin susceptive, thus no antibiotic therapy changes had occurred.

DISCUSSION

Nicolau Syndrome is an acute and destructive condition after injections. It was first described and named by Dr. Stefan Nicolau in 1925. Although he described this syndrome as a result of arterial damage in his study, there have been many theories on the pathophysiological pathway (4). This pathology seems to occur most frequently after NSAID injections (3), however that the necrosis may also be seen after penicillin, vitamin K, thiocolchicosid, lidocaine, naltrexone, DPT vaccine and mesotherapy injections (5-7). Ezzedine et al. (8) suggest diclofenac, as a potent cox inhibitor - thus inhibiting prostaglandin synthesis, increases the formation of necrosis. Another theory suggests that perineural, intra-arterial, and periarterial injections cause local pain, and vasospasm secondary to the local pain leads to sympathetic nerve stimulation followed by necrosis (6,9). NS rises to the occasion with pain and erythema and they are followed by the bullous lesions and tissue necrosis (10). Necrosis may not be limited to subcutaneous adipose tissue. In the literature, extensive damage to deep muscle tissue and even limb losses due to ischemia of the whole limb have been reported (4,11). Saputo and Bruni (12) reported that 80 of their 102 cases were under the age 12. They suggested that any small arterial diameter has a tendency to build up thrombosis, which leads to the tissue necrosis. Nonetheless, our study group composed of only adults (a mean of 60 years), making their suggestion contradictory. Furthermore, the irritative features of the injection materials are thought to cause abscess and necrosis (13).

Some medical treatments have been defined to treat NS, such as heparin, topical betamethazone, and vasoactive agents (14). We have our reasons to believe that early surgical intervention is more advantageous for these patients, since the etiology is not clear, patients' appeal for medical attention is late, and most importantly the general medical condition of these patients is not suitable for a long-lasting hospitalization.

As one can see from all of the cases, our clinical approach to NS was surgical, that is, debridement and reconstruction of the wound according to the plastic surgery basic principles. The basic difference between primary versus flap reconstruction was the size of the necrosis. In the primary reconstruction group the mean defect size was 4.25x3.5x4.25 cm. This mean size was calculated as 11.25x12.25x5.25 cm in the flap reconstruction group. Kocman et al. (15) put forward the free style perforator flaps for reconstruction of the gluteal region. They believe their technique offers better aesthetic results. However, a perforator flap requires meticulous dissection of the pedicle, thus extending the operation time. Owing to the fact that we reconstructed our patients either primary closure or with fasciocutaneous flaps, our average operation time was 64 minutes, making our intervention significantly shorter. When we consider the patients with multiple comorbidities, this result gains importance. Furthermore, none of our patients experienced postoperative complications. In addition to its safety, long term aesthetic results were pretty satisfactory, which takes our technique one step ahead.

When the defects were observed, the average depth was 4.75 cm (4-6 cm), being deeper than an ordinary green 21 Gauge needle, which is 3.8 cm long. The basic problem here might be the injection into a superficial plane rather than muscle, such as the fat tissue, which has a scarce vascular support. Our group was constituted by obese patients, supporting the latter proposition. Dadacı et al. (16) did find similar results in their own research. NSAIDs, such as diclofenac sodium, may cause cytotoxic effects by altering local pH levels and activating the preapoptotic pathways. Therefore, even a proper injection inside the musculature may cause local destruction (6,17,18). On the

Table 1. Patient demographics and clinical progression

Gender, n (%)	
Male	2 (12.5)
Female	14 (87.5)
Age (years), mean±SD	60±14.4 (35-78)
Body mass index (kg/m^2), mean±SD	33±1.4 (31.4-35.2)
Comorbidities, n (%)	
Primary Hypertension	6 (37.5)
Diabetes Mellitus	10 (62.5)
Hypothyroidism	2 (12.5)
Necrosis Localization, n (%)	
Gluteal region	11 (68.8)
Tight	4 (25.0)
Periumbilical	1 (6.2)
Defect size (cm)	
Minimum	2x2x4
Maximum	10x20x4
Mean	8x8x5
Surgical Treatment, n (%)	
Debridement + Primary repair	8 (50.0)
Debridement + Fasciocutaneous rotation flap	6 (37.5)
Primary reconstruction + NPWT + Delayed primary repair	2 (12.5)

SD: standard deviation, NPWT: negative pressure wound therapy

other hand, the necrosis mimics a perforasome as its demarcation line passes over the injection area. The basic pathology here might be the injury of the arterial plexus at the hypodermal level by the injected agent, causing embolization of the perforators nourishing the skin, leading a perforasome-like necrosis area.

There is still no preventive method against NS development. The Lesser technique that is used for IM injections seems inadequate (19). This technique emphasizes to aspirate first when the needle is at the desired plane, then to inject in order not to damage any vasculature. In order to prevent NS, a modified version of the above explained technique can be used, the "Z-shaped Injection". In this technique the skin and subdermal contents are pushed downwards with the nondominant hand, thus reducing the distance between the skin and the muscular plane, thereupon increasing the possibility to inject the drug into the muscle. The needle is inserted at angle of 90 degrees and aspirated for at least 5 seconds before injecting the drug. A maximum of 5 ml is injected at a single time. The needle exits all tissues at an angle of 90 degrees. By this way, the drug leakage to subcutaneous tissues is prevented (20).

CONCLUSION

There is still no proven method to prevent NS development, however, injecting with the Z-shaped technique, using a longer needle in obese patients, and choosing different injection locations (deltoid, thigh, etc.) in selected patients possibly lessen the frequency of tissue necrosis. Another option is to be aware of this pathology and prescribe drugs per oral in suitable patients. There isn't any unique way to cure NS when it is developed, but early recognition, aggressive surgical debridement and reconstruction offers an efficient treatment and a rapid recovery.

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Medeniyet University Faculty of Medicine (24.06.2020, 0406).

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A Rare Cause of Female Gender Dysphoria: Report of Three Cases with Low Percentage of Turner Mosaicism

Kadın Cinsiyet Disforisinin Nadir Bir Nedeni: Düşük Yüzdeli Turner Mozaisizmli Üç Olgu Raporu

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ABSTRACT

Gender dysphoria is a condition caused by a mismatch between the gender assigned at birth and gender identity. Gender development disorders include situations where congenital chromosomal, gonadal, or anatomically gender-related physical features are atypical. In the studies conducted mostly by karyotype analysis, it is reported that the rate of chromosomal abnormality is very low in people with gender dysphoria. In Turner mosaicism, gender dysphoria is not a common finding. In this case series, we examined the phenotype and genotype characteristics of the three cases identified as Turner mosaicism, who applied with gender dysphoria. The patients' complaints were feeling like a male, negative thoughts about being a female, being uncomfortable with feminine body image, wanting to have a male body. None of our 3 cases had Turner stigmata however their chromosomal or FISH analyses showed that one of them was 45,X/46 XX/47,XXX and two of them were 45 X/46 XX karyotype.
Keywords: Turner's syndrome; gender dysphoria; karyotype; FISH.

ÖZ

Cinsiyet disforisi, doğumda belirlenen cinsiyet ile cinsiyet kimliği arasındaki uyumsuzluğun neden olduğu bir durumdur. Cinsiyet gelişim bozuklukları, konjenital kromozomal, gonadal veya anatomik olarak cinsiyetle ilişkili fiziksel özelliklerin atipik olduğu durumları içerir. Çoğunlukla karyotip analizi ile yapılan çalışmalarda cinsiyet disforisi olan kişilerde kromozomal anormallik oranının çok düşük olduğu bildirilmektedir. Turner mozaisizminde cinsiyet disforisi yaygın bir bulgu değildir. Bu olgu serisinde, cinsiyet disforisi ile başvuran Turner mozaisizmi olarak tanımlanan üç olgunun fenotip ve genotip özelliklerini inceledik. Hastaların şikayetleri erkek gibi hissetmek, kadın olmakla ilgili olumsuz düşünceler, kadın beden imajından rahatsız olmak, erkek bedene sahip olmak istemek şeklindeydi. 3 olgumuzun hiçbirinde Turner stigmatası yoktu ancak kromozomal veya FISH analizleri, birinde 45,X/46 XX/47,XXX ve ikisinde 45 X/46 XX olarak tespit edildi.
Anahtar kelimeler: Turner sendromu; cinsiyet disforisi; karyotip; FISH.

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INTRODUCTION

Gender dysphoria (GD) can be described as a conflict between a person's assigned gender and gender identity (1). People with GD may be very uncomfortable with their assigned gender, sometimes the reason is their body (particularly developments during puberty) and sometimes it is the expected roles of their assigned gender (2). Contrarily, disorders of sexual development (DSD) refer to a range of congenital conditions including the chromosomal anomalies, gonads, and/or genitalia (3). It is reported that 5% of people diagnosed with gender development disorders have GD. Although GD and DSD are distinct entities, they can coexist. Thus, when evaluating an individual with suspected GD, it is crucial to know whether he/she also has a DSD (2).

To make a diagnose of "Gender Identity Disorder", according to DSM IV-TR, and "Transsexualism" (F64.0) according to ICD-10, GD should not be related with an abnormality of gender chromosomes, or another disorder of sex development. DSM-5, removed accompanying DSD in GD diagnosis category from exclusion criterion for the diagnosis, and suggested that if they are present, should be indicated (1,4). However, some DSD types such as complete androgen insensitivity syndrome (CAIS), XY gonadal dysgenesis and Klinefelter syndrome may remain undiagnosed since clinical symptoms do not manifest until adolescence. In these cases, the diagnosis of GD may therefore forego doubt of a DSD, particularly in persons who's gender identity concerns appear in early period of life (2,5).

Turner syndrome (TS), one of the most frequent chromosomal disorder in female live births, is characterized by partial or complete lack of one X chromosome and its estimated incidence is 1/2500. Even though classic TS karyotype is 45,X, 30-40% of the remaining have a mosaic type with a second cell line (45,X/46,XX, 45,X/47,XYY, 45,X/46,XY, 45,X/47,XXX and 46,X,delXq) (6,7).

Some patients may be diagnosed at birth because of dysmorphic findings but the diagnosis may be delayed until childhood, adolescence or later (6,7). Clinical features of TS are short stature, characteristic stigmata, primary amenorrhea and infertility. The characteristic phenotypic findings of the disease are, low-set ears, low nuchal hair line, cubitus valgus, high palate, small mandibula, nail hypoplasia, short 4th metacarpal bone, swelling of the hands and feet in the neonatal period, discrete nipples and wide thoracic cage. Also system anomalies including cardiac and renal anomalies, gastrointestinal and dermatological problems, neoplasms, hypothyroidism, vision disorders, hearing loss (6) neurodevelopmental and behavioral problems are also general findings in patients with TS (8).

In the literature, very few mosaic TS in chromosome analysis of broad GD series have been reported. In this case series, we examined the phenotype and genotype characteristics of the three cases identified as Turner mosaicism, who applied with GD.

CASE REPORT

Routine hemogram, biochemistry, thyroid functions as well as antibodies and hormone profile were normal in all cases. Pelvic ultrasonography (USG) showed multiple anechoic follicles in bilateral ovaries in all cases.

CASE 1: 15 years and 9 months old girl patient applied with complaints of liking people of her own sex and gender dissatisfaction which are present for 2-3 years. During childhood period, the patient behaved in accordance with her gender, played with her girlfriends and girls' toys. During puberty, the patient started to like girls more and more. Desire to have a male body arose. She attempted suicide by taking drugs at the age of 15. The patient had spontaneous pubarche, thelarche, and menarche at 12 years with irregular menses. She had a gynecoid waist/pelvis ratio and big breasts but mild hirsutism. She had no Turner stigmata. Results of an echocardiogram and renal USG were normal. MTHFR C677T homozygous mutation and PAI-I:4G-5G mutation were detected in the thrombophilia panel. Other physical and laboratory values are given at Table 1. Karyotype analysis was performed from the peripheral blood and low percentage of a mosaicism was detected (46, XX[63] / 45, X[2]) (Figure 1a and 1b). FISH (X,Y) analysis was performed using CEP X (DXZ1), CEP Y (DYZ3) (Cytocell) probe and revealed nuc ish(CEP X x 1)[8]/(CEP X x 2)[490]/(CEP X x 3)[2] chromosomal composition.

CASE 2: 12 years and 4 months old girl admitted with negative thoughts about being a female, and being uncomfortable with feminine body shape and feeling like a male with increasing qualifications characteristics for the last 6 months. She had spontaneous pubarche, thelarche and menarche at 11 years with irregular menses. Karyotype analysis was performed from the peripheral blood and 46,XX karyotype was detected (Figure 1c). Because of the case's masculine self-perception FISH (X,Y) analysis was performed using CEP X (DXZ1), CEP Y (DYZ3) (Cytocell) probe and nuc ish(CEP X x 1)[4]/(CEP X x 2)[146] chromosomal composition was detected.

CASE 3: 17 years old girl applied with complaints that started in adolescence such as being sexually interested in girls, feeling uncomfortable with breasts and menstruation, and wanting to have a male body. She had spontaneous pubarche, thelarche and menarche at 13 years with regular menses. She had no Turner stigmata and hirsutism.

Karyotype analysis was performed from the peripheral blood and 46,XX karyotype was detected (Figure 1d). Due to her masculine feelings, FISH (X,Y) analysis was performed using CEP X (DXZ1), CEP Y (DYZ3) (Cytocell) probe and nuc ish(CEP X x 1)[6]/(CEP X x 2)[294] chromosomal composition was detected in interphase nuclei.

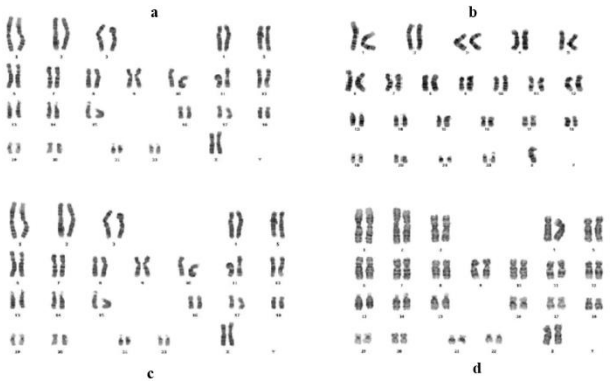

Figure 1. Karyotype analysis of cases using conventional G banding techniques, a-b) Case 1, c) Case 2, d) Case 3

DISCUSSION

Considering the studies conducted with GD and transgender patients using conventional G banding techniques, an overall rate of chromosomal abnormalities in 11/481 trans females (2.3%) was reported (5,9-12). (One of these, about mosaic TS with 45,X[10]/47,XXX[6]/46,XX[98] karyotype and without pubertal or hormonal disturbance (5).) Bağcaz et al. (13) performed G banding from 154 female-to-male transsexualism cases and TS was not represented in their sample. Pang et al. (14) have performed molecular

Table 1. Physical and laboratory values of cases

	Case 1	Case 2	Case 3
Age	15 year 9 month	12 year 4 month	17 year 3 month
Height (SDS)	1.8	0.13	1.6
Weight (SDS)	2.78	0.47	1.08
BMI (SDS)	2.26	0.57	0.23
Tanner stage	5	4	5
Menarche start	12	11	13
FSH (mIU/mL)	6.65	4.86	7.58
LH (mIU/mL)	10.16	6.34	5.18
E2 (pg/mL)	55.5	84	58
Testosterone	0.64	0.32	0.01
Karyotype	46 XX	45,X(4)/46 XX(146)	46 XX
FISH	45,X(8)/ 46 XX(490)/47,XXX(2)		45XO(6)/46XX(294)
Turner stigmata	absent	absent	absent
Hırsutism	mild	absent	absent
Irregular menses	yes	yes	no
Additional findings	MTHFR C677T homozygous mutation and PAI-I:4G-5G mutation	Bicuspid Aorta	

BMI: body mass index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, FISH: fluorescence in situ hybridization, SDS: standard deviation score

karyotyping from 128 Australians with GD and reported in a normal karyotype 117/128 (92.1%) of the patients, TS was not represented in their sample. So they suggested that molecular karyotyping has minimal clinical benefit in the routine management of GD. In another study, performed between 2000 and 2016 years in the Barcelona and Málaga (Spain); the G-banding techniques and high-resolution microarrays were performed from 444 male-to-females and 273 female-to-males and significantly higher Klinefelter syndrome frequency (1.13%) was detected but TS was not represented (15).

In another study, 80 adult women with TS were evaluated for the psychosocial and sexual function and it was concluded that most affected women report being heterosexual despite they are less likely than their peers to have sexual relationships and to be at an older age (16). Regarding these studies, Turner mosaicism has been reported to a neglectable extent in the wide range of sex dysphoria. Due to the absence of karyotyping and FISH analyses in most studies, patients who are low mosaic Turner's are missed. Although karyotype analysis were normal in our 2nd and 3rd patients, low mosaicism was detected in FISH analyses of these cases. Our case had a low percentage of a cases mosaicism on peripheral blood, they had clinical findings related with this karyotype. May this situation be explained by the ratio of mosaicism being different in other tissues? To obtain more certain knowledge additional studies in large series should be performed. The most interesting observation of us is that despite this sparsity of relationship between GD and TS, the cases reported in these series are the 3 GD cases who applied consequently during the last year to our pediatric endocrinology outpatient clinic and our 13 years experience about GD comprises of less than 10 cases!

Many of the mosaic patients may not have Turner stigmata and their heights may be normal. Patients may have normal puberty, menarche timing and even normal fertility (6,7). There are some correlations between karyotype and phenotype. TS patients with 45,X/46,XX or 45,X/47,XXX mosaic karyotype are presumably to have spontaneous menarche and fertility. TS patients with 45,X/46,XX karyotype are marginally taller than other patients with TS (17). Our patient's heights were all (although one of them slightly) above mean. All 3 patients had normal menarche time, and our patients 1 and 2 had menstrual irregularities. Polycystic ovary (PCO) image was available in pelvic USG. Few cases have been reported with mosaicism and polycystic ovary syndrome (PCOS) comorbidity (18). Approximately 22-33% of the general population show PCOs on pelvic ultrasound. Classic PCOS, on the other hand, is found in approximately 5-10% of the general population with its clinical and biochemical properties (19). In a study of patients with GD including 69 female-to-male transsexualism cases, 40 (58%) were found to have PCOS. Female-to-male transsexual patients have a high prevalence of PCOS and hyperandrogenaemia (20). In our patients, testosterone levels were slightly high. In another study, a significantly higher prevalence of PCO and PCOS in lesbians compared with heterosexual women was reported. Lesbian women with either PCO or PCOS had more prominent hyperandrogenism than heterosexual women with either PCO or PCOS (21). It was reported that women with PCOS have psychological gender identification problems. Severity and duration of PCOS can negatively affect the self-image of cases, cause a disturbed recognition with the female-gender scheme and related with it, social roles (22).

It was reported that due to the high frequency of chromosomal mosaicism in TS, this syndrome is an interesting model for evaluation the relation between nondisjunction and MTHFR gene polymorphisms. Santos et al. (23) reported increasing frequency of the MTHFR 677C > T homozygote alteration in patients with TS. According to our result, the case with 45,X/46,XX/47,XXX genotype had homozygote both MTHFR 677C > T and PAI variation. Importance of pericentromeric DNA methylation for chromosomal stabilization and segregation. They announced that the

MTHFR 677C > T homozygote alteration may be a contributing risk factor to somatic chromosomal non-disjunction (23). Also in our case 1, homozygous MTHFRC677T and 4G/5G variation in PAI were detected. Does the mosaic Turner karyotyping might have caused the homozygous MTHFRC677T variation that may be contributing risk factor to somatic chromosomal non-disjunction. To obtain more certain knowledge, additional studies including large series should be performed.

We offer interesting cases in this regard and we have mentioned in detail the patient's clinical profile. Even if these patients have normal phenotype, low percentage of Turner mosaicism should be considered. Early karyotype and FISH analysis in children with such cases can provide early detection of the mosaicism, thus more effective treatment strategy for the management of the disease can be performed. In clinically suspected cases, karyotype analysis should be confirmed with the FISH method even if it is normal.

Informed Consent: Written informed consent was obtained from the patient's parents for publication of this case report and the accompanying images.

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ERRATUM

doi: 10.18678/dtfd.748816

Yalçın A, Keleş H, Kahraman T, Bozkurt MF, Aydın H. Protective effects of ellagic acid against chemotherapy-induced hepatotoxicity. Duzce Med J. 2020;22(2):124-30.

The material and methods section of the abstract for the article has been corrected as following:

Material and Methods: Twenty-four Sprague-Dawley rats (180-220 gr) were separated into four equal groups. A single dose of 150 mg/kg CP was given intraperitoneally to generate hepatotoxicity. Different doses (50 and 75 mg/kg) of EA were administered orally 20 minutes before, 4 and 8 hours after CP administration. The histopathological evaluation of liver tissues and immunohistochemical evaluation for caspase-3 were conducted as well as the serum biochemical analyses.

Gereç ve Yöntemler: Yirmi dört adet Sprague-Dawley türü sıçan (180-220 gr) dört eşit gruba ayrıldı. Hepatotoksisite oluşturmak için intraperitonal olarak tek doz 150 mg/kg CP verildi. CP uygulamasından 20 dakika önce ve 4 ila 8 saat sonra oral yolla farklı dozlarda (50 ve 75 mg/kg) EA uygulandı. Serumun biyokimyasal analizlerinin yanı sıra karaciğer dokularının histopatolojik değerlendirmesi ve kaspaz-3 için immünohistokimyasal değerlendirme yapıldı.

AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of 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) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
- Compliance of the articles with the scientific and ethical rules is responsibility of authors.

SUBMISSION FILES

Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information and e-mail addresses of at least two reviewers suggested in relation to the subject of the article and not related to the authors and their institutions should be written. Editors' right to choose the reviewers are reserved.

Title Page: It must include the title of article (English and Turkish), short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the article has been presented previously in a scientific meeting; the name, date and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

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Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
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- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

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.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.


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