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İstanbul Tıp Fakültesi Dergisi

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Journal of Istanbul Faculty of Medicine
İstanbul Tıp Fakültesi Dergisi

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Abstract: An English and a Turkish abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. The abstract of Research articles should be structured with subheadings (Objective, Materials and Methods, Results, and Conclusion). Abstracts of Case Reports and Reviews should be unstructured. Please check Table 1 below for word count specifications.

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Manuscript types

Research articles: This is the most important type of article since it provides new information based on original research. The main text of research articles should be structured with Introduction, Material and Method, Results, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for research articles.

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INSTRUCTION TO AUTHORS

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Invited review articles: Invited reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. The invited reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The main text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Invited Review Articles.

Case reports: There is limited space for case reports in the journal and reports on rare cases or conditions that constitute challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not included in the literature, and interesting and educative case reports are accepted for publication. The text should include Introduction, Case Presentation, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Case Reports.

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and figure legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format)

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Research Article	3500	250 (Structured)	50	6	7 or tatal of 15 images
Invited Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1000	200	15	2	10 or total of 20 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	1	1

through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of research articles should be mentioned in the Discussion section before the conclusion paragraph.

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When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

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Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts accepted for publication, not published yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub ahead of print articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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CASE REPORT

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EDITORYAL

Dear Colleagues:

Welcome to the January 2022 issue of the Journal of Istanbul Faculty of Medicine-İstanbul Tıp Fakültesi Dergisi. Starting from this issue, we will publish the Journal of Istanbul Faculty of Medicine with a new logo, reflecting our Faculty's rich tradition and deep roots with the Journal. We express our gratitude to "Dr. Gökhan Canaz," who has generously gifted this new artwork, for his valuable and timeless contribution to our Journal.

As we complete a year filled with good news for our journal and start a new year, we are publishing our latest issue, hoping that the progressive trend of our journal will continue in the new year.

Although the past year was spent in the shadow of the pandemic declared by the World Health Organization in March 2020, we began publishing more articles in each issue. Consequently, our Journal's numbers of citations and readings have increased compared to previous years. Furthermore, the Journal of Istanbul Faculty of Medicine-İstanbul Tıp Fakültesi Dergisi was accepted for indexing by DOAJ, EBSCO, and SCOPUS. All these developments will increase our journal's international visibility and number of citations.

Our next goal is to be indexed by PubMed® and MEDLINE, which contains over 33 million citations for biomedical literature from life science journals and online books. We believe that the acceptance of our journal's applications for indexing by EBSCO, DOAJ, and SCOPUS will help us achieve this goal.

This issue consists of 17 original articles and four case reports, which we hope you will read with interest. At the beginning of these articles, there are those related to COVID-19, as is the case worldwide. Thereafter, clinical studies, laboratory studies, and community studies are listed.

Dear Readers, the online article submission system of the Journal of Istanbul Faculty of Medicine was changed in October 2020 and is now available through DergiPark. With this new system, it is simple to sign up and submit an article.

Please visit us online at iupress.istanbul.edu.tr/en/journal/jmed/home, and keep in touch with us by following us on Twitter@iutfd, Facebook İstanbul Tıp Fakültesi Dergisi, #istanbultıpfakültesidergisi, and Linkedin #jour nalofistanbulfacultyofmedicine.

I would like to wish you a happy and prosperous new year in 2022, and we look forward to receiving your valuable submissions. Thank you in advance for your contributions.

Sincerely,

Prof. Dr. Birsen Karaman
Prof. Dr. Ayşe Kubat Üzüm
Editors in Chief Journal of Istanbul Faculty of Medicine, JMED

İSTANBUL ÜNİVERSİTESİ YAYINEVİ

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.934417
İst Tıp Fak Derg 2022 / J Ist Faculty Med 2022

PATIENT CHARACTERISTICS AND RISK FACTORS FOR MORTALITY IN 504 HOSPITALIZED PATIENTS DUE TO COVID-19

COVID-19 NEDENİYLE HASTANEYE YATIRILMIŞ 504 HASTANIN ÖZELLİKLERİ VE MORTALİTE AÇISINDAN RİSK FAKTÖRLERİ

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Cite this article as: Medetalibeyoglu A, Ezirmik E, Senkal N, Bayrakdar S, Aktar I, Akas R, et al. Patient characteristics and risk factors for mortality in 504 patient characteristics and risk factors for mortality in 504 hospitalized patients due to COVID-19. J Ist Faculty Med 2022;85(1):1-8. doi: 10.26650/IUITFD.932128

ABSTRACT

Objective: In this study, we aimed to analyze the demographic characteristics, symptoms, and comorbidities of 504 patients hospitalized for COVID-19. We also sought to describe the relationship between these features and intensive care unit (ICU) admission and mortality.

Materials and Methods: This study is a descriptive study involving 504 COVID-19 patients hospitalized between 16.03.2020 and 07.05.2020 at Istanbul Universitys' Istanbul Faculty of Medicine Hospital. Information about the patients was obtained from the hospital automation system and evaluated retrospectively.

Results: The average age of the 504 patients was 56±15.14, and 59.1% of them were male. The proportion of the patients admitted into ICU 11.9% and for 8.52% of them the disease resulted in death. Real time polymerase chain reaction (RT-PCR) test results were positive for 60.5% of the patients. The median time spent in the hospital was eight days. Fifty six percent of the patients had at least one accompanying comorbid disease, with hypertension (39.3%) and diabetes (20.8%) being the most common. Being 65 years old or older (p<0.001), days spent in the hospital (p<0.001), presence of at least one comorbidity (p=0.009), hypertension (p=0.003), coronary artery disease (p=0.004), congestive heart failure (p=0.005) and dyspnea (p<0.001) were all factors found in those admitted to ICU.

ÖZET

Amaç: Bu araştırmada, COVID-19 nedeniyle tedavi almak üzere hastaneye yatırılan 504 hastanın demografik özellikleri, semptomları ve komorbiditeleri incelenerek; bu özelliklerin yoğun bakım ünitesine yatış ve mortalite ile ilişkisini ortaya koymak amaçlanmıştır.

Gereç ve Yöntem: Bu araştırma, 16.03.2020-07.05.2020 tarihleri arasında COVID-19 tedavisi almak üzere İstanbul Üniversitesi İstanbul Tıp Fakültesi Hastanesi'ne yatırılan 504 hastanın dahil edildiği tanımlayıcı tipte bir araştırmadır. Hastalara ait bilgiler hastane otomasyon sisteminden alınarak retrospektif olarak değerlendirilmiştir.

Bulgular: Beşyüz dört hastanın yaş ortalaması 56±15,14 yıl, hastaların %59,1'i erkekti. Yoğun bakım ünitesine yatışı olan hastaların oranı %11,9; ölen hastaların oranı %8,52 idi. Hastaların %60,5'inin test sonucu pozitifti. Hastanede kalınan sürenin ortancası sekiz gündü. Hastaların %56'sının en az bir komorbid hastalığı vardı; hipertansiyon (%39,3) ve diyabet (%20,8) en sık eşlik eden komorbiditelerdi. Altmış beş yaş ve üzeri olmak (p<0,001), hastanede kalınan gün sayısı (p<0,001), en az bir komorbidite varlığı (p=0,009), hipertansiyon (p=0,003), koroner arter hastalığı (p=0,004), konjestif kalp yetmezliği (p=0,005) ve dispne (p<0,001), yoğun bakıma yatış ile ilişkili bulunmuştur.

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Conclusion: COVID-19 infection leading to high morbidity-mortality rates and an increased requirement for ICU admission is mainly seen among older patients and those who have dyspnea. During the process of analyzing patients suspected of COVID-19 who are admitted to hospital, it is crucial to consider both the patient's age and any respiratory symptoms. Such a clinical evaluation is crucial for a better understanding of the course of the disease.

Keywords: COVID-19, mortality, admission to intensive care unit, COVID-19 risk factors

Sonuç: Yüksek morbidite-mortalite oranlarına ve yoğun bakım ünitesine yatış ihtiyacının artmasına neden COVID-19, özellikle yaşlı hastalarda ve dispnesi olan hastalarda daha yüksek mortalite oranlarına neden olmaktadır. Hastaneye başvuran COVID-19 şüpheli hastalar değerlendirilirken özellikle hastanın yaşı ve solunum sistemi semptomları göz önünde bulundurularak klinik değerlendirilmesinin yapılması hastalığın seyri açısından önem taşımaktadır.

Anahtar Kelimeler: COVID-19, mortalite, yoğun bakım ünitesine kabul, COVID-19 risk faktörü

INTRODUCTION

The World Health Organization (WHO) declared the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak to be a pandemic on 11 March 2020 due to a continuous increase in the number of cases outside China (1). In Turkey, the first COVID-19 case was identified on the same date (2). The COVID-19 pandemic caused by the SARS-CoV-2 virus leads to high morbidity and mortality rates, long hospitalization , and increased necessity for ICU admissions. (3, 4). There were 138,411,980 confirmed COVID-19 cases and 2,974,642 deaths worldwide as of 15 April 2021 (5). There were 4,086,957 confirmed COVID-19 cases and 35,031 deaths in Turkey up until the same date (6). COVID-19 fatality rate demonstrates variability depending on countries and regions and it ranges between 0-26.8% (7-13).

The most common symptoms of COVID-19 infection are fever, cough, fatigue, malaise, shortness of breath, and myalgia (7, 8, 14). Hypertension, diabetes, chronic heart disease, and chronic obstructive pulmonary disease (COPD) are the most common comorbidities accompanying COVID-19 (7, 15).

When the factors associated with COVID-19 mortality were analyzed, older age, male gender, hypertension, and the presence of diabetes were found to significantly correlate with mortality (7, 16). Pneumonia, kidney damage, shock status, heart failure, and acute respiratory distress syndrome (ARDS) constitute the main clinical factors affecting COVID-19 mortality. In most patients laboratory parameters associated with COVID-19 mortality were reported as follows: leukocytosis, neutrophilia, elevated levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST), creatinine, lactate dehydrogenase (LDH), procalcitonin, C-reactive protein (CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP), ferritin, D-dimer, lymphopenia, thrombocytopenia, along with decreased levels of albumin (7, 8, 17, 18).

In this study, we aimed to analyze the demographic characteristics, symptoms, and comorbidities of 504 patients hospitalized for COVID-19 . We also sought to describe the relationship between these features and intensive care unit (ICU) admission and mortality.

MATERIALS AND METHODS

The present descriptive study was approved by Istanbul Universitys' Istanbul Faculty of Medicine Clinical Research Ethics Committee, and written informed consent was waived (Date: 11.05.2020, No: 81246). We evaluated adult patients who were suspected of having COVID-19 and were hospitalized between 16.03.2020 and 07.05.2020. Patients who died or were discharged were removed from our study. We excluded patients who had missing data yet were still hospitalized. We collected the data according to age, gender, smoking habits, symptoms (fever, dry cough, dyspnea, fatigue, sputum, nausea, diarrhea, anosmia), contact history, PCR results, days spent in hospital, comorbid diseases (hypertension, diabetes, coronary artery disease, chronic obstructive pulmonary disease, congestive heart failure, malignancy) and patient outcome. We obtained the information from the hospital automation system. We investigated the effect of the baseline characteristics of the patients on ICU admission and death.

Continuous variables were presented as median (interquartile range) or mean±standard deviation, according to whether the distribution was normal or not. Categorical variables were presented as percentages. The compliance of the numerical values to the normal distribution was examined using histograms or analytic methods (Kolmogorov-Smirnov/Shapiro Wilk test). The student t-test, Mann-Whitney U test, Chi-square test, or Fisher's exact test (when chi-square test assumption does not hold due to low expected cell counts) were used to compare the differences between the groups. Univariable and multivariable logistic regression models were used to investigate the risk factors associated with in-hospital death. Considering the total number of deaths (n=43) in our study and to avoid overfitting in the model, four variables were chosen for multivariable analysis based on previous findings and clinical constraints. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. An overall p-value of less than 0.05 was considered to show a statistically significant result. Statistical analyses were performed using the SPSS software v22 (IBM Inc, USA).

RESULTS

We analyzed the data of 504 patients who remained in the study after excluding 93 of 597 patients hospitalized between 16.03.2020 and 07.05.2020. The mean age was 56±15.14 years, ranging from 20 to 98 years, and most of the patients were male (59.1%). Sixty patients (11.9%) were admitted to ICU and 43 patients (8.5%) died. The percentage of positive PCR results was 60.5%. The median time from the onset of symptoms to hospitalization was five days (3-7). The median time spent in hospital was eight days (5-11). The most common symptoms were fatigue (91.1%), dry cough (82.3%) and fever (70.6%), less common symptoms were dyspnea (42.5%), nausea (16.7%), diarrhea (12.1%), anosmia (7.9%) and sputum (3%). The percentage of people with at least one comorbid disease was 56%. Hypertension, the most common comorbid disease, was seen in 197 patients (39.3%), followed by diabetes in 105 patients (20.8%) (Table 1).

Table 1: COVID-19 patients' (ICU patients and non-ICU patients) baseline demographic and clinical characteristics

Characteristics	n=504 (%)
Age, median (IQR)	56 (46-67)
Age ≥65 years	143 (28.4%)
Gender	
Female	206 (40.9%)
Male	298 (59.1%)
RT-PCR positive	305 (60.5%)
Exposure history	172 (34.1%)
Smoker	62 (12.3%)
Symptoms	
Fever	356 (70.6%)
Cough	415 (82.3%)
Dispnea	214 (42.5%)
Fatigue	459 (91.1%)
Sputum	15 (3%)
Nausea	84 (16.7%)
Diarhea	61 (12.1%)
Anosmia	40 (7.9%)
Comorbidities	282 (56%)
Hypertension	198 (39.3%)
Diabetes	105 (20.8%)
COPD	59 (11.7%)
Coronary artery disease	55 (10.9%)
Congestive heart failure	30 (6%)
Malignancy	43 (8.5%)

ICU: Intensive care unit, IQR: Interquartile range, RT-PCR: Real time polymerase chain reaction, COPD: Chronic obstructive pulmonary disease

We found that older age (≥65 years) was associated with ICU admission (p<0.001). In addition, days spent in hospital (p<0.001), at least one comorbidity (p=0.009), hypertension (p=0.003), coronary artery disease (p=0.004), congestive heart disease (p=0.005), and dyspnea (p<0.001) were found to be statistically significant (Table 2). We also found that older age (≥65 years) (p=0.001), days spent in hospital (p=0.001), ICU admission (p<0.001), at least one comorbidity (p=0.011), hypertension (p=0.020), coronary artery disease (p=0.017) and dyspnea (p<0.001) were significantly associated with death. In contrast, the connection between patient mortality and fever (p=0.026) and dry cough (p=0.024) was statistically less significant (Table 3).

In univariate analysis, the odds of in-hospital death were higher in patients with hypertension (p=0.023) or coronary artery disease (p=0.002). Age, days spent in hospital, ICU admission, and dyspnea were also associated with death. In multivariate analysis, older age and presence of dyspnea were associated with increased likelihood of death. There was no significant association between gender and ICU admission or mortality. Moreover , there was no significant association between PCR results and ICU admission or mortality (Table 4).

DISCUSSION

COVID-19 affects elderly patients more seriously by causing more severe clinical symptoms and higher mortality rates (19, 20). COVID-19 case-fatality rate increases rapidly with age. While the case fatality rate is 3.6% of cases in those aged between 60 and 69, it rises to 8% in patients between 70 and 79 years and to 14.8% in cases aged 80 years or older (20). In the first case series reported from China, the proportion of the elderly patients among all patients was 15.1% (21). In Turkey, the proportion of patients aged 65 or older is 11%. However, 73% of overall deaths were in this age group (22).

In this study, patients aged 65 or older form 67.4% of the deaths and 24.7% of the discharged patients (p<0.001). In univariate analysis, being 65 years or older was found to be a factor that increases mortality risk by 6.3 times (95% CI 3.2-12.3; p<0.001). It was calculated that being 65 years or older increases the mortality risk by 4.94 times in multivariate analysis (95% CI 2.31-10.58; p<0.001). It has also been suggested in many previous studies that older age is an important risk factor for COVID-19 mortality (13, 23-29). Being 65 years or older has been stated as a risk factor for ICU admission and this outcome is in keeping with the literature (4, 29, 30).

Table 2: Baseline demographic and clinical characteristics of COVID-19 patients according to ICU admission rates

Characteristics	Total (n=504)	ICU - (n=444)	ICU + (n=60)	p value
Age, mean (SD)	56.41±15.14	55.02±14.76	66.71±13.94	**<0.001**
Age ≥65 years	143 (28.4%)	108 (24.3%)	35 (58.3%)	**<0.001**
Gender (male)	298 (59.1%)	257 (57.9%)	41 (68.3%)	0.122
RT-PCR positive	305 (60.5%)	266 (59.9%)	39 (65%)	0.449
Exposure history	172 (34.1%)	160 (36%)	12 (20%)	**0.014**
Smoker	62 (12.3%)	57 (12.8%)	5 (8.3%)	0.319
Days from symptom onset to hospitalization, median (IQR)	5 (3-7)	5 (3-7)	5 (3-7)	0.916
Days in hospital, median (IQR)	8 (5-11)	7 (5-10)	14.5 (9-21)	**<0.001**
Comorbidities	282 (56%)	239 (53.8%)	43 (71.7%)	**0.009**
Hypertension	198 (39.3%)	164 (36.9%)	34 (56.7%)	**0.003**
Diabetes	105 (20.8%)	165 (69.6%)	13 (21.7%)	0.866
COPD	59 (11.7%)	48 (10.8%)	11 (18.3%)	0.089
Coronary artery disease	55 (10.9%)	42 (9.5%)	13 (21.7%)	**0.004**
Congestive heart failure	30 (6%)	21 (4.7%)	9 (15%)	**0.005**
Malignancy	43 (8.5%)	38 (8.6%)	5 (8.3%)	0.953
Symptoms				
Fever	356 (70.6%)	317 (71.4%)	39 (65%)	0.307
Dry cough	415 (82.3%)	367 (82.7%)	48 (80%)	0.612
Dyspnea	214 (42.5%)	165 (37.2%)	49 (81.7%)	**<0.001**
Fatigue	459 (91.1%)	405 (91.2%)	54 (90%)	0.756
Sputum	15 (3%)	14 (3.2%)	1 (1.7%)	1
Nausea	84 (16.7%)	78 (17.6%)	6 (10%)	0.140
Diarrhea	61 (12.1%)	57 (12.8%)	4 (6.7%)	0.169
Anosmia	40 (7.9%)	38 (8.6%)	2 (3.3%)	0.207

ICU: Intensive care unit, SD: Standard deviation, IQR: Interquartile range, RT-PCR: Real time polymerase chain reaction, COPD: Chronic obstructive pulmonary disease

The overall percentage of patients who had at least one comorbidity (hypertension (39.3%) and diabetes (20.8%) being the most prevalent) was found to be 56%. The results suggest that the presence of comorbidity strongly correlates with ICU admission and mortality (p=0.009, p=0.011). On ICU admission, hypertension, coronary artery disease and congestive heart failure (p=0.003, p=0.004, p=0.005) and on mortality, hypertension and coronary artery disease (p=0.020, p=0.017) were found to be gravely significant comorbidities. However, in multivariate analysis, there was no significant statistical correlation found between these comorbidities and mortality. Similarly, in the previous studies the most common comorbidities accompanying COVID-19 were found as follows: hypertension, diabetes, coronary artery disease (26, 31, 32). The presence of multiple comorbidities was described as the most important risk factor for severe disease progression and mortality (25, 31, 32). In a study conducted on patients aged 60 or older, the case fatality rate was found to be 1.4% in cases with no comorbidities. However, it was 13.2% in patients with cardiovascular disease, 9.2% in those with diabetes, 8.4% in those with hypertension, 8% in cases with chronic lung disease, and 7.6% in those with cancer (25). A greater severity of pneumonia, enzyme release related to tissue damage, inflammatory responses, and dysregulations of glucose metabolism were only observed in COVID-19 patients who had diabetes (33). According to multivariate analysis

Table 3: Baseline demographic and clinical characteristics of the patients who were discharged and who died due to COVID-19

Characteristics	Total (n=504)	Discharged (n=461)	Died (n=43)	p value
Age, mean (SD)	56.41±15.14	55.19±14.69	69.53±13.69	**<0.001**
Age ≥65 years	143 (28.4%)	114 (24.7%)	29 (67.4%)	**<0.001**
Gender (male)	298 (59.1%)	270 (58.6%)	28 (65.1%)	0.404
RT-PCR positive	305 (60.5%)	278 (60.3%)	27 (62.8%)	0.750
Exposure history	172 (34.1%)	160 (35.1%)	10(23.3%)	0.116
Smoker	62 (12.3%)	56 (12.1%)	6 (14%)	0.730
Days from symptom onset to hospitalized, median (IQR)	5 (3-7)	5 (3-7)	5 (3-7)	0.202
Days in hospital, median (IQR)	8 (5-11)	7 (5-10)	12 (7-17)	**0.001**
ICU admission	60 (11.9%)	24 (5.2%)	36 (83.7%)	**<0.001**
Comorbidities	282 (56%)	250 (54.2%)	32 (74.4%)	**0.011**
Hypertension	198 (39.3%)	174 (37.7%)	24 (55.8%)	**0.020**
Diabetes	105 (20.8%)	97 (21%)	8 (18.6%)	0.707
COPD	59 (11.7%)	53 (11.5%)	6 (14%)	0.632
Coronary artery disease	55 (10.9%)	45 (9.8%)	10 (23.3%)	**0.017**
Congestive heart failure	30 (6%)	25 (5.4%)	5 (11.6%)	0.165
Malignancy	43 (8.5%)	37 (8%)	6 (14%)	0.246
Symptoms				
Fever	356 (70.6%)	332 (72%)	24 (55.8%)	**0.026**
Dry cough	415 (82.3%)	385 (83.5%)	30 (69.8%)	**0.024**
Dispnea	214 (42.5%)	177 (38.4%)	37 (86%)	**<0.001**
Fatigue	459 (91.1%)	421 (91.3%)	38 (88.4%)	0.572
Sputum	15 (3%)	15 (3.3%)	0	0.629
Nausea	84 (16.7%)	78 (16.9%)	6 (14%)	0.618
Diarrhea	61 (12.1%)	57 (12.4%)	4 (9.3%)	0.556
Anosmia	40 (7.9%)	39 (8.5%)	1 (2.3%)	0.236

ICU: Intensive care unit, SD: Standard deviation, IQR: Interquartile range, RT-PCR: Real time polymerase chain reaction, COPD: Chronic obstructive pulmonary disease

results from another study from Malesia, while diabetes in COVID-19 patients increased the risk of mortality by 12.2, having hypertension increased mortality by 3.5 (34).

According to this study, there was no significant difference in terms of gender among the ICU and non-ICU patient groups (p=0.122). There was also no significant difference between the deceasead and surviving patients groups in terms of gender (p=0.404). However, this outcome shows consistency with other study results suggesting that gender is a risk factor for COVID-19 mortality and severe COVID-19 infection (23, 35, 36). On the other hand, multiple studies reported that males are at greater risk of severe disease progression and ICU admission due to COVID-19 infection (7, 29, 34, 37). Men carry an increased risk of severe COVID-19 infection by 1.73 times (95% CI 1.50-2.01) (37). The fact that the male gender was not found to be a significant risk factor was considered to be potentially related to the lack of homogeneity in gender distribution or an inadequate number of patients included in the study.

Our study concluded that smoking is not a notable risk factor for ICU admission due to COVID-19 and COVID-19 mortality (p=0.319, p=0.730). There are complementary results with this outcome in other studies (28, 38, 39).

Table 4: Logistic regression analysis results associated with COVID-19 mortality

	Univariable OR (95% CI)	p value	Multivariable OR (95% CI)	p value
Demographics and characteristics				
Age	1.068 (1.044-1.093)	<0.001		
Age ≥65 years	6.305 (3.220-12.347)	<0.001	4.946 (2.311-10.582)	**<0.001**
Days in hospital	1.079 (1.035-1.126)	<0.001		
ICU admission	93.642 (37.772-232.158)	<0.001		
Comorbidities	2.455 (1.208-4.990)	0.013		
Hypertension	2.083 (1.109-3.914)	0.023	0.975 (0.444-2.143)	0.950
Coronary artery disease	4.467 (1.764-11.307)	0.002	0.906 (0.357-2.302)	0.836
Fever	0.491 (0.260-0.926)	0.028		
Cough	0.456 (0.227-0.913)	0.027		
Dyspnea	9.895 (4.093-23.921)	<0.001	7.944 (3.206-19.668)	**<0.001**

ICU: Intensive care unit, OR: Odds ratio, CI: Confidence interval

In this study, the average number of days spent in hospital was eight. In terms of length of hospitalization a statistically significant difference was found between patients who were admitted into ICU and those who were not. Patients who were admitted into ICU had longer hospitalization times (7 days; 14.5 days, p<0.001). In univariate analysis, the number of days spent in hospital was found to correspond with mortality (95% CI 1.035-1.126). Likewise, in other studies, it was reported that patients with severe COVID-19 infection spent more days in hospital (p<0.001) (40). Among the patients admitted to hospital, the average number of days spent there were four days for patients treated on COVID-19 wards, eight days for patients treated in ICU but who did not need invasive mechanical ventilation (IMV), and 15 days for patients treated in ICU who needed IMV (41).

The presence of dyspnea was found to be a risk factor for both ICU admission due to COVID-19 and COVID-19 mortality (p<0.001, p<0.001). According to the multivariate analysis results, dyspnea escalates the likelihood of multivariate mortality by 7.9 times (95% CI 3.2-19.6; p<0.001). Furthermore, as stated in another study, dyspnea raises the risk of mortality in multivariate patients by 4.52 times. (95% CI 3.15-6.48; p<0.001) (42).

COVID-19 infection leading to high morbidity-mortality rates and an increased requirement for ICU admission also causes higher mortality rates among older patients and those with dyspnea. While analyzing patients suspected of having COVID-19 who are admitted to hospital, it is crucial for an understanding of the course of the disease to consider the patient's age and any respiratory symptoms during the clinical evaluation.

The limitations of the study

Several limitations of this study should be noted. First of all, it is a single-centered study with a limited number of patients. Secondly, baseline demographic and clinical features of the patients were obtained from the hospital automation system. Moreover, since the laboratory results of the patients were not included, it could not be evaluated whether these parameters are effective on ICU admission and mortality. Although all these factors constitute the limitations of this study, the fact that there was no lack of data, that the study included a considerable number of patients based on the dates given, and that certain risk factors associated with COVID-19 mortality were predicted are major strengths of this study.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 11.05.2020, No: 81246).

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İSTANBUL ÜNİVERSİTESİ YAYINEVİ

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AN INTEGRATED CARE MODEL BASED ON HOSPITAL AND HOME DURING THE COVID-19 PANDEMIC: TELEHEALTH

COVID-19 PANDEMİSİNDE HASTANE VE EVDE KONTROLLÜ HASTA YÖNETİMİ SAĞLAYAN ENTEGRE BİR MODEL: TELESAĞLIK

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ABSTRACT

Objective: In this study, we aimed to present the details of a successfully implemented telehealth model in a university hospital during the COVID-19 pandemic.

Materials and Methods: Istanbul Faculty of Medicine is a university hospital where the first confirmed case of COVID-19 in Turkey was detected. In IFM, patients who were diagnosed with COVID-19 and received outpatient or inpatient treatment were followed up by telehealth for 21-28 days after leaving the hospital. The distinguishing features of this service are the provision of remote outpatient clinical monitoring personally by physicians and the use of web-based IP information technologies.

Results: Between March 15 and July 1, 2020, 1,042 individuals were followed up at least once, 860 patients for 21 days or more by the 26 physicians providing the telehealth service. A total of 11,736 calls were made by the physicians and 7,342 of those calls were answered and a total of 1,086 calls were made by patients. The median number of calls per patient was 4 (1-23). The median duration of the completed calls was 2.8 min (<1–50 min). During these follow-ups patients were informed about the importance of isolation. Most of the patients expressed their satisfaction with these follow-ups by thanking the calling physician.

Conclusion: In a pandemic such as COVID-19, telehealth services may increase adherence to treatment and isolation precautions among patients with diseases that require follow-up without hospitalization after diagnosis. Telehealth will facilitate early recognition of symptoms that may require hospitalization, ensuring these patients receive the care they need. Therefore, this approach should be widely adopted.

Keywords: COVID-19, telehealth, telemedicine, Turkey

ÖZET

Amaç: Bu makalede bir üniversite hastanesinde COVID-19 pandemisi sırasında başarıyla uygulanmış olan telesağlık modelinin anlatılması amaçlanmıştır.

Gereç ve Yöntem: İstanbul Tıp Fakültesi (İTF), Türkiye'de ilk doğrulanmış COVID-19 vakasının tespit edildiği bir üniversite hastanesidir. İTF'de COVID-19 tanısı almış ve ayaktan veya yatarak tedavisi düzenlenen hastaların hastaneden ayrılışından itibaren 21-28 günlük bir telesağlık izlemi yapılmıştır. Bu hizmetin örneklerinden farkı bizzat hekim tarafından uzaktan poliklinik izlemi olarak sunulmasıdır ve web-tabanlı, IP bilgi-iletişim teknolojisi kullanılmıştır.

Bulgular: 15 Mart-1 Temmuz 2020 tarihleri arasında tele-sağlık hizmeti ile 1.042 kişinin en az bir izlemi yapılmış olup 860 hastanın ise 21 gün ve üzerinde izlemi 26 hekim tarafından gerçekleştirilmiştir. Hekimler tarafından bu süreçte toplam 11.736 çağrı yapılmış ve bu çağrıların 7.342'si cevaplanmıştır. Ayrıca hastalar tarafından da toplam 1.086 arama yapılmıştır. Hasta başına medyan arama sayısı 4 (1-23) ve tamamlanan aramaların medyan süresi 2,8 dakika olarak saptanmıştır (<1-50 dakika). Bu izlemlerde hastalar izolasyonun önemi hakkında da bilgilendirilmiştir. Hastaların çoğu bu takiplerden memnuniyetini arayan hekime teşekkür ederek ifade ettiler.

Sonuç: COVID-19 gibi bir pandemide telesağlık hizmetinin tanı sonrası hastaneye yatış olmaksızın takip edilebilecek hastalarda tedavi ve izolasyon önlemlerine uyumu arttıracağı söylenebilir. Telesağlık, hastaneye yatış gerektirebilecek semptomları erken fark edip bu hastaların ihtiyaç duydukları bakımı almalarını sağlayacaktır. Tüm bu durumlar göz önüne alındığında bu yaklaşım daha yaygın olarak benimsenmelidir.

Anahtar Kelimeler: COVID-19, tele-sağlık, teletıp, Türkiye

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INTRODUCTION

Since appearing in China, the COVID-19 pandemic has affected millions of people worldwide, including people in Turkey. It seems that it will continue to do so until an effective treatment or vaccine is discovered. Since antiquity, humanity has faced many pandemics caused by infectious agents. In this and coming centuries, we may face more pandemics due to zoonotic diseases caused by existing or novel agents.

On the other hand, our technological age is opening doors to new opportunities in the fight against microorganisms, such as telehealth. Telehealth, or e-health, is the use of information and communication technologies for the diagnosis and treatment of medical conditions, as well as for health promotion and disease prevention (1). These systems encompass a wide spectrum of applications, from obtaining information from healthcare professionals to remote patient monitoring by having patients measure various health parameters such as blood pressure, oxygen saturation, pulse, temperature, and blood glucose in their homes and sending the data to healthcare professionals (2). The use of telehealth in healthcare services reduces the risk of healthcare associated infections in older and chronically ill patients due to repeated follow-up appointments and prevents unnecessary hospital/emergency admissions by people who are healthy or have mild infections (3).

The use of data communication technologies in the delivery of healthcare services is increasing, and successful examples of their use in infectious diseases have been reported in the last 10 years. Telehealth services have also been used to perform contact tracing during the Ebola epidemic and to provide consultation in SARS and influenza epidemics (4).

During the COVID-19 pandemic, it was determined that disease transmission occurs primarily through contact and droplets and that asymptomatic individuals also play a role in transmission (5, 6). This demonstrates the need to first reduce contact number and duration, followed by the use of personal protective equipment, waste management, and hygiene measures (7). Telehealth is ideally suited to these features of the pandemic and as a result, delivery of telehealth services has been increased worldwide as well as in Turkey during the pandemic. In Turkey, the use of digital systems in health began with a national action plan developed in the early 2000s (8). Since then they have been widely used, especially in the field of imaging. For the COVID-19 pandemic, the Ministry of Health (MoH) provided patient and contact tracing using two electronic monitoring systems, the Public Health Management System (PHMS) and Contact and Isolation Tracing System. All possible/confirmed COVID-19 patients were recorded in the PHMS by hospitals which provided diagnostic and treatment services and using these records, primary health care professionals were able to identify patients' close contacts to ensure isolation and generate a transmission tree.

The goal in COVID-19 pandemic management in Turkey was to prevent the demand for healthcare services to exceed the capacity of the healthcare system (9). As for the hospitals that would treat COVID-19 patients, those with at least two specialists each in the fields of Infectious Diseases and Clinical Microbiology, Pulmonary Diseases, and Internal Medicine and with level three adult intensive care beds were designated as "the pandemic hospitals" (10). Which services would be provided in primary care and at pandemic hospitals were organized based on the COVID-19 guidelines updated regularly by the MoH and used since the start of the pandemic. In the management of patients with suspected or confirmed COVID-19, patients who are discharged from hospital after treatment or who are treated at home have been followed up by primary family physicians and district health directorates until their isolation period is completed. Moreover, tracing, isolation, and screening of these patients' contacts and their follow-up until completing their isolation periods are conducted by district health directorates (11).

In the pandemic hospitals, patients with suspected COVID-19 undergo diagnostic tests and treatment is planned for those whose diagnosis is confirmed. Patient management is based on the MoH treatment algorithm. The guideline recommends inpatient treatment for patients with tachypnea (>24 beats/min), oxygen saturation <93% in room air, imaging findings of bilateral pneumonia, and signs of poor prognosis in blood tests (lymphocyte <800/μL or C-reactive protein >10 or ferritin >500 ng/mL or D-dimer >1000 ng/mL) and outpatient care for patients with uncomplicated/mild disease (12).

Istanbul Faculty of Medicine and the pandemic

Istanbul Faculty of Medicine (IFM) is a university hospital where the first confirmed case of COVID-19 in Turkey was detected. With the interventions conducted and pioneered during the pandemic, it has become one of the leading institutions in Turkey for hospital-level pandemic management. Following the first case detected at IFM on March 11, 2020, a total of 1,572 cases were diagnosed and treated as COVID-19 till June (13). In terms of disease management, patients diagnosed as having COVID-19 were either treated as inpatients or outpatients based on their clinical presentation and test results. The most important way to reduce the transmission of COVID-19 is to limit contact (7). Accordingly, in an application not previously used in Turkey, our hospital used digital communication tools for patient management and follow-up for 28 days after discharge or home treatment. These patients still continue to be monitored quarterly in a dedicated

COVID follow-up outpatient clinic established in our hospital. In this study, we aimed to present the details of a successfully implemented telehealth model in the university hospital during the COVID-19 pandemic.

MATERIALS AND METHODS

This article describes the organizational processes of the telehealth service model applied in the COVID-19 pandemic in a university hospital.

Telehealth at IFM

The telehealth service at IFM was planned to perform outpatient clinic follow-up of patients who presented to IFM and were subsequently treated at home and after discharge for patients who were hospitalized and treated in IFM, provided by the physician using web-based, IP information/communication technology. The aim of this service was to prevent unnecessary hospital admissions by maintaining continuity of care for confirmed COVID-19 patients, as well as avoid delays in case they required admission to the emergency department.

Telehealth implementation

Preparation phase

The process of implementing telehealth at IFM started in March 2020 and was coordinated and managed by the Department of Public Health. First, a management team was established, and leader and assistant faculty members were identified to be in charge of the system. A coordinator from the information technology unit was designated for the telehealth system infrastructure requirements. The leader and assistant faculty members prepared the COVID-19 follow-up protocol, the history and follow-up forms, and trained the physicians who would be delivering telehealth services, prior to its implementation. While creating the history form and determining follow-up duration, a faculty member from the Department of Infectious Diseases and Clinical Microbiology was consulted and the guidelines published by the Ministry of Health were considered. The participating physicians were trained on using the system, communication skills, and the algorithm to apply. Resident physicians (n=26) were appointed under the supervision of faculty member physicians from the department (n=5). This task-sharing aimed to facilitate rapid feedback about patients' potential health problems in daily follow-up, the generation of daily reports for each followed patient at the end of the day, and process evaluation. This process worked almost like online daily round visits. Two pulmonologists in the team undertook responsibility for intervening in cases requiring consultation. Considering the patients' ages and internet access, the decision was made to provide the service via a web-based phone system to make the system inclusive of all patients. The preparation, implementation, and evaluation of telehealth management were performed according to the administrative process shown in Table 1.

Implementation

The algorithm shown in Figure 1 was used to ensure the standardization of telehealth calls. When using the history and follow-up form, in the first interview the patients were asked about their sociodemographic characteristics, habits, physician-diagnosed chronic diseases, regularly used medicines, and contact and travel history. In subsequent calls, the physicians questioned the patients about their symptoms and compliance to isolation measures and provided information about their health status and isolation (14).

To enrol in the telehealth system, the patients were first informed that the follow-up team would contact them after they had left the hospital, and their written consent was obtained. The contact information and electronic health records of consenting patients were reported to the home follow-up coordinator in accordance with the

Table 1: Administrative process

- Establishing a telehealth management team
- Determining the scope of the provided healthcare services
- Creating history and follow-up forms to be used while providing the healthcare service
- Identifying and training the team of service providers
- Installing software on physicians' computers for use of the web-based phone system
- Obtaining patients' verbal consent to be included in the service at time of hospital presentation
- Ensuring patients are called from an institutional line
- Ensuring patients are called by the same physician
- Not imposing any additional costs to patients
- Providing consultation if patients need information from specialists related to the disease
- Providing patients the telephone numbers of physicians to address social and psychological impacts
- Reporting and evaluating daily patient follow-ups
- Organizing periodic evaluation meetings of the telehealth follow-up team
- Preparing weekly reports and informing the hospital administration about the process and cases
- Increasing adherence to isolation precautions and treatment among COVID-19 patients

Personal Data Protection Law and the Patients' Rights Act. A pool of patients was generated, and a physician was appointed to follow-up each patient for 21-28 days. A follow-up frequency was determined based on the presence and severity of active symptoms. Older patients, patients with comorbid diseases, and those with ongoing active symptoms were followed up daily, while patients who were in a stable condition and those with no active symptoms and in good general condition were followed up weekly.

Evaluation

After the resident physician followed up the COVID-19 patients receiving the telehealth service, they prepared a summary report at the end of the day to present to the supervising physician. The supervising physician monitored the process by providing daily feedback to the residents specific to each patient. Moreover, patient data was collected in a common file without including patient names and was presented weekly to hospital administration to facilitate the process management. In addition to basic descriptive information, these reports included information such as weekly changes in the patients' symptoms.

RESULTS

During the telehealth calls, the "patient follow-up algorithm" was used and the patients were followed by using the "history and follow up forms" (Figure 1). Between

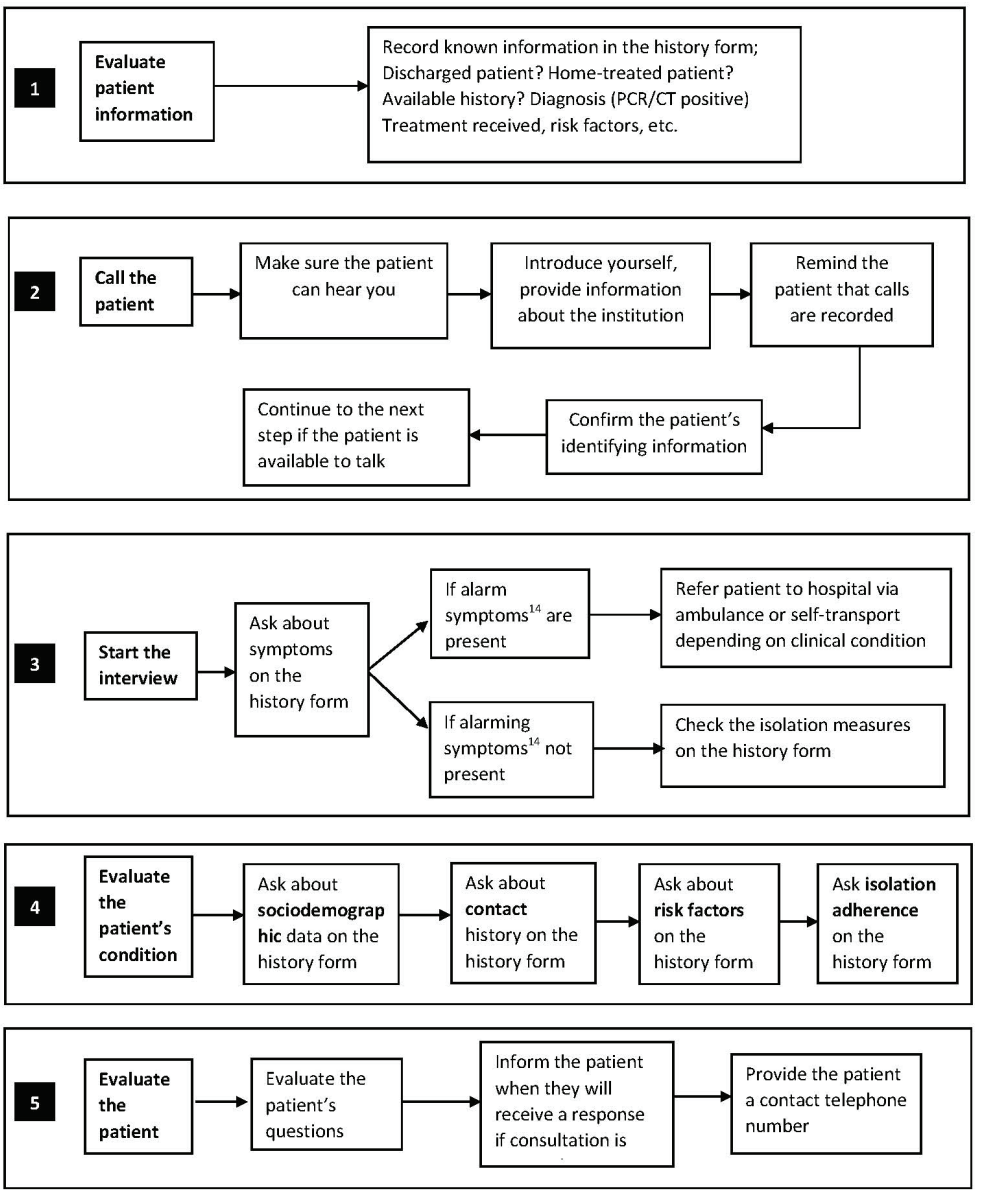

Figure 1: Telehealth patient follow-up algorithm

March 15 and July 1, 2020, a total of 1,207 individuals were enrolled in follow-up and 1,042 individuals were followed up at least once by the 26 physicians providing the telehealth service. Of the 165 individuals not followed up, 109 could not be reached by phone number they provided, 40 could not be followed for reasons such as hospitalization, and 16 declined to participate despite having initially given verbal consent. Of these 1,042 patients, there were 860 patients who have been followed up for 21 days or more. Of the 182 patients who were followed up for less than 21 days 36 were hospitalized, 32 declined to continue the follow-up, 4 died, and 110 left the follow-up early due to other reasons related to the patient.

A total of 11,736 calls were made by the physicians and 7,342 of those calls were answered. The number of calls per patient was 4 (1-23). The median duration of the completed calls was 2.8 min (<1–50 min). In addition, the patients were also able to call the physician, and a total of 1,086 such calls were made by 302 patients.

The scope of the telehealth service included medical care, diagnosis, consultation, and treatment, as well as health education and providing patients with medical information they wanted to learn about. In this regard, in addition to recording the patients' histories and monitoring their symptoms and isolation, the patients were also educated about the drugs they were using to treat COVID-19. When required, coordination was established between the patient and primary care physician. To address social needs, communication was established between the patients and teams volunteering in their areas. Patients requiring psychological support were referred to a free psychological counselling service provided by Provincial Directorate of Health. During these follow-ups, the patients asked questions such as when their isolation would end and how they would understand if they had recovered, and they asked for help learning their PCR test results. In particular, patients were informed about the importance of isolation and what precautions to take in crowded families. Most of the patients expressed their satisfaction with these follow-ups by thanking the calling physician. For instance, a physician made the following report about a patient with mild symptoms who was scheduled for less frequent calls: *"...they were very happy to be called, they said they wanted to be called every day, as if they were bored and overwhelmed. I am planning to call them tomorrow, too, and ask how they are doing."*

DISCUSSION

During a pandemic, rapid increases in patient numbers and emergency admissions are a threat to health systems and, most importantly, to healthcare providers. The guidance provided remotely by professionals in telehealth services is important because it prevents unnecessary emergency admissions and thereby reduces the patient burden. On the other hand, it will reduce the infection risk to healthcare professionals as well as their patient load (3, 15, 16). Thus, in situations such as the COVID-19 pandemic, it can be used successfully in hospital and home-based service delivery models.

The fact that the telehealth service provided by our hospital was free of charge was an advantage that increased patient adherence. No payment was made to the service providers. This decreased the motivation of the participating resident physicians during the process. However, in many countries, healthcare delivered by telehealth services has been or is being integrated into remuneration systems using different approaches (17, 18). In our country and many other countries without this integration, including telehealth services in the health insurance reimbursement system may be a solution to this problem (3, 15).

In the telehealth system, the patient's active participation in the process is important. Health data obtained from patients are recorded solely on the basis of self-report. Ensuring that measurements such as blood glucose, temperature, and blood pressure made by the patients are seen by the physician via an integrated system will facilitate objective decision-making (3, 15). Disease duration is long in chronic conditions. Therefore, patients can self-monitor via simple measurements such as blood pressure and blood glucose levels, and this information can be used in telehealth. In acute diseases, however, it is not always possible for patients to reach the necessary knowledge level in a short amount of time and be able to perform critical measurements. In such acute cases, management depends heavily on the person's age, education, health literacy, and level of technology use. Especially for individuals able to use smartphones and applications, communication has become easier. Those who do not use such technologies are able to describe some of their symptoms as better or worse than the day before.

There may occasionally be problems ensuring that patients trust the system. Such problems were also encountered in this study and were overcome by informing the patients before they left the hospital that they would be called for follow-up and ensuring that the patients were called from an institutional line and by the same physician each time.

In a pandemic such as COVID-19, expecting telehealth services to completely replace face-to-face care is not reasonable in terms of the patient–physician communication and ethical considerations. However, it can be said to increase adherence to treatment and isolation precautions among patients with diseases that require follow-up without hospitalization after diagnosis. Telehealth will

also enable critical patients to receive the care they need by preventing unnecessary hospital admissions, and therefore this approach should be more widely adopted.

Ethics Committee Approval: This study was approved by the Clinical Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 14.08.2020, No: 135872).

Informed Consent: Written consent was obtained from the participants.

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THE EFFECT OF AGMATINE ON SPIKE-AND-WAVE DISCHARGES IN A GENETIC MODEL OF ABSENCE EPILEPSY

AGMATİNİN GENETİK BİR ABSANS EPİLEPSİ MODELİNDE DİKEN-VE-DALGA DEŞARJLAR ÜZERİNE ETKİSİ

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ABSTRACT

Objective: Studies on the actions of exogenous agmatine in experimental models have shown its anti-convulsant effects. However, there are no findings regarding the influence of agmatine on absence epilepsy. Therefore, we investigated the effects of the agmatine in the occurrence of spike-and-wave discharges (SWDs) in the Wistar Albino Glaxo Rijswijk rats (WAG/Rij) model of genetic absence epilepsy.

Materials and Methods: Three different doses (20, 40 or 80 mg/kg) of agmatine or saline were administered intraperitoneally to the adult WAG/Rij rats, and electroencephalography (EEG) was recorded for 2.5 hours. The number and the mean and cumulative durations of SWDs were measured. The SWD frequency characteristics were quantified by means of average power-spectra of the first 2nd segments of the SWD complexes before and after the effective dose of agmatine.

Results: Agmatine, administered 80 mg/kg systemically, temporarily increased the number and cumulative duration of SWDs compared with saline injected WAG/Rij rats. This effect of agmatine, however, was not associated with any change in the frequency characteristics of the SWD complexes. There was no effect on neither the incidence nor the cumulative duration of SWDs when agmatine administered in lower doses (20 or 40 mg/kg).

Conclusion: Higher doses of agmatine temporarily and slightly increase the incidence of SWDs in WAG/Rij rats, which may suggest its possible aggravating activity in absence epilepsy patients through its activity on adrenergic, serotonergic and AMPA receptors.

Keywords: Agmatine, absence epilepsy, wistar albino glaxo rijswijk rats

ÖZET

Amaç: Deneysel modellerde yapılan araştırmalar ekzojen agmatinin anti-konvülzan etkilerinin olduğunu göstermiştir. Ancak, agmatinin absans epilepsisi üzerindeki etkisine dair herhangi bir veri bulunmamaktadır. Bu çalışmada, agmatinin absans epilepsinin genetik bir modeli olan Wistar Albino Glaxo Rijswijk (WAG/Rij) ırkı sıçanlarda Diken-ve-Dalga Deşarjlar (DDD) üzerine olan etkilerini araştırdık.

Gereç ve Yöntem: Yetişkin WAG/Rij sıçanlarda periton içine 3 farklı doz agmatin (20, 40 ya da 80 mg/kg) ya da serum fizyolojik uygulandı ve 2,5 saat boyunca elektroensefalografi (EEG) kaydedildi. Elde edilen EEG kayıtlarında DDD'lerin sayısı, ortalama ve kümülatif süreleri değerlendirildi. DDD'lerin frekans özellikleri, etkili agmatin dozundan önce ve sonra, DDD aktivitesinin ilk iki saniyelik bölümlerinin ortalama güç spektrumları hesaplanarak karşılaştırıldı.

Bulgular: WAG/Rij sıçanlara sistemik olarak uygulanan 80 mg/kg agmatin DDD'lerin sayısını ve kümülatif süresini serum fizyolojik uygulanan gruba göre geçici olarak arttırdı. Ancak, agmatinin bu etkisi DDD'lerin frekans özelliklerindeki bir değişiklikle ilişki bulunmadı. Daha düşük dozlarda uygulanan agmatin (20 veya 40 mg/kg) ise DDD'lerin sayı ve süresine değişikliğe yol açmadı.

Sonuç: Yüksek doz agmatin WAG/Rij ırkı sıçanlarda DDD insidansını geçiçi olarak arttırmaktadır. Bu sonuçlar, agmatinin absans epilepsisi hastalarında adrenerjik, serotonerjik ve AMPA reseptörleri aracılığı ile olası bir kötüleştirici etki gösterebileceğini işaret etmektedir.

Anahtar Kelimeler: Agmatin, absans epilepsi, wistar albino glaxo rijswijk ırkı sıçanlar

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INTRODUCTION

Typical absence seizures are accompanied by regular, synchronous, bilateral spike-and-wave discharges (SWDs) in the electroencephalogram (EEG) and occur in various genetic generalized epilepsy syndromes such as childhood absence epilepsy and juvenile absence epilepsy (1). The fully inbred strain Wistar-Albino-Glaxo from Rijswijk rats (WAG/Rij) is a well-validated model of generalized genetic epilepsy, and it has been developed to particularly model the childhood absence epilepsy (2). All individuals of this strain exhibit spontaneous SWDs in the EEG similar to those in human absence epilepsy (1,3). This strain has been widely used in studies that aim to elucidate neurobiological mechanisms of SWDs and epileptogenesis and to evaluate the action mechanisms of antiepileptic drugs (2,4).

Electrophysiological and EEG–fMRI studies in genetic rodent models and human subjects revealed that SWDs are generated within the cortico-thalamo-cortical neuronal networks (1,4,5). Animal models demonstrated that SWDs begin within the focal, hyperexcitable area within deep layers of the perioral somatosensory cortex (S1po) before propagating to the thalamic nuclei (6,7). Electrophysiological studies have shown that sensory relay nuclei of the thalamus such as the ventral-postero-medial (VPM) and the posterior thalamic nuclei (Po) and the GABA-ergic reticular thalamic nucleus (RTN), the main inhibitory nucleus of the thalamus, seem to play major roles in the generation of SWDs (2,8). The thalamus is reciprocally connected with the cortex through glutamatergic excitatory pathways, while both thalamo-cortical and cortico-thalamic pathways have collateral glutamatergic projections to the GABA-ergic RTN (2,4). Due to this neurochemical profile, both ionotropic and metabotropic receptors of both neurotransmitter systems are widely expressed in the cortex and the thalamus, and strongly influence the occurrence of SWDs (2,4,9,10). However, it has also been demonstrated that monoamines such as noradrenalin, adrenalin and serotonin also modulate the cortico-thalamo-cortical circuitry, hence affecting the occurrence of SWDs (1,11-13).

Agmatine, an endogenous polyamine naturally synthesized from amino acid L-arginine by the enzyme arginine decarboxylase, is found in the synaptic terminals and cytoplasm of neurons and has been suggested to modulate many ion channels, receptors and downstream signaling pathways (14-16). Although daily diet provides only small amounts of polyamines, high levels of agmatine are present in fermented alcoholic beverages and in protein-rich foods (17). Agmatine activates G-protein coupled receptors (GPCRs), including α2-adrenergic ($\alpha_{2A}R_S$) and serotonergic (5-$HT_{1A/1B}$ and 5-HT_2) receptors. In addition to its interaction with AMPA type glutamate receptors ($AMPAR_S$) and blocking effect on NMDA type glutamate receptors ($NMDAR_S$), it may also inhibit voltage-dependent calcium channels and nitric oxide synthase (NOS) (15,18-20).

Exogenous agmatine administration in experimental models revealed its neuroprotective effects in various neurological disorders (15). In addition to its anti-depressant, anti-oxidant and anti-inflammatory activity, agmatine also plays an anti-convulsant role against pentylenetetrazole (PTZ), maximal electroshock seizure (MES) and glutamate-induced seizure models in rodents (14,20-22). However, there are no findings about the influence of agmatine in absence epilepsy. Considering its modulatory action on multiple neurotransmitter receptors and ion channels that may play a role in the pathophysiology of SWDs, we investigated the effects of agmatine on the SWDs in WAG/Rij rats.

MATERIALS AND METHODS

Animal subjects and drugs

Adult male WAG/Rij rats (6-8 month-old, weighing 240-350 g) from the breeding colony of the Experimental Medical Research and Application Center of Kocaeli University were used in the experiments. The animals were housed under standard laboratory conditions on a 12/12 h light/dark cycle (lights on at 7:00 a.m.) at 21±2°C and were allowed for free access to food and water in groups of 3-4 rats per cage. All experimental procedures were performed in accordance with the regulations of the Animal Research Ethics Committee in Turkey (Date: 06.06.2006, No:26220) and approved by Kocaeli University Animal Research Ethics Committee (protocol no: 1/3-2010).

The rats were randomly assigned in four groups (n=9, each group) in order to evaluate the dose-dependent effects of agmatine on the occurrence of SWDs. Group-1 (Saline) was treated with physiological saline only; Group-2 (Ag-20) received 20 mg/kg, Group-3 (Ag-40) 40 mg/kg and Group-4 (Ag-80) 80 mg/kg agmatine. Agmatine sulphate (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in physiological saline at the concentration of 20, 40 or 80 mg/ml on the day of the experiment. Drugs or saline were injected intraperitoneally (1 ml/kg; ip) by using needles of 27G. The doses of agmatine are based on the previous report (23).

Stereotaxic surgery

Stereotaxic surgery was performed as previously reported (24). Briefly, rats were anesthetized with ketamine (100 mg/kg, ip) and xylazine (10 mg/kg, ip). When rats were fully anesthetized, the head was placed in a stereotaxic frame (Stoelting Model 51600, Stoelting Co., IL, USA) with the skull surface flat and bregma at 0 mm. Epidural recording electrodes (MS333/2A; Plastic One, USA) were implanted on the frontal cortex (AP+2.0 mm and L+3.5 mm from bregma), occipital cortex (AP-6.0 mm and L+4.0

mm from bregma), and the ground electrode was placed on the cerebellum after a longitudinal incision was cut over the skull. The electrodes were fixed to the skull with dental acrylic. All coordinates were obtained from the stereotaxic atlas of Paxinos and Watson (25). Following stereotaxic surgery, the animals were housed singly in Plexiglas cages and were allowed a one-week recovery period prior to EEG recordings.

EEG recording and analysis

One day before the experiment, the rats were habituated to the EEG recording system. The next day, after a 30 min adaptation period, the baseline EEG was recorded for one hour. Rats were injected with either a single dose of saline or with 20 mg/kg, 40 mg/kg or 80 mg/kg agmatine. The EEG was recorded continuously 150 min after the injections. To avoid the effects of circadian rhythm on the occurrence of SWDs, EEG recordings of all animals were performed at the same time of the day (between 9.00 a.m. and 12.30 p.m). The electrical activity of the cortex was amplified using Powerlab 8S System (ADI Instruments, UK) BioAmp ML-136 module, filtered between 0.3 and 120 Hz, digitized at 1000 samples/s and recorded *via* LabChart v7.

A SWD complex was identified if its duration was longer than first second with a characteristic train of sharp large-amplitude spikes and slow waves (26). The first and last spike of an SWD complex with an amplitude at least twice the background amplitude of EEG was accepted as the SWD onset and offset. The number and mean duration of SWDs was measured for five periods of 30 min within the 150 min after the injection of the saline or the agmatine. The cumulative durations of SWDs were calculated as the sum of the individual durations of all SWDs present over 30 min intervals. The baseline SWDs were analyzed over one hour period, divided into two 30 min intervals. The sum of the two 30 min intervals was accepted as baseline level.

The spectral characteristics of SWDs were analyzed by computing the power spectra computed by using the Fast Fourier Transform (FFT) (The MathWorks MATLAB 9.5, Natick, USA) (27). The average power-spectra of the first 2nd segments of the SWDs were computed for randomly selected 15 SWD complexes of each animal in each group.

Statistical analyses

All statistical analyses were performed with GraphPad Prism version 8.00 (GraphPad Software, San Diego, USA). Data were assessed for normality using a Shapiro-Wilk normality test. Results found to be normally distributed ($p>0.05$) were expressed as mean±SEM. A two-way ANOVA (two factors: "Group" and "Time") followed by the post-hoc Bonferroni tests was used to analyze the number, mean duration and cumulative duration of SWDs among six-recording intervals and four experimental groups. For the comparison of the SWD spectra before and after the administration of the effective dose of agmatine (80 mg/kg) was carried out by a paired Student's t-test. The level of statistical significance was considered to be $p<0.05$. The results are presented as "FDFn, DFd = F value, p value" for ANOVA and "p value" for post-hoc Bonferroni and t-tests.

RESULTS

All WAG/Rij rats displayed characteristic 7-9 Hz SWD complexes in their baseline EEG recordings (Figure 1). As seen in Figure 2 and 3, the number (measured as event number per 30 min period) and the cumulative duration of SWDs decreased over time in all conditions including saline injected group (Time effect: $F(5,192)=27.91$, $p<0.0001$; $F(5,192)=22.67$, $p<0.001$, respectively). There was also a significant effect of the group on both the number of SWDs and the cumulative duration of SWDs (Group effect: $F(3,192)=5.426$, $p=0.0013$ and $F(3,192)=3.876$, $p=0.01$, respectively). The post-hoc Bonferroni tests

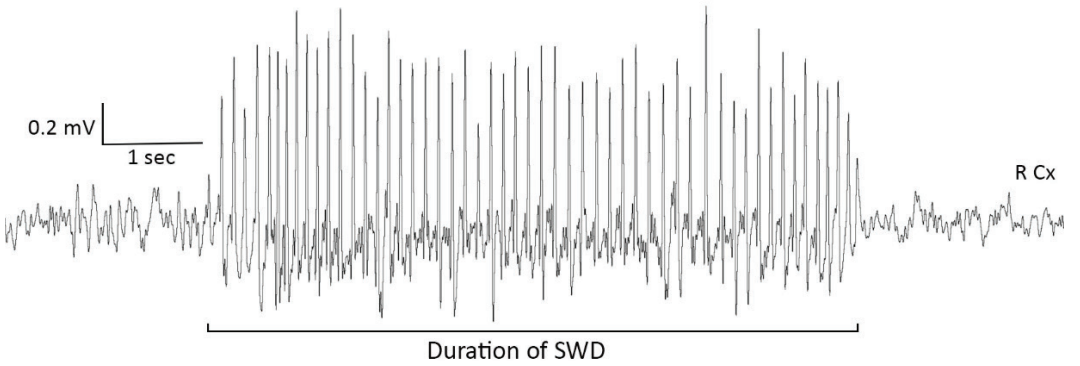

Figure 1: The representative electrographic pattern of an SWD recorded from the right cortex in a WAG/Rij rat. Voltage/time scale 0.2 mV/1 sec. R Cx: Right cortex

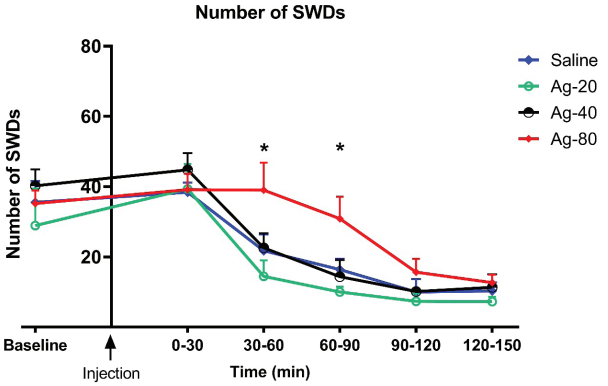

Figure 2: The effect of agmatine on the number of SWDs. The statistical analysis revealed a significant difference among four groups (Group effect: F (3,192)=5.426, p=0.0013). The post-hoc Bonferroni tests showed that this difference was due to the higher number of SWDs during the 30-60 min and 60-90 min post-injection recording periods (p=0.0292) than saline treated WAG/Rij rats. Data are expressed as mean±SEM. *: p<0.05

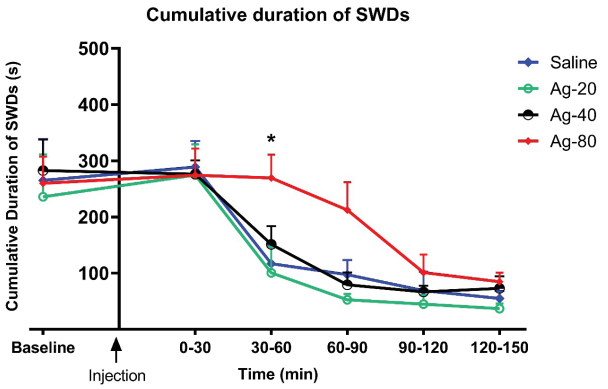

Figure 3: The effect of agmatine on the cumulative duration of SWDs. The statistical analysis revealed a significant difference among four groups (Group effect: F (3,192)=3.876, p=0.01). The post-hoc Bonferroni tests showed that this difference was due to the higher number of SWDs during the 30-60 min post-injection recording periods (p=0.0205) than saline treated WAG/Rij rats. Data are expressed as mean±SEM. *: p<0.05

showed that the first effect was due to the higher number of SWDs during the 30-60 min and 60-90 min post-injection recording periods (p=0.0292, p=0.0484, respectively) than saline treated WAG/Rij rats. In line with this, the group effect on cumulative duration of SWDs was also due to the higher values obtained in the Ag-80 group compared with the saline group during the 30-60 post-injection recording period (p=0.0205). No difference was observed among the groups in terms of the mean duration of SWDs (Group effect: F (3,192)=2.409, p=0.0684) (Figure 4).

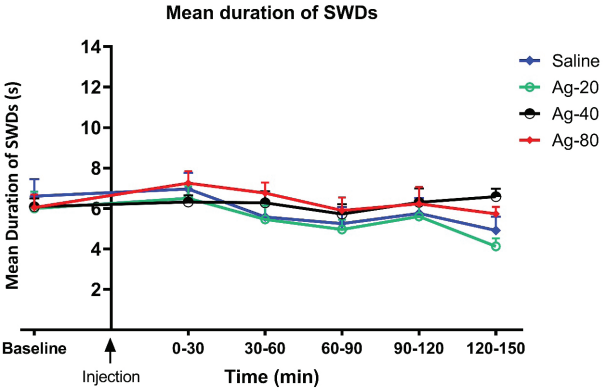

Figure 4: The effect of agmatine on the mean duration of SWDs. No difference was observed among the four groups (Group effect: F (3,192)=2.409, p=0.0684). Data are expressed as mean±SEM

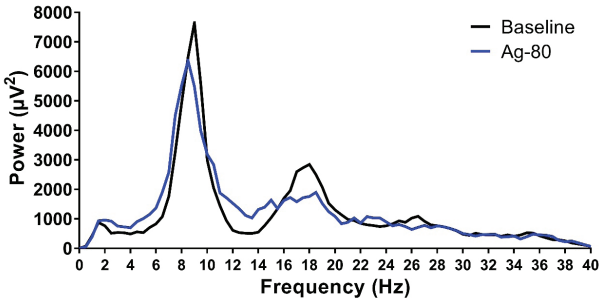

Figure 5: The power spectra of the first 2 s of the SWD complexes in the baseline and the 30-90 min post-injection periods of the Ag-80 group

For the effective dose of agmatine, which introduced significant increase in the SWD number and cumulative duration, the frequency characteristics of the SWDs in the baseline and the 30-90 min post-injection periods of the Ag-80 group were compared (Figure 5). The peak frequency in the power spectra computed by averaging the FFT magnitudes of the first 2nd of 15 SWD segments in each condition was 8.83±0.12 Hz for baseline EEG and 8.61±0.14 Hz for SWD segments recorded 30-90 min after 80 mg/kg agmatine injection. Paired t-test revealed no significant difference in the SWD frequencies between both periods (p=0.225).

DISCUSSION

All WAG/Rij rats displayed characteristic 7-9 Hz SWD complexes in their baseline EEG recordings and a decrease in the number, and cumulative duration of SWDs was observed in all treatment groups including the injection of saline or various doses of agmatine along the 150 min EEG recordings. Such phenomenon was previously described in genetic absence epilepsy rats, and is assigned to the circadian change of the vigilance

state along the day that influences the number of SWDs (3,28,29). In order to exclude such circadian effect, post-hoc comparisons were carried out between each of the agmatine administered groups and the saline applied control group, which displayed that agmatine, administered 80 mg/kg systemically, temporarily increased number (30-90 min after injection) and cumulative duration (30-60 min) of SWDs compared with the saline injected WAG/Rij rats. This effect of agmatine, however, was not associated with any change in the frequency characteristics of the SWD complexes.

Studies in genetic absence epilepsy models suggested that $AMPAR_S$ are recruited during absence seizures (4). Intracerebroventricular (ICV) injection of AMPA dose-dependently increased the incidence of SWDs (30,31), whereas ICV injection or focal administration of AMPAR antagonists into S1po (initial site of SWDs) or RTN induced a dose-dependent decrease in the incidence and duration of SWDs in WAG/Rij rats (9,30). Moreover, elevated AMPAR proteins, GluA1 and GluA2 in the somatosensory cortex plasma membrane of Genetic Absence Epilepsy Rats from Strasbourg (GAERS), another genetic model of absence epilepsy, have been reported, which suggested their contribution to hyperexcitability in somatosensory cortex and therefore to SWD generation (32). Agmatine is a neuromodulator capable of interacting with several types of glutamate receptors (20). For example, rapid antidepressant actions of agmatine have been shown to involve activation of $AMPAR_S$ due to the fast increase in AMPAR subunit GluA1 (33). Therefore, such a modulatory effect of agmatine on AMPARs may play a role in the aggravating effect of higher doses of agmatine on SWDs in our study.

Additionally, exogenous agmatine is able to increase monoamines and subsequently activate multiple postsynaptic GPCRs, including $\alpha_{2A}R_S$ and $5\text{-}HT_{1A/1B}$ receptors, which couple to a variety of second messenger systems (14,34). The anti-convulsant effects of agmatine have been found to be associated with its effects on $\alpha_{2A}R_S$ in addition to NMDARs and NO in PTZ induced seizures (14,22). In contrast to this effect, the $\alpha_{2A}R_S$ agonist clonidine inhibits the release of noradrenaline, and it has been reported that this leads to increased incidence and duration of SWDs in a dose-dependent manner in WAG/Rij rats (13). On the contrary, the $\alpha_{2A}R_S$ antagonist atipamezole is reported to cause a dose-dependent suppression of SWD activity in GAERS rats (11). The effects of agmatine on the convulsive vs. absence seizures in opposite directions, in terms of suppressing convulsive seizures in contrast to aggravating absence seizures, may depend on this mechanism associated with the $\alpha_{2A}R_S$. Finally, activation of $5\text{-}HT_{1A}$ receptors by receptor agonists or increase in endogenous 5-HT concentration has been reported to cause dose dependent increase in the cumulative duration and number of spike-wave discharges while ICV injection of $5\text{-}HT_{1A}$ receptor agonist causes dose dependent increase in WAG/Rij rats (12,35). Hence, the agmatine effect on $5\text{-}HT_{1A}$ receptors may also contribute to its aggravating activity on SWDs.

In contrast to the above mentioned mechanisms, however, agmatine can act as an NMDAR antagonist and NOS inhibitor (14,20). These effects would be expected to result in the suppression rather than aggravation of the SWDs as both competitive and non-competitive antagonists of NMDARs reduce the number of SWDs in WAG/Rij rats (10,36). To the contrary, injections of NMDA increase the number of SWDs (10). Additionally, agmatine inhibits all isoforms of NOS, and reduces production of the neuromodulator NO (19,20), and it is known that NO donors and biological precursor of NO increase the incidence of SWDs in WAG/Rij rats whereas inhibitors of neuronal NOS decrease the number of SWDs (37,38). While agmatine is known as a potent NMDAR antagonist and NOS inhibitor, the report on that $5\text{-}HT_{1A}$ receptor agonists counterbalance the decrease in number and duration of SWDs caused by NMDA antagonists (35), point to the importance or weight of other mechanisms in SWD generation. Within such a framework, present findings may indicate that agmatine's effects at high doses on multiple other receptor types including the $5\text{-}HT_{1A}$, $\alpha_{2A}R_S$ and AMPARs dominate those of the NMDARs and NOS inhibition on SWD generation.

Summing up, the present study shows that the higher doses of agmatine temporarily and slightly increase the incidence of SWDs in WAG/Rij rats, which may suggest its possible aggravating activity in absence epilepsy patients through its activity on adrenergic, serotonergic and AMPA receptors.

Ethics Committee Approval: This study was approved by the Kocaeli University Animal Experiments Local Ethics Committee (KOU-HAYDEK 2/3-2010).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Ö.A., T.U., F.A., N.A., A.K.; Data Acquisition- Ö.A., A.K.; Data Analysis/Interpretation- Ö.A.; Drafting Manuscript- Ö.A.; Critical Revision of Manuscript- T.U., F.A., N.A., A.K.; Final Approval and Accountability Ö.A., T.U., F.A., N.A., A.K.

Conflict of Interest: Authors declared no conflict of interest.

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BONE HEALTH AND GROWTH IN SPINAL MUSCULAR ATROPHY TYPE 2 AND 3

SPİNAL MÜSKÜLER ATROFİ TİP 2 VE 3'TE KEMİK SAĞLIĞI VE BÜYÜME

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ABSTRACT

Objective: Spinal muscular atrophy is a lower motor neuron disease, but other parts of the body could be affected. This study compared bone mineral density with bone metabolism and physical growth rates in patients diagnosed with spinal muscular atrophy type 2 and type 3.

Materials and Methods: Twenty-six patients with spinal muscular atrophy were included in the study (15 patients for type 2 and 11 for type 3). Weights and heights of patients were measured, standard deviation scores were determined, and the body-mass index was calculated. Motor function and pubertal assessment were performed. Serum calcium, phosphorus, alkaline phosphatase, parathormone, and 25-hydroxyvitamin vitamin D levels were compared. Spine radiography for scoliosis and bone densitometry for bone mineral density were performed, and volumetric bone mineral density was calculated for age and sex.

Results: Medians of height standard deviation scores were significantly lower in type 2 patients. There was no difference between the two groups in terms of serum calcium, phosphorus, alkaline phosphatase, parathormone, and 25-hydroxyvitamin D levels. The ratio of scoliosis was higher in type 2 patients as was its severity, but Z-scores of volumetric bone mineral density was lower in the same group.

Conclusion: This study showed that bone mineralization and growth rates were significantly lower in spinal muscular atrophy, mainly in type 2. Further studies are needed to evaluate bone health in spinal muscular atrophy patients.

Keywords: Spinal muscular atrophy, bone mineral density, bone health

ÖZET

Amaç: Spinal müsküler atrofi, alt motor nöron hastalığıdır, ancak kemik sağlığı ve diğer birçok organ sistemi etkilenebilir. Bu çalışmada, tip 2 ve 3 spinal müsküler atrofi tanısı alan hastalarda kemik mineral yoğunluğu kemik metabolizması ve fiziksel büyüme oranları karşılaştırıldı.

Gereç ve Yöntemler: Çalışmaya spinal müsküler atrofisi olan 26 hasta dahil edildi (tip 2; 15, tip 3; 11 hasta). Hastaların ağırlıkları ve boyları ölçülerek standart sapma skorları belirlendi ve vücut kitle indeksi hesaplandı. Motor fonksiyon ve pubertal değerlendirme yapıldı. Serum kalsiyum, fosfor, alkalen fosfataz, parathormon ve 25-hidroksivitamin D seviyeleri karşılaştırıldı. Skolyoz için omurga radyografisi ve kemik mineral yoğunluğu için kemik dansitometrisi yapılarak yaş ve cinsiyete göre hacimsel kemik mineral yoğunluğu hesaplandı.

Bulgular: Tip 2 hastalarda medyan boy standart sapma skorları anlamlı olarak daha düşüktü. İki grup arasında serum kalsiyum, fosfor, alkalen fosfataz, parathormon ve 25-hidroksivitamin D seviyeleri açısından fark yoktu. Tip 2 hastalarda skolyoz oranı ve şiddeti daha yüksek, volümetrik kemik mineral yoğunluğu Z skoru daha düşüktü.

Sonuç: Bu çalışma, kemik mineralizasyonunun ve büyüme oranlarının, özellikle tip 2'de olmak üzere, spinal müsküler atrofide önemli ölçüde daha düşük olduğunu göstermiştir. Spinal müsküler atrofi hastalarında kemik sağlığını değerlendiren daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Spinal müsküler atrofi, kemik mineral yoğunluğu, kemik sağlığı

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INTRODUCTION

Low bone mineral density (BMD) and recurrent bone fractures are an important problems in spinal muscular atrophy (SMA) patients (1). Various mechanisms are responsible for these conditions. Immobility negatively affects bone mineralization and results in low BMD, osteopenia, and fractures (2, 3). Some studies on the relationship between the survival motor neuron (SMN) protein and osteoclast and bone remodeling have been published, but data on bone health and indicators of bone involvement in SMA patients remain limited (4). A recent study showed that bone resorptions and vertebral fractures increased in these patients and low levels of serum 25-hydroxyvitamin D [25(OH)D] and high levels of parathormone (PTH) were observed (5).

In our study, we aimed to assess and compare patients with SMA type 2 and type 3 in terms of bone mineral density, bone metabolism, fractures, localization and severity of scoliosis, growth, and pubertal features. To achieve this, both the volumetric method and biochemical analysis were used simultaneously to obtain reliable results about bone health, and as such our study will contribute to the literature.

MATERIALS AND METHODS

Patients

In this cross-sectional and single-centered study, 26 patients who had been followed up by the Department of Pediatric Neurology and diagnosed with SMA, including 15 patients of type 2 and 11 of type 3, were enrolled.

The inclusion criteria were defined as follows: 1) 18 months to 18 years of age; 2) clinically and genetically diagnosed as SMA type 2 and 3; 3) not having any chronic disease except for SMA; 4) not receiving any treatment that affects bone mineralization such as systemic steroids or bisphosphonates; 5) not suffering from congenital fractures. This study was approved by the Bioethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 24.10.2019, No: 1213). Written informed consent was obtained from the parents.

Endocrinologic assessment

Height and weight were measured for all patients using a wall-mounted or infant scale calibrated Harpenden Stadiometer (Holtain Ltd.) and electronic scale (sensitivity of 0,1 kg). If children could not stand, the length was measured from the heels to the top of the head, and weight was calculated by subtracting the parental weight from the total weight measured on the scale. An infant scale was used to weigh children under two years and when patients could not stand independently. Body mass index (BMI) was calculated as [weight (kilogram)/height (meter)^2]. According to national data, the standard deviation score (SDS) of all auxological measurements was calculated (6). Patients were categorized as obese (BMI SDS >2), overweight (BMI SDS between 1–2), normal-weight (BMI SDS between -2 and 1), and underweight (BMI SDS <-2) (7).

Marshall and Tanner's staging were used to assess puberty. The onset of puberty was defined as breast Tanner stage 2 in girls and testicular volume of ≥4 ml in boys (8). The pubertal assessment was performed by a pediatric endocrinologist (EKO, APO, and FB). The rates of prepubertal and pubertal patients were determined in both groups, and volumetric bone mineral density (vBMD) was compared according to these two categories.

Assessment of motor function

The patients' motor function was evaluated according to the ability to perform the activities itemised in the Hammersmith Functional Motor Scale Expanded (HFMSE) test (9). For each item, if the patient was unable to complete it, the score was '0'. If achieved, the degree of success was scored as either '1' or '2'. If all steps of the test were achieved, the total score was 66.

Imaging of left hand and wrist and spine

X-rays of the patients' left hands and wrists were taken. A single observer assessed bone age (BA) according to the Greulich Pyle Method (10).

X-rays of the whole spine anteroposterior (AP) and lateral views were performed to determine whether the patients had scoliosis. Concurring with the Scoliosis Research Society, patients were diagnosed with scoliosis if the curvature was more than 10° Cobb's angle. The severity of each patient's scoliosis was determined in accordance with The International Scientific Society on Scoliosis Orthopedic and Rehabilitation Treatment (SOSORT) guideline in 2016 (11).

In an earlier study, the fastest progression of scoliosis had been shown to be around ten years of age (12). Thus, for assessing the relationship between scoliosis and age, we examined patients in two groups, under and above the age of ten.

Lateral thoracolumbar spine imaging was performed and reported by a single experienced reader to evaluate whether vertebral fracture was present.

Evaluation of vitamin D status, bone mineral density, and osteoporosis

Serum [25(OH)D] concentrations were performed using the electro-chemiluminescence immunoassay (ECLIA) method (Cobas e601 autoanalyzer, Roche Diagnostic GmbH, Mannheim, Germany). None of the patients were on vitamin D replacement, and samples were collected in the winter. Vitamin D deficiency was defined as a serum [25(OH)D] level of <12 ng/mL (<30 nmol/L) and in-

sufficiency as a level between 12 and 20 ng/mL (30-50 nmol/L). Levels higher than 20 ng/mL (50 nmol/L) were accepted as sufficient (13).

The bone mineral density of L1 to L4 lumbar spine was measured either by a Lunar DPX IQ with a pediatric software package or Hologic QDR 4500 (Hologic, Inc, Waltham, Mass) scanner. Hologic® Conversion Tables were used to switch from a Lunar DPX IQ to a Hologic densitometer (14). We calculated volumetric bone mineral density (vBMD) (gram/cm^3) of patients using the formula of Carter et al. [Bone Mineral Content (BMC)/area$^{1.5}$] (15). The vBMD Z-scores were calculated according to Turkish boys and girls of similar age using national data (16).

According to the International Society for Clinical Densitometry (ISCD) 2019 criteria, diagnosis of osteoporosis in children and adolescents should not be made based on densitometric criteria alone (17). Based on these criteria, we made a diagnosis of osteoporosis if the BMD Z-score was less than or equal to -2.0 with a history of significant clinical fracture, which was defined as one or more of the following: 1) ≥2 fractures of long bone by ten years of age; 2) ≥3 fractures of long bone at any age. Also, if patients had vertebral compression fractures, it was accepted to be diagnosed for osteoporosis.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) Statistic Base 21.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Shapiro-Wilk test was used for the evaluation of the distribution of variables. In the descriptive analysis, median; inter quarter range (IQR) are used when appropriate. Variables were compared with the Chi-squared test or Fisher's exact test where applicable. Student's t-test or Mann-Whitney U test was used to compare the continuous data between the two groups. P-value was considered as significant with ≤0.05.

RESULTS

Demographic data

The median age of the patients was 7.2 (1.8-17.2) years in SMA type 2 (n=15) and 7.7 (2.7-17.3) years in SMA type 3 (n=11). The female/male ratio was 7:8 and 8:3 in type 2 and type 3 patients, respectively (Table 1). Median values of bone age were 6.0 and 8.0 years in type 2 and type 3, respectively.

All patients in type 2 were non-ambulatory, and in type 3 one patient was non-ambulatory with the remainder being ambulatory.

Anthropometric measurements and puberty

The anthropometric parameters of our cohort are outlined in Table 1. Median of height SDS was significantly lower in type 2 (p=0.03). Medians of BMI and weight SDS were not significantly different between the two groups (p=0.64 and 0.12, respectively).

Both groups were compared in terms of puberty; six of 15 patients were pubertal in type 2 (40%), five of 11 patients were pubertal in type 3 (45%). The pubertal stages of the pubertal patients were compatible with their chronological age. Precocious or delayed puberty was not observed in either type. There were no significant differences in terms of pubertal features between the two groups (p=0.91) (Table 1).

Motor function (HFMSE score)

The median HFMSE score in SMA type 2 and type 3 patients was 9 and 46, respectively (p<0.01). HFMSE score was above 30 points in all patients except one in type 3. In contrast, this score was below 30 in all patients except one in type 2.

Laboratory analysis

The serum levels of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), and parathormone (PTH) were within normal limits. Levels of serum [25(OH)D] did not differ significantly between the two groups. The median serum level was 16.7 vs. 17.3 ng/ml in type 2 and type 3, respectively.

Scoliosis

Scoliosis was observed in 60% of patients in type 2. Cobb's angle ranged from 45° to 100° in these patients, all of them classified as having severe or very severe scoliosis. Scoliosis (Cobbs angle 98°) was detected in only one patient in type 3, this patient being the one who was non-ambulatory. Three patients that had spinal curvature between 0-9° were not included in the scoliosis group.

Scoliosis was observed in all type 2 patients who were ten years of age or older; the frequency of scoliosis declined to 25% in patients between 5 and 10 years of age. No patient under five years of age had scoliosis in this group. According to Fisher's exact test, the differences between these three age groups in type 2 were considered significant (p=0.007). The mean of Cobb's angle was 15° and 76° in patients who were <10 and ≥10 years old, respectively. This difference was statistically significant (p=0.004) (Table 2).

Bone mineral density

Z-scores of areal BMD were lower in type 2 patients (p=0.018). In type 2 and type 3 patients, median vBMD (gram/cm^3) was 0.065 and 0.087, median Z-scores of vBMD were -2.6, and -0.69, respectively. These results were also statistically significant (p=0.018 and p=0.03, respectively) (Table 1).

Osteoporosis and bone fractures

None of the patients had a bone fracture history except one SMA type 2 patient who had a vertebral fracture. Thus, according to ISCD 2019 criteria, we had just one patient who fulfilled the criteria for osteoporosis.

Table 1. Clinical and laboratory characteristics of patients with spinal muscular atrophy type 2 and 3 (Values are given as median and range (min-max))

	Type 2 (n=15)	Type 3 (n=11)	p value
Gender (F/M)	7/8	8/3	
Age (years)	7.2 (1.8-17.2)	7.7 (2.7-17.3)	0.44[a]
Weight SDS	-1.7 (-7.27-1.61)	-0.06 (-2.34-1.47)	0.12[a]
Height SDS	-2.7 (-7.25-0.85)	-0.35 (-4.6-0.35)	**0.03[a]**
BMI SDS	-0.44 (-6.52-2.82)	0.04 (-1.8-1.77)	0.64[a]
BMI SDS groups			0.32[b]
Normal (BMI SDS -2 – 1)	8	8	
Overweight (BMI SDS 1-2)	3	3	
Obese (BMI SDS >2)	2	-	
Underweight (BMI SDS <-2)	2	-	
Pubertal stage (Tanner)			0.91[b]
I	9	6	
II-III-IV	3	2	
V	3	3	
Ambulation status			**<0.01[c]**
Non-ambulatory	15	1	
Ambulatory	-	10	
HFMSE score (median)	9	46	**<0.01[a]**
Scoliosis (Cobb's angle)			**0.013[b]**
No scoliosis (<10°)	6	10	
Severe, severe to very severe (≥41° -≤55°)	2	0	
Very severe (≥56°)	7	1	
Number of fractures	1	0	
vBMD (g/cm^3)	0.065 (0.033-0.182)	0.087 (0.071-0.149)	**0.018[a]**
vBMD SDS	-2.6 (-6.1-10.0)	-0.69 (-2.3-3.6)	**0.03[a]**
Ca (mg/dL) (normal ranges: 9.2-11)	9.8 (9.4-10.9)	10 (9.4-10.2)	0.72[a]
P (mg/dL) (normal ranges: 3.2-5.8)	4.7 (3.5-6.0)	4.9 (3.3-6.1)	0.61[a]
ALP (U/L) (normal ranges: 70-270)	130 (63-205)	145 (40-200)	0.5[a]
25-OH D (ng/mL)	16.7 (5.0-78.0)	17.3 (5.6-32.4)	0.51[a]
Vit D status			0.61[b]
Normal (>20 ng/mL)	6	4	
Insufficiency (12-20 ng/mL)	7	4	
Deficiency (<12 ng/mL)	3	3	
PTH (pg/mL) (normal range: 15-65)	23.1 (12.7-50.4)	25 (3.4-74.9)	0.79[a]

[a]: Mann-Whitney U Test, [b]: Chi Squared Test, [c]: Fisher's Exact Test, F: female, M: male, SDS: standard deviation score, BMI: body mass index, vBMD: volumetric bone mineral density, g/cm^3: gram per cubic centimeter, Ca: calcium, P: phosphorus, ALP: alkaline phosphatase, 25-OH D: 25 dihydroxy vitamin D, PTH: parathormone, mg/dL: milligrams per deciliter, ng/mL: nanogram per milliliter, PTH: parathormone, pg/mL: picogram per milliliter

Table 2: Ratio of scoliosis and median of Cobb's angles in <10 and ≥10 years old patients with SMA type 2

SMA type 2 (n=15)	Ratio of scoliosis			Cobb's angle				
	n	%	p value	Median / mean	Std dev	min	max	p value
<10 years old (n=8)	6	25	**0.007**	0° / 15°	29.8	0°	78°	**0.004**
≥10 years old (n=7)	7	100		75° / 76°	10.8	47°	100°	

SMA: spinal muscular atrophy, std dev: standard deviation, min: minimum, max: maximum

DISCUSSION

This study observed that bone density, height and vitamin D levels were negatively affected, especially in type 2 patients. In addition, we observed that especially type 2 patients over the age of 10 had severe or very severe scoliosis. Therefore, bone health monitoring of SMA patients should be done carefully.

In a study conducted by Martinez et al., Z-scores of weights were between ≥-2 to ≤2 in 81% of patients, regardless of SMA subtypes (18). Median Z-scores of height and BMI were -1.37 and 0.15, respectively. The differences between type 2 and 3 were not specified. In our study, regardless of SMA subtypes, median SDS values of weight, height, and BMI were similar to those in Martinez et al.'s study (-1.7, -1.2, and 0.3, respectively). Median SDS of weight and BMI were lower in type 2 patients; however, these differences were not statistically significant. Type 2 patients were shorter in height than type 3 patients; this situation could be explained by the higher severity of scoliosis in type 2 patients. It may be due to inadequate energy intake, and there is a need for further studies to yield this condition.

In our study, median chronological and bone age were 7.2 vs. 7.7 in type 2 patients and 6.0 vs. 8.0 in type 3 patients, respectively. We observed a strong positive correlation between chronological and bone age of all patients ($r=0.99$, $p<0.01$). However, two patients in type 2 had a slightly lower bone age, and one patient in type 3 had a slightly higher bone age. This result showed that bone age is affected by chronological age rather than BMD.

In our study, six patients were pubertal (40%) in type 2 and five patients (45.5%) in type 3. No patient had precocious or delayed puberty. The median age and the rate of pubertal patients were similar in both subtypes of SMA. For this, the effects of puberty and chronological age on bone tissue mass and bone mineralization were homogeneous in both subtypes.

Vai et al. showed that serum calcium, phosphorus, and ALP levels were normal in 30 patients with SMA type 2 and 3. Also, the PTH levels were normal; however, two children had slightly higher levels than the normal limit (5). Similarly, Baranello et al. followed up 32 SMA type 2 and type 3 patients between the ages of 18 and 36 months in three visits. At each visit, they demonstrated that serum calcium, phosphorus, and ALP levels were normal (19). The Median levels of these laboratory tests were also normal in our study, and there was no significant difference between type 2 and 3. The median PTH levels were 22.5 pg/mL and 33.5 pg/mL in type 2 and type 3 patients, respectively. This difference was not statistically significant.

Low levels of [25(OH)D] (below 20 ng/mL) were shown in 36.6% of SMA patients by Vai et al. The frequency of Vitamin D insufficiency (below 20 ng/mL) was higher in our patient group compared to other studies; levels of [25(OH)D] were below 20 ng/mL in 16 of 26 patients (61.5%) and were normal (above 20 ng/mL) in 10 patients. However, the median level was similar in both types of SMA, 16.32, 16.05 ng/mL, respectively.

Scoliosis is a crucial problem that limits the quality of life in patients with SMA. Advancing age is a significant risk factor for the occurrence of scoliosis. Granata et al. showed that the mean age at the onset of scoliosis in SMA type 2 patients was 4.3 years, and this study showed that the frequency and severity of scoliosis increased by age (12). An annual increase of >12° degrees was observed in type 2 patients, and the fastest progression was at around ten years of age. In our study, we detected scoliosis in all type 2 patients above ten years of age. No patient under five years of age had scoliosis in type 2. A positive correlation between the severity of scoliosis and advancing age in SMA type 2 patients was observed; it was statistically significant ($r=0.749$, $p<0.01$). Since only one patient with type 3 SMA had scoliosis, it is difficult to comment on the relationship between scoliosis and age in type 3 patients. Type 2 patients whose paraspinal muscles are weaker and exposed to body weight for a prolonged period with advanced age might suffer higher severity of scoliosis.

Bone mineral density is considered to be the standard method to assess bone quality (20). Wassermann et al. studied 85 patients with SMA. The mean Z-score of BMD of type 2 patients was lower than that in type 3 patients,

-2.5 vs. -0.2, respectively (21). The lowest mean Z-score of BMD was observed in type 1 patients. In contrast to Wassermann's report, in a study published in 2004 by Kinali et al., BMD was normal in all 12 SMA type 2 and 3 patients aged under 17 years (22). Both studies used areal BMD for bone mineralization. However, especially in children of growing age, areal BMD may not precisely be expressed by bone mineral content. VBMD was used to investigate bone mineral density in several clinical studies (23).

Baranello et al. performed bone mineral apparent density (BMAD=vBMD) in 32 prepubertal SMA type 2 and 3 patients, and mean Z-scores of BMAD (vBMD) were -0.7 regardless of SMA subtypes at the beginning of the study (19). In our study, both groups were compared in terms of vBMD and the Z-score of vBMD. Our cohort's median of vBMD values were 0.065 and 0.087 gr/cm^{3,} respectively, in type 2 and 3 patients. We corrected vBMD values according to age and sex and calculated the Z-score of vBMD for each patient. Median Z-scores of vBMD were -2.6 and -0.69, respectively, in type 2 and 3 patients. These results showed that bone mineralization in SMA type 2 patients was lower in our cohort. The difference between type 2 and 3 patients was statistically significant.

In a prospective study conducted by Baranello et al., initial Z-scores of BMAD were below -2 in 18% of patients, while this ratio was 37% at 36th month. Our study found that this rate was 46.6%, with the low BMD ratio being 66.6% in type 2 and 18.2% in type 3. There was a positive correlation between chronological age and Z-score of vBMD for the whole group (r=0.47, p=0.015). A strong correlation was observed in type 3 (r=0.755, p=0.007); no correlation was found in type 2 patients.

Boot et al. studied the effect of puberty on BMD in girls and boys. Five hundred children and adolescents were enrolled in this study, and age-corrected values were used. A positive correlation between puberty and vBMD was observed (24). We compared the bone density of prepubertal and pubertal SMA patients, and we found that the Z-score of vBMD increased by pubertal stages. Low bone mineral density (Z-score of vBMD ≤-2.0) was observed in 7 of 9 prepubertal patients with type 2 (77.7%) and 2 of 6 prepubertal patients with type 3 (33.3%).

Wassermann et al. showed that Z-scores of BMD in non-ambulatory type 1 and 2 patients were lower than ambulatory type 3 patients. Similarly, in a study by Khatri et al. of 8 SMA patients, the Z-score of BMD was significantly lower in non-ambulatory SMA patients than in ambulatory (25). Our study found that the Z-score of vBMD was significantly lower in type 2 patients than in type 3. The ambulation status of the patients could explain these results. All type 2 and only one type 3 patients with SMA were non-ambulatory in our cohort. Additionally, puberty and median age, which were considered to affect BMD, were similar in both groups. The HFMSE scores of patients were compatible with ambulation. The median of this score was significantly different between the groups.

Bone fractures are severe complications of SMA patients. In a recent study, the fracture rate in SMA type 2 patients was 46.9%, and 38 of 81 patients had one or more fractures with various localization. This rate was 51.5% in SMA type 3a, and 17 of 33 patients had fractures (26). In our study, osteoporosis was diagnosed in only one patient who had a fracture of thoracic vertebrae.

The low number of patients included in our study is the most crucial limitation of our research. The patients who took part in our research all live in the same geographical area, and they are followed up by a single center. The results may not reflect the whole of society. Multicenter studies containing a large number of patients should be conducted . The volumetric method to evaluate BMD was used in our study, which gives it great strength given that some other methods for observing SMA type 2 patients who have severe scoliosis could be misleading for BMD.

CONCLUSION

Bone health is significantly affected to varying degrees in patients with SMA. We observed that type 2 patients were shorter in height than type 3. Serum Ca, P, ALP, and PTH levels were normal despite severe bone mineral deficiency; however, serum [25(OH)D] levels were lower in both types of patients. The ratio and severity of scoliosis increased by age in SMA type 2. Z-scores of vBMD were very low in patients with type 2. The vBMD was correlated with Tanner stages, bone age, height, and weight. The main limitation of our study is that the number of patients is low. Studies involving more patients are needed.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 24.10.2019, No: 1213).

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THE EFFECT OF USE OF DIFFERENT DOSES OF URSODEOXYCOLIC ACID ON GALLSTONE FORMATION AFTER SLEEVE GASTRECTOMY

SLEEVE GASTREKTOMİ SONRASI FARKLI DOZLARDA URSODEOKSİKOLİK ASİT KULLANIMININ SAFRA TAŞI OLUŞUMUNA ETKİSİ

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ABSTRACT

Objective: To evaluate the effects of using different doses of ursodeoxycholic acid (UDCA) for six months in prevention of gallstones after Laparoscopic Sleeve Gastrectomy (LSG) on the formation of gallstones in the 6th and 12th months.

Materials and Methods: The data of patients who underwent LSG for morbid obesity were analyzed. Three groups of 20 people were formed. These were the control group, Group I who were treated with UCDA 500 mg/day for six months after LSG, and Group II who were treated with UCDA 1000 mg/day for 6 months after LSG. Demographic characteristics, co-morbid diseases, cholesterol and triglyceride differences before and after surgery, excess weight loss (EWL%), total weight loss (TWL%), body mass index (BMI), and abdominal ultrasound (US) data for the occurrence of cholelithiasis (CL) preoperatively and at six and 12 months after surgery were collected.

Results: The results of tests taken on these patients in the sixth month revealed that the stone detection rate in the US results of the control group was significantly higher than that in Group II (p=0.001; p<0.01). There was no statistically significant difference between the control group and Group I (p=0.149), and between Group I and Group II (p=0.066). The results of tests taken in the 12th month showed that the stone detection rate in the US results of the control group was significantly higher than Group I and Group II (p=0.010; p<0.05). There was no statistically significant difference between Group I and Group II.

ÖZET

Amaç: Laparoskopik Sleeve Gastrektomi (LSG) sonrası safra taşlarının önlenmesinde altı ay boyunca farklı dozlarda ursodeoxycholic acid (UDCA) kullanımının 6. ve 12. aylarda safra taşı oluşumuna etkilerini değerlendirmek.

Gereç ve Yöntem: Morbid obezite nedeniyle LSG uygulanan hastaların verileri analiz edildi. 20'şer kişilik üç grup oluşturuldu: Kontrol grubu; LSG sonrası altı ay süreyle UCDA 500 mg/gün ile tedavi edilen Grup I; LSG sonrası altı ay süreyle UCDA 1000 mg/gün ile tedavi edilen Grup II. Demografik özellikler, eşlik eden hastalıklar, ameliyat öncesi ve sonrası kolesterol ve trigliserit farklılıkları, aşırı kilo kaybı (%EWL), toplam kilo kaybı (%TWL), vücut kitle indeksi (BMI) ve abdominal ultrason (US) verileri ameliyat öncesi ve ameliyattan altı ve 12 ay sonra kolelitiazis (CL) gelişimi değerlendirildi.

Bulgular: Olguların 6. ay sonuçlarında kontrol grubunun US sonuçlarında taş tespit oranı Grup II'ye göre anlamlı olarak yüksek bulundu (p=0,001; p<0,01). Kontrol grubu ile Grup I arasında (p=0,149), Grup I ile Grup II arasında (p=0,066) istatistiksel olarak anlamlı fark yoktu. Olguların 12. ay sonuçlarında kontrol grubunun US sonuçlarında taş tespit oranı Grup I ve Grup II'ye göre anlamlı olarak yüksek bulundu (p=0,010; p<0,05). Grup I ve Grup II arasında istatistiksel olarak anlamlı fark yoktu.

Sonuç: Bu çalışma sonucunda hastalara LSG sonrası 1000 mg/gün UDCA verilmesini öneriyoruz.

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Conclusion: As a result of this study, we recommend that patients be given 1000 mg/day UDCA after LSG.

Keywords: Cholelithiasis, laparoscopic sleeve gastrectomy, ursodeoxycholic acid

Anahtar Kelimeler: Kolelitiazis, laparoskopik sleeve gastrektomi, ursodeoksikolik asit

INTRODUCTION

While cholelithiasis (CL) incidence is around 5% in the general population, this incidence can rise to 45% in the obese population, especially in female patients (1-3). It is known today that bariatric surgical methods cause gallstones in patients. Additionally, bariatric methods can cause a-symptomatic gallstones to become symptomatic (such as biliary colic, cholecystitis, cholangitis, or pancreatitis) (4).

Obesity can be defined as the excessive accumulation of fat in the body. Body fat is between 15-20% in males with an average body weight and 25-30% in females. Since it is not easy to determine body fat percentage, obesity is defined as being overweight rather than being excessively fat. According to the World Health Organization (WHO) obesity is classified by calculating the body mass index (BMI=Weight [kg]/Height [m²]). Accordingly, BMI=25.0-29.9 kg/m² is classified as overweight, and BMI≥30 kg/m² is classified as obesity (5). 30-34.9 kg/m² is defined as Grade 1 obesity, 35-39.9 kg/m² is defined as Grade 2 obesity, and ≥40 kg/m² is defined as Grade 3, or morbid obesity (5).

It has been reported that gallstones can increase due to metabolic disorders such as diabetes mellitus (DM) and to excess estrogen, which can increase the cholesterol content of bile (6). In studies conducted on this topic, the difference between the presence of chronic diseases and the formation of gallstones was found to be statistically significant (7, 8).

Changes in cholesterol metabolism involving rapid weight loss increase the cholesterol concentration in bile. Undissolved cholesterol crystallizes in company with a glycoprotein that stimulates cholesterol crystal aggregation. This particularly occurs with calcium (Ca^{+2}) and mucin (9) which increase 10-20 times in bile after bariatric surgery with an unknown mechanism (9, 10). Various rates of gallstone formation incidence have been reported after different bariatric procedures. Although the incidence of symptomatic gallstones was reported to be higher in LSG compared to Roux-en-Y Gastric Bypass (RYGB) in only one of the studies conducted to date, no significant difference was found between them (11). Gallstone development has been reported at a rate of 10-25% after a calorie-restricted diet (12, 13). Morbidly obese patients were observed to develop gallstones at a rate of 35-38% when they lost weight after bariatric surgery (9, 14, 15).

With ursodeoxycholic acid (UDCA), it is possible to medically prevent CL, which occurs as a result of rapid weight loss. In the prevention of gallstones, UDCA is a secondary bile acid that acts by increasing the flow rate of bile and decreasing its lithogenicity. Use of UDCA for three to six months was found to effectively prevent CL formation up to 24 months after surgery (Odds Ratio (OR) 0.43) (16). In all studies conducted to date, though the relationship between UDCA use and gallbladder stone formation has been sought, the relationship of the latter with the dose of UDCA has not been investigated (17, 18).

In this study, we aimed to evaluate the effects of using different doses of UDCA for six months in the prevention of gallstones after LSG. We checked degrees of gallstone formation after 6 and 12 months.

MATERIALS AND METHODS

This study was planned as a prospective, randomized, controlled study. Patients who underwent LSG due to morbid obesity at the General Surgery Clinic in Fatih Sultan Mehmet Training and Research Hospital between July 2018 and July 2019 were evaluated. Approval from the hospital ethics committee was obtained, as was informed consent from all patients. (FSMEAH-KAEH 17073117-050.06). The patients were randomly grouped into 3 groups of 20 each using the website www.randomizer.org. Those who did not receive UDCA treatment after LSG were defined as the control group, those who received UCDA 500 mg/day for 6 months after LSG were defined as Group I, and those who received UCDA 1000 mg/day for 6 months after LSG were defined as Group II. Before LSG, US was performed in all patients to detect the presence of stones in the gallbladder. The demographic characteristics of the patients, DM, HT, pre and postoperative cholesterol and triglyceride differences, EWL%, TWL%, and BMI values were analyzed. In addition, abdominal US reports were collected to evaluate CL formation before surgery and at 6 and 12 months after surgery.

Inclusion criteria

All patients above 18 years of age who had had LSG for the first time due to obesity and who had not had gallbladder surgery before were included in the study.

Exclusion criteria

- Detection of gallstones in US before LSG
- History of previous bariatric surgery or gall bladder surgery
- Presence of hypersensitivity to active or auxiliary components of UDCA

- Inflammatory Bowel Disease (IBD) and pathologies involving the small intestines and liver that affect the enterohepatic circulation of bile salts (ileal resection and stoma, extra/intrahepatic cholestasis, severe liver disease)
- Pregnancy or use of contraception
- Failure to obtain informed consent

RESULTS

A total of 60 patients , 73.3% (n=44) of whom were female and 26.7% (n=16) of whom were male, were included in the study. The ages of the patients included in the study ranged from 19 to 61, and the mean age was 36.58±10.82. The median BMI was 43.8.

A comparison of demographic data such as age, gender, BMI and presence of DM revealed that no difference was found between the groups. However, the rate of hypertensive patients was coincidentally higher in Group I (Table 1).

No significant difference was found between these three groups in terms of EWL%, TWL%, cholesterol and triglyceride differences (Table 2).

A statistically significant difference was found between the postoperative 6th month US results of the cases according to UDCA use (p=0.001; p<0.01). In the control group, the rate of CL on US was found to be significantly higher than in Group II (p=0.001; p<0.01). In regards to stone formation, there was no significant difference between the control group and Group I (p=0.149) and Group I and Group II (p=0.149) (p=0.066).

The difference between the distribution of postoperative 1st year US results according to UDCA use was statistically significant (p=0.010; p<0.05). The incidence of CL was found to be significantly higher in those who did not use UDCA (Table 3).

Statistical analyses

The NCSS (NumberCruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard

Table 1: Evaluation of ursodeoxycholic acid intake according to descriptive characteristics

			UDCA intake status			Test value
		Total	Control group (n=20)	Group I (n=20)	Group II (n=20)	p
Age	Min-Max (Median)	19-61 (34)	23-52 (31.5)	24-61 (36.5)	19-57 (31)	F=0.839
	Mean±SD	36.58±10.82	35.30±9.58	39.15±11.32	35.30±11.54	[a]**0.437**
Gender	Female, n (%)	44 (73.3)	14 (70.0)	14 (70.0)	16 (80.0)	χ^2=0.682
	Male, n (%)	16 (26.7)	6 (30.0)	6 (30.0)	4 (20.0)	[b]**0.711**
Diabetes	No, n (%)	48 (80.0)	17 (85.0)	16 (80.0)	15 (75.0)	χ^2=0.684
	Yes, n (%)	12 (20.0)	3 (15.0)	4 (20.0)	5 (25.0)	[c]**0.917**
Hypertension	No, n (%)	49 (81.7)	20 (100.0)	13 (65.0)	16 (80.0)	χ^2=8.624
	Yes, n (%)	11 (18.3)	0 (0.0)	7 (35.0)	4 (20.0)	[c]**0.011***
BMI		43.8	43.2	45.6	42.4	[b]0.154

[a]: Oneway ANOVA, [b]: Pearson Chi-Square Test, [c]: Fisher-Freeman-Halton Test, *: p<0.05

Table 2: Evaluation of EWL, TWL, cholesterol, and triglyceride differences according to the status of ursodeoxycholic acid intake

			UDCA intake status			Test value
		Total	Control group (n=20)	Group I (n=20)	Group II (n=20)	p
EWL%	Mean±SD	59.22±13.21	60.45±13.89	59.20±14.23	58.00±11.95	[a]**0.846**
TWL%	Mean±SD	30.08±5.83	30.25±6.77	29.85±5.44	30.15±5.48	[a]**0.976**
Cholesterol difference	Mean±SD	-1.36±30.99	3.73±29.81	-9.45±32.14	1.65±30.89	[a]**0.357**
Triglyceride difference	Mean±SD	-48.27±67.99	-55.40±72.40	-47.10±52.49	-42.30±79.18	[b]**0.555**

[a]: One-way ANOVA, [b]: Kruskal Wallis Test

Table 3: Evaluation of ultrasound results according to the status of ursodeoxycholic acid intake

			UDCA intake status			Test value
		Total	Control group (n=20)	Group I (n=20)	Group II (n=20)	p
Postoperative 6th month US result	**No stone**, n (%)	38 (63.3)	9 (45.0)	14 (70.0)	15 (75.0)	χ^2=15.742
	Mud, n (%)	8 (13.3)	1 (5.0)	2 (10.0)	5 (25.0)	[a]**0.001****
	Stone present, n (%)	14 (23.3)	10 (50.0)	4 (20.0)	0 (0.0)	
	No stone, n (%)	40 (66.7)	9 (45.0)	15 (75.0)	16 (80.0)	χ^2=11.538
Postoperative	**Mud**, n (%)	5 (8.3)	1 (5.0)	1 (5.0)	3 (15.0)	[a]**0.010***
1st year US result	**Stone present**, n (%)	15 (25.0)	10 (50.0)	4 (20.0)	1 (5.0)	

[a]: Fisher Freeman Halton Test, *: p=0.05, **: p=0.001

deviation, median, frequency, percentage, minimum, maximum) were used while evaluating the study data. Conformity of the quantitative data to a normal distribution was tested both by the Shapiro-Wilk test and by graphical methods. One-way analysis of variance and Bonferroni-corrected binary evaluations were used for comparing more than two groups of quantitative variables with normal distribution. Kruskal-Wallis test and Dunn-Bonferroni test were used for comparing more than two groups of quantitative variables that did not show normal distribution. Pearson chi-square test and Fisher-Freeman-Halton exact test were used to compare qualitative data. Statistical significance was accepted as $p<0.05$.

DISCUSSION

In this study, the effect of using different doses of UDCA after primary LSG on gallstone formation was investigated. While the development of gallstones decreased with the use of 1000 mg/day UDCA in the 6th month evaluation, it was determined that any dose of UDCA use in the 1st year was protective compared to the control group.

Today, surgery has become the first choice worldwide for those seeking permanent effects in the treatment of obesity. Studies are therefore aimed at increasing the effectiveness of this option and reducing its potential complications (19).

LSG surgery is a completely restrictive method with entero-endocrine system and transit through the normal physiological pathway. Details of CL development mechanism after LSG remain unclear. Low-calorie and low-fat diets cause the development of gallstones by affecting the bile-lipid composition. The bile-lipid content depends on the amount of cholesterol entering the liver. UDCA prevents supersaturation of bile and cholesterol stone formation by inhibiting cholesterol secretion into bile (20).

UDCA as a preventive agent for gallstone formation during weight loss was applied for the first time by Broomfield et al. in 1998 (21). Sugerman et al. and Miller et al. showed that gallstone formation was reduced if patients used UDCA for 6 months after restrictive bariatric surgery (22, 23). A meta-analysis showed that UDCA use significantly reduced gallstone formation compared to the placebo group (8.8% and 27.7%, respectively). As a result, it was reported that UDCA can effectively prevent CL after bariatric methods (16).

Despite these supportive reports on the use of UDCA, there is still no consensus regarding its routine use in prophylaxis. This may be due to the lack of different criteria defining appropriate indications for the use of UDCA in the light of the risk factors. Gastrointestinal side effects (nausea, vomiting, diarrhea or constipation) may affect patient compliance (24). Although the observed side effects seem to be the biggest obstacle to the continuity of use of the medicine, none of the side effects mentioned above were observed in this study.

Today, the guidelines of the American Association of Clinical Endocrinologists (AACE), the Turkish Obesity Surgery Society (TOSS), and the American Society for Metabolic and Bariatric Surgery (ASMBS) recommend performing routine US for the formation of CL after bariatric surgery, especially in the first six months (25). In our study, we investigated the presence of mud/stone in the control US at six and 12 months and its relationship with different doses of UDCA.

Symptomatic gallstones were seen in three (5%) of the 60 patients and these were operated on. These three patients were in the control group. Apart from these three patients in the group which did not receive UDCA after LGS, 19 patients were observed to be asymptomatic even though stones or mud were detected on US.

Regarding the key role of cholesterol in hepatic synthesis, dietary intake, and bile acid synthesis, the effect made

by UDCA in the difference between cholesterol and triglyceride values in the preoperative and postoperative serum and the formation of gallstones was investigated. Differences in cholesterol and triglyceride levels were ignored, as there was no statistical difference in stone formation between the groups.

No correlation was found between gallstone formation after LSG and comorbidities such as DM and hypertension (HT). Since there was no significant difference between the groups, the effects on stone formation in terms of DM were ignored. Although the rate of HT in Group I was found to be higher than that in the other two groups, we think that it may be ignored since there is as yet no study in the literature which shows a relationship between hypertension and gallstones.

Although studies have reported that EWL% has predictive value for CL development in obese patients, Moon et al., Shiffman et al., and De Oliveira et al. reported no significant difference in mean EWL% with symptomatic and asymptomatic gallstones after LSG (3, 24, 26, 27). In our study, no significant difference was found between the groups in terms of EWL% and TWL%, and the relationship with stone formation was ignored.

This study has some limitations. Although periodic US follow-ups were required for the development of CL for a longer period, US was only performed at 6 and 12 months in this study. As a result, this may lead to an incorrect assessment of the timing of CL development and an underestimation of gallstone formation. Since weight loss continues until the 18th month, it would be ideal to perform US control for stone formation until that time. The last of the limitations, and perhaps the most important, is the small sample size.

CONCLUSION

As a result of our study, it was determined that the use of 500 mg/day UDCA for six months after LSG reduced CL formation in the 1st year, but did not significantly reduce CL formation in the first six months. It was determined that use of 1000 mg/day UDCA for six months after LSG significantly decreased CL formation in the 6th and 12th months. Therefore, we recommend that patients be given 1000 mg/day UDCA after LSG. However, due to its low number of patients, we believe that this result should be supported by studies with larger sample sizes.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Health Sciences University, Istanbul Fatih Sultan Mehmet Training and Research Hospital (Date: 03.09.2019, No: 12030).

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EVALUATION OF THE FREQUENCY OF TROCAR SITE HERNIA AFTER LAPAROSCOPIC SLEEVE GASTRECTOMY

LAPAROSKOPİK SLEEVE GASTREKTOMİ AMELİYATI SONRASI TROKAR YERİ HERNİSİ GELİŞİM SIKLIĞININ ARAŞTIRILMASI

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ABSTRACT

Objective: Today, laparoscopic sleeve gastrectomy (LSG) has become the most frequently performed bariatric surgical method. One of the complications seen after LSG is trocar site hernia (TSH). There is no clear information about the rate of TSH detected radiologically after LSG. A thick abdominal wall and the failure to adequately expose the fascial defect related to this, as well as mobility limitations due to excessive subcutaneous fatty tissue, are the reasons for increased incidence of TSH.

Materials and Methods: The demographic characteristics and postoperative weight loss of patients who underwent LSG procedures and whose fascial defects in the trocar region were repaired with the Carter-Thomason Suture Passer (CTSP) in our clinic between January 2015 and June 2017 were evaluated. TSH evaluation was performed both through physical examination and superficial USG by a general surgeon who had radiological training on concurrent superficial abdominal ultrasonography (USG). Detected TSHs were divided into two groups: symptomatic and asymptomatic.

Results: A total of 61 patients were included in the study. The mean period after operation was calculated as 36 months (min 20, max 52). TSH was detected in seven (11.5%) of 61 patients, two of whom had symptomatic and five of whom had asymptomatic TSH. Being over 40 years of age and having a calculated body mass index (BMI) value greater than 30 kg/m^2 during measurement were found to be the factors that significantly increased the incidence of TSH ($p<0.05$).

ÖZET

Amaç: Günümüzde laparoskopik sleeve gastrektomi (LSG) en sık uygulanan obezite cerrahisi yöntemi haline gelmiştir. LSG sonrası görülen komplikasyonlardan biri de trokar yeri fıtığıdır (TSH). LSG sonrası radyolojik olarak saptanan TSH oranı hakkında net bir bilgi yoktur. Kalın karın duvarı ve buna bağlı fasya defektinin yeterince ortaya konulamaması, cilt altı yağ dokusunun fazla olmasına bağlı hareket kısıtlılığı, TSH insidansının artmasıyla suçlanan nedenlerdir.

Gereç ve Yöntem: Ocak 2015-Haziran 2017 tarihleri arasında kliniğimizde LSG ameliyatı yapılan ve trokar bölgesindeki fasya defektleri Carter-Thomason Sütür Geçirici (CTSP) ile onarılan hastaların demografik özellikleri ve ameliyat sonrası kilo kayıpları değerlendirildi. TSH değerlendirmesi, eş zamanlı yüzeyel abdominal ultrasonografi (USG) konusunda radyolojik eğitim almış bir genel cerrah tarafından hem fizik muayene hem de yüzeyel USG ile yapıldı. Tespit edilen TSH'ler semptomatik ve asemptomatik olmak üzere iki gruba ayrıldı.

Bulgular: Çalışmaya toplam 61 hasta dahil edildi. Ameliyat sonrası ortalama takip süresi 36 ay (min 20, max 52 ay) olarak hesaplandı. 61 hastanın yedisinde (%11,5) TSH (ikisi semptomatik, beşi asemptomatik) saptandı. Kırk yaşın üzerinde olmak ve ölçüm sırasında hesaplanan vücut kitle indeksi (VKİ) değerinin 30 kg/m^2'nin üzerinde olması TSH insidansını anlamlı olarak artıran faktörler olarak bulundu ($p<0,05$).

Sonuç: Sleeve Gastrektomi sonrası TSH oranını artıran faktörler ileri yaş ve yetersiz kilo kaybıdır.

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Conclusions: Advanced age and inadequate weight loss are the factors that increase the rate of TSH after Sleeve Gastrectomy.

Keywords: Morbid obesity, laparoscopic sleeve gastrectomy, trocar site hernia, Carter-Thomason suture passer

Anahtar Kelimeler: Morbid obezite, laparoskopik sleeve gastrektomi, trokar yeri fıtığı, Carter-Thomason sütür geçirici

INTRODUCTION

The frequency of the application of laparoscopic techniques in abdominal surgery increases day by day. The main benefits of laparoscopy consist of decreased postoperative pain, rapid return to daily activities and short hospitalization time (1, 2). The rate of trocar site-related complications was found to be 2.1/1000 in laparoscopy (3).

The causes of trocar site hernia (TSH) include factors such as trocar size, type of trocar, pre-existing fascial defect, and aspect of trocar entry (4, 5). It is known that the frequency of TSH development is even higher in obese patients (6). On the other hand, no clear ratio has been determined in the literature. It is shown in the literature that obesity is associated with the increase in trocar site-related complications; long trocar need, a thick abdominal wall, and mobility limitations due to excessive subcutaneous fatty tissue are some of the reasons for this condition (7).

In this study, we aimed to determine the frequency of TSH and the factors affecting the development of TSH in patients whose fascial defects of 10 mm or more were repaired with the Carter-Thomason Suture Passer (CTSP) after laparoscopic sleeve gastrectomy (LSG).

MATERIALS AND METHODS

This study included 61 patients who applied to the obesity follow-up outpatient clinic for any complaints or routine follow-ups over a 3-month period or who were called by telephone for follow-up and who underwent laparoscopic sleeve gastrectomy for morbid obesity between January 2015 and June 2017. This study was approved by the Clinical Research Ethical Committee of the Health Sciences University, Istanbul Fatih Sultan Mehmet Training and Research Hospital (Date: 12.09.2019, No: 80).

Patients with symptoms associated with trocar site hernia were also included in the study, while patients with multiple bariatric surgical procedures or abdominal operations were excluded. Physical examinations to detect hernias were performed both in standing and supine positions for all patients and also during the application of increased abdominal pressure using the Valsalva maneuver. All abdominal incision sites were examined. On physical examination, a hernia was defined as a bulging during the Valsalva maneuver and palpation of the fascial defect. USG was performed by a clinician who had previously received superficial USG evaluation training for trocar site hernia with a GE pro 500, 3 MHz, and abdominal incision sites were investigated for the presence of hernias (Figure 1, 2).

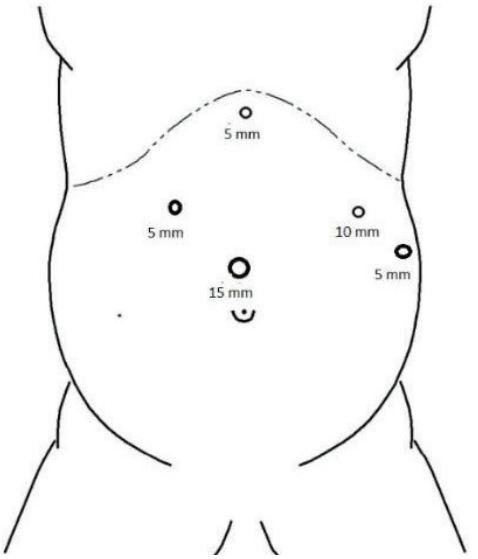

Figure 1: LSG trocar entry localizations

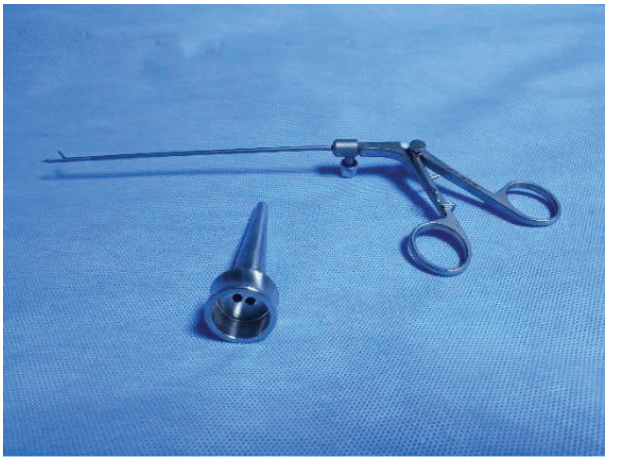

Figure 2: Carter-Thomason suture passer (CTSP)

The trocar entry localizations of the patients were all the same and are as follows: one 15-mm camera trocar (supraumbilical), one 10-mm working trocar (left upper quadrant), two 5-mm working trocars (right and left upper quadrant), and one 5-mm liver retractor. Trocar entry localizations are shown in Figure 3. During the fascia closure procedure, a No. 0 absorbable suture (PolyglactinYü-ce Vicryl®, Tekirdağ, Turkey) was used with

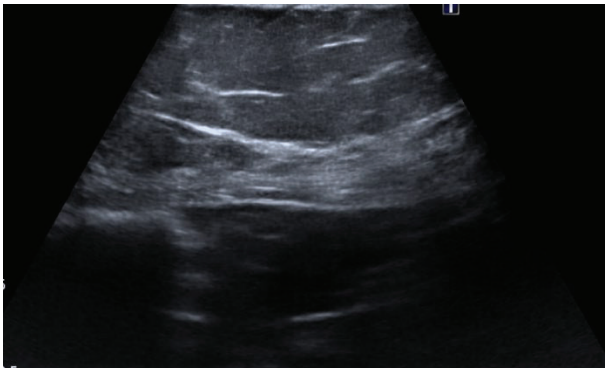

Figure 3: Normal superficial USG image from 5 cm superolateral to the umbilicus

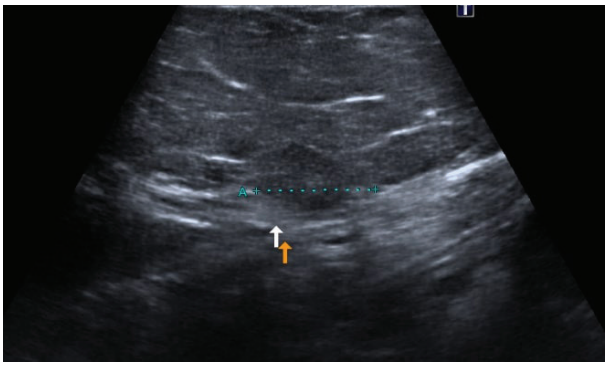

Figure 4: Superficial USG image of TSH

the CTSP (Figure 4). The 10- and 15-mm trocars were removed and a 10-mm reusable metal tube was placed in place of the trocar (Figure 4). Guided by the inserted metal tube, with the help of CTSP through the upper hole on the tube, No. 0 absorbable sutures (PolyglactinYü-ce Vicryl®, Tekirdağ, Turkey) were sent into the abdomen with camera. The metal tube with guide holes allowed the suture to advance through the fascia and peritoneum. Following this, CTSP was sent through the lower guide hole on the metal tube and the No. 0 Vicryl suture, which was advanced into the abdomen, was caught and taken out of the abdomen. The metal tube was slid out of the abdomen, taking care not to cause the suture to protrude. The suture was securely tied and the fascia closure procedure was terminated. The collected data were recorded in a pre-established database with age, gender, Diabetes Mellitus (DM), postoperative follow-up period, BMI at the time of operation and BMI during superficial USG.

Statistical analysis

When evaluating the findings obtained in this study, the IBM SPSS Statistics 22 (SPSS IBM, USA) program was used for statistical analysis. When evaluating the study data, the Shapiro Wilks test was used for the suitability of the parameters for normal distribution. In addition to the descriptive statistical methods (mean, standard deviation, frequency), the Student t-test was used for the two-group comparisons of the quantitative parameters with normal distribution, and the Mann Whitney U test was used for the two-group comparisons of the quantitative parameters without normal distribution. The Chi-square test and Fisher's exact test were used for the comparison of the qualitative data. The level of significance was set at $p<0.05$.

RESULTS

Between January 2015 and June 2017, a total of 61 patients (41 females and 20 males) aged between 21 and 64 years were included in the study. The demographic and descriptive characteristics of the cases are summarized in the table below (Table 1).

Table 1: Distribution of study parameters

	n, Mean±SD or %
Age	45.16±10.12
Follow-up period	39.2±7.91
BMI at the time of operation (kg/m²)	49.68±6.35
BMI during measurement (kg/m²)	33.06±6.95
BMI at the time of operation	
<50	33, 54.1%
≥50	28, 45.9%
Asymptomatic TSH	5, 8.2%
Symptomatic TSH	2, 3.3%
TSH development status	
No	54, 88.5%
Yes	7, 11.5%
Hernia region (n=7)	
Upper right quadrant	1, 14.3%
Supraumbilical	6, 85.7%

BMI: Body mass index, TSH: Trocar site herni

The follow-up period of the patients included in the study ranged from 20 to 52 months, with a mean of 39.2±7.91 and a median of 36.

The BMI values of the patients at the time of operation ranged from 36.85 to 69.06, with a mean of 49.68±6.35. The BMI values during measurement ranged from 17.42 to 48.49 and the mean value was calculated as 33.06±6.95.

During TSH evaluation, no TSH was detected in 96.7% of the patients upon physical examination at rest and Valsalva position, while it was detected in 3.3% of them upon

Table 2: Evaluation of study parameters in terms of TSH development status

	TSH development status		
	No	Yes	p
	Mean±SD	Mean±SD	
Age	44.24±10.16	52.29±6.87	[1]0.047*
Follow-up period	38.76±7.99 (36)	42.57±6.8 (45)	[2]0.364
Weight at the time of operation	131.87±20.09	132.86±12.32	[1]0.900
Weight during TSH evaluation	85.2±17.29	107.43±20.1	[1]0.003*
BMI at the time of operation	49.33±6.58	52.4±3.35	[1]0.231
BMI during TSH evaluation	31.85±5.91	42.43±7.69	[1]0.000*
BMI difference	17.48±5.55	9.97±7.61	[1]0.002*
	n (%)	n (%)	
Asymptomatic	54 (100%)	5 (71.4%)	[3]0.011*
Symptomatic	0 (0%)	2 (28.6%)	
Diabetes mellitus			
Yes	6 (11%)	2 (29%)	[3]0.225*
No	48 (89%)	5 (71%)	

[1]: Student t-test, [2]: Mann Whitney U test, [3]: Fisher's exact test, *: p<0.05
BMI: Body mass index, TSH: Trocar site hernia

physical examination. Of the patients with hernia, 14.3% had TSH in the right upper quadrant and 85.7% in the supraumbilical trocar site.

The incidence of TSH was found to be significantly lower in the patients with adequate weight loss compared to those without adequate weight loss after operation (p<0.05) (Table 2).

DISCUSSION

In our study, despite the repair of all fascial defects of 10 mm or more after LSG procedure, we determined the rate of development of symptomatic (detected upon physical examination) and asymptomatic (not detected upon physical examination, can only be revealed in superficial USG) TSH as 11.5%. Among these cases, the rate of symptomatic TSH was only 3.3%. Asymptomatic TSH, which could not be detected upon physical examination, was detected in 8.2% of the patients.

In the literature, the incidence of TSH was reported to be between 0.23% and 3.1% in the general population (8). This rate was found to be higher in obese patients. In correlation with this, the incidence of TSH was found to be higher in our study.

The incidence of incisional hernia development in obese patients with higher BMI rates was found to be increased by Uslu et al. (9). However, there are no studies in the literature with a high level of evidence that can clearly show the incidence of trocar site hernia in obese patients. In our study, this rate was found to be 11.5%. In this study, while the frequency of symptomatic TSH was found to be consistent with the literature, we found that this frequency increased in superficial USG applied to asymptomatic patients. We found that TSH was more common in patients who could not lose weight after bariatric surgery. We think that the reason for the higher TSH frequency in this study compared to the literature is due to the high number of patients who could not achieve adequate weight loss among the patients included in the study.

Iranmanesh et al. were able to reach 23 of 70 patients who underwent bariatric surgery and in whose operation the CTSP and NeoClose suture passer were used as fascia closure devices at the one-year follow-up in their study. They did not encounter any TSH upon physical examination and abdominal ultrasonography during the one-year follow-up of these 23 patients. Considering this situation, we thought that the incidence of TSH increased as the follow-up period was prolonged (10).

In the literature, weight loss failure was described as <50% Excess Weight Loss (EWL) postoperatively, less than 20% loss of preoperative total body weight, and BMI above 35 kg/m^2 (11). In our study, the group described as unable to lose weight was formed in light of this information.

Full-thickness closure of all anatomical walls is very difficult in the obese patient group. Hand-assisted closure is often not successful and also causes time loss (12). The use of new laparoscopic trocar site closure techniques instead of the traditional hand-assisted fascia closure technique enables the peritoneal and fascial leaves to be closed together. At the same time, the repair of trocar site defects in obese patients can be performed in a much safer and easier way thanks to new laparoscopic trocar site closure techniques (13).

Many risk factors have been identified in the development of incisional hernia or TSH. Some of those are advanced age, gender (male>female), nutritional status, diabetes, anemia, steroid treatment, renal failure, cancer, and wound infections (14). We found that the frequency of TSH development was higher in advanced age patients in our study. At the same time, we thought that the frequency of TSH development was higher in patients with less postoperative weight loss due to higher intraabdominal pressure.

In the literature, it is recommended to close the fascial defects of the trocars with a thickness of 10 mm and more as the risk of developing TSH is high (15, 16). Contrary to this information, in our study, we found that a patient developed hernia from a 5-mm trocar area. In the study group where we evaluated a small number of cases, it was not possible to make any further assumptions about 5-mm trocar site hernia. However, randomized studies with a higher number of patients should investigate the factors that affect the risk of hernia in these areas.

BIAS

Some of the most important drawbacks of this study are that it was not prospectively designed, the number of the patients was relatively low, and the procedures were performed by more than one general surgeon.

In the literature, there is no clear ratio showing the incidence of TSH development after LSG procedure in morbidly obese patients.

CONCLUSION

In patients who did not lose enough weight after laparoscopic sleeve gastrectomy and in elderly patients, the rate of development of trocar site hernia is higher and most of these patients are asymptomatic. In these patients, physical examination is not successful in the evaluation of trocar site hernia and superficial USG is recommended.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Health Sciences University, Istanbul Fatih Sultan Mehmet Training and Research Hospital (Date: 12.09.2019, No: 80).

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FACTORS PREDICTING RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED GASTRIC CANCER

LOKAL İLERİ MİDE KANSERİNDE NEOADJUVAN KEMOTERAPİYE YANITI ÖNGÖREN FAKTÖRLER

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ABSTRACT

Objective: In the treatment of local advanced gastric cancer (LAGC), it is recommended to start with neoadjuvant chemotherapy (NAC). Although the benefits of NAC have been shown, it is still not fully understood which patients respond better. The aim of this study was to investigate the effect of hematological and histopathological parameters on the response to chemotherapy.

Materials and Methods: A retrospective examination was made of 38 patients who underwent surgery for LAGC after receiving NAC. Evaluations were made by comparing the demographic characteristics, histopathological characteristics in an endoscopic biopsy of the tumor, preoperative hemoglobin levels, and neutrophil-lymphocyte ratios with the postoperative pathological response to determine which tumor characteristics gave a better response.

Results: In the postoperative histopathological evaluation, there was a pathological complete response to chemotherapy in two patients (6%), grade 1 in 9 patients (24%), grade 2 in 13 patients (34%), and grade 3 in 14 patients (36%). A statistically significant relationship was determined between the histopathological absence of perineural invasion and pathological complete response (p=0.023).

Conclusion: A relationship between perineural invasion and poor response to chemotherapy was determined. Although not

ÖZET

Amaç: Lokal ileri mide kanserinde (LİMK) tedaviye neoadjuvan kemoterapi (NAK) ile başlanması önerilir. Ancak NAK'nin faydaları gösterilmiş olsa da hangi hasta gurubunun tedaviye daha iyi yanıt verdiği tam olarak anlaşılamamıştır. Çalışmamızda hematolojik ve histopatolojik parametrelerin kemoterapi yanıtı üzerine etkisi araştırılmıştır.

Yöntem ve Gereç: Bu çalışmada lokal ileri evre mide kanseri nedeni ile neoadjuvan kemoterapi görüp sonrasında ameliyat edilen 38 hasta retrospektif olarak incelendi. Ameliyat sonrası patolojik yanıt ile hastanın demografik özellikleri, tümörün endoskopik biyopsideki histopatolojik özellikleri, preoperatif hemoglobin seviyesi, nötrofil lenfosit oranı karşılaştırılıp hangi özellikteki tümörlerin daha iyi yanıt verdiği değerlendirildi.

Bulgular: Ameliyat sonrası yapılan histopatolojik değerlendirmede 2 (%6) hastada patolojik tam yanıt, 9 (%24) hastada grade 1 yanıt, 13 (%34) hastada grade 2 yanıt ve 14 hastada (%36) grade 3 yanıt mevcuttu. Patolojik tam yanıt ile histopatolojik olarak perinöral invazyon olmaması istatistiksel olarak anlamlı ilişki gösterdi (p=0,023).

Sonuç: Çalışmamızda NAK alan LİMK'li hastalarda perinöral invazyon varlığının kemoterapiye kötü yanıt ile ilişkisi olduğu görülmüştür. Ayrıca lenfovaskular invazyon ve düşük grade mevcudiyetinde istatistiksel olarak anlamlı olmasa da kemoterapiye kötü yanıt gözlenmiştir.

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at a statistically significant level, there was also observed to be a poor response to chemotherapy in the presence of low grade and lymphovascular invasion.

Keywords: Local advanced gastric cancer, neoadjuvant chemotherapy response, pathological complete response, perineural invasion

Anahtar Kelimeler: Lokal ileri mide kanseri, neoadjuvan kemoterapi yanıtı, patolojik tam yanıt, perinöral invazyon

INTRODUCTION

Gastric cancer is the fifth most commonly diagnosed cancer worldwide, and the third most common cause of cancer-related death (1). Although 5-year survival is >90% when determined at the early stage, approximately two-thirds of patients are diagnosed at an advanced stage and survival decreases to <50% for these patients (2-4).

The chance of a cure with surgery is high in early-stage gastric cancer, but it is recommended to start treatment of local advanced gastric cancer (LAGC) with neoadjuvant chemotherapy (NAC). According to the National Comprehensive Cancer Network (NCCN) guidelines, NAC or neoadjuvant chemoradiotherapy is recommended for LAGC patients in any group at clinical grade ≥T2 (5). The aim of NAC is to down-stage the tumor before resection, inhibit micrometastasis, increase the R0 resection rate and reduce the risk of recurrence and metastasis. Tumors which are not suitable for resection can be shrunk with NAC to become suitable for operation. Consequently, while survival rates increase with NAC, the radical resection rate and the risk of postoperative recurrence and metastasis decrease (6, 7).

Many studies have been conducted to determine the factors affecting prognosis in operable gastric cancer, and the TNM grading system is accepted as the most important independent prognostic factor by the American Joint Committee on Cancer (AJCC) (8). In addition, the presence of lymph node metastasis without serosal involvement, the extent of lymphatic spread, invasion depth, tumor macroscopic type, histological growth pattern, the presence of lymphovascular invasion (LVI), tumor region, grade, and resection type, and patient age have also been shown to have an effect on prognosis (9-11).

Although studies have shown that reaching pathological complete response (pCR) with NAC provides a survival advantage in LAGC, there have been no studies investigating in which conditions there is an increase in pCR (12, 13). The pCR rate with neoadjuvant treatment in gastric and gastroesophageal junction cancers varies between 15% and 30%, and the chance of R0 resection has been reported to be 70-77% (14, 15). There are also studies showing an increase in survival with a partial response even if pCR is not obtained (16). However, not all LAGCs benefit from NAC, and approximately 15% of patients show tumor progression (17). While NAC provides an improvement in prognosis for patients with increasing pCR and the chance of R0 resection, it may cause a delay in surgery and disease progression in the patient group who do not respond or have an insufficient response (13). The determination of the patient groups for which NAC is more effective will allow the selection of subgroups who will benefit from treatment and eliminate unwanted delays for other groups, thereby providing more benefit to the prognosis of patients.

The aim of this study was to investigate the tumor characteristics in gastric cancer that respond better to NAC.

MATERIALS AND METHODS

Data collection

The study included 38 patients who received NAC at Medipol University Hospital and then underwent surgery at Medipol University Hospital and Gaziosmanpasa Training and Research Hospital between January 2015 and April 2019, reviewed retrospectively.

Approval for this study was granted from Gaziosmanpasa Training and Research Hospital Ethics Committee for Clinical Studies (Date: 28.05.2020, No: 80). The study was performed in accordance with the Declaration of Helsinki.

The study inclusion criteria were as follows: 1) Histopathological diagnosis of adenocarcinoma, and 2) A clinical LAGC diagnosis (≥T2, Nany). Patients were excluded from the study if they had distant metastasis, and other exclusion criteria were as follows: 1) Non adenocarcinoma histology, 2) Emergency or palliative resection during treatment, 3) The patient refused to complete the neoadjuvant treatment, 4) There were contraindications for NAC and surgery, 5) A history of gastric surgery.

Evaluation was made of 38 patients diagnosed with LAGC after the exclusion of 31 patients following further tests; 9 were determined to have metastasis, 10 with bleeding, seven with partial obstruction, and five applied to have emergency surgery because of perforation. No patient abandoned treatment. All patients underwent preoperative metastasis scanning, and before NAC, evaluation with gastroscopy and abdominal computed tomography (CT). All the patients were diagnosed with adenocarcinoma, and in seven patients there was signet ring cell differentiation.

The majority of patients received chemotherapy with the FOLT protocol and the FOLFOX regimen was applied to only three patients. The data flow chart is shown in Figure 1.

Data were retrieved from the medical records of each patient in respect of medical history, age, sex, chemotherapy regimen of NAC, surgical method, and pathology results (e.g. histological type, tumor size, histological grade, perineural invasion (PNI), LVI and laboratory data.) The tumor size (T stage), lymph node status (N stage), presence of metastasis (M stage) and the American Joint Committee on Cancer (AJCC) stage for each patient were obtained from the cancer registry data (5). Tumor size was assessed in three groups as <4.5 cm, 4.5-8 cm and >8 cm (18).

In histological evaluation, the data obtained from all patients were included in the statistics and histological type, grade, presence of perineural invasion, presence of lymphovascular invasion and human epidermal growth factor receptor 2 (HER 2) status were included in the evaluation. Both the WHO classification and the Lauren classification were used for histological evaluation. In the Lauren classification, it was evaluated in 3 groups as diffuse intestinal and mixed type, as it was made in 1965 and is still ongoing. According to the WHO classification, it was

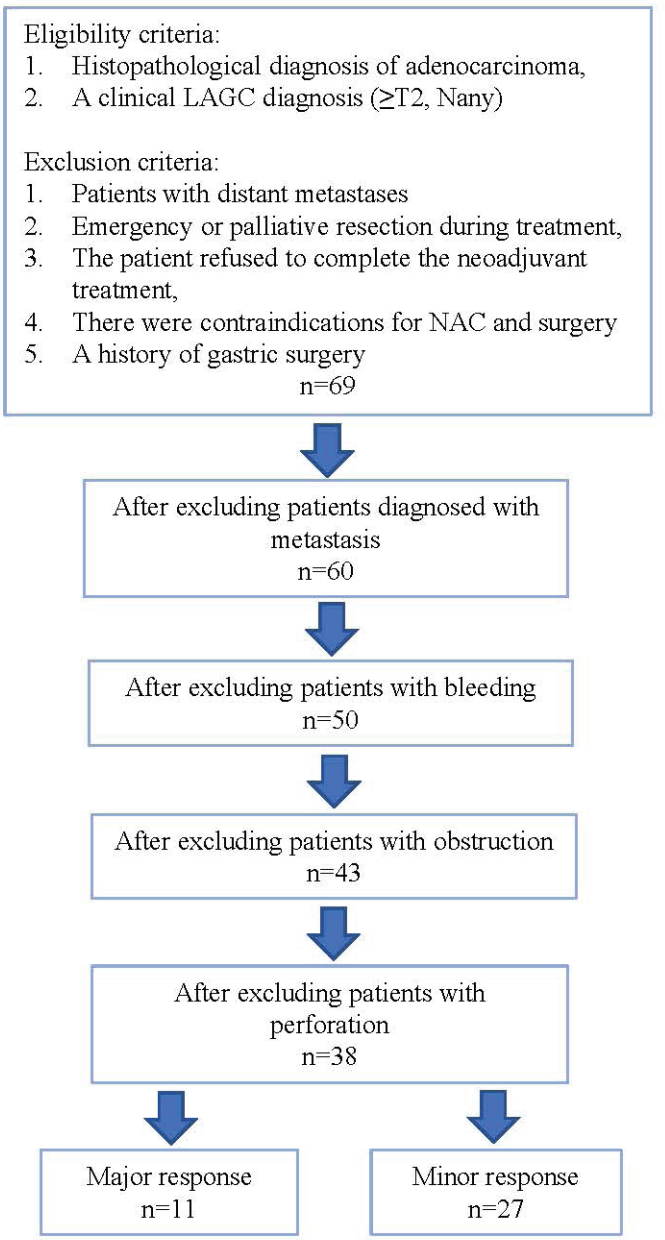

Figure 1: The data flow chart

graded in 3 groups as well-differentiated, moderately differentiated and poorly differentiated (19, 20). In the HER 2 scoring, 0 was accepted as no reaction in tumor cells or incomplete reaction in ≤10% of tumor cells, 1 as paleness in >10% of the tumor cells and incomplete membranous reaction that was difficult to differentiate, 2 as incomplete weak or moderate level membranous reaction in >10% of the tumor cells or a complete strong membranous reaction in ≤10% of the tumor cells, and 3 as a uniform strong membranous reaction in >10% of the tumor cells. Scores 0 and 1 were accepted as negative, Score 2 as suspected positive, and Score 3 as a positive reaction. Patients with Score 2 were then evaluated according to fluorescent *in situ* hybridization (FISH), and were included in the negative or positive group according to the results (21).

Chemotherapy

For neoadjuvant treatment, the patients were administered the FLOT regimen (docetaxel (50 mg/m^2), oxaliplatin (85 mg/m^2), and LV (200 mg/m^2) with short-term infusional FU (2600 mg/m^2 as a 24-hour infusion) or the FOLFOX regimen (oxaliplatin 85 mg/m^2 IV + leucovorin 400 mg/m^2 IV + fluorouracil 400 mg/m^2 IV bolus + fluorouracil 2400 mg/m^2 IV) (22, 23).

Surgery

The surgical technique applied to all the patients was D2 lymphadenectomy with curative total gastrectomy. D2 lymph node dissection including the 1, 2, 3, 4, 5, 6, 7, 8a, 9, 11p, 11d and 12 were performed so that at least 15 lymph nodes were removed (24).

Assessment of chemotherapy response

Following retrospective collection of patient demographic data, such as age and gender, the radiological, gastroscopic, and histopathological findings before and after NAC were compared with the pathological response. In the histopathological examination, evaluations were made of tumor grade, differentiation, PNI, and LVI, and the presence of HER 2 and necrosis to determine which tumors with which characteristics benefited most from NAC.

The College of American Pathologists Tumor Regression Grading (CAP-TRG) system was used to evaluate the response to NAC. Classification in this system is defined as Grade 0: No viable cancer cells (complete response); Grade 1: Single cells or small groups of cancer cells (moderate response); Grade 2: Residual cancer cells with evident tumor regression but more than single cells or rare small groups of cancer cells (minimal response); Grade 3: Minimal or no tumor killed or extensive residual cancer (poor response) (25). The response to treatment in the histopathology samples according to the CAP-TRG system is shown in Figure 2.

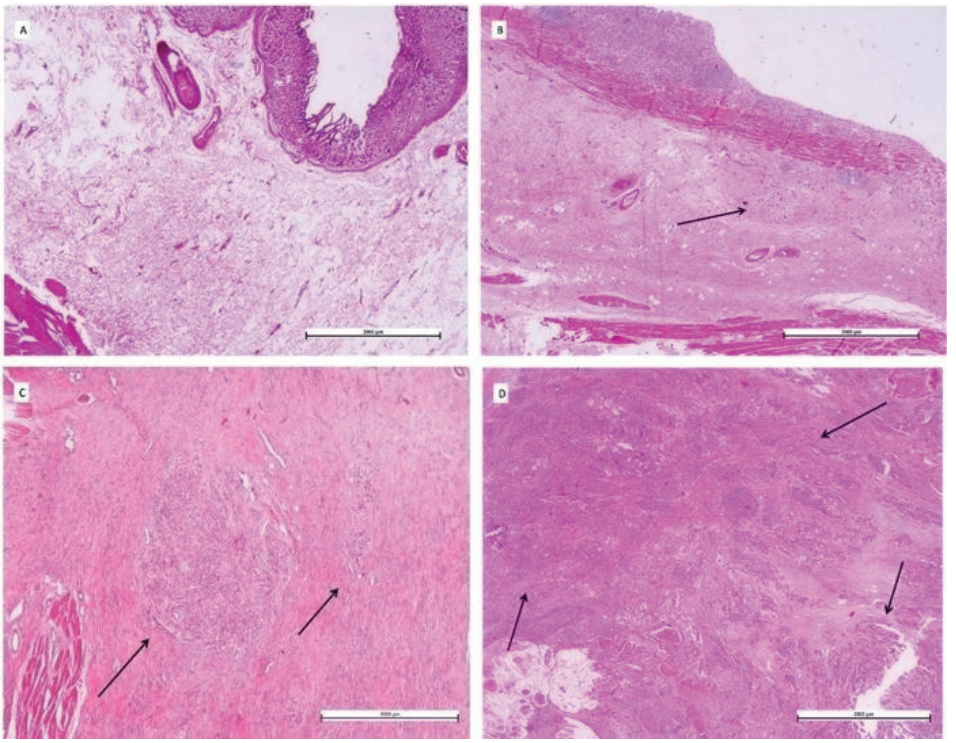

Figure 2: The response rates to asses neoadjuvant chemotherapy according to the College of American Pathologists Tumor Regression Grading (CAP-TRG) **A)** CAP-TRG Grade 0 (pCR), **B)** CAP-TRG Grade 1, **C)** CAP-TRG Grade 2, **D)** CAP-TRG Grade 3 (H&E x 20).

All subsequent analyses were made after separating the patients into major response (grades 0 and 1) and minor response (grades 2 and 3) (26).

From the hemoglobin level in the routine blood tests performed one week before NAC, the neutrophil-lymphocyte rate (NLR) was calculated as the ratio of absolute neutrophil count to absolute lymphocyte count, and the correlation of NLR to response to NAC was evaluated.

Clinical outcomes

Prognosis was analyzed with the disease-free survival rate (defined as the time elapsed between surgery with curative intent and reappearance of disease, either locally or distant in months) and the overall survival rate (defined as the time elapsed between surgery with curative intent and death due to disease in months).

Follow-up

In the first two years after surgery, patients were followed up regularly at three or four-month intervals and then every six months in the third year, and annually thereafter. During the follow-up visits, patients underwent physical examination, complete blood tests, chest radiography and CT or magnetic resonance imaging (MRI) as clinically indicated. Upper gastrointestinal endoscopy was used to verify locoregional recurrence.

Statistical analysis

All statistical analyses were performed using the SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA). Conformity of continuous variables to normal distribution was assessed with the Shapiro Wilk test. Normally distributed parametric data were presented as mean ± standard deviation (SD) values. In the comparison of variables showing normal distribution, according to the major and minor response groups, the Student's t-test was used and for those not showing normal distribution, the Mann Whitney U-test. The Chi-square test and Fisher's Exact test were applied in the analysis of categorical data. To determine variables with an effect on the groups, Logistic Regression Analysis was performed. The survival analysis was calculated using the Kaplan–Meier method, and the log-rank test was used for the univariate analysis. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Evaluation was made of 38 patients diagnosed with LAGC. There was a PCR to chemotherapy in 2 (6%) patients, Grade 1 in 9 (24%) patients, Grade 2 in 13 (34%), and Grade 3 in 14 (36%). The relationships between the pathological response and the laboratory findings, demographic and histopathological data are shown in Table 1.

When the statistical results were examined, a significant relationship between the presence of PNI and a poor response to NAC in univariate analysis ($p=0.023$) was observed. In logistic regression analysis, only the presence of PNI was found to be related to the absence of tumor regression (OR: 9.692;(CI 1.074-87.437); $p=0.043$). A large proportion of patients with a major response were Her 2 negative, high grade and without LVI, but this was not statistically meaningful ($p>0.05$). No statistically significant relationship was seen between hemoglobin levels and NLR and pathological response ($p=0.625$ and $p=0.727$, respectively). In patients with pCR, there was no PNI and LVI, and the tumor grade was high in these patients. There was regression in tumor size and the possibility of R0 resection was obtained in all patients. No relationship was determined between major response and overall survival (OS), disease-free survival (DFS), metastasis and recurrence ($p>0.05$). The follow-up periods, DFS, OS, metastasis, and mortality rates are shown in Table 2. No metastasis or mortality was seen in the patients with pathological complete response. The multivariate analysis results are shown in Table 3. Overall survival according to histopathological response groups is summarized in Figure 3.

DISCUSSION

Significant changes have happened in oncological therapy in recent years, and are reported to improve prognosis, especially in local advanced stage tumors with neoadjuvant therapy (27). Neoadjuvant treatment of aggressive solid organ malignancies has many proven advantages. Early treatment of distant microscopic disease is provided with NAC, and shrinkage can occur in the tumor increasing resectability (28). There are publications in LAGC with NAC, there is an increase up to 30% in pCR and R0 resection up to 100%, although the complete response rates are not high in most researches (16, 29, 30). In this regard, it is important to determine which patients will be preferred by NAC. Factors affecting NAC response have been researched in our study for this purpose. Our study assessed in which patient group the pathological response was better, and R0 resection could be done in all patients after NAC even if it was not pCR.

Shrinkage of the tumor to obtain R0 resection in patients diagnosed with LAGC has an effect on prognosis and facilitates surgery. In the MAGIC study, 503 patients diagnosed with ≥T2 stomach, gastroesophageal junction, and distal esophagus adenocarcinoma were randomized into two groups. One group had surgery only and the patients in the other group were given NAC, then operated on, and were subsequently administered adjuvant chemotherapy. The results of that study showed that pCR was not obtained with NAC but the resected tumors were significantly smaller in size and less nodal metastasis was determined. Although 58% of the patients could

Table 1: Summary of the demographic, histopathological and laboratory findings of the patients according to response

		Major response n (11)		Minor response n (27)		Total n (38)		p value
		mean±SD		mean±SD		mean±SD		
Age (year)		62.41±10.54		58.52±11.84		59.65±11.48		0.356[a]
NAC_lf (10^9/L)		2.39±1.28		1.88±0.68		2.03±0.91		0.278[a]
NAC_nt (10^9/L)		4.68±2.63		5.04±2.42		4.94±2.46		0.751[a]
NLR		2.89±1.25		3.07±1.59		3.02±1.48		0.727[a]
HB (g/dl)		12.37±2		12.03±1.89		12.13±1.90		0.625[b]
		n	%	n	%	n	%	p value
Gender (%)								
	Male	10	90.9	21	77.8	31	81.6	0.648[b]
	Female	1	9.1	6	22.2	7	18.4	
Tumor localization								
	Proximal	2	18.2	6	22.2	8	21.1	0.852[c]
	Distal	6	54.5	12	44.4	18	47.4	
	EGJ	3	27.3	9	33.3	12	31.6	
Tumor size (cm)								
	<4.5 CM	7	63.6	11	40.7	18	47.4	0.427[c]
	4.5-8 CM	3	27.3	13	48.1	16	42.1	
	>8 CM	1	9.1	3	11.1	4	10.5	
Stage								
	Stage 2a	1	9.1	0	0	1	2.6	0.174[c]
	Stage 2b	0	0	4	14.8	4	10.5	
	Stage 3	8	72.7	21	77.8	29	76.3	
	Stage 4a	2	18.2	2	7.4	4	10.5	
Lauren classification								
	Intestinal	3	27.3	9	33.3	12	31.6	0.644[c]
	Diffuse	5	45.5	8	29.6	13	34.2	
	Mixed	3	27.3	19	0.37	13	34.2	
Differentiation								
	Well-moderate	4	36.4	9	33.3	13	34.2	1.00[b]
	Poorly	7	63.6	18	66.7	25	65.8	
Grade								
	Low-moderate	2	18.2	11	40.7	13	34.2	0.268[b]
	High	9	81.8	16	59.3	25	65.8	
PNI								
	Absent	9	0.9	13	48.1	22	59.5	**0.028[b]***
	Present	1	0.1	14	51.9	15	40.5	
LVI								
	Absent	7	0.7	9	33.3	16	43.2	0.067[b]
	Present	3	0.3	18	66.7	21	56.8	
HER 2								
	Absent	10	90.9	20	0.87	30	88.2	1.00[b]
	Present	1	9.1	3	0.13	4	11.8	

[a]: Mann Whitney U test, [b]: Fisher Exact test, [c]: Chi-Square test, *: refers to the higher ratio ($p<0.05$).
SD: Standard deviation, NAC_lf: One week before NAC the lymphocyte ratio. NAC_nt: One week before NAC the neutrophil ratio, NLR: neutrophil to lymphocyte ratio, HB: hemoglobin, EGJ: esophagogastric junction, HER 2: Human epidermal growth factor receptor-2, PNI: perineural invasion, LVI: lymphovascular invasion

Table 2: Follow up times of the patients, disease free survival (DFS), mean survival (OS) and findings of metastasis and death

		Major response n (11)		Minor response n (27)		Total n (38)		p value
		mean±SD		mean±SD		mean±SD		
Follow-up (month)		18,45±10,73		20,15±13,54		19,66±12,67		0.899[a]
DFS (month)		17.36±9.94		18.37±13.66		18.08±12.57		0.874[a]
		n	%	n	%	n	%	
Cancer-related death								1.00[b]
	Absent	7	63.6	16	59.3	23	60.5	
	Present	4	36.4	11	40.7	15	39.5	
Metastasis								0.720[c]
	Absent	9	81.8	20	74.1	29	76.3	
	Present	2	18.2	7	25.9	9	23.7	
Recurrence								1.00[b]
	Absent	10	90.9	25	92.6	35	92.1	
	Present	1	9.1	2	7.4	3	7.9	

[a]: Mann Whitney U test, [b]: Fisher Exact test, [c]: Chi-Square test, DFS: Disease free survival

Table 3: Multivariate analysis

	B	S.E.	Wald	Sig.	Exp (B)	95% C.I.for EXP(B)	
						Lower	Upper
NLR-categorical (>2.06)	0.142	0.837	0.029	0.866	1.152	0.224	5.939
PNI	1.659	1.206	1.891	0.169	5.254	0.494	55.899
LVI	1.107	0.925	1.434	0.231	3.026	0.494	18.527
Grade	-1.019	0.987	1.065	0.302	0.361	0.052	2.500
Constant	1.613	0.619	6.796	0.009	5.016		

NLR: neutrophil to lymphocyte rate, PNI: perineural invasion, LVI: lymphovascular invasion, S.E.: standard error

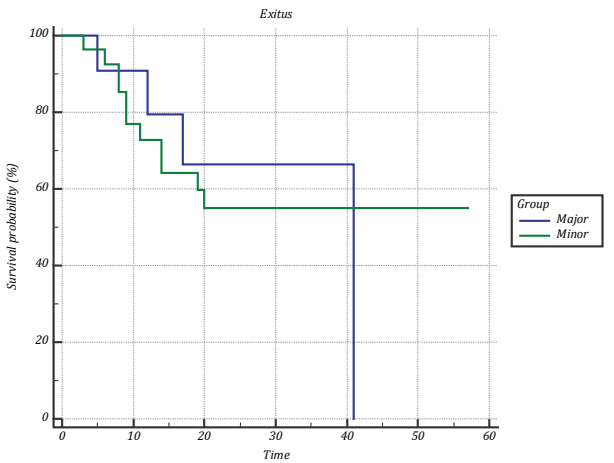

Figure 3: Overall survival according to histopathological response groups
Survival time does not differ between minor and major response groups (p=0.876).

not complete treatment in the postoperative period, OS and DFS were better in the group that received chemotherapy (31).

In a study by Ychou et al, 224 patients were randomized to surgery and NAC+adjuvant chemotherapy groups. The R0 resection rate was seen to increase with NAC, and there was an improvement in OS and DFS rates (32).

Although many studies have shown an increase in survival and R0 resection rates with neoadjuvant treatment in gastric cancer, as stated above, the factors affecting the increase in pathological response have not been extensively investigated. There are few studies in the literature on this subject. Li et al investigated the effect of HER 2, Ki67 and p53 proteins on NAC response and concluded that Ki67 was an independent determinant of NAC efficacy (33). In another study, HER 2 and p53 were found to be predictive factors of the response to neoadjuvant

chemotherapy with the FOLFOX regimen (34). The histopathological factors predicting NAC response in LAGC were investigated in another study and the most important predictors affecting pathological response were reported to be tumor differentiation and tumor size (35). In 2017, Molina et al. examined histopathological parameters predicting NAC response in LAGC, and found that LVI, PNI, and high grade were associated with a poor response to treatment. No relationship was determined between NAC response and age, gender, inflammatory reaction in the tumor, and desmoplasia (26).

It is more difficult to obtain pCR in LAGC than in other solid organ tumors. The important question here is to determine which patients will have a better response to treatment. From the available data, it can be thought that there will be a better response to NAC in certain subgroups of gastric cancer.

The results of the current study showed a relationship between major pathological response and the absence of PNI. Furthermore, although not statistically significant, there was a better response to NAC in patients without LVI. In contrast to findings in literature, a better clinical response was observed in high grade tumors in the current study (26). However, no relationship was determined with age, gender, tumor localization, grade or size.

There are studies in the literature that have shown that of the hematological parameters, NLR is correlated with the long-term results in lung, colorectal, pancreas and stomach cancers and that an increased NLR is predictive of mortality. There are also studies that have shown an increase in lymphocyte count and an increase in anti-cancer activity associated with lymphocyte-mediated antibodies, and therefore claimed a potential increase in pCR (36, 37). However, the effect of NLR on pathological response has only been evaluated in one study. In that study of breast cancer patients receiving neoadjuvant chemotherapy, an increase in pCR when NLR was <2.06 (38-40) was determined.

When starting the current study, it was predicted that in addition to hemoglobin level and histopathological features such as PNI, LVI, grade and differentiation, NLR, as an inflammatory indicator, would be a factor affecting NAC response, but no relationship was determined between NLR and pathological response. The lymphocyte count was higher in the patient group showing a major response, but this was not reflected in the NLR. Predictive importance of NLR could not be determined in this study. Nevertheless, it could be predictive of NAC response in stomach cancer and in other malignancies such as breast cancer and lung cancer, and there can be considered to be a need for re-evaluation in further studies with larger patient numbers.

There were some limitations to this study, primarily that the endpoint was limited to pCR. The follow-up periods of the patients, OS and DFS were added to the study but long-term clinical results were not evaluated. As a limited number of patients were included in this study there is a need for further more extensive studies to reach significant results. The same chemotherapy regime wasn't applied to all of the patients. Although this did not affect the results , it can be assessed as a limitation. It is thought that with more robust evidence similar results will be obtained and should be supported with studies that will determine the parameters predicting NAC response.

In conclusion, the results of this study demonstrated that PNI is a parameter predicting response to chemotherapy in LAGC patients receiving NAC. In the clinical evaluation, the absence of LVI and high grade were correlated with good response. Given the fact that an increased lymphocyte count stimulates the immune response, and it has not been previously investigated as a factor with predictive value in gastric cancer, NLR was investigated as it can be easily calculated from routine blood tests in all patients at no extra cost, but it was not found to be statistically significant. However, further studies with a higher number of patients are needed for PNI and other parameters to be used in clinical practice.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul Gaziosmanpasa Training and Research Hospital. (Date: 28.05.2020, No:80)

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ELEVATED TRIGLYCERIDE GLUCOSE INDEX IS RELATED TO THE PRESENCE OF HEART FAILURE

YÜKSEK TRİGLİSERİD GLUKOZ İNDEKSİ KALP YETMEZLİĞİ VARLIĞIYLA İLİŞKİLİDİR

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ABSTRACT

Objective: Previous studies have shown a significant association between insulin resistance (IR) measured by using different methods and heart failure (HF). In recent years, the triglyceride glucose (TyG) index has been used to measure IR, and there are several reports showing that the TyG index indicates conditions such as metabolic syndrome (MetS) and atherosclerotic process. However, there is no study investigating the association of the TyG index with HF. Therefore, we aimed to evaluate the role of the TyG index in HF presence and its relationship with HF severity in this study.

Materials and Methods: Sixty-nine subjects matched for age and gender were analyzed retrospectively. The TyG index was used to measure IR and was calculated by the formula Ln [fasting triglycerides (mg/dl) x fasting glucose (mg/dl)/2]. The severity of HF was assessed by New York Heart Association (NYHA) functional class, left ventricular ejection fraction (LVEF) and N- terminal prohormone brain natriuretic peptide (NT-proBNP).

Results: HF patients had higher TyG index (9.11±0.59 vs. 8.55±0.55; p<0.001) but there was no correlation between TyG index with HF severity identified by NYHA functional class, LVEF and NT-proBNP. The ROC curve showed the cut-off point of the TyG index in determining HF as 9.19 with 71% sensitivity and 51% specificity (AUC:0.745, p<0.001).

Conclusion: TyG index may be a useful marker for diagnosis of HF, but is not correlated with HF severity.

Keywords: Heart failure, insulin resistance, triglyceride glucose index

ÖZET

Amaç: Önceki çalışmalar, farklı yöntemler kullanılarak ölçülen insülin direnci (IR) ile kalp yetmezliği (KY) arasında önemli bir ilişki olduğunu göstermiştir. Son yıllarda, trigliserid glikoz (TyG) indeksi IR'ni ölçmek için kullanılmaktadır ve TyG indeksinin, metabolik sendrom (MetS) ve aterosklerotik süreç gibi durumları gösterdiğine dair birkaç rapor vardır. Ancak TyG indeksinin KY ile ilişkisini araştıran herhangi bir çalışma yoktur. Bu nedenle, biz bu çalışmada TyG indeksinin KY varlığındaki rolünü ve KY şiddeti ile ilişkisini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Yaş ve cinsiyet uyumlu 69 hasta geriye dönük olarak incelendi. IR'ni ölçmek için TyG indeksi kullanıldı ve Ln [açlık trigliseridleri (mg/dl) x açlık glikozu (mg/dl)/2] formülüyle hesaplandı. KY'nin şiddeti, New York Kalp Derneği (NYHA) fonksiyonel sınıfı, sol ventriküler ejeksiyon fraksiyonu (LVEF) ve N-terminal prohormon beyin natriüretik peptidi (NT-proBNP) ile değerlendirildi.

Bulgular: KY hastaları daha yüksek TyG indeksine sahipti (9,11±0,59'a karşı 8,55±0,55; p<0,001). Ancak NYHA fonksiyonel sınıfı, LVEF, NT-proBNP ile tanımlanan KY şiddeti ile TyG indeksi arasında herhangi bir korelasyon yoktu. ROC eğrisi, KY'nin belirlenmesinde TyG indeksinin kesme noktasını %71 duyarlılık ve %51 özgüllük ile 9,19 olarak gösterdi (AUC:0,745, p<0,001).

Sonuç: TyG indeksi, KY tanısı için yararlı bir belirteç olabilir ancak KY ciddiyeti ile ilişkili değildir.

Anahtar Kelimeler: Kalp yetmezliği, insülin direnci, trigliserid glikoz indeksi

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INTRODUCTION

Heart failure (HF) is one of the leading causes of mortality and morbidity worldwide. Although the pathophysiological mechanisms underlying HF are still not fully understood, previous animal and human studies have shown a significant relation between HF and insulin resistance (IR) (1). Currently, real-life data studies have reported that HF is associated with hyperglycemia or chronic hyperglycemic conditions such as IR, diabetes and that the incidence of HF is higher in patients with these conditions (2).

The triglyceride glucose (TyG) index has been derived from fasting triglyceride and glucose, and several studies have shown the relationship between the TyG index with hypertension, diabetes, metabolic syndrome (MetS), arterial stiffness and coronary artery calcification. In addition, the TyG index may be an indicator for determining the presence and severity of coronary artery disease, carotid atherosclerosis and ischemic stroke according to the recent study results (3-5). However, no study investigating the relationship between the TyG index and HF has yet been reported in the literature. Hence, we aimed to evaluate the role of the TyG index in patients with HF in this study.

MATERIALS AND METHODS

Study population

In this study, age and gender matched 69 subjects who were admitted to our cardiology outpatient clinic were analyzed retrospectively. Acute coronary syndrome, acute myocarditis/pericarditis, malignancy, acute and chronic infections, autoimmune diseases, severe hematological disorders, systemic inflammatory diseases, chronic renal failure (glomerular filtration rate calculated by Cockcroft-Gault formula <50 mL/min/1.73 m^2), severe liver failure and pregnancy were defined as exclusion criteria. The subjects aged 18-90 years with a diagnosis of HF for at least 6 months and left ventricular ejection fraction (LVEF) <40% were included in the patient group (n=34). The New York Heart Association (NYHA) functional classification was determined by cardiologists who were blind to the patients' clinical data. The subjects aged 18-90 with LVEF>50%, but no known history of heart disease or clinical findings were included in the control group (n=35). This study was approved by our institutional ethical committee in accordance with the Declaration of Helsinki (2021/01, protocol no:020-4331).

Data collection and definitions

Previously recorded data such as demographic, medical history, medications and the laboratory findings including fasting blood glucose (FBG), renal function tests, lipid parameters, complete blood counts and serum N- terminal prohormone brain natriuretic peptide (NT-proBNP) concentrations were re-analyzed. The TyG index was calculated by the formula Ln [fasting triglycerides (mg/dl) xfasting glucose (mg/dl)/2] (6). LVEF calculated by modified biplanar Simpson method of the study population was scanned retrospectively.

Hypertension was defined in accordance with the criteria of World Health Organization (WHO) (7). The diagnosis of diabetes was based on the American Diabetes Association criteria (8). National Cholesterol Education Programme guidelines were used for the definition of dyslipidemia (9).

Statistical analysis

Statistical analysis was performed using SPSS version 21.0 software (SPSS, Inc., Chicago, Ill., USA). In expressing the values, number (percentage) for categorical variables and mean ± standard deviation for continuous variables were used. Kolmogorov-Smirnov test was done to determine the normal distribution. Student's t-test or chi-square test was performed to compare variables between groups where appropriate. The relationship between TyG index and other variables was evaluated using Pearson's correlation analysis. In the advanced stage, the association between the TyG index with variables, including HF severity indicators, was assessed by multiple logistic regression after the adjustment for any potential confounding. Curve analysis receiver operating characteristics analysis (ROC) was used to predict the ability of the TyG index to detect the presence of HF.

RESULTS

The baseline characteristics of the study population are presented in Table 1. The incidences of comorbidity such as hypertension, diabetes, hyperlipidemia and vital signs including systolic blood pressure, diastolic blood pressure, and heart rate did not differ between the groups. Laboratory parameters including FBG, HbA1c, sodium, creatinine, triglyceride, HDL-C, white blood cells (WBC), albumin and NT-proBNP showed significant differences in the groups ($p<0.05$). However, there was no significant difference in total cholesterol, LDL-C and hemoglobin levels. The TyG index was higher in HF patients compared to controls (9.11±0.59 vs 8.55±0.55; $p<0.001$). The ROC curve generated from the TyG index resulted in the area under the curve (AUC) of 0.745 to indicate HF presence and and the cut-off point calculated based on this curve was 9.19 with 71% sensitivity and 51% specificity (Figure 1).

In Pearson's correlation analysis, a significant relationship was found between the TyG index with systolic blood pressure, total cholesterol, creatinine, as well as its components FBG and triglycerides (Table 2). However, the TyG index was not significantly correlated with body mass index, diastolic blood pressure, heart rate, LVEF, hemoglobin A1c (HbA1c), LDL-C, HDL-C, hemoglobin, WBC, albumin and, NYHA functional class, LVEF or NT-proBNP

Table 1: Baseline characteristics and medications of study population

Variables	Subjects with HF (n=34)	Subjects without HF (n=35)	p
Mean age (years)	64.17±9.57	62.57±9.22	0.481
Males, n (%)	16 (47)	16 (46)	0.911
Body mass index (kg/m^2)	28.06±4.98	28.42±4.55	0.751
Hypertension, n (%)	15 (44)	13 (37)	0.555
Diabetes, n (%)	14 (41)	9 (26)	0.173
Hyperlipidemia, n (%)	12 (35)	6 (17)	0.086
Current smoking, n (%)	3 (9)	7 (20)	0.187
Systolic blood pressure (mmHg)	119.00±13.24	120.43±14.67	0.672
Diastolic blood pressure (mmHg)	75.94±10.35	74.29±8.23	0.463
Heart rate (beats/minute)	75.21±13.33	69.83±11.62	0.078
LVEF (%)	26.60±4.55	59.26±3.58	<0.001
Atrial fibrillation, n (%)	10 (29)	-	-
NYHA III-IV, n (%)	20 (58)	-	-
Ischemic etiology, n (%)	19 (56)	-	-
ACEI/ARB, n (%)	25 (74)	12 (34)	0.001
Beta-blockers, n (%)	31 (91)	3 (9)	<0.001
Statins, n (%)	12 (35)	2 (6)	0.002
Antiplatelets, n (%)	32 (94)	9 (26)	<0.001
Diuretics, n (%)	23 (67)		
Digitalis, n (%)	9 (26)		

LVEF: left ventricular ejection fraction, NYHA: New York Heart Association, ACEI: Angiotensin-converting enzyme inhibitor, ARB: Angiotensin receptor blocker, HF: heart failure

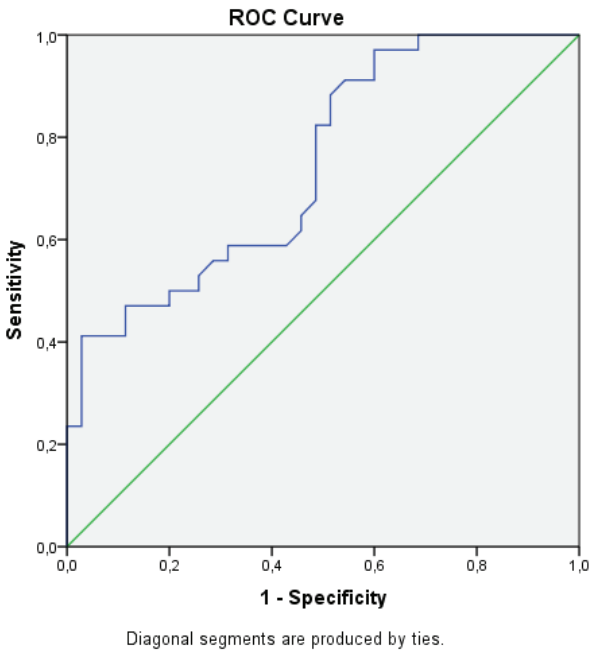

Figure 1: Receiver operator characteristic curve (ROC) of TyG index in predicting the presence of heart failure
TyG index: triglyceride glucose index

used in predicting HF severity and prognosis (Table 2, Figure 2). In addition, TyG index did not differ significantly in HF subgroups divided as ischemic and non-ischemic (Figure 3). In the multiple logistic regression analysis, it was found that none of the variables used in the model was independently associated with the TyG index, but the combination of these variables could strongly indicate a high TyG index as presented in Table 3 (95% CI:3.366-8.157; p<0.001).

DISCUSSION

In this study, we found that the patients with HF had a higher TyG index, but did not correlate with parameters indicating HF severity, including LVEF, NT-proBNP, and NYHA functional class. After adjusting the confounding factors by multiple logistic regression analysis, we observed that none of the clinical variables alone was associated with the TyG index, but their combination strongly indicated the high TyG index.

IR is defined as the impairment of cells' ability to respond to the action of insulin in the transport of glucose from the bloodstream to target tissues (10). IR is related to dyslipidemic conditions and patients with IR are typ-

Table 2: The laboratory data of study population

Variables	Subjects with HF (n=34)	Subjects without HF (n=35)	p
FBG (mg/dl)	117.02±28.56	97.26±12.59	<0.001
HbA1c	6.45±1.05	5.59±0.80	<0.001
Creatinine (mg/dl)	0.96±0.17	0.75±0.13	<0.001
Sodium (mEq/L)	139.14±3.54	140.63±1.97	0.003
T-chol (mg/dl)	189.71±46.63	193.20±41.54	0.743
TG (mg/dl)	167.35±78.06	119.69±55.32	<0.001
LDL-C (mg/dl)	109.97±30.98	116.48±33.98	0.408
HDL-C (mg/dl)	40.67±7.48	52.57±13.38	<0.001
Hemoglobin (g/dl)	13.61±1.29	14.16±1.25	0.076
WBC (cells/μL)	9.23±2.00	6.98±1.54	<0.001
Albumin (g/dL)	4.25±0.26	4.57±0.23	<0.001
NT-proBNP (pg/ml)	613.71±433.70	97.32±49.78	<0.001
TyG index	9.11±0.59	8.55±0.55	<0.001

FBG: fasting blood glucose, HbA1c: hemoglobin A1c, T-chol: total cholesterol, TG: triglycerides, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, WBC: white blood cells, NT-proBNP: N-terminal prohormone brain natriuretic peptide, TyG index: triglyceride glucose index, HF: heart failure

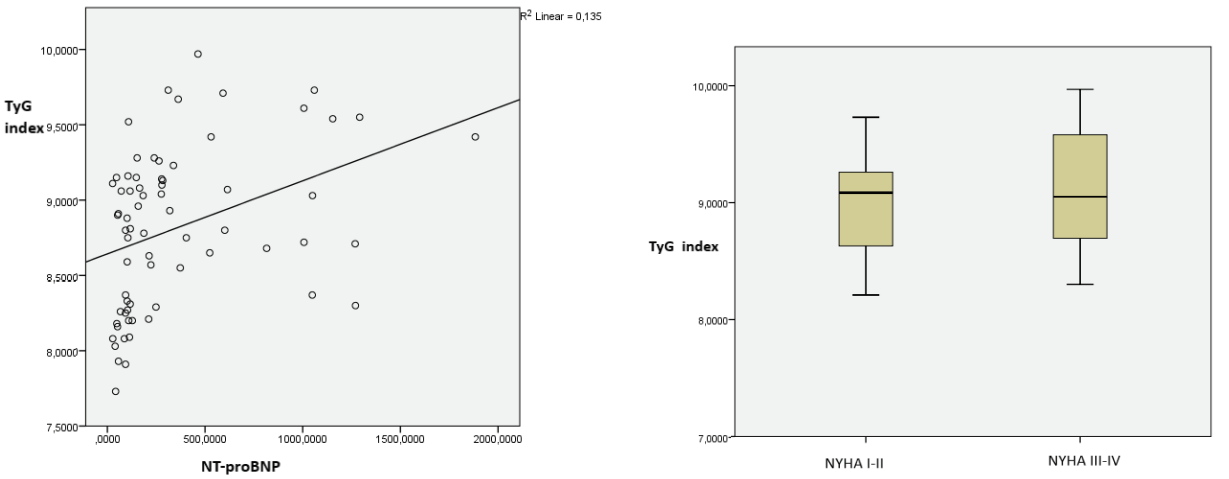

Figure 2: The relationship between TyG index with NT-proBNP and NYHA functional class
TyG index: triglyceride glucose index, NT-proBNP: N- terminal pro hormone brain natriuretic peptide, NYHA: New York Heart Association

ically characterized by high triglycerides, small dense LDL particles and low HDL-C levels. Also, triglycerides and triglyceride-rich lipoproteins play a major role in atherosclerosis, which is the main etiologic factor of HF by reducing of endothelial function, increasing of proinflammatory cytokines expression, inducing of monocyte-platelet activation and raising of fibrinogen levels (11,12). However, there is increasing evidence of the direct effects of IR on heart failure itself (13). The major responsible mechanisms are listed as follows: (i) TNF-α is a pre-inflammatory cytokine associated with HF and it has been reported that TNF-α may affect the glucose metabolism. Indeed, a study showed that patients with IR had higher TNF-α levels compared to insulin-sensitive patients; (ii) In IR, both plasma and cardiac free fatty acids (FFA) and triglycerides concentrations increase and their accumulation in myocardial cells may result in apoptosis and fibrosis due to lipotoxic effects. Moreover, FFA may trigger IR by activation of Toll-like Receptor 4 and the innate immune response; (iii) Activa-

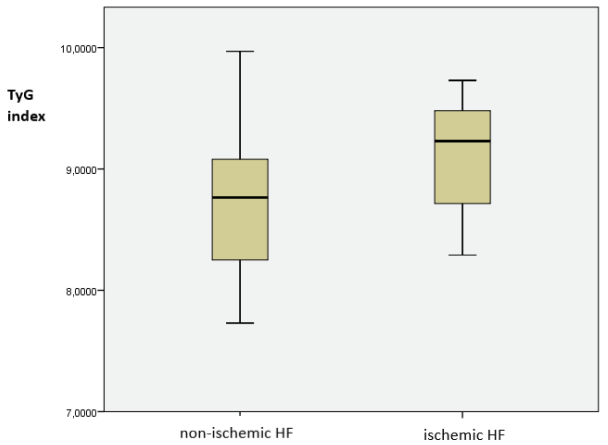

Figure 3: The TyG index levels according to underlying etiology in heart failure
TyG index: triglyceride glucose index, HF: heart failure

Table 3: Correlation between the TyG index and clinical variables

Variables	r	p
Systolic blood pressure	0.364	0.034
FBG	0.553	<0.001
Creatinine	0.420	0.010
T-chol	0.345	0.045
TG	0.567	<0.001

FPG: fasting blood glucose, T-chol: total cholesterol, TG: triglycerides, TyG index: triglyceride glucose index

tion of the renin-angiotensin II-aldosterone (RAAS) and sympathetic nervous system have been associated with impairment of glucose metabolism, and the interaction between IR and RAAS may contribute to HF through abnormal aldosterone release by triggering of TNF-α and IL-6 (iv) IR causes to endothelial dysfunction by reducing NO release in endothelial cells, and this may result in hypoxia and inhibition of angiogenesis, leading to myocardial cell apoptosis (v) The other culprit mechanisms are mitochondrial dysfunction and endoplasmic reticulum stress. Hyperglycemia may trigger inflammatory responses through mitochondrial dysfunction in oxidative stress, resulting in cell apoptosis. In addition, it has been

Table 4: Multiple logistic regression analysis for TyG index

Model	Unstandardized coefficients		Standardized coefficients	t	p	95.0% Confidence interval for B	
	B	Std.Error	ßeta			Lower bound	Upper bound
Constant	5.761	1.148	-	5.018	<0.001	3.366	8.157
LVEF	0.004	0.011	0.128	0.359	0.721	-0.018	0.026
FBG	0.007	0.003	0.401	2.075	0.051	0.000	0.013
T-chol	0.002	0.003	0.194	0.685	0.501	-0.004	0.008
TG	0.002	0.001	0.346	1.952	0.065	0.000	0.004
Systolic blood pressure	0.004	0.008	0.101	0.463	0.648	-0.013	0.020
Diastolic blood pressure	-0.004	0.010	-0.080	-0.353	0.727	-0.025	0.018
HbA1c	0.074	0.099	0.161	0.745	0.465	-0.133	0.280
Creatinine	0.022	0.504	0.078	0.441	0.664	-0.830	1.275
LDL-C	0.001	0.004	0.038	0.158	0.876	-0.007	0.008
HDL-C	0.006	0.013	0.093	0.454	0.655	-0.021	0.033
Hemoglobin	0.044	0.068	0.118	0.647	0.525	-0.098	0.185
NT-proBNP	0.000	0.000	-0.036	-0.131	0.897	-0.001	0.001
Ischemia	-0.095	0.202	-0.100	-0.471	0.643	-0.517	0.327
NYHA III-IV	0.165	0.238	0.172	0.694	0.496	-0.331	0.661

LVEF: left ventricular ejection fraction, FBG: fasting blood glucose, T-chol: total cholesterol, TG: triglycerides, HbA1c: hemoglobin A1c LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, NT-proBNP: N- terminal prohormone brain natriuretic peptide, NYHA: New York Heart Association, TyG index: triglyceride glucose index

reported that miR 19a-3p, 144-5p, miR-34a and miR-21 polymorphisms responsible for glycolipid metabolism disorders are associated with myocardial dysfunction (11,14-16). Considering the effects of glycolipid disorders on HF, the TyG index derived from triglycerides, and glucose is likely to indicate the presence of HF.

In the first studies investigating the relationship between HF and hyperglycemia, IR was measured using the euglycemic-hyperglycemic clamp method, and one study showed impaired insulin-mediated glucose uptake into skeletal muscle and liver muscle tissue in HF patients (15). In later studies, IR was measured by non-invasive methods such as the homeostatic model assessment of insulin resistance (HOMA-IR) score. In a study using HOMA-IR, HF patients had a higher IR than healthy volunteers. Another study conducted with 12,606 subjects, Atherosclerosis Risk in Communities (ARIC) study, showed the relationship between HOMA-IR and increased risk of HF (17,18). Unlike these studies, we used the TyG index for the first time to evaluate IR in HF patients and confirmed the relationship between HF presence and IR with a different method.

Several studies have reported the relationship between HF severity and IR. In a study, HF patients with IR had a worse 6-minute walking test than patients without IR (19). In another study, the relationship between insulin sensitivity and HF severity was evaluated with peak VO_2, which is used to determine functional exercise capacity, and lower peak VO_2 levels were observed in patients with reduced insulin sensitivity (20). However, we could not find any correlation between TyG index and HF severity in our study. In contrast to these studies, we used the NYHA functional classification, which is more subjective and based on patients' symptoms due to retrospective design. Also in these studies, the use of digoxin and diuretics, which improve functional capacity, was higher and the mean age was lower compared to our patients. In addition, the decrease in physiological functional capacity with aging may lead to defining the NYHA as class more exaggerated in our patients. Moreover, IR may reduce the glucose uptake into skeletal muscle, which may increase fatigue by reducing skeletal muscle strength, and this may be more pronounced in older ages. Finally, prolongation of HF exposure time may increase the severity of diastolic dysfunction in our patients, and it may contribute to progression of HF symptoms independently from IR (21).

The other parameter used for evaluation of HF severity was LVEF in this study. In addition, we investigated the importance of etiology in the TyG index, but we failed showing the correlation between the TyG index and these parameters. Indeed, one study did not show any relationship between IR with LVEF and HF etiology (22). In another study, abnormalities in insulin metabolism were found to be similar in patients diagnosed with ischemic or dilated cardiomyopathy, and it has been claimed that IR may develop as a part of a neurohormonal and metabolic response in HF rather than atherosclerotic disease (15). In addition, there was no correlation between the TyG index and variables with confounding effects such as blood pressure, age, and body mass index in multiple logistic regression analysis in our study. The absence of any relationship between these parameters and IR in the study conducted by Suskin et al supports our study results (19). However, many mechanisms may be responsible for the relationship between HF and IR, and it is not clear which mechanism explains this relationship more, and in our study, multiple logistic regression analysis showed the interaction of many factors with each other for high TyG index in HF.

There are also studies investigating the relationship between IR and NT-proBNP levels in hyperglycemic patients. A study found lower BNP levels in HF patients with diabetes than in non-diabetic HF patients, and lower levels of NT-proBNP were associated with less HF symptoms and HF severity. The researchers explained this status as beta-adrenergic receptor antagonism may increase plasma BNP levels and its increased levels may lead to a decrease in IR (19,23). In another study, there was an inverse association between NT-proBNP and HOMA-IR, and the insulin sensitivity index was an independent predictor of plasma NT-proBNP levels in HF patients. This has been associated with the lipolytic effects of BNP, and it has been hypothesized that decreased natriuretic peptide signals may cause IR and MetS by increasing lipid accumulation in adipose tissue and skeletal muscle (24). Unlike these studies, we could not find any association between the TyG index and NT-proBNP. The different result of our study at this point may be due to the heterogeneous distribution of comorbidities closely related to IR and MetS, such as hypertension, diabetes, hyperlipidemia or the differences in visceral fat accumulation or the intensive use of beta blockers.

There were some limitations to be addressed in our study. The study was a relatively small sample, and most patients were under HF treatment, which could affect glucose metabolism. HOMA-IR was not analyzed and compared with the TyG index as insulin levels could not be measured. Due to the lack of data, confounding factors such as exercise and diet habits, participation in a cardiac rehabilitation program, and cardiorespiratory fitness were not included in this study.

In conclusion, TyG index can be used for diagnosis in patients with HF symptoms, but not for HF severity. Howev-

er, larger studies are needed to determine the relationship between TyG index and HF.

Ethics Committee Approval: This study was approved by the Non-Invasive Clinical Research Ethical Committee of the, Pamukkale University (Date: 05.01.2021 No: 01).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.Ç.Ş., G.N.; Data Acquisition- S.Ç.Ş., G.N.; Data Analysis/Interpretation- M.G.G.; Drafting Manuscript- S.Ç.Ş., G.N., M.G.G.; Critical Revision of Manuscript- S.Ç.Ş., G.N., M.G.G.; Approval and Accountability- S.Ç.Ş., G.N., M.G.G.

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EVALUATION OF PTEN AND PI3K/AKT EXPRESSIONS IN STANOZOLOL-TREATED RAT KIDNEYS

STANOZOLOL UYGULANAN SIÇAN BÖBREĞİNDE PTEN VE PI3K/AKT EKSPRESYONLARININ DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Stanozolol is an anabolic androgenic steroid (AAS) that is widely used by teenagers and athletes in bodybuilding, sports, and athletics. The potential effects of stanozolol in kidney functions have not been defined. In this study we investigated the expression of tumor suppressor protein phosphatase and tensin homolog (*Pten*) and mRNA levels of phosphatidylinositol 4,5-bisphosphate 3 kinase (*Pi3k*) and the protein kinase B (*Akt1*) signaling pathway in rat kidneys treated by stanozolol.

Materials and Methods: Rats were randomized to 5 groups as control, solvent control, steroid (stanozolol), solvent-exercise, and steroid-exercise. Subcutaneous injection of stanozolol (5 mg/kg) was applied for 28 days and swimming exercise was performed 20 min/day, 5 days/week in exercise groups. Expression of PTEN was evaluated by immunohistochemistry. Also, *Pten*, *Pi3k*, and *Akt1* mRNA expressions were analyzed via RT-PCR.

Results: Mesangial cells and renal tubules in the control, solvent control, and solvent exercise groups showed strong (+++) PTEN reactivity against weak PTEN reactivity in the steroid group. Moderate PTEN reactivity was detected in cells of the steroid exercise group. *Pten* mRNA expression was significantly decreased, and *Pi3k* and *Akt1* mRNA expression were significantly increased in the steroid group versus other groups ($p<0.001$). *Pten* expression showed increase while *Pi3k* and *Akt1* expression showed decrease with exercise treatment ($p<0.05$).

Conclusion: Our findings suggest that AAS usage may inhibit PTEN expression in kidneys, which can be associated with increased *Pi3k* and *Akt1* mRNA levels. Exercise performed with AAS usage can be protective on stanozolol-exposed kidneys due to increased levels of PTEN expression and decreased levels of *Pi3k* and *Akt1*.

Keywords: Stanozolol, kidney, *PTEN, PI3K, Akt1*

ÖZET

Amaç: Stanozolol, gençler ve sporcular tarafından vücut geliştirme, spor ve atletizmde yaygın kullanımı olan bir anabolik androjenik steroiddir (AAS). Stanozololün böbrek fonksiyonlarındaki potansiyel etkileri tanımlanmamıştır. Bu çalışmada, stanozolol ile tedavi edilen sıçan böbreklerinde tümör baskılayıcı protein fosfotaz tensin homolog (*Pten*), Phosphatidylinositol-3-kinase (*Pi3k*) ve *protein kinaz B* (*Akt1*)'nin mRNA düzeylerinin ekspresyonunu araştırdık.

Gereç ve Yöntem: Sıçanlar, kontrol, çözücü kontrol, steroid, çözücü kontrol egzersiz, steroid egzersiz olarak 5 gruba ayrıldı. Yirmi sekiz gün boyunca subkutan stanozolol enjeksiyonu (5 mg/kg) uygulandı ve egzersiz gruplarındaki hayvanlara 20 dk/gün, 5 gün/hafta yüzme egzersizleri yaptırıldı. PTEN ekspresyonu immünohistokimya ile değerlendirildi. Ayrıca *Pten*, *Pi3k* ve *Akt1* mRNA ekspresyonları RT-PCR yoluyla analiz edildi.

Bulgular: Kontrol, çözücü kontrol ve çözücü kontrol egzersiz gruplarındaki mezanjiyal hücreler ve böbrek tübülleri, steroid grubunda saptanan zayıf PTEN reaktivitesine karşı güçlü (+++) PTEN reaktivitesi gösterdi. Steroid egzersiz grubunun mezanjiyal ve tübül hücrelerinde orta düzeyde PTEN reaktivitesi saptandı. *Pten* mRNA ekspresyonu, steroid grubunda kontrol ve diğer gruplara göre anlamlı düşüş gösterdi, *Pi3k* ve *Akt1* mRNA ekspresyonu anlamlı olarak arttı ($p<0.001$). Egzersiz tedavisi ile *Pten* ekspresyonu artış, *Pi3k* ve *Akt1* ekspresyonu azalma gösterdi ($p<0.05$).

Sonuç: Bulgularımız, AAS kullanımının, artan *Pi3k* ve *Akt1* mRNA seviyeleri ile ilişkili olabilecek böbreklerde PTEN ekspresyonunu inhibe edebileceğini düşündürmektedir. AAS kullanımı ile yapılan egzersiz, PTEN ekspresyon seviyelerinin artması ve *Pi3k* ve *Akt1* seviyelerinin düşmesi nedeniyle stanozolole maruz kalan böbrekler üzerinde koruyucu olabilir.

Anahtar Kelimeler: Stanozolol, böbrek, *PTEN, PI3K, Akt1*

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INTRODUCTION

Anabolic androgenic steroids (AAS), which are synthetic testosterone derivatives, exert their androgenic effects via enhancing anabolic activity. These effects cause increases in muscle growth and strength, improving athletic performance. AASs are prescribed for hypogonadism. They prevent muscle mass loss during medical use, however, these types of chemicals are used by non-expert athletes due to their anabolic effects (1-3). An abusive dose of AAS is 10–100 times higher than its pharmacological dose. These higher doses cause several adverse effects that include hepatotoxicity, reproductive system problems, cardiovascular problems, psychiatric and behavioral disorders, and cancer (4). It has been documented that AASs have different types of adverse effects, such as acute kidney injury, chronic kidney diseases, glomerular toxicity, etc., in kidneys (5). One of the adverse effects of AAS misuse is renal failure in bodybuilders and athletes (6, 7). It has been reported that an increase in athletes' serum creatinine levels may be due to AAS abuse. Serum creatinine, blood urine nitrogen (BUN), and uric acid levels can increase through steroid use (8). In some cases, Wilm's tumor development was reported in athletes (9). It has been reported that administration of stanozolol can cause renal failure in some cases. Stanozolol is a C17α-alkylated derivative of testosterone and is used as an AAS (3, 4, 10-13).

The *PTEN*/*PI3K*/*AKT1* pathway is an important modulator of cell proliferation and cell death (14). The *PTEN/PI3K/AKT1* signaling inhibition suppresses cellular adhesion and induces apoptosis (15). Tumor suppressor *PTEN* (the phosphatase and tensin homolog gene) dysfunction has an important role in different types of cancer. Mutations, deletions, and protein folding disruptions may cause *PTEN* function loss. *PTEN* dysfunction negatively affects the phosphoinositide 3-kinase (*PI3K*) signaling pathway that causes phosphatidylinositol (3-5)-triphosphate accumulation. These accumulations result in inhibition of the *PI3K/AKT1* pathway (16). In different studies and case reports it has been reported that AASs cause kidney failure, however, the potential effects of stanozolol in kidney functions have not been evaluated. It has been known that exercise has benefits in the whole-body system and regular exercise activity has a preventive role against kidney cancer (17, 18).

In this study, we aimed to clarify the effects of stanozolol on the expression of the tumor suppressor protein PTEN and the levels of *Pten*, *Pi3k*, and *Akt1* mRNA in rat kidneys, and also if there is a potential protective effect of exercise on kidneys.

MATERIALS AND METHODS

Animal study

Study procedures were approved by the Istanbul University Institutional Animal Care and Use Committee (protocol number of Animals Ethics approval: HADYEK; 2013-100). Thirty-four 8 month-old, 230 gr. Sprague Dawley rats were used in this study. The animals were divided into 5 groups: group I - control group (n=5), group II - solvent (propylene glycol) control (n=5), group III - steroid (stanozolol 5 mg/kg) (n=8), group IV - solvent exercise (n=8), group V - steroid exercise (n=8). Subcutaneous injections of stanozolol (5 mg/kg) and vehicle propylene glycol (1 ml/kg) were applied for 28 days, once a day. Through the experiments, swimming exercise was applied to the exercise groups for 20 minute a day, 5 days/week. After animal scarification under anesthesia, one of the kidneys was fixed with 10% neutral buffered formalin and the other was stored at -80°C after being frozen with liquid nitrogen.

Total RNA extraction and cDNA synthesis

Twenty mg tissue samples obtained from animals were incubated with RNA Stabilization Reagent (RNAlater; Qiagen), then TissueLyser II (Qiagen) was added. RNA purification was performed with RNeasy Mini Kit (Qiagen) in QIAcube (Qiagen) and the manufacturer's instructions were followed. Reverse transcription to cDNA was performed with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). cDNA quality was assessed with an Epoch Spectrophotometer System and Take3 Plate (BioTek, Winooski, VT, USA).

Relative quantification of the gene expression

StepOnePlus Real-Time PCR System technology (Applied Biosystems) was used to evaluate *Pten*, *Pi3k*, and *Akt1* mRNA expression analyses with TaqMan Probe-based technology (Primer Design Ltd., Southampton, UK). Study results were shown as relative fold increase/decrease compared with the control animals using the $2^{-\Delta\Delta Ct}$ method (Livak, KJ and Schmittgen, TD). The primer sequences are shown in Table 1. All assays were done in triplicate and on three different days. For each sample, 100 ng cDNA was added into the PCR mix with 1 µL of

Table 1: The primer sequences for real time PCR

Gene	Forward (5′–3′)	Reverse (5′–3′)	Assay ID
Pten	TTGGCGGTGTCATAATGTCT	GCAGAAAGACTTGAAGGCGTA	Rn00477208
Pı3k	AACACAGAAGACCAATACTC	TTCGCCATCTACCACTAC	Rn01769524
Akt1	GTGGCAAGATGTGTATGAG	CTGGCTGAGTAGGAGAAC	Rn00583646
β-actin	TGGTGGGTATGGGTCAGAAG	GACAATGCCGTGTTCAATGG	Rn00667869

Primer Perfect Probe and QuantiTect Probe PCR Master mix (Qiagen). Beta-actin was used as a reference. Total reaction mix was 20 µL for each sample. The cycle procedure was heating for 2 min, 95°C for 10 min, then 40 cycles of 15 sec. at 94°C and 60 sec. at 60°C (19).

Immunohistochemistry

PTEN expression was evaluated by the immunohistochemistry method. Kidney tissues were fixed with 10% formaldehyde and embedded in paraffin. Sections from the paraffin block (approximately 2–3 µm) were taken with a microtome and emplaced on charged microscope slides. After overnight incubation at 56°C, sections passed one by one decreasing alcohol series for the rehydration process. Then sections were incubated in 5% hydrogen peroxide (in methanol) for 15 min. for blockage of endogen peroxidase. Sections were washed with PBS. 10% citrate buffer incubation in a microwave was performed 3 times for 5 min for the antigen retrieval process. After cooling of sections, 1/100 diluted PTEN primary antibody (Abbiotec 251264) was added onto sections and incubated overnight at +4°C. Then sections were washed with PBS and subsequently biotin-labeled secondary antibody and DAB chromogen solutions were performed for 10 min each. After washing with distillated water, sections were dyed with Mayer's hematoxylin for 30 seconds. Then sections were washed with tap water, covered with coverglass, and scored under light microscopy. In the immunohistochemistry evaluation, the tissue samples of each animal were examined and the data were evaluated. PTEN antibody staining intensity was scored according to the overall intensity with 3 levels (+ = weak staining; ++ = moderate staining; and +++ = strong staining).

Statistical analysis

We used SPSS version 20 software (IBM Corp., Armonk, NY, USA) for statistical analyses for gene expression evaluation only between all 6 groups. Mean value and standard deviation were calculated. Comparison of means was performed by ANOVA one-way analysis of variance and the Tukey test. Differences were considered significant if $p<0.05$.

RESULTS

Gene expression

The gene expression levels of all groups are summarized in Table 2. In our study, upon completion of the treatment, the expression of *Pten, Pi3k,* and *Akt1* mRNA was measured using RT-PCR. *Pten* mRNA expression downregulated significantly in the steroid group ($p<0.05$) compared to other experimental groups. In the steroid exercise group, *Pten* upregulated significantly compared to the steroid group ($p<0.001$) (Figure 1). A significant in-

Table 2: Gene expression levels of treated animal groups

	PTEN		PI3K		AKT	
	Mean	SD	Mean	SD	Mean	SD
Groups						
Control	2.02	±0.28	1.37	±0.13	1.29	±0.17
Solvent control	1.78	±0.21	1.41	±0.26	1.58	±0.22
Steroid	0.57	±0.03	3.04	±0.48	3.21	±0.39
Solvent exercise	2.17	±0.31	1.46	±0. 23	1.19	±0.16
Steroid exercise	1.3	±0.18	2.05	±0.3	2.26	±0.24

PTEN: phosphatase and tensin homolog, PI3K: phosphoinositide 3-kinase, AKT: protein kinaz B, SD: Standard deviation

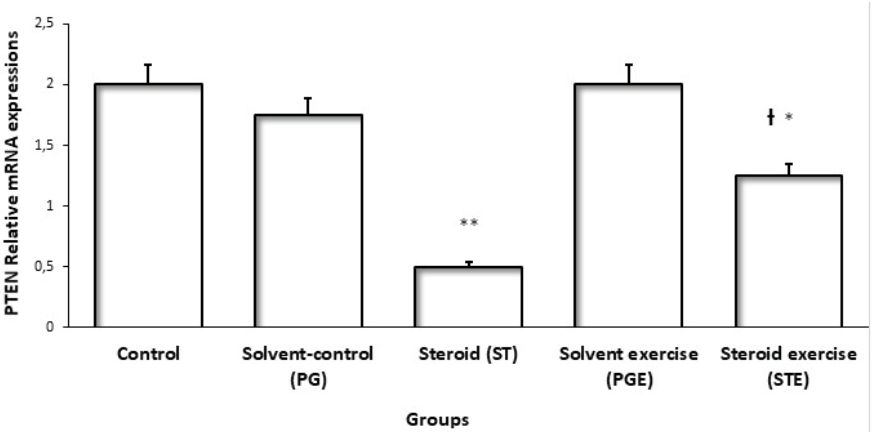

Figure 1: Compares the *Pten* mRNA expression levels. Significant changes steroid versus other groups (**$p<0.05$), and steroid exercise versus steroid group (*$p<0.001$), steroid exercise versus other groups (ŧ$p<0.001$).

crease in *Pi3k* mRNA expression was seen in the steroid group compared to other groups (p<0.05). A significant increase in *Pi3k* mRNA expression was also seen in the steroid exercise group compared with the control, solvent control, and solvent exercise groups (p<0.001), but decrease was detected compared with the steroid group (p<0.001) (Figure 2). The steroid treatment group led to a significant increase in *Akt1* mRNA expression compared with the other groups (p<0.05). The steroid exercise treatment group led to a significant increase in *Akt1* mRNA expression compared with the control, solvent control, and solvent exercise groups (p<0.001) but decreased compared with the steroid group (p<0.001) (Figure 3).

Immunohistochemistry

The results of the PTEN immunoreactivity are summarized in Table 3. Similar reactivity was detected in light

Table 3: PTEN immunohistochemistry reactivity scoring

Pten reactivity	Control	Solvent control	Steroid	Solvent exercise	Steroid exercise
Proximal tubules	-	-	-	-	-
Distal tubules	+++	+++	+	++	++
Mesangial cells	+++	+++	-	+++	-
Medullar tubules	++	++	+	++	++

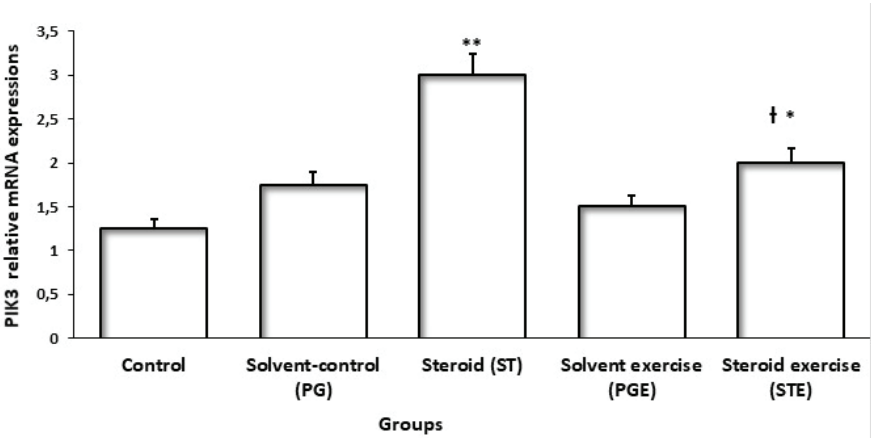

Figure 2: Compares the *Pi3k* mRNA expression levels. Significant changes steroid versus other groups (**p<0.05), and steroid exercise versus steroid group (*p<0.001), steroid exercise versus other groups (†p<0.001).

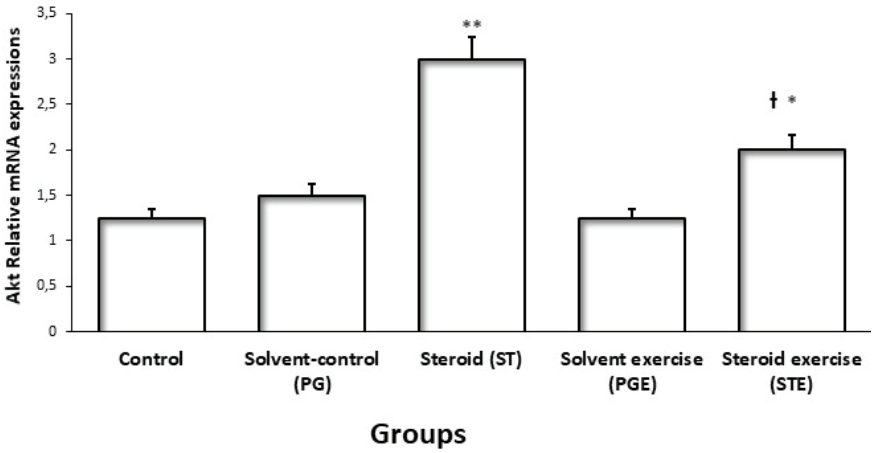

Figure 3: Compares the *Akt1* mRNA expression levels. Significant changes steroid versus other groups (**p<0.05), and steroid exercise versus steroid group (*p<0.001), steroid exercise versus other groups (†p<0.001).

microscopic evaluation of the control, solvent control, and solvent exercise groups. In these groups, glomerular mesangial cells had a strong (+++) PTEN reaction. Cells of distal tubules had strong (+++) immunoreactivity but proximal tubules did not show any reaction. Tubules of medulla showed a moderate (++) reaction. In the steroid-treated group, mesangial cells and proximal tubule cells did not show PTEN immunoreactivity. A few distal tubules showed weak (+) reaction. Medullary tubules also had weak (+) immunoreactivity. In the steroid exercise group, no immunoreactivity was detected in mesangial cells or proximal tubules. But moderate (++) reaction in distal tubules and medullary tubules was detected (Figure 4).

DISCUSSION

Synthetic AAS Stanozolol is a substance that has functions like testosterone. This chemical is used to enhance athletic performance and improve aesthetics (20). Higher doses of AAS can cause several types of adverse effects on the cardiovascular system, reproductive system, urinary system, and hepatic system, and mental health problems (21). AAS mimics testosterone's physiological effect by inducing an altered expression on DNA sequences. Reports showed a close relationship between AASs and cancer formation, progression, and metastases. The metabolites of AASs are inducers of cell proliferation. Like testosterone, AASs are metabolized to 17 beta-estradiol and play an important role in estrogen-dependent can-

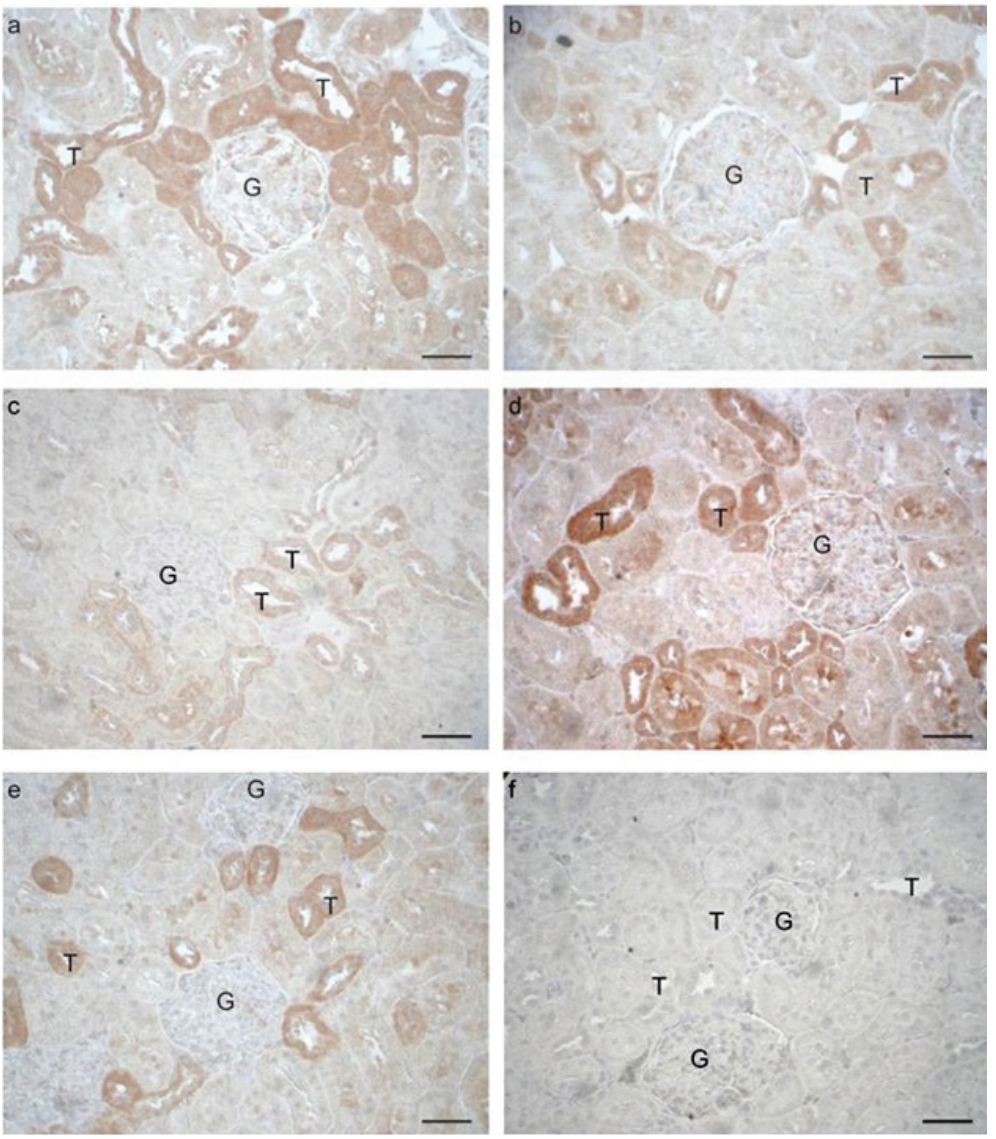

Figure 4: PTEN immunoreactivity, a) Control group, b) solvent control group, c) steroid group, d) solvent exercise group, e) steroid exercise group, G: Glomerulus, T: tubules

cer mechanisms. Additionally, reactive oxygen species (ROS) are increased during AASs catabolism. This can cause genotoxicity and the formation of adenomas (22-25). The misuse of AASs can cause kidney toxicity and increased kidney cancer formation risk (12, 26, 27). Chronic AAS usage can promote apoptosis via oxidative stress in kidney tissue. It has also been reported that AASs induce genetic damage in kidney tissue (27-29). Pak et al. reported that the kidney carcinogenesis mechanism could be triggered by AAS usage through the STAT5 pathway (26). However, AASs' adverse effects on kidney tissue have not been clarified yet. The adverse effects of stanozolol on kidneys have been evaluated in different studies. An excessive dose of stanozolol increased the serum creatinine levels, and induced oxidative stress (4, 10).

One of the major regulating molecular mechanisms of cell proliferation and survival is the *PTEN/PI3K/AKT1* pathway. *AKT1* is an anti-apoptotic factor and its overexpression causes cell cycle arrest and inhibition of cellular death. *AKT1* regulates the apoptosis mechanism via inactivating the pro-apoptotic proteins. Alterations in this pathway are associated with several diseases, including cancer. It has been shown that *PI3K/AKT1* signaling is disrupted in many cancer types, such as breast cancer, colon cancer, ovarian cancer, pancreatic cancer, and prostate cancer. *PTEN* inactivation is associated with *AKT1* activation, which causes tumor cell proliferation. *PTEN* function loss might be the cause of mutation, deletion, or epigenetic modulations at high frequency in many primary and metastatic human cancers (30, 31).

Mutations of the *PTEN* gene and disrupted expression of PTEN were reported in several studies. Nassif et al., reported the reduced or absent immunohistochemical reaction of PTEN in primary sporadic colorectal cancer (32). Also, immunohistochemical and RT-PCR analysis has shown a complete loss of *PTEN* mRNA expression in anaplastic thyroid cancers (33). Wang et al. reported mutations in *PTEN* genes in late-stage bladder carcinoma, and Wu et al. also showed loss of *PTEN* expression in melanoma (34, 35). Kanamori et al. reported a negative correlation between *PTEN* and *AKT1* expression in endometrial carcinoma cells (36). Breuksch et al. demonstrated that *PTEN* function loss can play a role in kidney tumor progression (37). In our study, we detected loss of PTEN expression in mesangial cells and cells of kidney tubules due to stanozolol treatment. Our results are consistent with the referenced literature. Several studies reported that androgens and AASs can cause cell proliferation and survival due to the activation of the PI3K/AKT pathway. Sirianni et al. evaluated the effects of nandrolone and stanozolol on breast cancer cell proliferation and detected increased *AKT1* phosphorylation and stimulation of the *PI3K/AKT1* and PLC/PKC pathways (38). It has been reported that androgens stimulate ovarian cancer cell survival via telomerase activity and the *PI3K/AKT1* pathway (39). In our study, the steroid-treated group showed increased mRNA expression of *Pi3k* and *Akt1*. This increased activation of *Pi3k/Akt1* expression is associated with decreased *Pten* expression.

Also, in the steroid exercise group, we detected increased expression of *Pten* with the decreased expression of *Pi3k* and *Akt1* against the steroid group. Physical inactivity is related to cancer development. Recent studies showed that physical activity is effective in reducing the risk of various cancers like pancreatic, colon, prostate, lung, ovarian, breast, and endometrial (40-42). Yu et al. have reported that exercise increased the *Pten* expression levels of mice skin cells compared with a sedentary control group and prevented the risk of skin cancer development. Also, benefits of regular exercise on the development of hepatocellular carcinoma were reported (43). There is no reported article about the protective effects of exercise on kidneys. Our findings suggest that exercise may have a protective effect on kidneys exposed to stanozolol.

As a result of our findings, we suggest that stanozolol usage can cause possible renal cancer development via decreased *Pten* expression and the increased *Pi3k/Akt1* pathway. According to our results, the *Pten* gene expression profile was in accordance with PTEN immunohistochemistry results. Daily exercise during AAS treatment may be beneficial to kidney health and can decrease the risk of cancer development. In this study, the inability to evaluate the parameters associated with kidney dysfunction in the blood in animals and the inability to examine the expression levels of genes and proteins belonging to more molecular pathways in the kidney are limiting factors. In conclusion, in more detailed and long-term chronic studies, the correlation between the *Pten* gene and protein expression levels will be investigated and even more molecular pathway elements can be evaluated.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: Ethics committee approval was received for this study from the Istanbul University Animal Experiments Local Ethics Committee (Date: 30.09.2013, No: 100).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study-Ç.S., M.K.; Data Acquisition- T.K., Ç.S., M.K.; Data Analysis/Interpretation-T.K., Ç.S., M.K.; Drafting Manuscript- T.K., Ç.S., M.K.; Critical Revision of Manuscript-T.K.,M.K.; Approval and Accountability-T.K., Ç.S., M.K.

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ANTIPROLIFERATIVE EFFECT OF *LACTOBACILLUS PLANTARUM* L4 STRAIN ISOLATED FROM CERVICOVAGINAL MICROFLORA ON HELA CANCER CELL LINE

SERVİKOVAJİNAL MİKROFLORADAN İZOLE EDİLEN *LACTOBACİLLUS PLANTARUM* L4 SUŞUNUN HELA KANSER HÜCRE HATTI ÜZERİNDEKİ ANTİPROLİFERATİF ETKİSİ

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ABSTRACT

Objective: *Lactobacillus* has been shown to inhibit proliferation of various cancer cells, but the effects of vaginal *Lactobacillus* on cervical cancer cells have rarely been reported. The goal of this investigation was to assess the anti-proliferative effect on cancer cell line HeLa (Human Cervical Carcinoma Cell) and potential probiotic properties of *Lactobacillus plantarum* L4 isolated from cervicovaginal flora of healthy women in Turkey.

Materials and Methods: Molecular identification of the species was performed by 16S rDNA analysis. Probiotic properties of the L4 strain were investigated by conventional methods. Human Interleukin-10 (IL-10) and Tumor Necrosis Factor- alpha (TNF-alpha) ELISA kits were used in the evelation of the immune modulator effect of the L4 strain. The antiproliferative effect of the L4 strain on the HeLA cell line was performed using the XTT kit.

Results: *L. plantarum* L4 strain exhibited strong probiotic properties. The L4 strain showed an anti-inflammatory effect on HeLa by reducing the production of TNF-α and increased IL-10 production. The greatest antiproliferative effect of *L. plantarum* L4 strain on HeLa cells was observed at the highest dose of the metabolite 0.0006 gr/ml with a death rate of 90-95% while the number of living cells was found to be between 5-10%. The strain showed no anticancer effect on human umbilical vein endothelial cells (HUVEC).

Conclusion: *L. plantarum* L4 strain, with strong probiotic properties, can be considered a promising treatment candidate for HPV cancer due to its immunomodulatory effect and high antiproliferative effect, even in very small doses.

Keywords: *Lactobacillus plantarum*, antiproliferative effect, HeLa, IL-10, TNF-α

ÖZET

Amaç: *Lactobacillus* spp. bakterilerinin çeşitli kanser hücrelerinin proliferasyonunu inhibe ettiği gösterilmiştir. Ancak, vajinal *Lactobacillus*'ların rahim ağzı kanseri hücreleri üzerindeki etkileri nadiren bildirilmiştir. Bu çalışmada Türkiye'de yaşayan sağlıklı kadınların servikovajinal florasından izole edilen *L. plantarum* L4 suşunun çeşitli probiyotik karakterleri ve HeLa kanser hücre hattı üzerindeki antiproliferatif etkisi araştırılmıştır.

Gereç ve Yöntemler: Türün moleküler tanımlaması 16S rDNA analizi ile gerçekleştirilmiştir. L4 suşunun probiyotik özellikleri geleneksel yöntemlerle belirlenmiştir. L4 suşunun immun modilatör etkisini belirlemede insan Interleukin-10 (IL-10) ve Tümör Nekroz Factor-alfa (TNF-α) ELISA kitleri kullanılmıştır. HeLA hücre hattı üzerindeki antiproliferatif etkisi XTT kiti kullanılarak gerçekleştirilmiştir.

Bulgular: L4 suşu güçlü probiyotik özellikler sergilemiştir. L4 suşunun, TNF-α üretimini azaltarak HeLa hücreleri üzerinde anti-inflamatuar bir etki gösterdi ve IL-10 üretiminin artırılmasını sağladı. *L. plantarum* L4 suşunun HeLa hücreleri üzerinde en büyük antiproliferatif etkinin metabolitin 0,0006 gr/ml'lik en yüksek dozunda %90-95 düzeyinde ölüm oranı gözlemlenirken canlı hücre sayısının %5-10 arasında olduğu görülmüştür. İnsan göbek ven endotel hücreleri (HUVEC) üzerinde herhangi bir antikanser etkisi olmamıştır.

Sonuç: Güçlü probiyotik özelliklere sahip *L. plantarum* L4 suşu, immunmodilatör etkisi ve çok küçük dozlarda bile yüksek bir antiproliferatif etki göstermesi sebebiyle HPV kanseri için umut verici bir tedavi adayı olarak kabul edilebilir.

Anahtar Kelimeler: *Lactobacillus plantarum*, antiproliferatif etki, HeLa, IL-10, TNF-α

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INTRODUCTION

Cervical cancer (CVC) is the fourth most common cancer in women in terms of mortality and morbidity, with 570,000 new cases seen worldwide each year. The number of people who lost their lives in 2018 due to CVC is 311,000 (1). Human papilloma virus (HPV) is the biggest cause of CVC. More than 200 HPV genotypes have been identified, and there are low-risk and high-risk types of the virus (2). Low-risk HPV types can generally cause benign abnormalities and genital warts, while HPV types in the high-risk category can cause cervical, vulvar, penile, anal, and oropharyngeal cancer types (3).

Almost all women are infected with HPV types at least once during their lifetime. Only some individuals have the risk of persistence of oncogenic HPV types, premalignancy and progression to invasive cervical cancer (3, 4). The changing clinical picture depends on the type of virus, the localization of the lesion, the immunological status of the individual (more severe in pregnant women and those with immunodeficiency) and the nature of the epithelium (5). Several risk factors have also been identified, including smoking, sexual and reproductive factors, and human immunodeficiency virus HIV infection (6). In recent studies, there has been increasing evidence that cervicovaginal microbiota may be an important cofactor in the etiology of CVC (7-10).

The vaginal microbiota (VM) of healthy premenopausal women predominates in terms of the *Lactobacillus* species (11-13). Bacteria of the genus *Lactobacillus* protect the host against various genital infections by blocking the adhesion of pathogens in the VM, producing hydrogen peroxide (H_2O_2), diacetyl, acetaldehyde, reuterine, bacteriocin and bacteriocin-like substances, and antimicrobial substances, controlling the proliferation of pathogenic bacteria and ensuring that microflora is in balance (14). *Lactobacillus* and accompanying dysbiosis deficiency in VM increase the risk of genital infection (15, 16). In contrast, the presence of *Lactobacillus* spp. in the VM has been shown to decrease HPV infection intrauterine intraepithelial neoplasia and CVC development rate (3, 16-19). Therefore, the presence of *Lactobacillus* spp. in the VM is of great importance for prevention of HPV infection and prevention of CVC development after infection (20, 21). Studies have shown that different *Lactobacillus* species isolated from VM have the ability to modulate cancer cell proliferation and apoptosis with different roles and protective characters (16, 22).

In this study, we investigated the probiotic properties of the *Lactobacillus plantarum* L4 strain isolated from VM, its effect on the production of anti-inflammatory and pro-inflammatory cytokines released by HeLa cells, and its antiproliferative effect on the HeLa cancer cell line.

MATERIALS AND METHODS

Microorganism and culture conditions

The *Lactobacillus* spp. L4 strain was isolated from VM of healthy women who applied to the Kırşehir Ahi Evran University Education and Research Hospital. The vaginal swab was taken from voluntary patients whose age range was 18-45 years, who had no symptoms of menopause, who were not protected by any birth control method, and who had not used antibiotics within three months. In this study, a voluntary consent form was read and signed before the swab sample was taken from the patient. The study was approved by the Kırıkkale University Ethics Committee (Date: 27.10.2014, No: 25/02).

The isolated *Lactobacillus* spp. L4 strain was developed in Man Rogosa Sharpe (MRS) (Merck, UK) liquid and solid media under anaerobic conditions at 37°C for 24-48 hours (23). For the biochemical identification of gram-positive and catalase negative colonies, Analytical Profile Index (API) 50 CHL assay (BioMerieux, Inc., France) was used (24). The API50 CHL assay was carried out according to the manufacturer's recommendations and the results was evaluated via https://apiweb.biomerieux.com/. The bacterial culture was stored at -80°C in a Tryptic Soy Broth (TSB) medium containing 20% (v/v) glycerol (25).

Amplification of 16S rDNA genes

Genomic DNA isolation of the strain was performed with a Thermo Scientific GeneJET Genomic DNA Purification Kit (Kit No: #K0721). The 16S rDNA was amplified by using universal primers (27F forward, 5'-AGA GTT TGA TCM TGG CTC AG-3' and 1429R reverse, 3'-GGT TAC CTT GTT ACG ACT T-5') (26). Polymerase chain reaction (PCR) was performed with a Thermo Fisher Scientific Arctic Thermal Cycler 5020.

Resistance to low pH and tolerance to bile-salt condition

The survival rate of the identified *Lactobacillus* spp. L4 strain at low pH and high bile salt was measured by modification from previous studies (27). The culture, activated for 18 hours in MRS broth, was centrifuged at 3000 x g for 15 minutes (4°C) and the cells were precipitated. The precipitate was washed twice with sterile phosphate buffered saline (phosphate-buffered saline (PBS) and resuspended in the phosphate buffer (pH 7.2) to 8.5±9.1 log CFU/ml. 100 µl from the prepared PBS buffer was injected into the MRS liquid media with pH 2.0, 2.5, and 3.0 and containing 0.3%, 0.5%, and 1% (w/v) bile salt (oxgall, Sigma) and incubated at 37°C for 3 hours. At hours 0 and 3 of the incubation, samples were taken from the L4 strain serial dilutions were made up to the 10^{-7} level, and cultured in triplicate on MRS solid media. After overnight incubation at 37°C, the L4 strain was counted as log cfu/mL by counting colonies in the control and test groups.

Survival rate (%) = (log cfu N1/log cfu N0)x100
N1: Number of live microorganisms in the test group
N0: The number of live microorganisms in the control group

Determination of antibiotic resistance

The antibiotic susceptibility of the isolated *Lactobacillus* spp. L4 strain was determined by the disk diffusion method against commonly used antibiotics (28). At the end of the incubation period, the diameters of the zones around the antibiotic discs were measured by caliper in millimeters. The antibiotic susceptibility levels of the strain were evaluated according to the 2007 criteria set by NCCLS (National Committee for Clinical Laboratory Standards) such as Resistant (R), Semi-sensitive (I) and Sensitive (S) (28).

Antagonistic activity

The antagonistic activity of the *Lactobacillus* spp. L4 strain against pathogenic microorganisms, which has clinical significance, was determined by the well diffusion method. Table 2 shows the development conditions and origins of indicator microorganisms and clinical preparations used in our study. Clinical isolates were obtained from the Kırşehir Ahi Evran University, Faculty of Medicine Microbiology Laboratory. The well diffusion method (Maldonado, 2012; Nami, 2014a) was carried out with minor modifications (29, 30).

Detection of hydrogen peroxide production

The H_2O_2 production capacity of the *Lactobacillus* spp. L4 strain was determined by a qualitative method. Plates of MRS agar that contained 5 mg/ml hemin, 1 mg/ml vitamin K, 0.01 mg/ml horseradish peroxidase (Sigma–Aldrich, USA) and 0.05 mg/ml 3,3′, 5,5′-tetramethylbenzidine (Sigma–Aldrich, USA) were spotted with a loop of the culture and incubated anaerobically at 37°C for 48 h. At the end of the incubation, Color changes in colonies were observed after subsequent incubation at room temperature. Colonies with H_2O_2 production capacity were classified as medium (brown), weak (light brown), or negative (white color) according to their density (blue) (28).

Auto-aggregation and co-aggregation assays

The method practiced by Juárez Tomás et al. was applied for determining Autoaggregation characteristics of *Lactobacillus* spp. L4 strain (31). Auto-aggregation percentage was calculated based on the formula 1- (A4/A0) x100. The same process was also prepared for use in the co-aggregation study for *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 strains. The coaggregation percentage was calculated by the following formula:

$$\text{Co} - \text{aggregation } \% = \frac{(\text{AX} + \text{AY}/2) - \text{A}(\text{x} + \text{y})}{\text{AX} + \text{AY}/2}\ x\ 100$$

x & y: 2 genera in the control tubes
x+y: Mixture

In order to observe the co-aggregation study, 500 ul of the L4 strain was mixed with the suspension containing *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 cell fluids (500 ul) at the same concentration, and after a short vortexing, the samples were mixed in the shaker (50 rpm) for 4 hours. Thereafter, a drop of Gram suspension taken from this suspension was monitored under 100x magnification under the light microscope (Leica DM500). Specimens were classified by the density of bacteria clusters (+1 to +4) (32, 33).

Adhesion to uroepithelial cells

Uroepithelial cells were obtained from the urine of healthy women with high epithelial cell density, who applied to Ahi Evran University Education and Research Hospital in Kırşehir. Uroepithelial cells and cultures activated overnight were washed twice with PBS and resuspended in PBS to achieve a cell density of 0.5 McF (625nm=0.08-0.1). Uroepithelial cells without bacterial culture were used as negative control. Prepared bacterial cells and uroepithelial cells were mixed in equal volumes and allowed to incubate at 37°C for 3 hours. After incubation, the mixture was washed with PBS and then resuspended with PBS to eliminate bacteria that were not adhered to the epithelial cells. In order to observe the capability of the L4 strain to adhere to epithelial cells, Gram staining of the mixture suspended with PBS was performed and the preparations were observed at 100x magnification under the light microscope (Leica DM500). The degree of adhesion of bacterial cells to epithelial cells is ranked between +1 and +4.

HeLa cell lines and development conditions

HeLa cell lines were used to determine the anticancer activity of the secretory metabolites of the vaginal isolates on tumor cell. HeLa cells are routinely developed in the RPMI 1640 medium containing 10% fetal bovine serum and gentamicin antibiotics (34). All experiments were conducted at 37°C at 5% CO_2 atmosphere (Nüve EC 160) (34, 35).

Preparing culture supernatants

In this study, the L4 strain developed at 37°C for 18 hours was centrifuged (5.000 g, 10 min, 4°C). The pH of the bacterial supernatants was adjusted to 7.2 (0.22 µm Milipore, USA). The lyophilization process of the sterilized supernatants was performed at -58°C in the lyophilization device (Labfreez FD-10-R) (36).

Immunomodulatory effect of *Lactobacillus* spp. L4

HeLa cells prepared at a ratio of $1x10^6$ were treated with the L4 strain in 96-well plates. They were incubated for 24 hours at 37°C in 5% CO_2. The amount of cytokines released by HeLa cells was measured using human Interleukin-10 (IL-10) and Tumor Necrosis Factor-α (TNF-α) ELISA kits (Life Tech). Measurements were carried out in

an ELISA reader device (Biobase BIOBASE-EL10A, China) at 450 nm. Cytokine levels were expressed as pg/ml of each cytokine. Experiments were performed in triplicates (37).

XTT (Cell proliferation kit) test of L4 strain

An XTT (Biological Industries, Israel) kit was used to evaluate the anti-proliferative action of the vaginal L4 strain on the HeLa cell line. HeLa cells grown in CO_2 incubator for 24 hours were incubated in (36) a serial diluted supernatant medium of the L4 strain for 72 hours. Each run was carried out with the blank control column and the cell control column. After incubation, XTT reagent (BIOTEK) was added and measurement was performed at 482 nm with the reader (36).

Statistical analyses

A completely randomized experimental design was used with three replications in 10x2 and 6x4 factorial arrangements. One-way analysis of variance was also used. Tukey HSD and Dunnet multiple comparison tests were used to find out which group originated the difference between the groups. The normality assumption in the analyses was examined by Kolmogorov-Smirnov and Shapiro Wilk tests. Statistical analyses were performed using SPSS (version 20.0, SPSS Inc, USA) statistical package program. In the analyses, the significance level was determined as $p<0.05$ and $p<0.01$.

RESULTS

Isolation and identification of *Lactobacillus* spp. L4 strain

The *Lactobacillus* spp. L4 strain we used in our study was isolated from the vaginal flora of healthy Turkish women aged 18-45 years. The L4 strain was obtained by a culture-based method in the MRS medium (26). According to morphological and biochemical properties, the L4 strain is gram positive, catalase negative, bacillus, and coke appearance. In our study, the 16S rDNA result was found to be compatible with the API 50 CHL test result (26). According to the 16S rDNA result, the NCBI (National Center for Biotechnology Information) gene bank number of strain L4 is MF155764 (26).

Resistance to low pH and tolerance to bile-salt condition

In our study, we investigated the resistance rate of the vaginal *Lactobacillus* spp. L4 strain in a low pH environment and it was found that the L4 strain maintained its viability at 63.5% at the end of the third hour in the pH 2.0 environment. The viability of L4 strains at pH 2.5 and 3.0 was 83.6% and 91.0%, respectively. The L4 strain was found to be highly resistant to different concentrations of bile salt simulating the small intestine system under in vitro conditions (Table 1).

Antibiotic resistance

The *Lactobacillus* spp. L4 strain was found to have high resistance to antibiotics commonly used in the treatment of various infectious diseases and was found to be resistant to ciprofloxacin gentamicin, tobramycin, amikacin aztreonam, and netilmicin antibiotics. The L4 strain was found to be susceptible to penicillin, ampicillin, cefazolin, tetracycline, rifampicin, chloramphenicol, erythromycin, clindamycin, cefoperazone, streptomycin, ceftazidime, and imipenem antibiotics. The L4 strain was also found to be resistant to vancomycin and teicoplanin antibiotics from the glycopeptide group, which is important for probiotics.

Antagonistic activity of L4 strain

In this study, the antagonistic activity of the *Lactobacillus* spp. L4 strain against pathogens that cause urogenital infections was observed to show antagonistic activity on all pathogen strains except *C. tropicalis* ATCC 13803 and *B. subtilis* W168 strains. In particular, the L4 strain was found to have a very good antimicrobial effect on *C. albicans*

Table 1: Survival rates of *Lactobacillus* spp. L4 strain in low pH and high bile environments

The survival count (log cfu/ml) and rate (%) of L4 strain in different ph value at the end of 3 hours			
	Start count**	**Count after application**	**Survival rate* (%)**
pH 2.0	8.64±1.1	5.49±2.1	63.5
pH 2.5	8.57±0.3	7.17±3.3	83.6
pH 3.0	8.91±3.5	8.11±3.1	91.0
The survival count (log cfu/ml) and rate (%) of L4 strain in different bile salt environments at the end of 3 hours			
0.3%	8.94±1.5	7.65±0.6	85.5
0.5%	8.85±0.9	6.42±4.1	72.5
1%	8.64±4.7	5.45±1.0	63.0

*Survival rate=Final (cfu/mL)/control (cfu/mL)x100.
**Viable count of vaginal strains determined at 0 h; the results are representative mean±SD of three independent experiments

ATCC 90028 and *K. pneumoniae* AEU5 strains isolated from the clinical samples (29, 30). Inhibition zone diameters of the L4 strain are given in Table 2.

Detection of hydrogen peroxide production

Lactobacillus spp. L4 was classified as a strong H_2O_2 producer because of the production of an intense blue color.

Auto-aggregation and co-aggregation assays

The auto-aggregation value of the *Lactobacillus* spp. L4 strain isolated from vaginal flora at the end of the 4th hour was 78.8%. In the co-aggregation study, the co-aggregation value of the L4 strain with *C. albicans* was found to be 54.4%, 42.7% with *E. coli*, and 27.6% with *P. aeruginosa*. Figure 1 shows the co-aggregation

Table 2: Antimicrobial activity against gastrointestinal and urogenital pathogens

Test organisms	Growth condition	Origin	L4 strain
E. coli	37°C, MacConkey agar	ATCC 25922	13±1.2
E. faecalis	37°C, MRS	ATCC29212	15±2.3
P. aeruginosa	37°C, MacConkey agar	ATCC 27853	14±1.6
S. aureus	37°C, blood agar	ATCC29213	12±3.4
B. subtilis	37°C, MPA	ATCC 6633	15±0.8
B. subtilis	37°C, MPA	W168	-
B. cereus	37°C, MPA	RSKK 709 ROMA	15±0.7
B. cereus	37°C, MPA	CU1065	17±2.4
C. tropicalis	28°C, MHA	ATCC 13803	-
C. albicans	28°C, MHA	ATCC 90028	18±1.8
C. albicans	28°C, MHA	ATCC 10098	15±0.7
C. albicans	28°C, MHA	Y-1200-NIH	17±1.9
C. glabrata AEU	28°C, MHA	Clinical isolate	14±2.3
E. coli AEU	37°C, MacConkey agar	Clinical isolate	13±0.5
E. coli AEU	37°C, MacConkey agar	Clinical isolate	16±1.6
E. faecalis AEU	37°C, MRS	Clinical isolate	12±2.7
K. pneumoniae AEU	37°C, MPA	Clinical isolate	18±2.1
P. mirabilis AEU	37°C, MacConkey agar	Clinical isolate	15±0.9
S. aureus AEU	37°C, blood agar	Clinical isolate	13±2.4

Values are the means±standard deviations of triplicate measurements. ATCC: American Type Culture Collection, Virginia, USA. RSKK: Refik Saydam National Type Culture Collection.

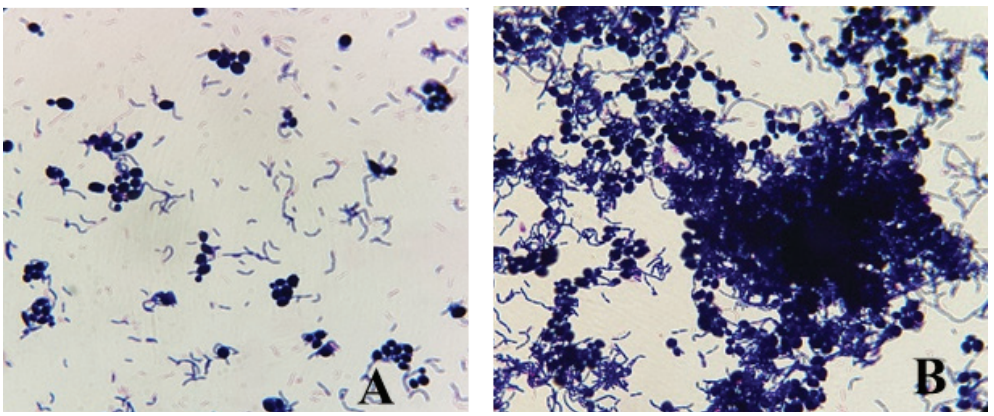

Figure 1: *Lactobacillus* spp. L4 strain *C. albicans* ATCC 10231 strain 1h (A) and 4h (B) co-aggregation at hour light microscope images (100x)

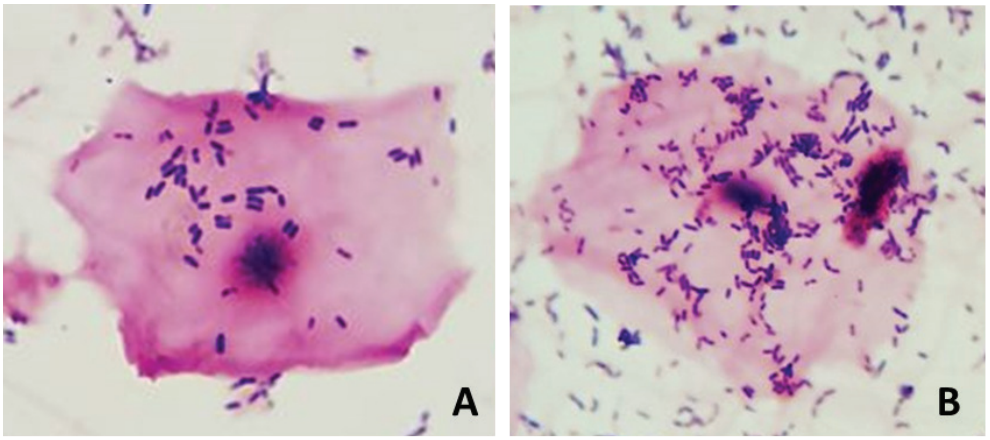

Figure 2: Binding of *Lactobacillus* spp. L4 strain to uropithelial cells, A: 1st hour, B: 4th hour 1h (A) and 4h (B) light microscope images (100x).

of the L4 strain with *C. albicans* ATCC 10231 at the end of the 4th hour.

Adhesion to uroepithelial cells

In our study, it was determined that the *Lactobacillus* spp. L4 strain had a high binding capacity to uroepithelial cells obtained from the urine of healthy women and the degree of binding was determined as (+4). The binding of the L4 strain to uroepithelial cells is given in Figure 2.

Immunomodulatory effect of L4 strain

Serving as an indicator of pro-inflammatory response, TNF-α was significantly decreased relative to control. In contrast, a significant increase in IL-10 production was observed in the sample treated with the L4 strain relative to the control (Figure 3).

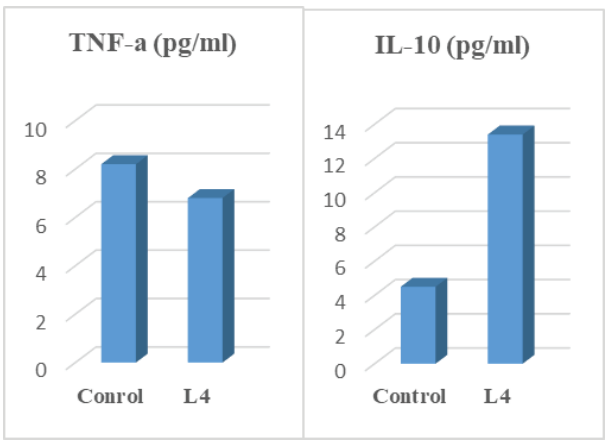

Figure 3: Effects of *Lactobacillus* spp. L4 strain on cytokine secretion by HeLa cells. The concentration of cytokine released by HeLa cells treated with 400 µg/ml of *L. plantaum* L4 strains was measured by ELISA to screen for changes in Tumor necrosis factor-α (TNF-α) and Interleukin-10 (IL-10) (n:5/group). *p<0.05: significant differences from the control.

The antiproliferative action of vaginal L4 strain on Hela cell line

The antiproliferative action of secretion metabolites of *Lactobacillus* spp. L4 isolated from vaginal microflora of healthy women on the HeLa cell line weas evaluated by the XTT method (72 h). With the LD50 (abbreviation of 50% lethal dose) program, in which the average lethal dose of a toxic substance is determined in toxicology, it was observed that L4 (LD_{50}: 0.0006 gr/ml) (36). As shown in Figure 2, deaths occurred at 90-95% at the highest metabolite dose of 0.0006 gr/ml, which showed the greatest antiproliferative action of the L4 strain on HeLa cells. When the antiproliferative effect of the L4 strain on HUVEC normal cells was also evaluated, it was seen that it had no effect on it. More than 94% of the HUVEC cells were found to grow well.

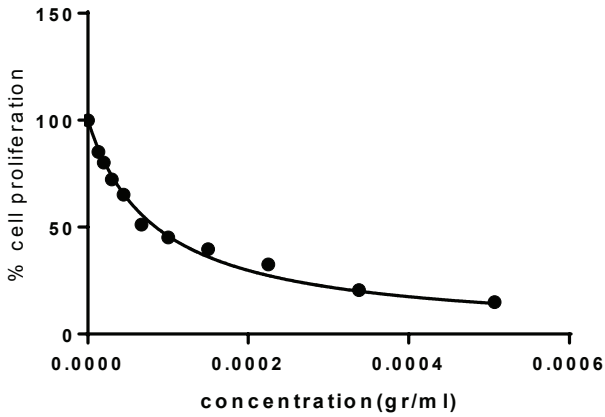

Figure 4: Antiproliferative effect of extracted metabolite products of *Lactobacillus* spp. L4 strain on HeLa cells using XTT cell proliferation kit.

DISCUSSION

Recent studies have also shown that probiotic vaginal *Lactobacillus* has an important protective role against urogenital infections and even has a protective and therapeutic role against various types of cancer, especially cervical cancer (9, 10, 16). Individual differences in *Lactobacillus* species are observed in the composition of women's vaginal flora in different geographical regions according to race and ethnicity (38). *L. crispatus*, *L. jensenii*, and *L. gasseri* are the predominant species in the vaginal microbiota that play a vital role in maintaining the balance of the vaginal microbiota, however *L. plantarum* is rare in vaginal flora (15). Each probiotic microorganism has its own biological effects and properties. Therefore, these organisms should be investigated in terms of various biological properties. The results show that this microorganism is susceptible to most antibiotics and can be regarded as a probiotic with optimal antagonistic and anticancer properties.

Probiotics were recently defined as live microorganisms which confer a health advantage on the host upon consumption in sufficient and suitable amounts (39). These two properties constitute the most important probiotic microorganism selection criteria. Several *Lactobacillus* strains lose their viability upon exposure to low pH for 3 h. (40). The L4 strain displayed high survival rates under low pH (91%) at pH 3.0 and high bile salt conditions (85.5%). Our outcomes on the viability of the L4 strain at pH 3.0 agree with the previous data (40, 41).

In our study, the *L. plantarum* L4 strain showed superior antagonistic activities against various pathogenic microorganisms (e.g. bacteria and fungi) causing gastrointestinal and urogenital infections. Previous studies have reported limited anti-pathogenic activity for this species (42-44). Vulvovaginal candidiasis (VVC) is one of the most common lower genital tract infections in women (45). In clinical studies, a variety of probiotic *Lactobacillus* spp. strains were found to be significantly effective in the treatment of VVC and in reducing recurrence. This activity is mainly attributed to the production of antimicrobial substances or organic acids (e.g. lactic acid) and metabolites such as hydrogen peroxide, which are toxic to *Candida* (46). VVC disease is likely to recur even in healthy individuals. Therefore, probiotic strains that have an antimicrobial effect on the *Candida* species are important in vaginal microflora. In our study, the *L. plantarum* L4 strain was found to be effective on the development of different *Candida* species.

Strains with a probiotic character should be sensitive to antibiotics administered in the clinic and should not have antibiotic resistance genes if they are resistant to antibiotics. In our study, the antibiotic susceptibility of the vaginal *Lactobacillus* spp. L4 strain was also tested. *Lactobacillus* has been shown to be resistant to aminoglycosides, beta-lactam antibiotics, cephalosporins, and glycopeptide antibiotics (46, 47). Similarly, in our study, it was found that the aminoglycosides were resistant to gentamicin, tobramycin, amikacin, and netilmicin antibiotics. Penicillin, ampicillin, cefazolin, tetracycline, rifampicin, chloramphenicol, erythromycin, clindamycin, cefoperazone, streptomycin, ceftazidime, and imipenem were also susceptible to antibiotics.

Vancomycin is one of the broad-spectrum antibiotics used in the treatment of multiple drug-resistant pathogens and various clinical infections. Therefore, resistance to vancomycin is important (29, 48). In our study, it was determined that the L4 strain showed high resistance to vancomycin and teicoplanin antibiotics from the glycopeptide group (49). This type of resistance doesn't cause concern for microorganisms with a probiotic character, as it differs from the inducible, transferable mechanism that may be in other bacteria, such as enterococci. Our results are similar to those reported previously (49, 50).

H_2O_2 is one of the important active compounds produced by vaginal *Lactobacillus*. Studies have shown that the *Lactobacillus* species with H_2O_2 production capacity can protect women against bacterial vaginosis infection (51, 52). In our study, the *L. plantarum* L4 strain was found to be a strong producer of H_2O_2. The L4 strain may be a suitable candidate for protection from genital infections.

Aggregation is an important feature of *Lactobacillus* because it can create a microenvironment around pathogens containing a high concentration of inhibiting agent and prevents the adherence of pathogens to the intestinal and/or vaginal epithelial cells. In this context, the *Lactobacillus* spp. L4 strain was determined to be a strain with high aggregation and co-aggregation ability. Cancer development and progression are thought to be associated with inflammation (53). In our study, it was observed that TNF-α value, which plays a role in proinflammatory response, significantly decreased compared to the control. In contrast, a significant increase in IL-10 production was observed in samples treated with the L4 strain relative to the control. IL-10 regulates the inflammatory response by suppressing the production of pro-inflammatory cytokines such as TNF-α (54).

This is a possible explanation for the decrease in TNF-αproduction observed in our study. It has been suggested that TNF-α is an inducer of proliferation, while IL-10 is an inhibitor of tumor growth. IL-10 plays an important role in the development of cervical cancer (55). In some IL-10 transfection studies, IL-10 has been shown to inhibit tumor growth and metastasis (56). Moreover, the decrease in IL-10 level is associated with the risk of cervical cancer (48). The results show that the L4 strain has an anti-inflammatory effect on HeLa cells and potentially

inhibits cell proliferation by reducing TNF-α production and increasing IL-10 production. This may explain the decrease in TNF-α production, which was also observed in our study. It has been suggested that TNF-α is a proliferation inducer and IL-10 is an inhibitor of tumor growth. IL-10 plays an important role in the development of CVC (55). The reduction in IL-10 level is associated with CVC (48). As a result, it shows that the L4 strain has an anti-inflammatory effect on HeLa cells and potentially inhibits cell proliferation by reducing TNF-α production and increasing IL-10 production.

The majority of anticancer studies of *Lactobacillus* are related to colorectal cancer (57, 58). Therefore, we decided to investigate the anticancer activity of the L4 strain, which has very good probiotic properties isolated from vaginal flora on HeLa cancer cells. In contrast, in this study the anticancer activity of the L4 strain on HeLa cancer cells was investigated. At the end of the study, it was found that even a very low dose of L4 strain metabolic products had a high antiproliferative effect on HeLa cells. In the present study, normal HUVEC cells were selected as controls. The results of the study showed that the L4 strain had no significant inhibitory or toxic effects on HUVEC normal cells.

The results of the meta-analysis conducted in 2019 show that there is a relationship between *Lactobacillus* species found in vaginal microbiota and HPV infection and HPV-related diseases, and it can be used as an aid in the treatment of these diseases (16, 20).

Studies investigating the anti-cancer activities of *L. plantarum* strains isolated from vaginal flora on cancer cell lines are very few. Nami et al. investigated the effect of the supernatant belonging to the *L. plantarum* 5BL strain on different cancer cell lines and observed the most important antiproliferative effect on HeLa cells (59). In the studies that different *Lactobacillus* species isolated from vaginal flora, the metabolites of the *L. acidophilus* 36YL strain have a reported cytotoxic effect on HT-29 and HeLa cells (29). Motevaselli et al. found that *L. gasseri* and *L. crispatus* strains showed a cytotoxic effect on HeLa cells (21). In another study, it was stated that the *L. fermentum* SK5 strain binds to HeLa, HT-29, and Caco-2 cells at rates of 92%, 93, and 93%, respectively and inhibits the growth of cells (28). Similarly, in our study, the greatest antiproliferative effect of the *Lactobacillus* spp. L4 strain on HeLa cells was observed as 90-95% mortality rate at the highest dose of metabolite 0.0006 g/ml, while the number of living cells was found to be between 5-10%.

CONCLUSION

Studies suggest that regular oral probiotic intake may play an effective role in gastrointestinal cancer treatment (20, 22). Vaginal probiotics may be effective in the development or prevention of gynecological cancers, as well as in vaginitis and HPV infection. The data we obtained from this study supported this result. In our study, the L4 strain with strong probiotic characters increased IL-10 cytokine production, proving that it plays an effective role on the system by inhibiting the production of TNF-α, whereas the metabolite of the *Lactobacillus* spp. L4 strain exhibited a high antiproliferative effect in bile in very small doses. The L4 strain is considered a promising treatment candidate for HPV cancer. However, additional research is needed to determine whether modulation of cervicovaginal microflora with probiotics is a preventative strategy or application to gynecological treatment.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Institutional Ethics Committee of Kırıkkale University (Date: 27.10.2014, No: 25/02).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.K., S.Y.A., E.K.; Data Acquisition- E.K.; Data Analysis/Interpretation- E.K., S.Y.A., E.K.; Drafting Manuscript- E.K.; Critical Revision of Manuscript- E.K., S.Y.A.; Approval and Accountability- E.K., S.Y.A., E.K.

Conflict of Interest: Authors declared no conflict of interest

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HIGH PREVALENCE OF CLASS I AND CLASS II INTEGRONS IN UROPATHOGENIC *E. COLI* STRAINS (UPECs) AND THEIR RELATIONSHIP WITH ANTIBIOTIC RESISTANCE, PHYLOGENY AND VIRULENCE

ÜROPATOJEN *E. COLI* (UPEC) SUŞLARINDA SINIF I VE SINIF II İNTEGRONLARIN YÜKSEK PREVALANSI İLE ANTİBİYOTİK DİRENCİ, FİLOGRUP VE VİRULANS İLİŞKİSİ

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ABSTRACT

Objective: Integrons, which are highly effective in capturing, integrating and expressing gene cassettes, play an important role in the dissemination of multiple antibiotic resistances. This study investigated the correlations of integrons in uropathogenic *E. coli* (UPEC) with antibiotic resistance, virulence and phylogeny and also the relationships of phylogroups with virulence and antibiotic resistance.

Materials and Methods: Fifty UPECs isolated from uncomplicated cystitis and uncomplicated pyelonephritis were investigated to detect the presence of class I, II and III integrons and phylogenetic grouping by the PCR method. Their statistical relationship with antibiotic resistance and virulence genes were investigated using our previous findings.

Results: Among 50 UPEC strains, 37 (74%), 22 (44%) and only one (2%) strain was shown to harbor class I, class II and class III integrons, respectively. Twenty one (42%) strains were found to carry both class I and class II integrons. The majority of the strains were grouped as phylogroup B2 (38%) and phylogroup E (38%). The presence of integrons was in association with only ampicillin resistance (p=0.014). Integrons was found to be related neither to virulence genes nor phylogroups; however, the presence of *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes was found

ÖZET

Amaç: Gen kasetlerinin yakalanması, integrasyonu ve ekspresyonunda oldukça etkin olan integronlar, çoğul antibiyotik direncinin yayılımında önemli bir rol oynarlar. Bu çalışmada, üropatojen *E. coli* (UPEC) suşlarında integron varlığının antibiyotik direnci, virülans ve filogenetik gruplar ile ve filogrupların, virulans ve antibiyotik direnci ile ilişkisi araştırılmıştır.

Gereç ve Yöntemler: Komplike olmayan sistit ve komplike olmayan piyelonefrit etkeni olarak izole edilen 50 UPEC suşu sınıf I, II ve III integronların varlığı ve filogruplarının belirlenmesi amacıyla PCR yöntemi ile incelenmiştir. Elde edilen sonuçlar istatistiki ilişki açısından daha önceki araştırmamızdan elde ettiğimiz bulgular ile incelenmiştir.

Bulgular: Elli UPEC suşunun 37'sinin (%74) sınıf I integron, 22'sinin (%44) sınıf II integron ve bir suşun (%2) ise sınıf III integron taşıdığı belirlenmiştir. Yirmi bir (%42) suşun ise sınıf I ve sınıf II integronları birlikte taşıdığı gösterilmiştir. Suşların çoğu B2 (%38) ve E (%38) filogruplarında sınıflandırılmıştır. İntegronların varlığı ile sadece ampisilin direnci ilişkili bulunmuştur (p=0,014). İntegronların varlığı ile virulans genleri veya filogruplar arasında bir ilişki bulunmamıştır; ancak *PAI* (p<0,001), *ompT* (p=0,035) ve *usp* (p<0,001) genlerinin varlığı ile B2 filogrubu arasında anlamlı ilişkili bulunmuştur. Filogrup E ile ko-trimoksazol direnci arasında

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to be significantly related to phylogroup B2. Phylogroup E was found to be statistically significantly correlated with co-trimoxazole resistance (p=0.043).

Conclusion: Consistent with previous studies, our results have proven that there is a strong association between antibiotic resistance and the presence of integrons (especially class I) in UPEC strains, and it has been shown that integrons became very prevalent globally.

Keywords: Uropathogenic *E. coli*, phylogroups, integrons, virulence factors, antibiotic resistance

anlamlı ilişkili bulunmuştur (p=0,043).

Sonuç: Bulgularımız literatürle de uyumlu olarak UPEC suşlarında antibiyotik direnci ile integronların (özellikle sınıf I) varlığı arasında güçlü bir ilişki olduğunu kanıtlamış ve tüm dünya genelinde integronların yaygın görüldüğü anlaşılmıştır.

Anahtar Kelimeler: Üropatojen *E. coli*, filogruplar, integronlar, virulans faktörleri, antibiyotik direnci

INTRODUCTION

Over the last decades, investigations have shown that integrons are highly effective in capturing, integrating and expressing gene cassettes, and play an important role in the dissemination of multiple antibiotic resistances within microbial populations. Integrons lack the specific mobilization machinery, but they are associated with insertion sequences, transposons and/or plasmids (1-4).

Integrons have an integrase gene (intI), an attachment site (attI), and a promoter region (Pc) which induces the expression of integrated gene cassettes (1, 5-7). Among the five different classes of integrons, class I and class II integrons are the most common and clinically important. Integrons are very common (22-95%) in enteric bacteria isolated from various infections, although they are also found to be present in commensal bacteria. Studies have shown that integrons horizontally transfer more than 130 different gene cassettes, which encode antibiotic resistance (8, 9).

Escherichia coli are classified into different pathogenicity groups according to specific virulence factors. One of these pathogroups is known as uropathogenic *E. coli* (UPEC). As one of the primary etiological agents of urinary tract infections (UTIs), UPECs account for 80-90% cases of community-acquired UTIs and 40-50 % of hospital-acquired UTIs. It is well known that UTIs are one of the most common infectious diseases, with nearly 150 million new cases diagnosed annually all around the world (7, 10, 11). It has been shown that UPEC is a heterogeneous group of strains composed of several virulence assortments and phylogroups. The majority of various virulence traits of UPECs are fimbrial and/or afimbrial adhesins, iron uptake systems such as siderophores, exotoxins and bacteriocins (12-16).

According to the presence/absence of *chuA* and *yjaA* and DNA fragment TspE4.C2, *E. coli* strains are classified into four main phylogroups (A, B1, B2 or D) (17); however in the last decade four new phylogroups were identified which have caused *E. coli* strains to be classified into eight groups (A, B1, B2, C, D, E, F and *Escherichia* clade I) (18). Previous studies have shown that UPECs are mostly grouped as phylogenetic groups B2 and D, and virulence factors described for UPECs are shown to be related to phylogenetic group B2 (6, 7, 19).

This study investigated the correlations of integrons in UPECs with antibiotic resistance, virulence and phylogeny and also the relationship between phylogroups and virulence.

MATERIALS AND METHODS

Strains

Fifty UPEC strains isolated from patients with different uncomplicated cystitis and uncomplicated pyelonephritis were included. These strains were isolated within the context of our previous study in which their virulence genes (*afa*, *aer*, *cnf1*, *sfa/foc*, *pap3/4*, *PAI*, *iroN*, *ompT*, *hly*, *usp*, *pap1/2*) and antibiotic susceptibilities [ampicillin (AMP), amoxicillin/clavulanate (AMC), cefuroxime (CXM), ceftriaxone (CRO), cefixime (CFX), gentamicin (GN), ciprofloxacin (CIP), ofloxacin (OFX), co-trimoxazole (SXT), nitrofurantoin (NIT) and aztreonam (ATM)] were investigated back then (15). We kept the bacteria at -80^{0}C for further examinations.

Detection of integrons and phylogroups

The DNA template from UPECs was prepared from overnight cultures in Tryptic soy broth (TSB) at 37°C. An extraction kit (GeneDireX, Taiwan) was used according to the manufacturer's instructions.

We investigated the presence of class I, class II and class III integrons on both genomic and plasmid DNAs as suggested in Ren et al. (20). For this purpose, all extracted DNAs were examined by multiplex polymerase chain reaction (PCR) for the presence of *intI*, *intII* and *intIII* genes (20).

Primers used in this research are shown in Table 1 (20, 21). A master mix kit (Genemark, Taiwan) was used in PCR assays. Mixtures (25 µL last volume) were prepared according to the manufacturer's suggestions (Genemark, Taiwan): 5 µL master mix, 2 µL DNA, 2 µL each primer (1 µL for each primer from 10 pmol concentration) and nuclease-free water.

Table 1: Primers used in integron PCR analysis

Genes	Primer sequence	Amplicon size	Reference
Class I int F	5′-CCT CCC GCA CGA TGA TC-3′	280 bp	(20, 21)
Class I int R	5′-TCC ACG CAT CGT CAG GC-3′		
Class II int F	5′- GTA GCA AAC GAG TGA CGA AAT G-3′	780 bp	(20)
Class II int R	5′- CAC GGA TAT GCG ACA AAA AGG T-3′		
Class III int F	5′ -GCC TCC GGC AGC GAC TTT CAG-3′	976 bp	(20)
Class III int R	5′-ACG GAT CTG CCA AAC CTG ACT-3′		

The reaction conditions for PCR amplification were as follows: initial denaturation for 4 min at 94°C; degradation for 45 sec at 94°C; annealing for 45 sec at 57°C; elongation for 55 sec at 72°C; final elongation for 4 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India) (20). The amplicons were stored at -20°C.

We could not find any strain harboring *intIII* gene for use as a positive control in PCR assays. Therefore, DNA sequence analysis was performed for confirmation of class III integron positive DNA samples by using the ABI3730 XL Genetic Analyzer device (GATC Biotech AG, Germany).

According to the study of Clermont et al., (18), phylogroups of UPEC strains were determined via two sequential PCR assays. In the first stage, phylogroups were determined according to the presence/absence of TspE4.C2, *chuA*, *yjaA* and *arpA* genes. Second multiplex PCR assays using *trpAgpC*, *ArpAgpE,* and *trpBA* primers were performed to distinguish the phylogroups D from E, E from *Escherichia* clade I, and A from C (18). All primers are shown in Table 2.

A Master mix kit (Genemark, Taiwan) was used in PCR. Mixtures (25 µL last volume) were prepared according to the manufacturer's suggestions (Genemark, Taiwan): 5 µL master mix, 2 µL DNA, 2 µL each primer (1 µL for each primer from 10 pmol concentration), and nuclease-free water.

The reaction conditions for PCR amplification were as follows: initial denaturation for 4 min at 94°C; degradation for 30 sec at 94°C; annealing for 30 sec at 56°C; elongation for 45 sec at 72°C; final elongation for 5 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India) (18). The amplicons were stored at -20°C.

All amplified products were separated by agarose gel electrophoresis in a 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL), visualized under UV light. After that products were electrophoresed for 40 min under 80

Table 2: Primers used in phylogroup PCR analysis

Genes	Primer sequence	Amplicon size	Reference
chuA.1bF	5′-ATGGTACCGGACGAACCACC-3′	288 bp	(18)
chuA.2bR	5′-TGCCGCCAGTACCAAAGACA-3′		
yjaA.1bF	5′-CAAACGTGAAGTGTCAGGAG-3′	211 bp	
yjaA.2bR	5′-AATGCGTTCCTCAACCTGTG-3′		
TspE4C2.1bF	5′-CACTATTCGTAAGGTCATCC-3′	152 bp	
TspE4C2.2bR	5′-AGTTTATCGCTGCGGGTCGC-3′		
AceK.fF	5′-AACGCTATTCGCCAGCTTGC-3′	400 bp	
ArpA1.rR	5′-TCTCCCCATACCGTACGCTA-3′		
trpAgpC.1	5′-AGTTTTATGCCCAGTGCGAG-3′	219 bp	
trpAgpC.2	5′-TCTGCGCCGGTCACGCCC-3′		
ArpAgpE.f	5′-GATTCCATCTTGTCAAAATATGCC-3′	301 bp	
ArpAgpE.r	5′-GAAAAGAAAAAGAATTCCCAAGAG-3′		
trpBA.f	5′-CGGCGATAAAGACATCTTCAC-3′	489 bp	
trpAgpC.1	5′-AGTTTTATGCCCAGTGCGAG-3′		

volts with 1XTBE electrophoretic liquid. The results were visualized and recorded using Hi-UV MAX transilluminator (HiMedia, India). DNA ladder (Genemark, Taiwan) labeled between 100-1000 bp was used.

Statistical analysis

The correlations of integrons with antibiotic resistance, virulence genes, and phylogroups were statistically analyzed. The categorical variables were reported as n (%) by using the Pearson Chi-Square test, and Fisher's exact test. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis, and a p-value <0.05 was considered as statistically significant.

This study was approved by the Istanbul Yeni Yuzyıl University Medical Faculty Research Ethics Committee (Date: 01.10.2018, No: 8).

RESULTS

Detection of integrons

Among 50 UPEC strains, 37 (74%) and 22 (44%) were shown to carry class I and class II integrons, respectively. Twenty-one (42%) of UPECs were found to possess both classes I and II integrons (Figure 1-2).

It was also shown that both *intI* and *intII* genes were encoded more frequently in plasmids rather than genomic DNA. The *intI* gene was shown to be harbored in 22 (44%) strains on genomic DNA and in 34 (68%) strains on plasmid DNA. Similarly, 14 (28%) and 19 (38%) of UPEC strains were found to possess *intII genes* in genomic and plasmid DNA, respectively. We found that only one strain was shown to carry the *intIII* gene which was encoded only on plasmid DNA. The result was confirmed with a sequence analyzing method.

Detection of phylogroups

The majority of the strains were grouped as phylogroup B2 (38%) and phylogroup E (38%), and the rest of the strains were defined as D (8%), A (2%), C (2%) and F (2%). Five (10%) strains could not be classified (Figure 3 and 4).

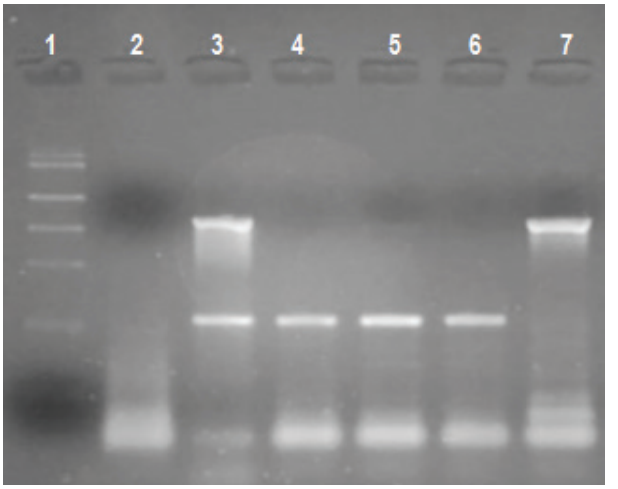

Figure 1: Agarose gel image of the *intI* and *intII* genes amplified by multiplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control *(intI*-280 bp *and intII*-780 bp), well 4-6: *intI* positive strains, well 7: *intII* positive strains

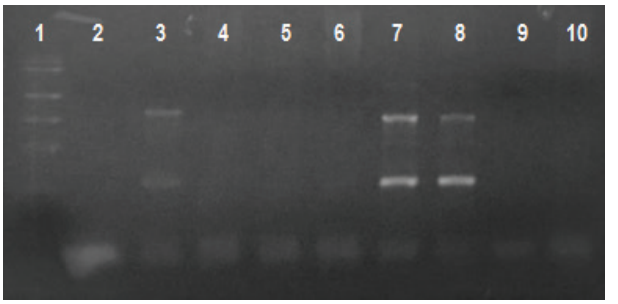

Figure 2: Agarose gel image of the *intI* and *intII* genes amplified by multiplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control (*intI*-280 bp and *intII*-780 bp), well 4-6: negative strains, well 7-8: *intI* and *intII* positive strains

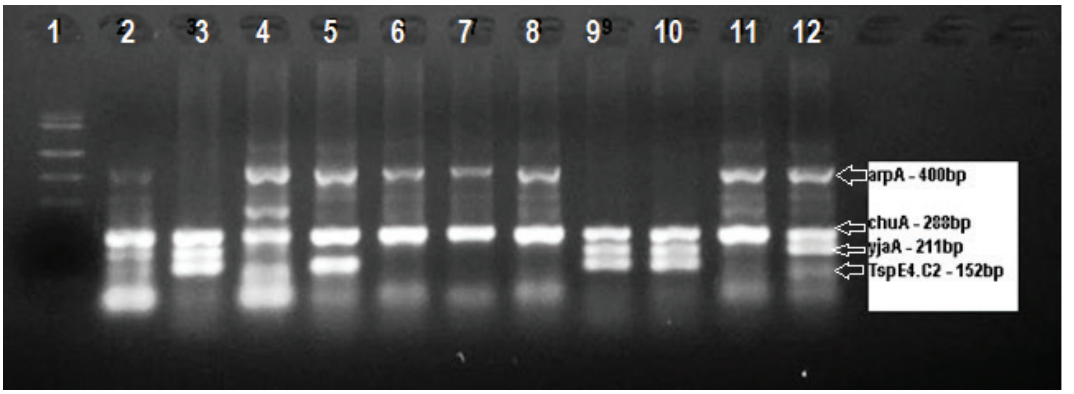

Figure 3: Agarose gel image of the *arpa*, *chuA*, *yjaA* and TspE4.C2 genes amplified by the multiplex PCR.

Well 1: DNA ladder, well 2: positive control, well 3: group B2, well 4-8: group E, well 9-10: group B2, well 11-12: group E.

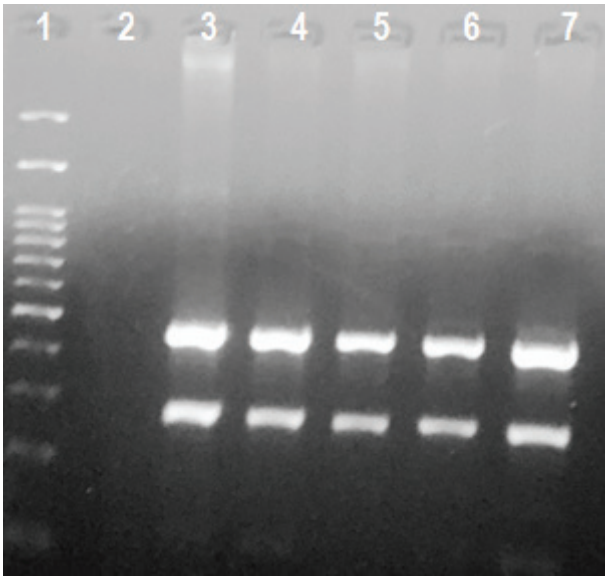

Figure 4: Agarose gel image of the multiplex PCR assays used for detection of E/cladeI groups.

Well 1: DNA ladder, well 2: negative control, well 3-7: *trpBA* (489 bp) and *ArpAgpE* (301 bp) positive strains which are classified as group E.

Statistical analysis

A statistically significant correlation (p=0.014) of the presence of integrons was found only to ampicillin resistance (Table 3).

In integron-bearing strains, the rate of the *usp* gene was shown to be lower than non-integron bearing strains and the difference was statistically significant (p=0.013) (Table 4). There was no statistically significant relationship between the presence of integrons and other virulence genes (p>0.05).

There was no statistically significant (p>0.05) relation between the presence of integrons and phylogroups.

Statistically significant relations between virulence genes and phylogroups were as follows: the presence of *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes were higher in group B2. The presence of *sfa/foc*, *PAI* and *usp* genes were found to be lower (p=0.035, p<0.001 and p<0.001, respectively) and the *ompT* (p=0.013) gene was higher in the E phylogroup than the others.

Only resistance to SXT was found to be statistically significantly (p=0.043) related to group E.

Table 3: Correlation between integrons and antibiotic resistance

	Integron positive (n=38)	Integron negative (n=12)	p-value[a]
AMP			
S	3 (7.89%)	5 (41.67%)	**0.014**[a]
R	35 (92.11%)	7 (58.33%)	
SXT			
S	20 (52.63%)	10 (83.33%)	0.091[a]
R	18 (47.37%)	2 (16.67%)	
CIP			
S	28 (73.68%)	11 (91.67%)	0.257[a]
R	10 (26.32%)	1 (8.33%)	
AMC			
S	30 (78.95%)	9 (75%)	>0.99[a]
R	8 (21.05%)	3 (25%)	
OFX			
S	28 (73.68%)	11 (91.67%)	0.257[a]
R	10 (26.32%)	1 (8.33%)	
CFX			
S	34 (89.47%)	11 (91.67%)	>0.99[a]
R	4 (10.53%)	1 (8.33%)	
ATM			
S	33 (86.84%)	12 (100%)	0.319[a]
R	5 (13.16%)	0 (0%)	
CXM			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
CRO			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
NIT			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
GN			
S	36 (94.74%)	12 (100%)	>0.99[a]
R	2 (5.26%)	0 (0%)	

Data are expressed as n (%), [a]: Fisher's Exact Test, S: susceptible, R: resistant

DISCUSSION

In the present study, 50 UPEC strains isolated from acute uncomplicated cystitis and acute uncomplicated pyelonephritis patients were investigated to determine the relations of integron classes (I-III) with antibiotic resistance, virulence genes and phylogeny. Moreover, the correlation of phylogeny, virulence and antibiotic resistance was also examined.

Table 4: Correlation between integrons and virulence genes

	Integron positive (n=38)	Integron negative (n=12)	p-value[a]
afa			
positive	2 (5.26%)	0 (0%)	>0.99[a]
negative	36 (94.74%)	12 (100%)	
aer			
positive	19 (50%)	5 (41.67%)	0.614[b]
negative	19 (50%)	7 (58.33%)	
cnf1			
positive	8 (21.05%)	4 (33.33%)	0.448[a]
negative	30 (78.95%)	8 (66.67%)	
sfa/foc			
positive	7 (18.42%)	4 (33.33%)	0.424[a]
negative	31 (81.58%)	8 (66.67%)	
pap$^{3/4}$			
positive	1 (2.63%)	1 (8.33%)	0.426[a]
negative	37 (97.37%)	11 (91.67%)	
iroN			
positive	14 (36.84%)	5 (41.67%)	>0.99[a]
negative	24 (63.16%)	7 (58.33%)	
PAI			
positive	17 (44.74%)	8 (66.67%)	0.185[b]
negative	21 (55.26%)	4 (33.33%)	
ompT			
positive	29 (76.32%)	10 (83.33%)	>0.99[a]
negative	9 (23.68%)	2 (16.67%)	
hly			
positive	2 (5.26%)	2 (16.67%)	0.240[a]
negative	36 (94.74%)	10 (83.33%)	
usp			
positive	16 (42.11%)	10 (83.33%)	**0.013**[b]
negative	22 (57.89%)	2 (16.67%)	
pap ½			
Positive	17 (44.74%)	7 (58.33%)	0.411[b]
Negative	21 (55.26%)	5 (41.67%)	

Data were expressed as n (%), [a]: Fisher's Exact Test, [b]: Pearson Chi-Square Test

Previous studies investigating the presence of bacterial integrons were mainly reported in various clinical isolates from Iran. Farshad et al. have shown that the prevalence of class I and class II integrons in strains isolated from urine samples of children were 6.25% and 10.4% respectively, and the class III integron was not detected (22). In another study from Iran, Falakian et al. reported that the prevalence of class I integrons was 49% in UPEC strains (10). The frequency of class I and class II integrons in UPEC strains were reported as 52% and 2.5%, respectively, by Khoramrooz et al. (5). Class III integron was not found in any of the isolates. They also showed a strong relationship between the presence of integrons and resistance to co-trimoxazole, ciprofloxacin, ceftazidime, and tetracycline resistance rates (5). Ebrahim-Saraie et al. reported that the prevalence of class I and class II integrons were 59.5% and 7.4%, respectively, in UPEC strains. While in the same study no class III integron was detected, resistance to sulfonamides and the presence of class I integron was found to be statistically related (2). In a study of Mirnezami et al. the prevalence of integrons in 100 UPEC strains were found to be 70% and 3% for class I and class II integrons, respectively. They also reported a strong relationship between the presence of class I integron and resistance to ampicillin, gentamicin, ciprofloxacin, co-trimoxasole, and nalidixic acid (23). Similar to previous studies, our study has shown that there was a high frequency of class I (74%) and class II integrons (44%). Also, the class III integron was detected in one of the UPEC strains.

In a study by El-Najjar et al. in Lebanon, it was shown that 30% of UPEC strains were positive for the class I integron. They also indicated that the prevalence of antibiotic resistance rates was higher in integron harboring strains (4). In Syria, Al-Assil et al. detected that 54.6% of UPEC strains harbored class I integrons and there was a strong correlation between multidrug resistance and class I integrons (24).

In a study from the USA, Solberg et al. reported that the detection rates of class I and class II integrons were 49% and 20%, respectively, in UPEC strains (25). Solberg et al. have declared a high prevalence rate of class II integrons when compared to the studies mentioned above. However, our results have shown a higher detection rate (44%) of class II integrons. In the study of Zeighami et al., 92.5% of UPEC and diarrheagenic *E. coli* strains (DEC) were found to carry integrons; the prevalence of class I and class II integrons were reported to be 85% and 2%, respectively. Class III integrons were not detected. As compared to UPEC, DEC was reported to carry integrons more frequently (94% for class I, 8% for class II) and they emphasized that enteric pathogens could act as a reservoir or donor of several antibiotic resistance genes which can be transferred to other *E. coli* pathogroups (26). Ochoa et al. in Mexico, have shown that class I and class II integron rates in multi-drug resistant (MDR) UPEC strains were 44% and 2%, respectively. They also showed that 48% and 9.5% of extended-drug resistant (XDR) UPECs

harbored class I and class II integrons, respectively, and none of these strains carried class III integron (7).

As seen above, the majority of the studies are mainly from the Middle-East (especially from Iran) and have shown higher frequencies of integrons. However, in a meta-analysis study, Halaji et al. emphasized that there is a strong correlation between class I integrons and high-level antibiotic resistance. In the same study, two issues were emphasized: firstly, the majority of the strains harbored class I integron isolated from hospital-acquired UTIs; and secondly, the frequency of class I integron carrying strains are high in Middle Eastern countries (27). Studies investigating the presence of integrons and their relation with antibiotic resistance in UPEC strains are limited in Turkey. In a multi-centered study, Çopur-Çiçek et al. reported that the prevalence of class I and class II integrons were 26% and 17%, respectively (28).

Numerous studies have examined the relationship between the presence of integrons, antibiotic resistance and phylogeny in *E. coli*. In a study by Poey and Lavina from Uruguay, UPEC strains isolated from pregnant women and children with urinary tract abnormalities were examined. The prevalence rates of class I and class II integrons were reported to be 22% and 8%, respectively. They have shown that the proportion of integron bearing strains were higher in children with urinary tract abnormalities due to recurrent antibiotic treatments. Resistance to ampicillin, cephalotin, and co-trimoxazole was found to be significantly associated with the presence of integrons. They also found a strong correlation between class I integron and phylogroup D (6). A similar approach was carried out by Oliveira-Pinto et al. from Brazil. They compared the presence of class I integrons in strains isolated from feces of healthy individuals who did not use any antibiotics and urine of women with community-acquired UTIs. They showed a higher incidence of class I integron in *E. coli* strains isolated from urine (65%) than commensal *E. coli* strains (12%); only urine strains were shown to be multi-resistant to all antibiotics tested. Most of the urine strains were grouped in phylogroup B2, however, 43% of commensal strains were shown to be assigned in phylogroup A (9). Gündoğdu et al. reported that the prevalence of class I and class II integrons were found to be 34.3% and 5.1%, respectively, and class III integrons were not detected in UPEC strains isolated from hospitalized patients in Austria. The prevalence of multi-drug resistance was shown to be significantly higher among integron positive strains and the majority (78%) of the strains were assigned to phylogroup B2 (29). Yekani et al., in Azerbaijan, showed that class I and class II integrons were found to be harbored in 64% and 4.5% of UPECs, respectively, and class III integrons were not found. The authors suggested that multi-drug resistance was significantly associated with the presence of class I integrons. Although strains were mostly shown to be assigned to phylogenetic group B2 (61%), a high prevalence of class I and class II integrons were detected among group B1 strains (3). In another study from Mexico examining multi-drug resistant UPECs, it was reported that class I integrons were distributed in phylogenetic groups A, B2, and D (40%, 44%, and 44%, respectively); class 2 integrons were distributed in phylogenetic groups B2 and D (3% and 2%, respectively) (7). Olivera-Pinto et al. from Brazil showed that 55% of UPEC isolates belonged to phylogroup B2, and to a lesser extent to group D, whereas most commensal *E. coli* isolates were grouped in phylogroup A (43%) (9). It seems that the presence of integrons is shown to be statistically significantly related to phylogroups B1, B2 and D in UPECs (3, 6, 9, 29).

In our study, 74% of the UPECs were shown to carry class I and/or class II. Furthermore, we found that the integrons were encoded mostly on plasmids. Previous studies have clearly shown that the presence of integrons is associated with particular multi-drug resistance patterns; in our study, the presence of integrons and ampicillin resistance were found to be correlated. According to our results, it was shown that the majority of the UPECs were distributed equally in groups B2 (38%) and E (38%). However, we did not find any statistically significant relationship between the presence of integrons and phylogroups. The phylogroup E and resistance to SXT were shown to be statistically correlated.

The limited number of studies showed the possible relations between the presence of integron and virulence factors. Poey and Lavina showed that class I integrons were found to take part in strains that carry P fimbria, yersiniabactin, and aerobactin systems (6). Düzgün et al. reported that there was a statistically significant relationship between the presence of class I integrons, *CTX-M* and *fim* genes in UPECs (30). In our study, there was no significant correlation between the presence of integrons and virulence genes.

Another issue about UPEC strains is to clarify the relationship of their virulence factors with phylogenetic distributions. Ochoa et al. concluded that most of the MDR-UPEC strains were grouped in D (55%) and B2 (39%) and the most frequent virulence genes in these groups were several fimbrial genes (*ecpA*, *fimH*, *csgA*, and *papGII*), an iron uptake gene (*chuA)* and a toxin gene (*hlyA)* (7). Similarly, in South Korea, Lee et al. indicated that most of the virulence genes (*fimH, sfa, pap, sfa, hly, feoB, irp2, iroN*) were found to be significantly higher in the strains grouped as B2 and D (31). Yılmaz and Aslantaş from Turkey showed that *E. coli* strains isolated from urine were mostly shown to be defined as group $B2_3$ (36%) and group A1 (21%); most strains were reported to carry at least one virulence gene (32). In line with other research,

we found that *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes were significantly related to group B2 while only the *ompT* gene was significantly related to group E (p=0.013).

In conclusion, unlike previous studies, we investigated the prevalence of integrons on genomic and plasmid DNAs separately. We have shown that they were more prevalent on plasmids (class I integron 68%, class II integron 38%) which highlights the common occurrence of horizontally transferring. To our knowledge, this is the first report detecting the presence of class III integron in UPEC strains isolated in Turkey and other countries from Europe. According to our results, the presence of integrons was found to be related neither to virulence genes nor phylogroups; however, the presence of *PAI*, *ompT,* and *usp* genes was found to be significantly related to phylogroups B2 and E.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Istanbul Yeni Yüzyıl University Medical Faculty Research Ethics Committee (Date: 01.10.2018, No: 8).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- D.G., F.K.Y., M.K.; Data Acquisition- D.G., F.K.Y., D.C., M.O.; Data Analysis/Interpretation- D.G., F.K.Y., D.C., C.M.; Drafting Manuscript- D.G., F.K.Y., M.K.; Critical Revision of Manuscript- D.G., F.K.Y., M.K.; Approval and Accountability- D.G., F.K.Y., F.D.C., M.O., A.C.M., M.A.K.

Conflict of Interest: Authors declared no conflict of interest

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THE TRANSITION OF ADOLESCENTS TO ADULT NEPHROLOGY CARE: A SURVEY ON YOUNG ADULTS' EXPERIENCE

ADOLESAN HASTALARIN ERİŞKİN NEFROLOJİ BÖLÜMÜNE DEVREDİLMESİ: GENÇ YETİŞKİNLERİN DENEYİMLERİ

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ABSTRACT

Objective: As increasing number of young people with renal diseases are reaching adulthood and issues related to their transition to adult units are getting more important. Failure to transfer adolescents under a well-designed transfer program can lead to a decline in attendance to an adult unit. We aim to share our experience and the views of the patients who went through a transition program.

Materials and Methods: We conducted a telephone questionnaire with 88 patients, who were asked to evaluate their concerns before their transition and their experience during the visit in the adult nephrology unit after the transition process.

Results: The mean age of participants was 19.21±0.97 years. Thirty patients (34%) had chronic kidney disease, and three of them were on dialysis. Three patients (3.4%) had a renal transplant at the time of transition. Fifty-one patients (58%) were felt anxious before the transition process. After their transfer to the adult unit, although 84 patients (95.5%) were satisfied to be in the adult unit, there were still 65 patients (74%) ready to return to the pediatric unit, if it was possible.

Conclusion: Transition to the adult unit is still a cause of concern for adolescent patients, even if they are transferred under a well-designed program. It is important to raise awareness about transition programs among pediatricians and to develop a pediatric-adult transition program for the medical and psychological well-being of patients.

Keywords: Adolescence, pediatrics, questionnaire, transition

ÖZET

Amaç: Böbrek hastalığı olan daha fazla sayıda genç hastanın erişkin dönemine ulaşmasıyla, bu hastaların erişkin bölümlerine devredilmesi de giderek önem kazanmaktadır. İyi planlanmış bir program ile devredilmeyen hastalarda erişkin bölümüne uyumda azalma görülebilir. Bu çalışmada devir programı ile erişkin bölümüne geçen hastaların tecrübe ve fikirlerini paylaşmayı hedefledik.

Gereç ve Yöntem: Seksen sekiz hastaya devir öncesi endişelerini ve devir süreci sonrasında erişkin nefroloji ünitesine ziyaret deneyimlerini değerlendiren bir telefon anketi uyguladık.

Bulgular: Hastaların ortalama yaşı 19,21±0,97 yıl idi. Erişkin bölümüne devir sırasında, otuz hasta (%34) kronik böbrek hastalığı ile izlenmekteydi, bu hastaların üçü (%3,4) diyaliz tedavisi alırken, üçü (%3,4) böbrek nakilliydi. Elli bir hasta (%58) devir öncesi endişeli olduğunu belirtti. Erişkin bölümüne ziyaretleri sonrasında, 84 hasta (%95,5) erişkin bölümündeki takipten memnun olduğunu belirtmesine rağmen, 65 hasta (%74) mümkün olsa yine de çocuk bölümüne geri dönmek istediklerini belirtti.

Sonuç: İyi tasarlanmış bir programla erişkin bölümüne geçmiş olsalar bile, devir adolesan hastalar için hala bir endişe kaynağıdır. Çocuk doktorları arasında devir programları hakkında farkındalık yaratmak ve hastaların tıbbi ve psikolojik iyiliği için bir pediyatrik-yetişkin devir programı geliştirmek önemlidir.

Anahtar Kelimeler: Adolesan, pediatri, anket, devir

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INTRODUCTION

More children with chronic diseases survive into adulthood with improvements in medicine, therefore the transition of adolescents into adult units is becoming an increasingly important issue (1-3). Because of the emotional, social and psychological changes during adolescence, health problems may become more apparent (4). Moreover, the expectations of adult units are more than the pediatric units in terms of taking the responsibility of their illness (2).

Medical transition is a planned movement of adolescents with chronic medical conditions from child-centered to adult-orientated healthcare units. The aim of a transition process is to ensure the continuity of lifelong functioning and well-being for young patients, who have special health care needs and those who do not. A timely and well-designed transition process should be specific to each patient and the timing is most appropriate between the ages of 18 and 21 years. The participants of a transition process are pediatricians, nurses, adult healthcare providers, patients and families (5). The coordination between the patient, family and the health care provider is important for the adolescent in order to assume responsibilities of adult roles and activities (6). When transferred without a transition process, poor health outcomes have been reported such as worsening of glycemic control in patients with diabetes mellitus, seizure control in those with epilepsy and graft failure in transplant recipients. Poor health outcomes were also reported in patients with pediatric cancers and cardiac surgery (7-9).

An appropriate and successful transition is very important in terms of future treatment and the compliance of patients with chronic illness.

We aim to share our experiences and views of the patients who went through a transition program.

MATERIALS AND METHODS

The transition process was achieved in a transition outpatient clinic with the participation of the patient, family, nurses, pediatric nephrologist and adult nephrologist. The study was conducted in accordance with the requirements of the Helsinki Declaration and was approved by the local Ethical Committee (Date: 02.10.2020 No: 1045). The recorded past medical history of the patients was summarized by a pediatric nephrologist and the medical records were reviewed and discussed by two nephrologists. The adult nephrologist, the patient and the family were introduced to each other. At the end of the interview, an appointment was scheduled for the patient in the adult nephrology unit.

We developed a telephone questionnaire of five questions evaluating the patients' concerns before transition and their experience about the visit to the adult nephrology unit after the transition process. We conducted the questionnaire for the patients who had at least one visit to the adult unit (Table 1).

Table 1: Questions in the interview

	Questions
1	Were you anxious about your transition to adult unit?
2	If it is avaiable, do you want to return to pediatric unit again?
3	Do you think the knowledge given about your illness was satisfactory?
4	Did you need to take a second opinion after transition?
5	Are you happy about adult unit follow up?

RESULTS

A total of 88 patients (44 male, 44 female) completed the questionnaire. The mean age of participants was 19.21±0.97 years (range: 18-22). Thirty patients (34%)

Table 2: Diagnosis of patients

Diagnosis	Patients with CKD (n=30)	Patients with renal tx (n=3)	Other patients (n=55)
Uropathy	17	2	8
Primary glomerulopathies	3	1	25
Hypertension			8
Orthostatic proteinuria			3
SB + NB	6		3
Nephrolithiasis	1		3
FMF			2
DM + proteinuria			2
Cystic disease			1
Unknown	3		

CKD: Chronic kidney disease, tx: transplantation, SB: Spina bifida, NB: Neurogenic bladder, FMF: Familial mediterranean fever, DM: Diabetes mellitus

were diagnosed with chronic kidney disease (CKD), and three of them were on dialysis. Three patients (3.4%) had a renal transplant from deceased donors at the time of transition. The diagnoses of the patients are shown in Table 2. Two patients with end stage renal disease (ESRD) had renal transplantation from living donors, within six months after being transferred to the adult clinic. When we evaluated the answers of the patients;

1. Fifty-one patients (58%) were anxious about the transition to the adult unit before the process. Their major concern was leaving the pediatric unit where they were known well.
2. Although most of them were satisfied, 74% of patients were ready to return to the pediatric unit, if possible.
3. 92% of patients were satisfied with the knowledge and answers given to him/her.
4. 84% of patients were not keen on consulting another doctor about their illness.
5. 95% of patients were satisfied with being followed-up under the adult unit (Table 3).

Table 3: Answers of the patients transferred to adult unit

88 patients (44 male, 44 female)	**Yes n (%)**	**No n (%)**
Concerned about transition	51 (58)	37 (42)
Return to pediatric unit again	65 (74)	23 (26)
Satisfactory knowledge about your illness	81 (92)	7 (8)
Need a second opinion about your illness	14 (16)	74 (84)
Pleased about adult unit follow up	84 (95.5)	4 (4.5)

Sixteen patients (18.2%), all with CKD, were older than 20 years of age at the time of transition and all of them were categorised into the anxious group based on their answers to question 1. However, they were all satisfied to be followed by the adult unit after the transition process.

DISCUSSION

As increasing number of young people with chronic illnesses are reaching adulthood and issues related to their transition to adult units are getting more important.

The American Academy of Pediatrics, The American Academy of Family Physicians, and American College of Physicians published a clinical report supporting the health care transition from adolescence to adulthood. The aim of the report was to ensure the continuity of lifelong functioning and well-being for young patients (6).

The studies showed that transfer of patients to adult clinics without a transition process has an adverse effect on their follow-up. O'Leary C et al. showed that only 22% of patients with celiac disease in childhood were admitted to an adult gastroenterology clinic and most of the patients received no medical or dietary supervision after leaving pediatric units (1). It is also reported that transition programs provide better HbAlc levels and health outcomes in patients with diabetes mellitus (7). In case of a poorly organized transition, suboptimal seizure control and an increased risk of sudden unexpected death were reported in patients with epilepsy (9).

With the advances in dialysis and renal transplantation, the prognosis of children with ESRD improved dramatically and most of them survive into adulthood (10). Adolescents constitute approximately 3% of the ESRD population. Non-adherence to diet, medical treatment and missed dialysis sessions are more common among these patients. In childhood, including infancy, the prognosis of the disease is the worst during the adolescence period. An inappropriate transition to adult clinic in patients with CKD and ESRD has been shown to adversely affect quality of life and prognosis (11,12).

In a study evaluating renal transplant patients, the decline in estimated glomerular filtration rate (eGFR) was significantly lower in the transition group compared to the non-transition group after three years (-11.3 vs -28.4). The rate of rejections was 34.6% in the non-transition group and 9.1% in the transition group. The results support positive effects of the transition process on eGFR and rate of acute rejections in renal transplant patients (13).

Some of the patients continue to be under pediatric follow-up longer than is actually required because of their concerns about leaving the pediatric unit. However, pediatricians are not experienced in adult-related issues such as contraception, pregnancy and employment (2). Tuffrey et al. have reported the following barriers in the transition process: The pediatric team is reluctant to transfer their patients as they consider adult units to be inferior, furthermore the adult health care providers have no experience of pediatric conditions and the families have concerns about the adult units and a loss of control (14). We were also reluctant to transfer our patients with diagnoses which adult nephrology team is not experienced such as cystinuria, hyperoxaluria and the patients who had CKD and a long follow-up time in pediatric unit.

Adolescents indicated that age should not be the only criteria for the timing of transfers and they wanted to be involved in the decision-making process for the transfer (15). For pediatric patients and their families, one of the most important periods in their lives is the time of the transition to adult units. The patients should be informed about the transition process, beginning from early adolescence (12–14 years) and the information about the

transition should be appropriate to his/her developmental stage (10). In a study evaluating 21 adolescents' views about the transition process from the patient's perspective, the patients emphasized that building new relationships with adult care doctors and adjusting to the adult model of care took a while and they missed the relationship with the pediatric doctors who knew them well (16).

We investigated the views of pediatric patients with renal disease who were transferred to the adult nephrology unit. The patients were informed about their transition, starting between the ages of 14-16 years and they were transferred to the adult unit between 18-22 years of age.

Although the sample size was small, interviews with 88 young adults allowed us to learn their concerns with regards to their transition process and their views after their visit to the adult unit. The survey results showed that more than half of the patients were anxious about the process before transition to the adult unit and their major concern was leaving the pediatric unit who knew them well. All the patients, who were older than 20 years and had CKD, were anxious and unwilling to leave the pediatric unit. It can be speculated that patients with chronic conditions and a long duration of follow-up in the pediatric unit have much tighter bonds with the pediatric team and greater difficulties during transition. Although they were anxious before the transition, all of them were satisfied to be under the follow-up of the adult unit after the transition process, which shows their adaptation.

After their visit to the adult unit, most of the patients were satisfied with being followed-up by the adult clinic; nevertheless 74% of the patients were still eager to return to the pediatric unit, which shows the strong relationship established between the patients and the pediatric team.

While the pediatricians are willing to speak more about the illness, the clinical presentation and the social events with the child and also the family, most of the adult doctors focus on the illness and the clinic of the adult patient and speak only with the patient. We also observed the same in our survey and found out that our patients were ready to be treated as an adult.

In a review of several transition studies, patient education and transition through a specific transition clinic were the most successful strategies in transition processes (7). Achieving a successful transition is possible by using a transition clinic, where the patient is seen both by pediatric and adult specialists together (10). We couldn't provide a separate transition clinic but we transferred our patients in an outpatient clinic, dedicated only to the transition process.

In conclusion, we showed that most of our patients were anxious about the process before their transition to the adult unit. After a successful transition program, they were ready to be treated as an adult patient and satisfied to be in the adult unit, however, most of the patients were still missing us.

It is important to raise awareness about transition programs among pediatricians and to develop a pediatric-adult transition program for the medical and psychological well-being of patients.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of Marmara University (Date: 02.10.2020 No: 1045).

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Author Contributions: Conception/Design of Study- N.Ç., H.A.; Data Acquisition- N.Ç.; Data Analysis/Interpretation- N.Ç., H.A.; Drafting Manuscript- N.Ç.; Critical Revision of Manuscript- H.A.; Approval and Accountability- N.Ç., H.A.

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A CROSS-SECTIONAL STUDY ON THE RELATIONSHIP BETWEEN SMARTPHONE ADDICTION AND DEPRESSION, ANXIETY AND SOCIAL APPEARANCE ANXIETY IN YOUNG ADULTS

GENÇ ERİŞKİNLERDE AKILLI TELEFON BAĞIMLILIĞI İLE DEPRESYON, ANKSİYETE VE SOSYAL GÖRÜNÜM KAYGISI ARASINDAKİ İLİŞKİ ÜZERİNE KESİTSEL BİR ÇALIŞMA

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ABSTRACT

Objective: The aim of this study was to determine the prevalence of smartphone addiction in young adults, and to investigate the relationship between smartphone use, depression, anxiety, and social appearance anxiety.

Materials and Methods: Sociodemographic data form, Smartphone Dependence Scale-Short Form, Beck Depression Inventory for Primary Care-Short Form, Beck Anxiety Scale, and Social Appearance Anxiety Scale were applied face to face in this cross-descriptive study.

Results: A total of 259 subjects, 128 (49.4%) female and 131 (50.6%) male, were included in the study. 25.9% of the participants were smartphone addicts. There was a high positive correlation between smartphone usage time and smartphone addiction score. The social appearance anxiety average scores of the social media users (30.8±13.9) were higher than those of subjects who do not use social media (28.8±11.3). Smartphone addiction scale had a positive correlation with anxiety and social appearance anxiety scores, while not with depression.

Conclusion: Our results revealed that the purpose of smartphone usage affects people more than smartphone usage or smartphone addiction. The internet, and especially programs related to sharing one's appearance and lifestyle and applications that create an unrealistic appearance that conforms to virtual norms, can add hard-to-repair prejudices to the mind. This situation should be seen as a threat, especially for the

ÖZET

Amaç: Bu çalışma ile, genç yetişkinlerde akıllı telefon bağımlılığının yaygınlığını belirlemek ve akıllı telefon kullanımı, depresyon, anksiyete ve sosyal görünüş kaygısı arasındaki ilişkiyi araştırmak amaçlandı.

Gereç ve Yöntem: Çapraz tanımlayıcı desende planlanan bu çalışmada sosyo-demografik veri formu, Akıllı Telefon Bağımlılık Ölçeği-Kısa Formu, Birinci Basamak için Beck Depresyon Envanteri-Kısa Formu, Beck Anksiyete Ölçeği ve Sosyal Görünüş Kaygısı Ölçeği yüz yüze uygulanmıştır.

Bulgular: Çalışmaya 128'i (%49,4) kadın ve 131 (%50,6) erkek olmak üzere toplam 259 kişi dahil edildi. Katılımcıların %25,9'u akıllı telefon bağımlısıydı. Akıllı telefon kullanım süresi ile akıllı telefon bağımlılığı puanı arasında yüksek bir pozitif korelasyon vardı. Sosyal medya kullanıcılarının sosyal görünüş kaygısı ortalama puanları (30,8±13,9), sosyal medya kullanmayanların puanlarından (28,8±11,3) istatistiksel olarak daha yüksekti. Akıllı telefon bağımlılığı ölçeği ile depresyon ölçek puanları arasında anlamlı ilişki yokken, anksiyete ve sosyal görünüş kaygısı puanları ile pozitif korelasyona sahipti.

Sonuç: Çalışmamızda, akıllı telefon kullanımının amacının, akıllı telefon kullanımı veya bağımlılığına kıyasla kişileri daha fazla etkileme olasılığının olduğunu ortaya koymuştur. İnternet ve özellikle de görünüş ve yaşam tarzının paylaşımıyla ilişkili programların, olduğundan farklı ve normlara uygun görünüşün yaratıldığı uygulamalarla desteklenmesi zihinlere tamiri zor ön yargılar ek-

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younger generation, and is a new sociological problem worth investigating.

Keywords: Smartphone addiction, social media, anxiety, depression, social appearance anxiety

leyebilir. Bu durum özellikle de genç nesil için bir tehdit olarak görülmelidir ve araştırılmaya değer yeni bir sosyolojik sorundur.

Anahtar Kelimeler: Akıllı telefon bağımlılığı, sosyal medya, anksiyete, depresyon, sosyal görünüş kaygısı

INTRODUCTION

In addition to facilitating communication, smartphones have become an important part of our lives in terms of having fun and making daily life easier. With the convenience provided by advancing smartphone technology, people can spend time on social media, manage their e-mail, use academic search databases, find answers to their questions easily via the internet, meet almost all their needs with online shopping, and access various entertainment tools through their smartphones. Although this versatile use of smartphones facilitates the life of human beings, it may negatively affect functionality in daily activities and relationships by predisposing individuals to become dependent on their smartphones, and increased use of smartphones is associated with various physical and psychiatric problems (1, 2). Smartphone addiction, which can be so severe as to make it difficult for the user to meet his or her own needs, can have a permanent negative impact. Excessive phone use, inability to keep away from the phone, and frequent control of the phone can be observed in smartphone addicts[3]. In addition to these, the results of various studies show that excessive use of smartphones and addiction to their content is related to anxiety disorder and depression (3, 4). According to data from the Turkey Statistical Institute, the percentage of a smartphone or mobile phone ownership grew from 53.7% in 2004 to 96.9% in 2016 (2, 5). The group with the most widespread use of smartphones is generation Y, born between 1977-1994, and generation Z, born in 1995 and later (6). In particular, generation Z is the generation most vulnerable to smartphone addiction, as it has grown up with today's technology and the internet age.

Body perception consists of physical appearance, health status, physical skills, and sexual attitude, and is formed by the individuals' feelings, thoughts, and intuitions about their own body (7, 8). The anxiety and stress that individuals feel when their physical appearance is being evaluated by others is called social appearance anxiety and is closely related to depression and anxiety (8, 9). It should be noted that social media applications, which have become easier to reach with the widespread use of smartphones, may also change body perception by causing individuals' dissatisfaction about their appearance. In addition, it should be noted that some mobile applications that enable changes in appearance might impair body perception over time. The given risk increases, especially when the physical appearance becomes easily criticized by anonymous identities as a result of the easy integration of mass media into daily life with technologies such as smartphones (10). This study's aim was to determine the frequency of smartphone addiction in the young adult population, in which smartphone use is the most common, and examine the relationship between smartphone use and depression, anxiety, and social appearance anxiety.

MATERIALS AND METHODS

The study, which was planned in a cross-sectional-descriptive design, was carried out for one month in August 2019 with the approval of the Çanakkale Onsekiz Mart University Faculty of Medicine Clinical Research Ethics Committee (Date:24.07.2019, No: 14), with young adults who applied to the Family Medicine outpatient clinic of a tertiary hospital, met the appropriate participation conditions, and gave consent. Sociodemographic data was obtained. Questions involving smartphone usage features prepared by practitioners, the Smartphone Addiction Scale-Short Form, the Beck Depression Inventory-Short Form for Primary Care, and the Beck Anxiety Scale and Social Appearance Anxiety Scale were administered face to face to 259 people between the ages of 18 and 45 who gave their consent, had a smartphone, and did not have any diagnosis that would hinder their assessment of reality (5, 8, 11, 12). The participants' smartphone usage duration was recorded objectively using the pre-installed measurement application on their phones. Smartphone users without this feature were not included in the study.

The Smartphone Addiction Scale-Short form is a 10-item scale using a 6-point Likert scale developed by Kwon et al. to measure the risk of smartphone addiction in young adults. The items of the scale were scored from 1 to 6. The total range of total score is 10-60. The increase in the score obtained from the test is evaluated in direct proportion as the risk of addiction increases. This is a single-factor scale without subscales. In the Korean sample, the cut-off score was determined as 31 for male and 33 for female. The Cronbach alpha coefficient of the original form's internal consistency and concurrent validity is 0.91 (13). The Turkish validity and reliability study of the scale was conducted by Noyan et al. and Cronbach's alpha coefficient of the scale was 0.867 and had a 0.926 reliability coefficient (5).

The Beck Depression Inventory for Primary Care (BDI-PC) performs depression screening under seven items using the symptoms of sadness, pessimism, past failure, self-dislike, self-criticalness, loss of interest, and suicidal thoughts or wishes. Each heading includes a four-digit rating from 0 to 3. The BDI-PC score is obtained by adding the highest scores in each item. Although no cut-off score is reported, the probability of depression in scores above 4 is evaluated as over 90% (11).

The Beck Anxiety Scale aims to determine the frequency and severity of anxiety symptoms experienced by individuals. The highest score that can be obtained from the scale, which consists of 21 items, is 63. Ulusoy et al., who completed the scale's Turkish validity and reliability study, identified the Cronbach alpha internal consistency as 0.93. In the scale scoring, 0-7 points are evaluated as no anxiety, 8-15 points as mild anxiety, 16-25 points as moderate anxiety, and 26-63 points as severe anxiety (12).

The Social Appearance Anxiety Scale was developed to measure the emotional, behavioral, and cognitive anxieties of individuals regarding their appearance based on self-reported answers. The scale has 16 Likert-type questions and was developed in 2008 by Hart et al. (14). The validity, reliability, and adaptation study of the scale to Turkish was carried out by Tayfun Dogan in 2010 (8). The internal consistency coefficient of the scale is 0.93 and the reliability coefficient is 0.88. The minimum score of the scale is 16 and the maximum score is 80. Higher scores indicate a higher level of social appearance anxiety (8).

After the interviews with the participants were completed, the research data was digitized. Frequency and percentage values were calculated for categorical variables, and mean and standard deviation values were calculated for continuous variables. Analyses were performed using Chi-square, Student t-test, ANOVA, Kruskal Wallis, Pearson, and Spearman correlation tests following data properties. Since the sample size was larger than 30, the normal distribution assumption was neglected in parametric tests based on the central limit theorem (15). $p<0.05$ was accepted as the limit of significance for all analyses and absolute p values were given for each analysis.

RESULTS

A total of 259 people (128 (49.4%) female, 131 (50.6%) male) were included in the study. The mean age of the participants was 30.7±7.0 [18-42]. While 55 (21.2%) individuals belong to generation Z, 204 (78.8%) were part of generation Y. 104 participants (40.2%) were single, 140 (54.1%) were married, and 15 (5.8%) were widowed. Regarding their educational status, 44 (17.0%) were primary school graduates, 61 (23.6%) were high school graduates, 119 (45.9%) were university graduates, and 35 (13.5%) had master's degrees and doctoral degrees.

Sixty-seven (25.9%) of the participants were smartphone addicts. Forty-two (62.7%) of the addicts were female and 25 (37.3%) were male; there was a significant difference between the genders ($\chi^2=6.363$; $p=0.01$). There was a significant negative correlation between smartphone addiction total scores and age ($r=-0.247$; $p<0.001$). The smartphone addiction rate in generation Z (45.5%) was significantly higher than that of generation Y (20.6%) ($\chi^2=13.968$; $p<0.001$). There was a statistically significant difference between marital status and smartphone addiction ($\chi^2=8.855$; $p=0.012$): 37 (35.6%) of the single participants, 28 of the married participants (20.0%), and 2 (13.3%) of the widowed participants were smartphone addicts. Eight (18.2%) of the primary school graduates, 13 (21.3%) of the high school graduates, 41 (34.5%) of the university graduates, and 5 (14.3%) of the master-doctoral graduates were smartphone-addicted, and there was a significant difference between the groups ($\chi^2=9.039$; $p=0.029$).

Of the participants, 211 (82.4%) reported using their smartphone to access social media, 95 (37.1%) to play video games, 181 (70.7%) to surf the web, 119 (46.5%) to do shopping-related activities, and 52 (20.4%) to meet new people. Smartphone addiction was higher in those who used their phone to access social media content, play video games, surf the web, do shopping-related activities, and meet new people ($\chi^2=16.207$; $p<0.001$, $\chi^2=14.949$; $p<0.001$, $\chi^2=13.198$; $p<0.001$, $\chi^2=15.601$; $p<0.001$ and $\chi^2=16.773$; $p<0.001$, respectively).

The mean screen time of the participants was 189.6±124.2 [30-960] minutes per day. Mean screen time (271.6±134.3 minutes per day) was significantly higher in patients with smartphone addiction than those without addiction (155.4±102.3 minutes per day) ($t=-6.654$; $p<0.001$). There was a high positive correlation between smartphone use time and smartphone addiction score ($r=0.512$; $p<0.001$). There was a significant negative correlation between age and the duration of smartphone use ($r=-0.324$; $p<0.001$). The daily smartphone use time of generation Z (251.5±129.9 minutes) was significantly higher than generation Y (172.8±117.5 minutes) ($t=3.594$; $p<0.001$). The mean screen time of females was 201.5±115.2 minutes, while the mean of males was 178.8±131.7 minutes ($t=1.293$; $p=0.198$). Smartphone use was found to be 221.0±134.1 minutes per day for singles, 163.4±15.2 minutes for married people, and 193.2±81.8 minutes for widows ($F=5.175$; $p=0.006$) (Table 1).

Sixty-five (35.7%) of the 253 participants who answered the BDI-PC had depressive symptoms above the threshold value. There was no significant difference between the genders ($\chi^2=0.294$; $p=0.587$). There was no significant difference between age and BDI-PC scores ($t=0.571$;

Table 1: The relationship between the participants' average smartphone usage duration and their sociodemographic features

Intended use of smartphone		Average smartphone usage duration (min/day)	Statistical analysis
Gender	Woman	201.5±115.2	t=1.293; p=0.198
	Man	178.8±131.7	
Generation	Y	172.8±117.5	t=-3.594; p<0.001
	Z	251.5±129.9	
Marital status	Single	221.1±134.1	F=5.175; p=0.006
	Married	163.4±115.2	
	Widowed	193.2±81.8	
Educational status	Primary school graduate	134.0±106.8	χ^2=23.239; p<0.001
	High school graduate	180.2±107.8	
	University graduate	227.9±142.7	
	Master-doctoral graduate	151.4±64.0	

p=0.569). Similarly, no statistically significant difference was found between marital status and educational status and the level of depressive symptoms (χ^2=2.633; p=0.268 and χ^2=6.429; p=0.093, respectively). The evaluation of Beck Anxiety Scale scores showed that 130 of the participants (50.2%) had no anxiety, 47 (18.1%) had mild anxiety, 45 (17.4%) had moderate anxiety, and 37 (14.3%) had severe anxiety. Anxiety was present in 82 (64.0%) of the female and 47 (35.9%) of the male (χ^2=29.056; p<0.001) participants. While there was no significant difference between marital status and anxiety scale results (χ^2=2.521; p=0.866), there was a significant negative correlation between educational status and anxiety (r=-0.140; p=0.024). The average screen usage time and statistical analysis of the participants classified according to the Smartphone Addiction Scale-Short form, BDI-PC, and Beck Anxiety Scale are shown in Table 2.

The mean social appearance anxiety score of the participants in the study was 30.4±11.8. While the mean score of females was 33.1±13.0, the mean score of males was 27.7±9.9 (t=3.797; p<0.001). While there is a significant negative correlation between social appearance anxiety scale total score and age (r=-0.094; p=0.023), there was no statistically significant difference between generations (t=0.928; p=0.356). The mean score of singles was 33.0±12.2, the mean score of married participants was 28.6±11.5, and the mean score of widows was 29.3±9.0, and this score was higher in singles than in married participants and widows (F=4.138; p=0.017). As the education level increased, the total scores of the social appearance anxiety scale increased significantly (r=0.148; p=0.018).

The social media users' mean social appearance anxiety score was 30.8±13.9, which was significantly higher

Table 2: The relationship between the participants' average smartphone usage duration and Smartphone Addiction, BDI-PC, and Beck Anxiety Scales

Scales		Average smartphone usage duration (min/day)	Statistical analysis
Smartphone addiction scale	Non addicts	271.6±134.3	t=-6.654; p<0.001
	Addicts	155.4±102.3	
Beck depression inventory for primary care	Non depressive	184.9±119.7	t=-0.738; p=0.435
	Depressive	201.2±201.4	
Beck anxiety scale	No anxiety	180.0±12.0	χ^2=1.294; p=0.005
	Mild anxiety	154.8±19.2	
	Moderate anxiety	214.2±23.6	
	Severe anxiety	237.4±21.7	

Table 3: The relationship between the participants' smartphone use purposes and social appearance anxiety scale mean scores

Intended use of smartphone		Social appearance anxiety score average	Statistical analysis
Social media	Users	30.8±13.9	t=-1.057; p=0.038
	Nonusers	28.8±11.3	
Gaming	Users	31.5±12.7	t=-1.046; p=0.297
	Nonusers	29.9±11.3	
Internet surfing	Users	31.5±12.3	t=-2.134; p=0.034
	Nonusers	28.0±10.4	
Shopping	Users	32.4±12.0	t=-2.450; p=0.015
	Nonusers	28.8±11.5	
To meet new people	Users	32.0±11.4	t=-1.058; p=0.291
	Nonusers	30.1±12.0	

than the mean score of 28.8±11.3 of those who did not use social media (t=-1.057; p=0.038). Similarly, the social appearance anxiety scale scores of those who use their smartphones for surfing the web and shopping were significantly higher than those who did not use smartphones for these purposes (t=-2.134; p=0.034 and t=-2.450; p=0.015). There was no statistically significant difference between the total mean score of the social appearance anxiety scale among those who used their smartphones to play video games and meet new people and the total mean scores of those who did not use smartphones for this purpose (t=-1.046; p=0.297 and t=-1.058; p=0.291) (Table 3).

While no significant correlation was observed between the smartphone addiction scale and depression scale (r=0.119; p=0.059), a statistically significant positive correlation was found between the anxiety scale and social appearance anxiety (r=0.203; p=0.001 and r=0.335; p<0.001 respectively).

DISCUSSION

The development of smartphones and their ensuing global popularity has changed the communication and information environment and had an impact on sociality, entertainment, access to information, and creating social identity. This situation has been accompanied by concerns about overuse and addiction. Smartphone addiction causes people to see their phones as a body part, leading to panic and stress in the absence of their smartphone. Smartphone addiction is a worldwide phenomenon that occurs in 9.3% to 48% of the population (16-18). In our study population, the percentage of smartphone addiction was found to be 25.9%, and this rate is quite high. This high rate may be related to the fact that smartphone addiction is more common at a young age and that the age of the people included in our study is younger than the general population and therefore more easily adaptable to innovations. Increasing frequency of smartphone use and screen time is also closely related to the severity of smartphone addiction (19). Our study supports this data in that a statistically significant positive correlation was observed between the duration of smartphone use and the smartphone addiction score.

Female participants were found to have a higher rate of smartphone addiction compared to males; similarly, female participants' mean duration of smartphone usage was higher than males. Different results have been obtained in terms of gender in different studies on this subject. While some studies have concluded that smartphone addiction is more common in males, the frequency is greater in the female gender in different studies (13, 20, 21). More studies are needed on the prevalence of smartphone addiction between the genders.

In our study, there was a negative correlation between smartphone addiction and age. One study found that younger age groups are more addicted to smartphones (22). Similarly, the mean duration of smartphone use was negatively correlated with age and decreased with age. This shows that young people integrate smartphones into their lives, and the risk of smartphone addiction increases as age decreases.

There was a significant difference between the marital status of the participants and their smartphone addiction. In our study, the highest rate of smartphone addiction and the highest mean duration of smartphone usage were observed in singles. This situation may be related to the relationship between age and marital status, and it suggests that marital status has a minimal effect on smartphone screen time and addiction.

A significant positive correlation between depression and anxiety and smart device addiction has been shown in the study of a mixed sample of 274 adults aged 16-59 years, which was applied in the general adult population to define the relationship between depression and anxiety and smartphone addiction (23). Similarly, a systematic review in 2019 on problematic smartphone use emphasized both the direction and possible explanations of the relationship between psychopathology and problematic smartphone use (1). Although many studies have shown a relationship between smartphone use and depression and anxiety, no significant relationship was found with depression in our study. This can be explained by the cultural differences and the characteristics of the sample group. Our results reveal the idea that general smartphone use has become an ordinary part of life. The purpose of using smartphones affects people, rather than smartphone use or addiction. There is a strong relationship between addiction and anxiety, and it is known that anxiety disorder contributes to the increment in addiction (24, 25). In our study, similar results were obtained. However, current data is insufficient to determine whether problematic use increases anxiety or whether existing anxiety triggers smartphone use in the direction of addiction.

In comparing participants' reasons for smartphone use and social appearance anxiety, the mean social appearance anxiety scores were higher, especially among social media users and individuals doing online shopping, than those who used smartphones for other purposes. Social appearance anxiety is positively related to social anxiety criteria and negative body image (26). Similar to the finding in our study, extensive research on women's body image emphasized that young women are less satisfied with their bodies and want to look slimmer and that they are in a mood called "normative discontentment" (27, 28). Given that the idealized female body is almost unattainable, the vast majority of women are likely to experience appearance anxiety to some extent. Similarly, as the ideal appearance depicted in the media becomes physically uniform and unreachable, physical dissatisfaction might rise in men (29). It is an important problem that the posts, which are prepared in an unrealistic way using make-up and digital interventions, are published on social media channels with the expectation that they will be liked. Users circulate these posts by sharing them, without questioning whether the images are natural or modified. While those shared on smartphones and social media can facilitate social comparison by providing the opportunity to learn about the looks and experiences of others, this can also increase social appearance anxiety.

CONCLUSION

Increased use of smartphones and social media has enabled people to create a new identity in the virtual world and interact with other users. Given the positive relationship between psychological health and quality of life, it should be kept in mind that there is a potential risk of long-term negative effects of social networks. Abuse of mobile applications, especially those associated with sharing looks and lifestyle, may lead to prejudices that are hard to overcome. This poses a threat particularly for the younger generation and emerges as a new sociological problem worth investigating. This study documented the possible positive relationship between smartphone addiction and social appearance anxiety, and both conditions need to be elaborated on in future studies.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Çanakkale Onsekiz Mart University (COMU) (Date: 24.07.2019 No: 14).

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UNNOTICED ETIOLOGY IN ORTHOPEDIC COMPLAINTS: CHRONIC MOUNTAIN SICKNESS

ORTOPEDİK ŞİKAYETLERDE FARK EDİLMEYEN ETİYOLOJİ: KRONİK DAĞ HASTALIĞI

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ABSTRACT

Objective: Chronic mountain sickness (CMS) is a clinical syndrome with symptoms of polycythemia that may interfere with other nonspecific diseases. The current study aimed to investigate the symptoms of patients living at high altitudes, who initially presented complaints that seemed to be common orthopedic problems, and to examine their relationship with chronic mountain sickness.

Materials and Methods: The prospectively collected data of 104 patients were retrospectively evaluated for serum hemoglobin (Hb) and hematocrit (Hct) levels, oxygen saturation (sO_2), Qinghai CMS questionnaire score, alcohol and tobacco use, "any history of acute mountain sickness, body mass index (BMI), blood pressure, heart rate, and duration of high-altitude living. Patients grouped according to the Qinghai score as healthy, mild, moderate or severe CMS. The groups were investigated in terms of parameters and demographic characteristics.

Results: Of the 104 patients, 33 (31.7%) had a mild CMS score ≥6 (28 patients, 6–10, 5 patients, 11–14 points); remaining patients had no CMS. The frequency of excessive erythrocytosis was 4.5% in men and 3.3% in women. There was a significant difference in Hb, Hct and sO_2 levels between the healthy and mild CMS groups ($p<0.001$). CMS score had a positive correlation with Hct (rho 0.381, $p<0.001$) and negative correlation between sO_2 levels (rho -0.432, $p<0.001$).

Conclusions: CMS can be observed at lower altitudes than described in literature. In individuals living in high altitudes, some of the CMS symptoms may be confused with real orthopedic symptoms and orthopedists working in these areas should increase their awareness on this issue. In order to make an appropriate approach, other symptoms related to CMS should be questioned in patients living at high altitudes.

Keywords: Chronic mountain sickness, orthopedics symptoms, muscle joint pain, hemoglobin, oxygen saturation

ÖZET

Amaç: Kronik dağ hastalığı (KDH), diğer spesifik olmayan hastalıklarla da karışabilen polisitemi semptomları olan klinik bir sendromdur. Bu çalışmada, yüksek rakımlarda yaşayan, başlangıçta sık görülen ortopedik problemler gibi görünen şikayetlerle hastaneye başvuran hastaların semptomlarını araştırmayı ve kronik dağ hastalığı ile ilişkilerini incelemeyi amaçladık.

Gereç ve Yöntemler: Prospektif olarak toplanan 104 hastanın verileri retrospektif olarak incelendi. Hastaların serum hemoglobin (Hb) ve hematokrit (Hct) seviyeleri, oksijen satürasyonu (sO_2), Qinghai KDH anket skoru, alkol ve tütün kullanımı, herhangi bir akut dağ hastalığı öyküsü, vücut kitle indeksi (BMI), kan basıncı, kalp atış hızı ve yüksek rakımda yaşam süresi kaydedildi. Hastalar; Qinghai skoruna göre sağlıklı, hafif, orta veya şiddetli KDH olarak gruplandırıldı. Gruplar parametreler ve demografik özellikler açısından incelendi.

Bulgular: Yüz dört hastadan 33'ünün (%31,7) ≥6 hafif KDH skoru vardı (28 hasta, 6-10, 5 hasta, 11-14 puan). Diğer hastalarda CMS saptanmadı. Aşırı eritrositoz sıklığı erkeklerde %4,5, kadınlarda %3,3 idi. Sağlıklı ve hafif CMS grupları arasında Hb, Hct ve sO_2 düzeyleri açısından anlamlı fark saptandı ($p<0,001$). KDH skorunun Hct (rho 0,381, $p<0,001$) ile pozitif, sO_2 seviyeleri arasında negatif korelasyon (rho -0,432, $p<0,001$) saptandı.

Sonuç: Kronik dağ hastalığı, literatürde tarif edilenden daha düşük rakımlarda gözlemlenebilir. Yüksek yerlerde yaşayan bireylerde CMS semptomlarının bazılarının gerçek ortopedik semptomlarla karışabileceğinden ve bu bölgelerde çalışan ortopedistler bu konudaki farkındalıklarını arttırmalıdırlar. Bireysel semptomlar diğer hastalıkların bulguları ile kesişebilir ve özellikle benzer yaygın semptomları olan hastalıklarda KDH gözden kaçabilir. Uygun bir yaklaşımın yapılabilmesi için yüksek rakımlarda yaşayan hastalarda KDH ile ilgili diğer semptomların da sorgulanması gerekir.

Anahtar Kelimeler: Kronik dağ hastalığı, ortopedik semptomlar, kas-eklem ağrısı, hemoglobin, oksijen satürasyonu

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INTRODUCTION

Chronic mountain sickness (CMS) was first described by Carlos Monge in 1928 as a disease presenting diverse symptoms (1, 2). According to the International Consensus on Chronic and Sub-Acute High-Altitude Diseases, CMS is a clinical syndrome characterized by excessive erythrocytosis (EE) and severe hypoxemia observed in people living at high altitudes (>2500 m) (3). Chronic hypoxia results in pulmonary hypertension and polycythemia, accompanied by headache, fatigue, dizziness, dyspnea, sleep disturbances, and bone and muscle pain (4).

CMS is a weakening and debilitating disease that may occasionally interfere with other nonspecific diseases (5). As the population and life at high altitudes increase, the prevalence of related diseases will also increase. For these reasons, appropriate methods for diagnosis, treatment, and care are needed for people at risk of developing CMS (6). However, similar symptoms with other etiologies hamper its clinical recognition. A wide variety of neurological, cardiological, vestibular, or orthopedic symptoms can be misleading if incompletely evaluated or underestimated. Regarding the general complaints that can be met orthopedically, bone and joint pain, muscle aches, and burning in the hands and feet are the main musculoskeletal system problems observed in CMS (6-9).

Since the underlying etiological cause of CMS is different, a detailed analysis of its symptoms and application of the current CMS diagnosis algorithm will increase the success rate of diagnosis and treatment, especially in areas where CMS is a possibility. Various end-organ involvement of the symptoms and their relationship with the disease have been examined in previous publications (10-12). However, there are no studies in literature about the incidence of common orthopedic symptoms.

This study aims to investigate individuals who live at high altitudes with CMS risk and evaluate the role of CMS as an etiology in patients presenting general orthopedic complaints.

MATERIALS AND METHODS

This is an institutional review board-approved study, and informed consent was obtained from all patients included in the study. The patient group comprised individuals living in settlements located at an altitude of 2400–2600 meters in the Sarikamis district of Kars province in Turkey. The data of 217 patients, who visited the orthopedic department between November 2015 and January 2017, were retrospectively analyzed. Inclusion criteria were musculoskeletal pain, joint pain, or a burning sensation of the palms and feet. Symptoms were not associated with any disease findings based on the physical examination or laboratory and radiological tests. The exclusion criteria included age <18 years and >65 years old, chronic interstitial lung disease, chronic heart disease, pregnancy, menopause, history of lung surgery, history of nose surgery, and living in high-altitude areas for less than two months. CMS is a clinical syndrome characterized by excessive erythrocytosis (EE) and severe hypoxemia and is seen in people living at high altitudes (>2500 m). It is diagnosed using the Qinghai score in the absence of any condition that may cause hypoxemia and EE (5). The variables analyzed included serum hemoglobin (Hb) and hematocrit (Hct) levels, the Qinghai CMS questionnaire score, oxygen saturation (sO_2), alcohol and tobacco use, any history of acute mountain sickness, body mass index (BMI), blood pressure, heart rate, and the duration of living at high altitudes. CMS was diagnosed based on the Qinghai CMS score that includes Hb levels and seven signs and symptoms (breathlessness/palpitations, sleep disturbances, cyanosis, vein dilatation, paresthesia, headache, and tinnitus) (Table 1). EE was diagnosed using the parameters of the Qinghai score (both EE symptoms and Hb concentration level). The high cut-off value for serum Hb was 21 g/dL in men and 19 g/dL in women (3). If the Hb level was equal to or higher than the cut-off value, the Hb score was scored as 3 points; if the level was below the cut-off value, it was scored as 0 points. Each sign or symptom was scored between 0 and 3 points according to its severity. Arterial blood pressure was measured with a left-sided digital blood pressure monitor after the patient had rested for 30 minutes (13). Heart rate and oxygen saturation were measured with a digital pulse oximeter (Contec med CMS50D digital pulse oximeter, China) placed on the right middle finger (14). Patients who smoked more than 100 cigarettes in the past 30 days were categorized as smokers (15), and those who consumed ≥20 g/day of pure alcohol were categorized as drinkers (16). Patients were grouped according to the Qinghai score. Individuals with a Qinghai CMS score of 0–5 points were considered healthy regarding CMS. In patients with Hb levels ≥ the cut-off value, a Qinghai score of 6–10 points was defined as mild CMS, 11–14 points as moderate CMS, and ≥15 points as severe CMS (17). The groups were investigated regarding the parameters and demographic characteristics, and these parameters were correlated with disease severity (Figure 1).

Statistical methods

The distribution of variables was measured using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to analyze independent quantitative data. The Chi-square test was used to analyze independent qualitative data, and Fischer's exact test was used when Chi-square test conditions were not met. The Spearman correlation analysis was used for correlation analysis. SPSS software (version 22.0, IBM Corp. Released 2013. Armonk, NY, USA) was used for the analyses. A p-value of <0.05 was considered significant.

Table 1: The Qinghai Score for CMS

Signs or symptoms	Score
Breathlessness and/or palpitations	0 No breathlessness/palpitations
	1 Mild breathlessness/ palpitations
	2 Moderate breathlessness/ palpitations
	3 Severe breathlessness/ palpitations
Sleep disturbance	0 Slept as well as usual
	1 Did not sleep as well as usual
	2 Woke up many times, poor night's sleep
	3 Could not sleep at all
Cyanosis	0 No cyanosis
	1 Mild cyanosis
	2 Moderate cyanosis
	3 Severe cyanosis
Dilatation of veins	0 No dilatation of veins
	1 Mild dilatation of veins
	2 Moderate dilatation of veins
	3 Severe dilatation of veins
Paresthesia	0 No paresthesia
	1 Mild paresthesia
	2 Moderate paresthesia
	3 Severe paresthesia
Headache	0 No headache
	1 Mild headache symptoms
	2 Moderate headache
	3 Severe headache, incapacitating
Tinnitus	0 No tinnitus
	1 Mild tinnitus
	2 Moderate tinnitus
	3 Severe tinnitus
Hemoglobin concentration	Men: <21 g/dL; score = 0 ≥21 g/dL; score = 3 Women: <19 g/dL; score = 0 ≥19 g/dL; score = 3
Assessment of CMS Severity with Total Qinghai Score	
Total score	CMS
0-5	Absent
6–10	Mild
11-14	Moderate
>15	Severe

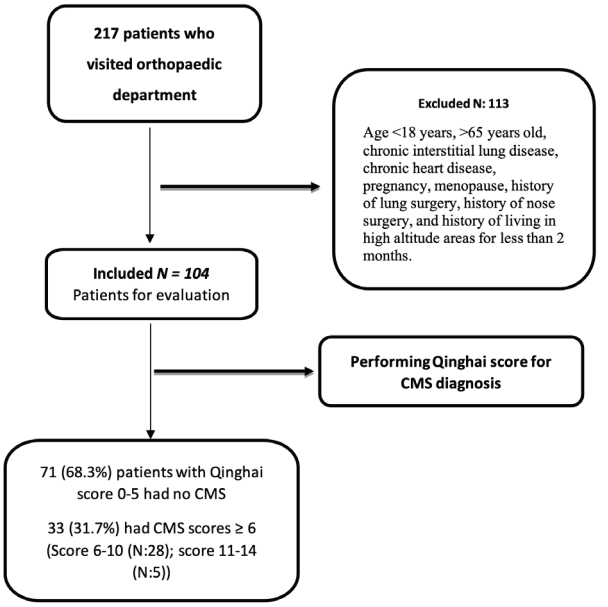

Figure 1: Flow chart of the study

RESULTS

One hundred and four patients met the inclusion criteria. Sixty were females (57.7%), and 44 were males (42.3%). The mean age of the cohort was 42.4±12.9 years old. The mean duration in the high-altitude was 38.1±17.2 months between 3 and 65 months. The mean CMS score was 4.6±2.9 (range, 1–14). The demographics, laboratory results, evaluated cardiac and respiratory findings, and Qinghai scores of the patients in the study group are given in Table 2.

The healthy group comprised 71 individuals, 35 were females (49.3%), and 36 were males (50.7%), and their Qinghai CMS scores were between 0 and 5 points. The mean Hb and Hct were 16.4±12.6 mg/dl and 44.4±5.2% in the healthy group, respectively. The mean sO_2 was 95.3±2.1, and the median CMS score was 3±1.3. The mean Hct was 47% in male patients, and 41.4% in female patients (p=0.873).

The remaining 33 (31.7%) patients in the cohort had CMS scores of ≥6. Twenty-eight patients' scores were between 6 and 10 and were diagnosed as mild CMS, and five patients' scores were between 11 and 14 and were diagnosed as moderate CMS. Among them, 25 were females (75.8%), and eight were males. The mean Hb and Hct were 16.9±2.2 mg/dl and 49.7±6.1% in the mild CMS group. The mean sO_2 was 93.1±2, and the median CMS score was 8.1±2. In the mild CMS group, mean Hb and Hct were 16.7 mg/dl and 46.7% in female, 19.5 mg/dl and 53.7% in male patients, respectively (p=0.218).

The Hb levels of only two male and two female patients were above the cut-off value for the diagnosis of EE. The

Table 2: The demographics, laboratory results, evaluated cardiac and respiratory findings, and Qinghai scores of the cohort

	n	%	Min-Max	Median	Mean±S.D
Age			18-65	45	42.4±12.9
Gender					
Female	60	57.7			
Male	44	42.3			
BMI			19.1-36.3	24.8	25.4±3.9
Alcohol	1	1			
Tobacco	20	19.2			
Time at high altitude[a]			3-65	41	38.1±17.2
Hemoglobin[b]			10.8-21.3	15.2	16.5±10.4
Hematocrit[c]			31.1-60	45	46.1±6
Systolic blood pressure[d]			90-170	130	126.4±18.4
Diastolic blood pressure[d]			50-100	70	74±12
Oxygen saturation[c]			88-99	95	94.6±2.4
Heart Rate[e]			56-124	72	76±12.3
Qinghai CMS score			1-14	4	4.6±2.9

[a]: month, [b]: mg/dl, [c]: %, [d]: mmHg, [e]: beat per minute
BMI: Body Mass Index, SD: standard deviation

frequency of EE was 4.5% in male and 3.3% in female patients (p=0.285).

The distribution of genders within groups was statistically different (p=0.011). The mean Hb, Hct, and sO_2 levels were significantly higher in the group with mild CMS than in the healthy group (p<0.01). There was no statistically significant difference between the groups with CMS scores of <5 and ≥6 regarding BMI, alcohol and tobacco use, the duration of living at high altitudes, systolic and diastolic blood pressure, and heart rate. The comparison of the groups is provided in Table 3.

Table 3: Statistical analysis of parameters between healthy and mild Chronic Mountain Sickness (CMS) groups

Parameters	CMS score ≤5			CMS score >6			p value
	n	%	Mean±SD (min-max)	n	%	Mean±SD (min-max)	
Age			41.1±14 **(18-65)**			45±9.7 **(20-61)**	0.148*
Gender							**0.011¥**
Female	35	49.3		25	75.8		
Male	36	50.7		8	24.2		
BMI			25.3±3.9 **(19.1-36.3)**			25.7±4 **(19.5-34.1)**	0.701*
Time at high altitude[a]			36.9±18.4 **(18-65)**			40.9±14.3 **(1-61)**	0.287*
Hemoglobin[b]			16.4±12.6 **(11-21)**			16.9±2.2 **(13-21)**	**0.000***
Hematocrit[c]			44.4±5.2 **(31-60)**			49.7±6.1 **(38-60)**	**0.000***
Systolic blood pressure[d]			125±20 **(90-170)**			129.2±13 **(100-150)**	0.310*
Diastolic blood pressure[d]			73.9±12.5 **(38-100)**			74.4±11.1 **(60-100)**	0.975*
Oxygen saturation[c]			95.3±2.1 **(88-99)**			93.1±2.3 **(88-97)**	**0.000***
Heart Rate[e]			76.6±12.4 **(60-124)**			74.8±12.2 **(56-107)**	0.545*
Qinghai CMS score			3±1.3			8.1±2	

¥: Chi-square test, *: Mann-Whitney U test, [a]: month, [b]: mg/dl, [c]: %, [d]: mmHg, [e]: beat per minute
BMI: Body Mass Index, SD: standard deviation

The correlation was found to be weakly positive between Hct and CMS scores (rho 0.381, p<0.001) and weakly negative between sO_2 values and CMS scores (rho −0.432, p<0.001). There was no significant correlation between the CMS score and the mean Hb level (rho −0.012, p=0.907).

DISCUSSION

The current study is the first to evaluate CMS in patients with common orthopedic complaints who live in a high-altitude province. Based on Qinghai CMS scores and laboratory findings, in residents living at around 2500 m altitude, 33 of 104 patients (32%) with common orthopedic symptoms had mild CMS. In addition to the disease being prevalent in women, a weak correlation was found between hematocrit, oxygen saturation, and disease severity.

Symptoms observed in CMS are headache, difficulty breathing, sleep disturbances, fatigue, palpitations, localized cyanosis, especially of the hands and lips, a burning sensation in the palms and feet, muscle and joint pain, loss of appetite, dizziness, attention deficit, and mental fatigue (18). Similar to the other symptoms mentioned, orthopedic symptoms are common complaints. Muscle and joint pain and burning in the hands and feet are common symptoms in orthopedic clinics and can be associated with different pathologies. Extremity problems and joint symptoms related to polycythemia and burning feet with erythromelalgia have been reported previously in different diseases (19, 20). In polycythemia, joint problems are generally caused by hyperuricemia and the development of synovitis in the joints with crystal deposition over time, while drug-induced peripheral neuropathy or hypoxia due to hyperviscosity is the reason for hand-foot burning (19-21). Orthopedic symptoms can be seen in CMS based on similar pathophysiology. However, when the disease is not known exactly, they can be unnoted. Its clinical findings and associated symptoms can be used to distinguish CMS from other etiologies. Most prominently, some or all of these symptoms should be present in patients at high altitudes. The clinical picture becomes less severe at lower altitudes and sometimes disappears, only to reappear at higher altitudes (6). In humans with better adaptation to chronic hypoxia, lower hemoglobin (Hb) values lead to a lower incidence of erythrocytosis and CMS. In contrast, EE and elevated Hb levels lead to pronounced clinical symptoms (17).

The definition and diagnostic criteria of CMS are debatable. One essential point that is not agreed upon in CMS is the Hb threshold value. The increase in Hb level is an adaptive mechanism following prolonged exposure to chronic hypoxia and is dependent on the severity of hypoxia and the altitude. For this reason, different Hb thresholds have been used according to the different altitude levels in studies related to CMS. The cut-off value of Hb was determined to be 21 g/dL for men and 19 g/dL for women by the World Congress on Mountain Medicine and High-Altitude Physiology (3). In past publications, the cut-off value of Hb was reported to be 2, 3, or 4 standard deviations above the mean level in the affected area (22). Jiang and colleagues reported that an Hb cut-off value of 20 g/dL, rather than 21 g/dL, was more appropriate for all altitudes. Because of these controversial points, some researchers suggest that the use of laboratory parameters, such as sO_2, carbon dioxide retention, and the Hb level, may be more appropriate for CMS scoring (23). We found the mean Hb value of 17.4 g/dL in the group with CMS and a significant correlation between mean Hct levels and CMS scores. In general, the low Hb and Hct compared with similar studies may be due to ethnic differences or lower altitudes.

The prevalence of CMS varies mainly according to the altitude with other affecting parameters like age, sex, altitude, the duration spent at high altitudes, smoking and alcohol use, chronic diseases, population, and ethnic characteristics (22). The most populated area at high-altitude is the Tibetan plateau. In a study, the prevalence of CMS in the Tibetan plateau was 17.8%, which is higher than that of previous studies (18). Spielvogel et al. found a prevalence of 5.2% in the La Paz area (3600 m) (24). De Ferrari and colleagues reported a prevalence of 6% in the Puno area (3800 m) (25). Monge and colleagues observed a prevalence of 15.4% in men aged 30–39 years in Cerro de Pasco (4380 m) (26). Although the prevalence of CMS varies in the range of 5% to 10% above 2500 m (5), the actual prevalence below 3000 m is unknown. In a study with a limited number of participants, the prevalence below 3000 m was 0%. However, there was a serious methodological limitation because Hb and blood values were not examined in the study (27). In addition, there is a physiological change at altitudes above 2500 m. At an altitude of 2500 to 3000 meters, the prevalence of acute mountain disease is from 8% to 25% (28-30). In our study, patients who applied to the hospital at an altitude of 2500 meters were included. The high rate of mild CMS (32%) found in our study was beyond the prevalence of the disease at that altitude. It showed the disease incidence in patients presenting with similar orthopedic complaints. The true prevalence can be determined by screening the asymptomatic population and symptomatic patients.

Chronic hypoxia and the resulting polycythemia play essential roles in the pathophysiology of CMS (31). Thus, CMS can be divided into two groups: primary CMS and secondary CMS. Primary CMS is caused by aggravated hypoxemia and increased erythrocyte concentration without an underlying cause. Secondary CMS occurs due to hypoxia caused by lung or heart disease (10). Therefore, in our study, to rule out secondary CMS, patients

with known heart or lung problems or associated illnesses were not included. Living at high altitudes for a long time and older age are also other important risk factors for the disease. Residing at high altitudes for longer than 60 months increases the CMS risk (5). In our study, CMS and healthy groups had a mean duration of 45 months and 37 months at high altitudes, respectively. The duration of the two groups reduced the risk-creating effect of time. Also, with the exclusion of patients over 65 years of age, attempts were made to minimize the effects of age.

CMS is a pathology seen at high altitudes and contains nonspecific symptoms. So, patients may refer to different outpatient clinics, and the diagnosis may be overlooked. Our study aims to draw attention to this rare pathology. Following the diagnosis, we referred our patients diagnosed with CMS to the department of internal medicine for their treatment.

Although we attempted to exclude secondary CMS in this study, it is difficult to achieve this completely. On the other hand, since the symptoms are similar whether CMS is primary or secondary, the complaints of individuals with chronic lung problems and heavy smokers living at high altitudes, which always seem orthopedic, may be related to CMS.

There are some limitations of our study. Since the seriousness of orthopedic complaints is not questioned within the Qinghai scoring system, the severity and duration of orthopedic symptoms were unknown. However, patients who were not associated with any disease findings based on their physical examination, laboratory and radiological tests were included in the study, and attempts were made to show the relationship of the disease to the patients. In diagnosing the disease, the subjective evaluation findings included in the scoring system create another limitation, despite the accompanying measurable evaluations. Since there was no aim to find the incidence of the disease, a scoring system was used by adding measurable values, such as oxygen saturation and blood pressure, to guide the etiology. However, further studies with larger patient cohorts and more detailed analyses are needed to investigate patient groups with similar complaints.

CONCLUSION

Many parameters play active roles in the development of CMS. When the symptoms are assessed individually, they may intersect with those of other diseases. Thus, the diagnosis of CMS may be missed. This study showed that CMS could occur at lower altitudes than those described in literature. Therefore, in the orthopedic clinic, this disease should be kept in mind, and patients should be questioned regarding the possibility of CMS to resolve the complaints of patients, which can be related to different etiopathogenesis.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Acıbadem University. (Date: 21.04.2021, No: 08)

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Author Contributions: Conception/Design of Study- K.K.; Data Acquisition- K.K.; Data Analysis/Interpretation- K.K., M.E., A.Y.; Drafting Manuscript- K.K.; Critical Revision of Manuscript- M.E., A.Y.; Approval and Accountability- K.K., M.E., A.Y.

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RESEARCH / ARAŞTIRMA
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THE ROLE OF B LYMPHOCYTES AND PRESENCE OF GERMINAL CENTERS IN PATIENTS WITH LOCALIZED PROVOKED VULVODYNIA

LOKALİZE PROVOKE VULVODİNİ HASTALARINDA B LENFOSİTLERİN VE GERMİNAL MERKEZ VARLIĞININ ROLÜ

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ABSTRACT

Objective: The aim of the study is to assess local immune activation through B lymphocytes density and the presence of germinal centers in the vestibular mucosa of patients with localized provoked vulvodynia (LPV).

Materials and Methods: Vestibulectomy specimens of 29 patients with LPV and colporrhaphy posterior specimens of 20 control women were included in the study. Immunohistochemical staining with CD20 antibodies for B lymphocytes were performed and subsequently, microscopical evaluation for B lymphocytes density and the presence of germinal centers was conducted.

Results: B lymphocyte densities were higher in the vestibular mucosa of patients with LPV compared to control women (87 vs 21, p<0.01). The presence of germinal centers was only observed in the specimens of patients with LPV (0.31 vs 0, p<0.011).

Conclusion: The presence of germinal centers with increased B lymphocyte density underlines a local immune activation in the vestibule of patients with LPV. An abnormal germinal center response can trigger the pathway to the development of autoimmunity through the emergence of an autoreactive B cell clone and autoantibodies. LPV can potentially emerge as an autoimmune disease of the vestibule. More studies are needed to understand the pathophysiology of LPV and its association with autoimmunity.

Keywords: Vulvar vestibulitis, B lymphocytes, germinal center

ÖZET

Amaç: Bu çalışmadaki amacımız, lokalize provoke vulvodinisi (LPV) olan hastaların vestibular mukozasında, B lenfosit yoğunluğu ve germinal merkez varlığına bakılarak lokal immün aktivasyonun olup olmadığını araştırmaktır.

Gereç ve Yöntem: LPV olup vestibülektomi ameliyatı geçiren 20 hastanın kolporafi posterior ameliyatı olan 20 kontrol hastanın vulvar vestibüler doku kesitleri incelenmiştir. B lenfositini göstermek için kullanılan CD20 antikoru ile yapılan immünohistokimyasal boyamayı takiben mikroskop altında B lenfosit yoğunluğuna ve germinal merkez varlığına bakılmıştır.

Bulgular: Kontrol vakaların kesitleri ile kıyaslandığında, LPV olan hastaların vulvar vestibüler mukoza kesitlerinde B lenfosit yoğunluğu daha yüksek izlenmiştir (LPV hasta 87 vs kontrol hasta 21, p<0,01). Germinal merkez varlığı sadece LPV olan hastaların vestibüler mukoza kesitlerinde izlenmiştir (LPV hasta 0,31 vs kontrol hasta 0, p<0,011).

Sonuç: LPV hastalığında, vestibüler mukozada artmış B lenfosit yoğunluğu ve beraberinde izlenen germinal merkez varlığı, lokal bir immün aktivasyonun olduğunu göstermektedir. Anormal germinal merkez cevabının olması durumunda, otoreaktif B lenfositleri ve bunun sonucunda otoimmün hastalıklar gelişebilmektedir. LPV'nin patofizyolojisinin ve otoimmünite ile ilişkisinin ortaya konmasında daha fazla sayıda çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Vulvar vestibülit, B lenfosit, germinal merkez

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INTRODUCTION

Vulvar vestibulitis syndrome (VVS), today described as localized provoked vulvodynia (LPV), is a disease showing three prominent characteristics: severe pain triggered by vestibular touch or vaginal entry, vestibular tenderness upon pressure and various degree of vestibular erythema (1, 2). With a prevalence up to 15% in premenopausal women, this disease seriously affects the sexuality and quality of life of patients because the pain can be triggered by any daily condition exercising pressure on the vestibule such as insertion of tampon, horse riding, wearing tight clothes but especially by vaginal penetration (3, 4).

The ethiopathogenesis of LPV is not fully understood. Many factors such as genetics, hormonal factors and inflammation are found associated with the disease (5). Lymphocytic infiltration in the vestibular mucosa has been described (6, 7). However, the absence of classic active inflammation suggests the hypothesis of an excessive response and deregulated inflammation (4, 8, 9).

A local inflammation mediated by increased inflammatory cytokine TNF-α was demonstrated in the vestibule of patients with LPV (10). Another study analyzing different immune cells in the vestibule showed a local immune activation: the formation of germinal centers with increased B lymphocytes density was found specific to LPV (4). In this study, the researchers looked for B lymphocytes and germinal centers in the vestibular mucosa and to assess if the local immune activation was observed in the patients.

MATERIALS AND METHODS

Study subjects

The study included 29 patients with LPV diagnosis and 20 age, sex and race-matched women with no history of vulvodynia. The archival vestibulectomy specimens of patients with LPV treated surgically in the gynecology clinic of Istanbul University, Istanbul Faculty of Medicine between 2009-2016 were used.

The inclusion criteria for LPV were: vulvar pain upon attempted vaginal intercourse or tampon insertion lasting more than one year; tenderness upon touch with a cotton tip in the vestibular area limited between Hart's line and hymen; failure of less than six months medical treatment (1-3). Exclusion criteria were: (1) use of antibiotics or immunosuppressive agents for the last 30 days; (2) clinically present microbial infection; (3) any neurologic disease or other pain syndrome (1-3).

Vestibular pain scores were obtained using a visual analog score ranging from 0 for no pain to 100 for severe intolerable pain: patients with LPV were asked to report the intensity of the pain they perceived while vestibular area was touched at 3, 5, 7 and 9 o'clock positions with a cotton tip. If the average of the 4 scores was higher than 36, surgical treatment (vestibulectomy) was offered to patients.

The control group involved age, sex and race-matched women who underwent posterior colporrhaphy for rectocele repair in the gynecology clinic of Istanbul University, Istanbul Faculty of Medicine between 2015-2016. The study was approved by the local ethical committee (Date: 12.09.2019, No: 80). All participants gave informed consent to participate in the study.

Immunohistochemistry evaluation

Tissues were fixed in 10% formalin for 24 hours then embedded in paraffin. Cut into 3-5 µm sections, embedded paraffin tissue blocks were placed on microscope slides. The slides were incubated in 56°C for one night to dry out the sections. Sections were deparaffinized with xylene solution for 30 minutes, immersed in acetone for 5 minutes and in 96% ethanol for 15 minutes, then washed with distilled water. Afterwards, to block the endogenous peroxidase activity, sections were incubated in 3% hydrogen peroxide solution in methanol at room temperature for 10 minutes.

Once the sections were ready for immunohistochemical staining, incubation with antibodies against CD20 for B cells (clone 760-2531,1:1000; Ventana Medical Systems Roche Group, USA) were performed according to the manufacturers' instructions. The sections were subsequently incubated with anti-mouse antibody followed by exposure to streptavidin, horseradish peroxidase (HRP) conjugate. Then, 3-amino-9-ethylcarbazole (AEC) was added as substrate. Finally, all sections were counterstained with hematoxylin. All the procedures were performed in the immunochemistry laboratory of the Pathology Department of the Istanbul University School of Medicine.

Tissue analyses

The immunohistochemical scoring was done under light microscope at x40 magnification (Nikon Eclipse E800). Antigen stainings were assessed for density and localization in the vestibular mucosa. The evaluation was performed by taking the mean number of positive cells observed per field from 2-4 high-power fields (hpf). The number of germinal centers formed by B cells in the sections were also counted.

Statistical analysis

The SPSS 20 software (IBM Corp, Armonk, NY) was used for statistical analyses. In addition to descriptive analysis, the Mann Whitney U test was used for the comparison between patient and controls. A p value of <0.05 was considered statistically significant.

RESULTS

All subjects were Caucasian, from low-middle class and age, sex and race-matched. There were no statistical differences in the demographic findings of the two study groups.

CD20 is a marker of B lymphocyte appearing in the pre-B-cell stage and disappearing with differentiation to plasma cells (11). Anti-CD20 antibodies showed the presence of B lymphocytes. When compared with the sections of controls, the researchers observed that B lymphocytes are statistically significantly higher in patients with LPV (Table 1). B lymphocytes were observed mostly in the stromal tissue of the mucosa (Figure 1).

The presence of germinal centers formed by B lymphocytes was observed only in patients with LPV (Figure 2). The number of germinal centers varied between 1-2 in 8 sections (27% of patients with LPV) (Table 1).

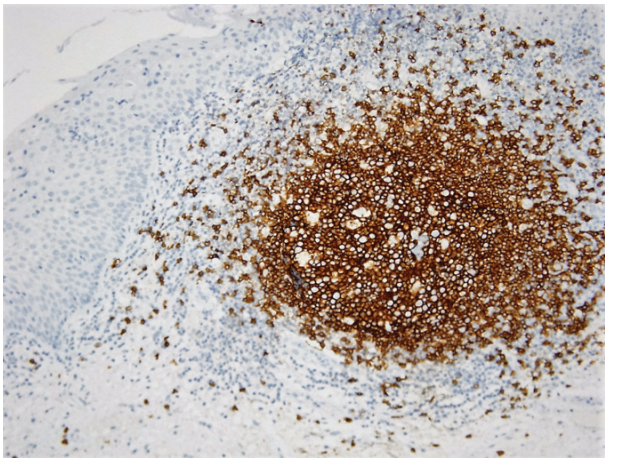

Figure 2: Germinal center in vulvar vestibular mucosa

A germinal center, formed by CD20+ immunostained B lymphocytes, in the sample of a patient with localized provoked vulvodynia. Histological sections were counterstained with hematoxylin and photomicrographed using x20 objective.

Table 1: The numbers of B lymphocytes and germinal centers in localized provoked vulvodynia

CellType	LPV (n=29) Mean (95%CI)[a]	Controls (n=20) Mean (95%CI)[a]	p value[b]
B lymphocytes	87 (58-129)	21 (12-32)	<0.001
Germinal centers	0.31 (0-2)	0 (0)	0.011

LPV: localized provoked vulvodynia, CI: confidence interval, [a]: Mean is obtained with cell counts per microscopy field, analyzed from 2-4 fields (x40 objective), [b]: Mann-Whitney U test

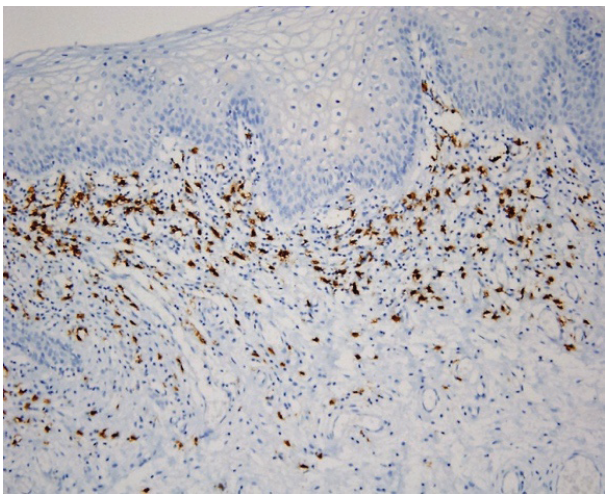

Figure 1: CD20(+) B lymphocytes in vulvar vestibular mucosa

Increased density of CD20+ B lymphocytes in a patient with localized provoked vulvodynia.
B lymphocytes were mostly observed mostly in the stromal tissue of the mucosa. Histological sections were counterstained with hematoxylin and photomicrographed using x20 objective.

DISCUSSION

This study is the first to assess local immune activation in the vestibulum of Turkish patients with LPV. The researchers observed significantly increased B lymphocytes in patients with LPV. The germinal centers were observed only in patients with LPV. In a previous study conducted on Finnish women, the comparison of vestibulectomy specimens between women with LPV and controls showed statistically significantly increased B lymphocytes in the vestibule of patients with LPV. The presence of germinal centers was also observed only in patients with LPV, like our results (4).

B lymphocytes are cells that recognize specific antigens via different immunoglobulins (Ig) anchored on the cell surface (12). Once a B lymphocyte recognizes an antigen, it differentiates into plasma cells that can secrete antibodies. Germinal centers are the microstructures where memory B cells and high-affinity antibodies secreting plasma cells are localized. It is important as it provides protection against reinfection (13). Recently, it was suggested that the germinal center response is the main pathway leading to autoantibodies found in autoimmune

diseases (14). Therefore, the germinal center response requires a very delicate balance and complex regulation to avoid the development of autoreactive B cell clones and subsequent autoimmunity (13).

The presence of germinal centers with increased B lymphocytes density in the vestibule of patients with LPV suggests a local immune activation. An 'unknown antigen' captured by B lymphocytes activated the humoral immunity and a germinal center response was given by the lymphoid tissue localized in the vestibule. Each time the vestibular mucosa is exposed to the 'unknown antigen', this cycle is triggered leading to a local inflammation in the vestibular mucosa, a feature demonstrated by previous studies (6). A constant exposure to the 'unknown antigen' contributes to the chronic persistence of the disease and the mainstay of the therapy will be avoiding the 'unknown antigen'.

A different perspective can be adopted for the ethiopathogenesis of LPV: an abnormal germinal center response following a single exposure of an 'unknown antigen' can be the starting point in LPV. The differentiation of B lymphocytes in the germinal centers can be unsuccessful if autoreactive B cells are developed in the end of this mutation process (13). Normally, the self-tolerance mechanisms in the germinal centers prevent the autoantibody production from these autoreactive B cells (15). However, an abnormal germinal center response with deficient self-tolerance mechanisms can't block this process and autoimmunity will subsequently develop. The increased B lymphocyte density found in the vestibule can in fact point out these autoreactive B cells clones. As the production of autoantibodies from these autoreactive B cells are found associated with autoimmune disease, LPV can potentially emerge as an autoimmune disease of the vestibule (15).

The main strength of the study is the high number of women involved in both patient (n=29) and control (n=20) groups. All specimens in the patient groups were obtained by the same surgeon, all specimens in the study were pathologically examined by one physician, making any inter-observer variation unlikely. The main limitation of the study is that any other autoimmunity antibodies were not assessed in both groups to support a stronger association between LPV and autoimmunity.

CONCLUSION

Localized provoked vulvodynia remains a disease where more studies are needed to understand its pathophysiology. The 'agent' triggering the activation of VALT is not elucidated. The presence of germinal centers with increased B lymphocytes density underlines a local immune activation in the vestibule of patients with LPV. An abnormal germinal center response can trigger the pathway to the development of autoimmunity through the emergence of autoreactive B cells clone and autoantibodies. The hypothesis of LPV being an autoimmune disease of the vestibule can be the subject of more studies that can reveal interesting findings. Hopefully, a better understanding of the disease will help in developing targeted therapeutics for LPV.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 26.02.2016, No: 290).

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THE EFFECT OF BLADDER PAIN SYNDROME/INTERSTITIAL CYSTITIS ON PARTNER SEXUAL FUNCTIONS

AĞRILI MESANE SENDROMU/İNTERSTİSYEL SİSTİTİN PARTNER SEXÜEL FONKSİYONLARINA ETKİSİ

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ABSTRACT

Objective: Bladder pain syndrome/Interstitial cystitis (BPS/IC) negatively affects both women's social life and sexual functions. The study researched if this situation can negatively affect the sexual functions of the male partners of these women.

Materials and Methods: The husbands of fifty female patients, who were treated for BPS/IC in our clinic, were contacted by telephone. The international index of erectile function-5 (IIEF-5), premature ejaculation diagnostic tool (PEDT), orgasmic function, sexual desire, sexual intercourse satisfaction and total satisfaction scores were used for the survey (Group 1). The same questionnaire was also used with fifty men whose wives did not have IC/BPS but who had applied to the urology outpatient clinic (Group 2). The scores of the participants were recorded and then the groups were compared.

Results: The median IIEF-5 score was 23 (22-24) in both groups (p=0.899). Both groups had a median orgasmic score of 8 (7-9) (p=0.980). While the Median PEDT score was 7.00 (4.00-12.25) in Group 1, it was 4.50 (3.00-6.00) in Group 2 (p=0.002). The men in Group 2 were significantly more advantageous than Group 1 in terms of sexual desire, sexual intercourse satisfaction and total satisfaction scores, (p=0.003, p=0.0001 and p=0.003, respectively).

Conclusion: Although BPS/IC does not have a significant effect on the IIEF-5 score of the husbands of women, it negatively affects the scores of sexual desire, sexual intercourse satisfaction, total satisfaction and premature ejaculation.

Keywords: Painful bladder, interstitial cystitis, partner sexual dysfunction

ÖZET

Amaç: Ağrılı mesane sendromu/İnterstisyel sistit (BPS/IC) kadınların gerek sosyal hayatlarına gerekse de seksüel fonksiyonlarına negatif etki etmektedir. Çalışmamızda bu durumun kadınların eşlerinin seksüel fonksiyonlarına etkisi olup olmadığını göstermeyi amaçladık.

Gereç ve Yöntem: Kliniğimizde BPS/IC nedeniyle tedavi gören 50 kadın hastanın eşlerine telefonla ulaşılarak hastaneye çağırılıp uluslararası erektil fonksiyon-5 (IIEF-5), prematür ejekülasyon (PEDT), orgazmik fonksiyon, seksüel istek, cinsel ilişki memnuniyet ve total memnuniyet skorları sorgulanmıştır (Grup 1). Aynı sorgulama eşinde interstisyel sistit olmayan ve üroloji polikliniğine başvuran diğer 50 erkek üzerinde de yapılmıştır (Grup 2). Katılımcıların skorları kayıt altına alınarak gruplar karşılaştırılmıştır.

Bulgular: Median IIEF-5 skoru her iki grupta da 23 (22-24) bulunmuştur (p=0,899). Her iki grubun median orgazmik skorunun 8 (7-9) olduğu görülmüştür (p=0,980). Median PEDT skoru Grup 1'de 7,00 (4,00-12,25) iken Grup 2'de 4,50 (3,00-6,00) bulunmuştur (p=0,002). Seksüel istek, cinsel ilişki memnuniyet ve total memnuniyet skorları bakımından da Grup 2'deki erkeklerin Grup 1'den anlamlı olarak avantajlı olduğu görülmüştür (sırasıyla, p=0,003, p=0,0001 ve p=0,003).

Sonuç: IC/BPS, kadınların eşlerinin IIEF-5 skoru üstünde anlamlı etki yapmasa da seksüel istek, cinsel ilişki memnuniyet, total memnuniyet ve prematür ejekülasyon skorlarını olumsuz yönde etkilemektedir.

Anahtar Kelimeler: Ağrılı mesane, interstisyel sistit, partner seksüel disfonksiyonu

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INTRODUCTION

Bladder pain syndrome/Interstitial cystitis (BPS/IC) is defined as a "persistent or recurrent chronic pelvic pain, pressure or discomfort perceived to be related to the urinary bladder accompanied by at least one other urinary symptom such as an urgent need to void or urinary frequency " by the International Continence Society (1). Recent studies indicate that the prevalence of BPS/IC in women is approximately 2.7-6.5% (2). Several treatment options of BPS/IC have been defined such as behavioral therapies, oral pharmacological agents, intravesical treatments, hydrodistension and various surgical procedures (3). This uncomfortable situation negatively affects the quality of life of women by causing significant problems both in their social activities and work life (4). One of the most important problems caused by BPS/IC in women is its negative effect on sexual functions (5,6). Chronic pain in the suprapubic area causes lack of interest to engage in a sexual relationship in women and this results in sexual avoidance, orgasm problems, and depression, which negatively affects the quality of life in the future (7). The negative effect of "sexual avoidance behavior" in women on the sexual functions of male partners or husbands of women has been shown in numerous studies in the psycho-sexual field (8). Although the negative effects of BPS/IC on the sexual functions of women were evaluated in studies, the literature data on how this situation affects the sexual functions of the male partner or husbands of women is quite limited. Therefore, this study will examine the sexual functions of husbands of women with BPS/IC and indicate to what extent their husbands' sexual functions were affected by this situation.

MATERIALS AND METHODS

After obtaining approval from the ethics committee of Keçiören Training and Research Hospital (Date:23.03.2021, No: 2012-KAEK-15/2274), a prospective cohort study was designed which included a total of one hundred sexually active male participants. To determine their sexual functions, the husbands of fifty female patients who were treated for BPS/IC for at least a year or more and followed-up in our clinic, were invited by telephone to participate in the study group (Group 1). In the histories of women with BPS/IC, pain or pressure sensations in the pelvic area, daily frequency or urgency symptoms, increased pain during bladder filling, and bladder pain symptoms that did not improve with antibiotic treatment were studied in detail. The exclusion criteria from this study were the presence of bladder cancer, presence of spinal cord damage or neurological disease that may cause bladder dysfunction (Parkinson's disease, multiple sclerosis, spina bifida), presence of genital herpes, presence of gynecological malignancy or radiation therapy to pelvic area. After evaluating the bladder diary results of the patients, urine analysis, urine culture, blood urea and creatinine values were examined. The presence of glomerulation or Hunner's ulcer was evaluated by performing cystoscopic imaging in all patients with BPS/IC, and punch biopsy was taken when necessary. Cystoscopy findings and biopsy results of the patients were classified using the European Society for the Study of Interstitial Cystitis (ESSIC) scale (9). The O'Leary scale, which was validated in Turkish version, was used to determine the symptom index of women with BPS/IC (10). This scale includes the interstitial cystitis symptom index (ICSI) and the interstitial cystitis problem index (ICPI). ICSI consists of a total of four questions that look at the frequency of urination during the daytime and at night, sudden urgency, nocturia , pelvic pain, and was evaluated from 0-20 points. The ICPI examines how many problems the symptoms questioned in ICSI cause in daily life and an evaluation was made in the range of 0-16 points. Fifty sexually active men from a similar age range and who applied to the urology outpatient clinic were included in the study and became a control group (Group 2). All patients in the groups were informed about the study and a written consent form was obtained. Sexual functions of men in Group 1 before BPS/IC symptoms in their wives and at least one year after the onset of BPS/IC symptoms were examined in detail. The previous sexual status of the men in Group 1 was made at the time of the diagnosis of BPS/IC by questioning the status of their partner before the onset of symptoms. Patients with co-morbidities that may adversely affect sexual functions such as hypertension, uncontrolled diabetes mellitus, dyslipidemia, alcoholism, and obesity were not included in the study. Another exclusion was the presence of erectile dysfunction secondary to any organic pathology previously revealed by penile Doppler Ultrasonography. In addition, the patients who had previous prostate surgery (transurethral prostatectomy, open prostatectomy, or radical prostatectomy), penile curvature surgery due to Peyronie's disease or congenital penile curvature, hypospadias or open urethroplasty were excluded from the study.

The participants included in the study were evaluated with the international index of erectile function form (IIEF) and the premature ejaculation diagnostic tool (PEDT), which were both validated in Turkish (11, 12). The first five questions (IIEF-5) in the IIEF form on erectile function were used for the survey study. Each of the questions in this area scored in the 0 to 5 point range. According to the scoring system, 5-7 points are considered severe erectile dysfunction (ED), 8-11 points as moderate ED, 12-16 points as mild-moderate ED, 17-21 points as mild ED, 22-25 points as no ED. The 9th and 10th questions of the IIEF form are related to orgasmic function and each of the questions is scored in the 0 to 5 point range and consists of a total of 0 to 10 points. The 11th and 12th questions of the IIEF form are about sexual desire and the questions are scored between 1 to 5 points and consist of 2 to10 points in total. The 6th, 7th and 8th questions of the IIEF form determine sexual intercourse satisfaction, each of the questions is scored between 0 to 5 points and consists of 0 to 15 points in total. The 13th and 14th questions

of the IIEF questionnaire determine the overall satisfaction score, and each of the questions is scored between 1 to 5 and consists of a total of 2 to 10 points. The PEDT form used in this study to determine the male premature ejaculation parameters consists of five questions with a total score in the range of 0 to 22 points. According to this scoring system, 0-8 points indicate the presence of low probability PE, 9-10 points indicate the possible presence of PE, and 11-22 points indicate the presence of PE.

The sexual function of the participants in Group 1 before the development of BPS/IC symptoms in their wives (Before BPS/IC-Group 1) and at least one year after the symptoms developed (After BPS/IC-Group 1) were compared. In addition, the pre-symptom and post-symptom sexual function scores of the participants in Group 1 were compared with Group 2 separately.

Statistical analysis

The Kolmogorow-Smirnov test was used for testing the distribution of variables, while continuous variables were compared using the Mann-Whitney U test and the Wilcoxon signed-rank test for univariate analyses. The median minimum, maximum, interquartile range, mean±standard deviation was used for defining variables. Data was analyzed with SPSS 25.0 (IBM Corp, Armonk, NY). software. Statistical significance was set at $p<0.05$.

RESULTS

The median age of Group 1 was 37.00 (28.75-46.00) years, and the median age of Group 2 was 34.00 (28.00-48.00) years (p=0.759). While the median body mass index (BMI) of the men in Group 1 was 25.00 (24.00-27.00) kg/m^2, it was 26.00 (24.00-27.25) kg/m^2 in Group 2 (p=0.837). ICSI score of the wives of the participants in Group 1 was 13.14±3.12 and the mean ICPI score was 11.10±2.35. ESSIC groups of women with BPS/IC were determined according to cystoscopic imaging results (Table 1).

The men in Group 1 stated that they had sexual intercourse with their wives a median of four (2-5) times in a month, while the men in Group 2 stated that they had sexual intercourse with a median of eight (6-8) times in a month, and the median number of sexual intercourse of the participants in Group 2 was significantly higher compared to Group 1 (p=0.0001).

There was no significant difference in mean IIEF-5 and mean orgasmic function scores before BPS/IC and after BPS/IC in men in Group 1 (22.20±3.31 vs 22.34±3.15, p=0.968 and 8.04±1.66 vs 7.60±2.50, p=0.585 respectively). While the mean PEDT score was 5.42±3.51 before BPS/IC, it was 8.12±5.02 after BPS/IC in Group 1 (p=0.002). In terms of sexual desire, intercourse satisfaction, and overall satisfaction, it was determined that the men in Group 1 were significantly more advantageous in the before BPS/IC period compared to the after BPS/IC period (p=0.002, p=0.585 and p=0.002 respectively) (Table 2). The median IIEF-5 score was 23 (9-25) in the before BPS/IC-Group 1, and it was 23 (8-25) in Group 2 (p=0.750). While the median PEDT score was 4 (2-17) in

Table 1: ESSIC groups and O'Leary scores of the women with BPS/IC in Group 1 (n=50)

ESSIC group	n (%)
1X	3 (6%)
1A	8 (16%)
1B	2 (4%)
1C	3 (6%)
2A	2 (4%)
2B	4 (8%)
2C	13 (26%)
3A	3 (6%)
3B	5 (10%)
3C	7 (14%)
ICSI score, mean±SD	13.14±3.12
ICPI score, mean±SD	11.10±2.35

ESSIC: European Society for the Study of Interstitial Cystitis, ICSI: interstitial cystitis symptom index, ICPI: interstitial cystitis problem index, SD: Standard deviation

Table 2: Comparison of sexual functions before and after BPS/IC in Group 1

Scores	Before BPS/IC-Group 1	After BPS/IC-Group 1	p value
IIEF-5, Mean±SD	22.20±3.31	22.34±3.15	0.968
PEDT, Mean±SD	5.42±3.51	8.12±5.02	**0.002**
Orgasmic function, Mean±SD	8.04±1.66	7.60±2.50	0.585
Sexual desire, Mean±SD	7.96±1.67	6.36±2.55	**0.002**
Intercourse satisfaction, Mean±SD	10.82±3.04	8.32±3.41	**0.003**
Overall satisfaction, Mean±SD	8.98±11.68	5.84±2.59	**0.002**

BPS/IC: Bladder pain syndrome/Interstitial cystitis, IIEF-5: International index of erectile function-5 score, PEDT: Premature ejaculation diagnostic tool, SD: Standard deviation

the before BPS/IC-Group 1, it was 4.5 (1-18) in Group 2 (p=0.955). Similarly no significant difference was found in terms of orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction scores between the before BPS/IC-Group 1 and Group 2 (p=0.825, p=0.803, p=0.939 and p=0.820 respectively) (Table 3).

The median IIEF-5 scores were 23 (22-24) in both the after BPS/IC-Group 1 and Group 2 (p=0.899). Similarly, the median orgasmic function scores were 8 (7-9) in the after BPS/IC-Group 1 and Group 2 (p=0.980) (Figure 1, Table 4). While the median PEDT score of men in the after BPS/IC-Group 1 was 1.00 (4.00-12.25), the median PEDT score of the men in Group 2 was 4.50 (3.00-6.00) (p=0.002). Similarly, the median sexual desire score was 7.00 (4.00-8.25) in men in the after BPS/IC-Group 1, and it was 8.00 (7.00-9.00) in the men in Group 2 (p=0.003). While the median sexual satisfaction score was 8.00 (5.00-11.25) in the after BPS/IC-Group 1, it was 12.00 (8.75-13.00) in Group 2 (p=0.0001). The median of total satisfaction score was 6 (3-8) in the after BPS/IC-Group 1, and it was 8 (6-9) in Group 2 (p=0.003) (Figure 2, Table 4).

Table 3: Comparison of sexual functions before BPS/IC - Group 1 and Group 2

Scores	Before BPS/IC-Group 1	Group 2	p value
IIEF-5, Med (min-max)	23 (9-25)	23 (8-25)	0.750
PEDT, Med (min-max)	4 (2-17)	4.5 (1-18)	0.955
Orgasmic function, Med (min-max)	8 (3-10)	8 (2-10)	0.852
Sexual desire, Med (min-max)	8 (2-10)	8 (2-10)	0.803
Intercourse satisfaction, Med (min-max)	12 (4-15)	12 (3-15)	0.939
Overall satisfaction, Med (min-max)	8 (3-9)	8 (2-10)	0.820

BPS/IC: Bladder pain syndrome/Interstitial cystitis, IIEF-5: International index of erectile function-5 score, PEDT: Premature ejaculation diagnostic tool, Med (min-max): Median (minimum-maximum)

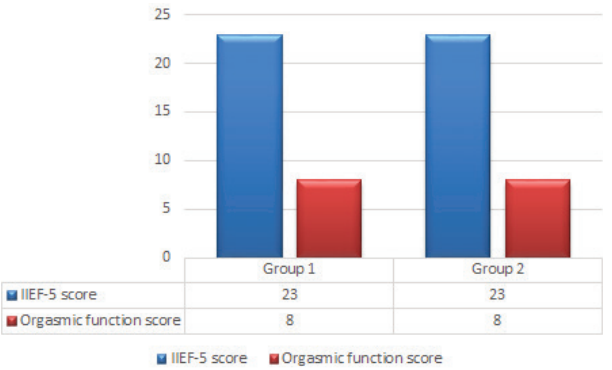

Figure 1: IIEF-5 and orgasmic function scores of the groups

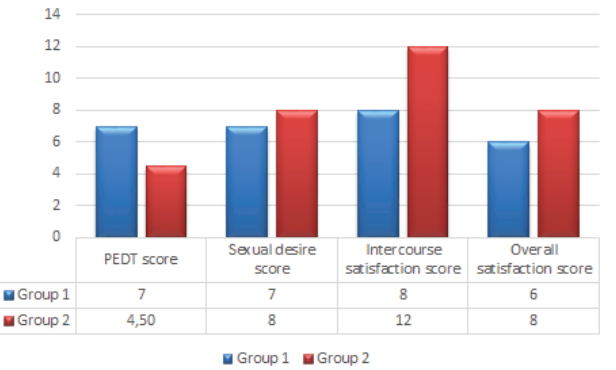

Figure 2: PEDT, sexual desire, intercourse satisfaction and overall satisfaction scores of the groups

Table 4: Comparison of sexual functions after BPS/IC - Group 1 and Group 2

Scores	After BPS/IC-Group 1	Group 2	p value
IIEF-5,Median (IQR)	23 (22-24)	23 (22-24)	0.899
PEDT, Median (IQR)	7.00 (4.00-12.25)	4.50 (3.00-6.00)	**0.002**
Orgasmic function, Median (IQR)	8 (7-9)	8 (7-9)	0.980
Sexual desire, Median (IQR)	7.00 (4.00-8.25)	8.00 (7.00-9.00)	**0.003**
Intercourse satisfaction, Median (IQR)	8.00 (5.00-11.25)	12.00 (8.75-13.00)	**0.0001**
Overall satisfaction, Median (IQR)	6 (3-8)	8 (6-9)	**0.003**

IQR: Interquartile range, IIEF-5: International index of erectile function-5 score, PEDT: Premature ejaculation diagnostic tool

DISCUSSION

Although, historically, male sexual health has been one of the primary areas of interest of urologists, recently women's sexual health has become one of the important research topics of urology (13). Although male and female sexual health is subject to examination separately, it is important to consider male and female factors together and to consider the partner effect when evaluating patients with sexual dysfunction (14). Recently, assorted studies reported the effect of the partner factor on sexual functions as a result of urological surgical procedures. These studies were conducted on the sexual functions of women and their partners especially after mid-urethral sling surgeries for female stress urinary incontinence (SUI). In studies conducted for this purpose, it indicated that the urinary incontinence in women also causes sexual dysfunction and improvement in sexual functions were achieved with successful incontinence surgery (15). Naumann et al. reported that a total of 73 patients applied for a single-incision mini-sling procedure and a success rate of 93.2% achieved (16). According to this study, the preoperative mean female sexual function index (FSFI) score of women was 23.86±5.67, while the postoperative FSFI score was 27.25±4.66 and significant improvement was observed in the postoperative period ($p<0.0001$). Despite the reported high success rates of mid-urethral sling procedures in the treatment of SUI and the improvement in sexual functions provided by it, vaginal mesh exposure/extrusion reported at a rate of 6-6.5% in these procedures which may cause serious sexual dysfunction in both women and their male partners (17). In an editorial view emphasizing this issue, Braubaker stated that the partner dyspareunia (hispareunia), which can be seen after vaginal surgeries, often develops secondary to vaginal mesh extrusion (18). According to this view, it has been reported that the penile laceration and even bleeding can be seen in the male partner during sexual intercourse, and this situation negatively affects their sexual functions. These results support the idea that the female sexual dysfunction caused by physiological or psychosomatic factors also negatively affect the sexual functions of the male partner, as we emphasized in this study. Consistent with the literature, in the present study, it was determined that PEDT, sexual desire, intercourse satisfaction, and overall satisfaction scores deteriorated significantly in men with the development of BPS/IC symptoms in their wives in Group 1. Factors that negatively affect partner sexual functions may be related to pain or discomfort during sexual intercourse, which is defined as hisparonia, this situation can also develop secondary to the behavior of avoiding sexual intercourse due to vaginal/pelvic pain felt by the female partner before, during, or after intercourse without any pain or discomfort in the penis of the male partner (19, 20). McCabe et al. emphasized the effect of sexual avoidance behavior in the evaluation of male and female sexual dysfunctions and pointed out the necessity of evaluating the male and female partner together in the implementation of treatment modalities (21). Similarly, Carvalho et al. reported that sexual desire was directly related to the psychological state of the partners during sexual activity and that partner treatment should be considered together in sexual dysfunction (8).

Like the physiological and psychological factors, we have mentioned, it has been stated in different studies that the presence of BPS/IC has a negative effect on female sexual functions and the development of sexual avoidance behavior in these women. In the study performed by Peters et al., 5000 randomized women without IC and 407 women with IC, reported that the female sexual dysfunction and sexual distress developed at a significantly higher rate than in women with IC (22). In another study conducted by Yoon et al., it was reported that BPS/IC negatively affected the sexual life of women secondary to the increase in symptoms such as frequency, urgency, and vulvodynia (23). In another similar study, Ottem et al. reported that the rate of dyspareunia was 72% in the group of women with BPS/IC, while it was 5% in the control group without BPS/IC ($p<0.0001$) (24). In the same study, it emphasized that the mean FSFI score was 20.2±9.6 in the IC/BPS group, while it was 29.9±6.3 in the control group, and that IC/BPS negatively affected women's sexual functions ($p<0.0001$). In another study conducted by Nickel et al. that included 128 patients with BPS/IC, it stated that the change in the mental and physical quality of life (QOL) scores of the patients was correlated with the change in their sexual functions ($r=0.46$, $p<0.0001$ and $r=0.29$, $p=0.0023$, respectively) (25). In a similar study, Bogart et al. reported that 75% of the 146,231 women with BPS/IC, who had sexual partners, of which 88% had ≥1 general sexual dysfunction symptoms and 90% had ≥1 BPS/IC specific symptoms within the last four weeks (26). In the same study they stated that the women with BPS/IC symptoms experienced very high levels of sexual dysfunction. In another study by Hung et al., 103 female patients with refractory BPS/IC were treated with intravesical hyaluronic acid (HA) and the effect of that treatment on sexual functions as well as the improvement in symptoms were examined (27). According to the logistic regression analysis, they reported that a baseline Pelvic Organ Prolapse/Urinary Incontinence Sexual Function Questionnaire (PISQ-9) score was negatively correlated with the duration of IC/BPS symptoms ($p=0.022$). At a result of their study intravesical HA may improve sexual function along with the reduction of IC/BPS symptoms. In another similar study, Agrawal et al. compared thirty-two female patients with BPS/IC and thirty-two control group patients without symptoms (28). This study reported that the mean FSFI scores of women with BPS/IC were significantly worse than the normal group and BPS/IC caused sexual dysfunction (18.678±4.531 vs. 28.05±4.318; $p<0.05$).

As stated in the aforementioned studies, BPS/IC emerges as an important factor causing sexual avoidance behavior and sexual dysfunction in women due to both the somatic pain it causes, and the depression and psychosomatic problems caused by the long-lasting discomfort. Like the literature data, in the present study, the men's sexual function scores whose wives did not have BPS/IC symptoms yet (Before BPS/IC-Group 1) was found to be similar compared to the normal population (Group 2). But it was observed that these men's PEDT, sexual desire, intercourse satisfaction, and overall satisfaction scores worsened significantly with the development of BPS/IC symptoms (After BPS/IC-Group 1) when compared to the normal population (Group 2). These results support the idea that the development of BPS/IC in women, as in other factors mentioned above, negatively affect the sexual functions of male partners. However, there are not enough studies investigating the effect of this disorder of women with BPS/IC on the sexual functions of their male partners. According to the literature research conducted, this study is the first randomized study to evaluate this issue in detail. These results indicate that the sexual avoidance behavior is higher in women with BPS/IC and secondary to this, the frequency of sexual activity is decreased in these couples. In the present study, the higher frequency of premature ejaculation in the husbands of women with BPS/IC compared to the control group may have developed secondary to the psychological stress caused by sexual avoidance behavior caused by dyspareunia or pelvic pain in women. Based on these results, it can be concluded that the dyspareunia and pelvic pain thatdevelop due to BPS/IC may cause various emotional and psychosomatic disorders in women, resulting in sexual dysfunction, and this situation leads to partner sexual dysfunction.

Limitations

The limited number of participants in this study is an important limitation. Additionally, the fact that sexual function was not questioned in female subjects, the relationship inventory was not included in the study, the sexual functions of women and their spouses were not examined according to BPS/IC phenotype subgroups and treatment responses can be considered as another limitation.

CONCLUSION

BPS/IC causes various degrees of sexual dysfunction in women. Especially dyspareunia and the emotional stress caused by it cause sexual avoidance behavior in women, which also leads to partner sexual dysfunction. Therefore, in the follow-up and treatment period of women with BPS/IC, the male partner sexual function should be evaluated in addition to female sexual function.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Kecioren Training and Research Hospital (Date:23.03.2021, No: 2012-KAEK-15/2274).

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RESEARCH / ARAŞTIRMA
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SEASONAL VARIATION IN HbA1c AND 25(OH) VITAMIN D CONCENTRATIONS IN THE TURKISH POPULATION: COULD RAMADAN BE CHANGING EXPECTATIONS?

TÜRK TOPLUMUNDA HbA1c VE 25(OH)D VİTAMİNİ KONSANTRASYONLARINDAKİ MEVSİMSEL DEĞİŞİM: RAMAZAN BEKLENTİLERİ DEĞİŞTİRİYOR OLABİLİR Mİ?

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ABSTRACT

Objective: In this study, the seasonal variations of HbA1c and serum 25(OH) vitamin D were investigated during different seasons in Istanbul.

Materials and Methods: The alterations in HbA1c and vitamin D were evaluated concerning age and sex. The study consisted of 86,835 samples, selected from the Istanbul Faculty of Medicine Electronic Medical Records.

Results: LC-MS/MS quantified 25(OH)D levels, and HbA1c were measured in HPLC. The HbA1c level in women was significantly lower than in men (p<0.001), and the HbA1c level was higher in summer compared with winter (p<0.001) and autumn (p=0.005). The 25(OH)D levels of all groups was 28.2±15.5 ng/mL with no difference between men and women. The highest vitamin-D levels were in summer, and the lowest were in winter.

Conclusion: Studies have shown that in both hemispheres of the world, HbA1c levels decrease when the air is warm and increase when the weather is cold. However, contrary to expectations, we witnessed that HbA1c wa s 0.3% higher in summer than in winter in Istanbul, where the temperature change over a year is 22.5°C, the coldest month is January, and the hottest month is August. A possible explanation of our findings could stem from the occurrence of Ramadan in the summer months between

ÖZET

Amaç: Bu çalışmada İstanbul'da farklı mevsimlerde HbA1c ve serum 25(OH) vitamin D'nin mevsimsel değişimleri araştırılmıştır.

Gereç ve Yöntem: Çalışmada kullanılan 86.835 örnek, İstanbul Tıp Fakültesi Elektronik Tıp Kayıtlarından seçilmiştir. HbA1c ve vitamin D düzeyindeki değişiklikler yaş ve cinsiyete göre değerlendirilmiştir. 25(OH)D seviyeleri LC-MS/MS, HbA1c ise HPLC yöntemleriyle ölçülmüştür.

Bulgular: HbA1c düzeyi kadınlarda erkeklere göre anlamlı olarak düşük (p<0,001) ve yaz aylarında, kış (p<0,001) ve sonbahar (p=0,005) ayları ile karşılaştırıldığında daha yüksek bulunmuştur. 25(OH)D ortalaması 28,2±15,5 ng/mL olup, erkekler ve kadınlar arasında fark bulunmamıştır. En yüksek D vitamini seviyelerinin yaz aylarında, en düşük ise kış aylarında olduğu görülmüştür.

Sonuç: Çalışmalar, dünyanın her iki yarım küresinde de hava ısındığında HbA1c seviyelerinin azaldığını, hava soğuk olduğunda ise arttığını göstermektedir. Ancak bir yıl içinde sıcaklık değişiminin 22,5°C olduğu, en soğuk ayın Ocak, en sıcak ayın ise Ağustos olduğu İstanbul'da HbA1c'nin beklenenin aksine yaz aylarında kış aylarına göre %0,3 daha yüksek olduğu görüldü. Bu durumun, 2015-2017 arasındaki yaz aylarında Ramazan ayının varlığından kaynaklanıyor olabileceği düşünüldü. Öte yandan HbA1c ile

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2015-2017. However, variations between HbA1c and 25(OH)D related to temperature changes during the year and age groups were detected.

Keywords: Diabetes mellitus, vitamin D, seasonal variation, HbA1c, Ramadan

25(OH)D arasında yıl ve yaş gruplarındaki sıcaklık değişimlerine bağlı farklılıklar tespit edildi.

Anahtar Kelimeler: Diabetes mellitus, vitamin D, mevsimsel değişiklikler, HbA1c, Ramazan

INTRODUCTION

Some metabolic factors and hormones demonstrate circannual patterns depending on seasonal environmental factors (1). Some metabolic changes with seasonal variations are seen in blood pressure, heart rate, and some serum metabolites such as serum lipids, glycated hemoglobin (HbA1c), vitamin D, and hormone levels.

Cold weather and some climate factors have been revealed to induce cyclical, seasonal variations. The body's standard thermoregulatory mechanism functions to preserve a stable internal state to survive, and the hypothalamus maintains this. In winter, the production of hormones such as glucagon, epinephrine, and cortisol levels increase with the effect of the cold temperature, which results in high blood glucose levels (2, 3). Recent studies have shown that increased body fat and consequently insulin resistance in winter are associated with high cortisol levels and increased tissue sensitivity of glucocorticoids (4). Several studies reported physiologic responses to cold temperatures (5, 6).

The hemoglobin A1c (HbA1c) test is essential for accomplishing adequate glucose monitoring in patients with type 1 or type 2 diabetes and screening tests to diagnose diabetes mellitus. The International Expert Committee and the American Diabetes Association (ADA)'s current diagnostic criteria suggest that A1c levels of 6.5% are indicative of type 2 diabetes, and a level between 5.7-6.4% indicates a high risk for progression to diabetes mellitus (7, 8). Although HbA1c has many advantages, such as being highly standardized with low biologic variation, it also shows poor validity with a sensitivity of 44% and a specificity of 79% at a diagnostic threshold value (6.5%) (9-11). However, the ADA recently recommended using repeated blood glucose measurements with the estimated average glucose (eAG) to make the best inference from individual data (12). Studies have shown that new-onset type 1 diabetes is mostly seen in winter in the northern hemisphere with seasonal changes (13). However, such seasonal patterns' significance is unclear due to the multifactorial etiology of diabetes (14). Seasonal changes also affect glycemic control and HbA1c levels in patients with diabetes. These alterations are associated with geographic regions based on temperature variations and latitudes (13, 15).

Vitamin D is an important factor in human metabolism, recently shown to have additional roles in insulin resistance, type 2 diabetes, cancer, immune system and cognitive dysfunction, and bone metabolism. Many studies have shown a link between insufficient vitamin D levels and diabetes, hypertension, coronary artery disease, and cancer development (16-19). In recent years, the growing public awareness of vitamin D functions, as well as bone metabolism, has led to increased vitamin D supplementation. Also, seasonal variations of vitamin D concentration depending on latitude, diet, and clothing habits have been investigated in previous studies (20, 21). Changes in HbA1c and 25(OH)D levels have been reported between seasons or months throughout the year and in both hemispheres (22-24).

In this study, we aimed to evaluate the seasonal variations of HbA1c and serum 25(OH) vitamin D concentrations during different seasons of the year in Istanbul and assess the alterations in HbA1c and serum 25(OH)D levels concerning age and sex.

MATERIALS AND METHODS

Study group

This study consisted of 86,835 subjects, selected from the Istanbul Faculty of Medicine Clinical Laboratory Electronic Medical Records between September 2015 and February 2018. The data selected were unidentified and only included age, sex, the month of blood sampling, and HbA1c and 25(OH)D levels. All subjects who participated in the study were residing in Istanbul (Latitude: North 41°00').

The blood samples were collected in vacutainer tubes containing dipotassium-EDTA (K2-EDTA) (Becton Dickinson, Plymouth, UK), for HbA1c and 25(OH)D assays. The subjects were categorized according their age and sex. According to age, the study group was classified in 6 subgroups: 0-5 years, 6-19 years, 20-39 years, 40-65 years, 65-80 years, and over 80 years. The study protocol was approved by the Ethical Committee of Istanbul Faculty of Medicine (Date: 29.03.2019, No: 06).

Methods

HbA1c levels were measured using cation-exchange high-performance liquid chromatography [HPLC] with Bio-Rad Turbo II (Bio-Rad, Richmond, California, USA) on the same day within four hours of blood sampling. All HbA1c results are expressed in percentage (%), using the certified method of the National Glycohemoglobin Standardization Program/Diabetes Control and Compli-

cations Trial (NGSP/DCCT). The precision results (CVs%) were 2.5, 4.0, and 2.3% at normal, high, and intermediate HbA1c levels, respectively. Based on ADA criteria, A1c levels of 6.5% were accepted as an indicator of type 2 diabetes and a level between 5.7-6.4% were accepted as an indicator of a high risk of progression to diabetes mellitus (pre-diabetes) (8).

The collected blood samples for vitamin D measurement were protected from light, centrifuged, and stored at -80°C until studied. 25(OH)D levels were quantified using the LC-MS/MS method with a Zivak Tandem Gold HPLC unit and a Zivak D2/D3 Tandem GOLD Triple Quadrupole MS/MS unit (Zivak Technologies, Turkey). The precision results (CVs %) were 7.2% and 7.3% at low (22.4±3.3 ng/mL) and high levels (82.3±11.8 ng/mL), respectively. Vitamin D deficiency is defined as 25(OH)D levels of below 20 ng/mL (50 mmol/L) according to the Endocrine Society (25).

The monthly and seasonal mean HbA1c and 25(OH)D levels for the entire study group were calculated using a maximum of one and half years' data, and evaluated for subgroups depending on age groups, sex, and temperature differences.

The mean monthly temperatures for different months were obtained from available meteorological information for Istanbul.

Statistical interpretation

The statistical analysis was performed using the SPSS 15 software program (SPSS, Chicago, IL, USA). A p value of <0.05 was considered statistically significant. The differences among multiple subgroups were determined using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test with post-hoc analysis. Multivariate logistic regression analysis was performed to determine the independent variables that were associated with Vitamin D and HbA1c.

RESULTS

The study comprised of 86,835 subjects (58.5% women, 40.5% men). The participants' mean age was 39.4±23.2 (range, 1-84) years. The women were slightly older than the men (40.1±21 years vs. 38.5±24.3 years, respectively). Of the study group, 12% (n=12243) were less than 5 years of age; 13% (n=12540) were aged 6-19 years; 21% (n=20568) were aged 20-39 years; 41% (n=39684) were aged 40-65 years, 11% (n=1800) were aged 65-80 years, and 2% were over 80 years of age.

The mean HbA1c concentration of all participants was 6.53±1.8%. The women's HbA1c levels were significantly lower than in men (6.42±1.8% vs. 6.64±1.8%; p<0.001) (Table 1).

Istanbul's average temperature obtained from available meteorologic information was the lowest in January (+4.0°C), and the highest in August (26.6°C). The HbA1c levels varied significantly across the different seasons. The average HbA1c level was higher in summer compared with winter (6.66±1.9% vs. 6.53±1.9%; p<0.001) and autumn (6.52±1.7%; p=0.005). The HbA1c levels in winter were also significantly different compared with spring (p=0.003). The monthly variations in HbA1c levels showed a peak in June and July, and lowered following October. Significant differences were obtained between January and June, July and August (p<0.001), and also between February and August (p<0.001) (Figure 1A).

When we investigated HbA1c levels according to the age groups, the HbA1c levels of the subjects aged 0-5

Table 1: HbA1c and 25(OH) vitamin D levels between sex and age subgroups

	Mean age (years±SD)	Sex distribution (%)	HbA1c (%)	25(OH) Vitamin D (ng/mL)
Women	41.1±21.0	58.5	6.42±1.80[x]	27.4±20.2
Men	38.5±24.3	40.5	6.64±1.80[x]	27.5±15.9
Age groups	**Mean age (years±SD)**	**Age groups distributions (%)**	**HbA1c (%)**	**25(OH) Vitamin D (ng/mL)**
0-5 years	2.71±1.5	12	5.99±1.5[a,b,c]	32.3±17.1[a,b]
6-19 years	11.4±4.2	13	6.29±2.2[a,b,c]	24.8±14.6[c,d]
20-39 years	30.3±5.9	21	5.70±1.1[a,b,c]	24.43±19.7[a,b,c]
40-65 years	52.7±7.1	41	6.80±1.9	27.00±18.8[d]
66-80 years	71.3±4.02	11	6.71±1.8	28.83±19.6[d]
>80 years	84.0±3.5	2	6.44±1.3	30.30±23.1

[x]: p<0.001: intergroup analysis, [a]: p<0.001: in comparison with group 3 (aged 40-65), [b]: p<0.001: in comparison with group 4 (aged 65-80), [c]: p>0.001: in comparison with group 5 (aged >80 years), [d]: p<0.001

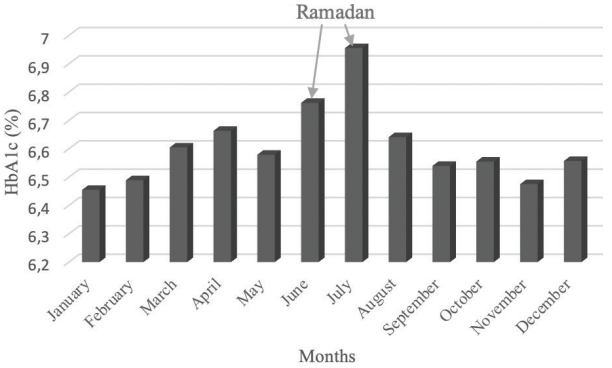

Figure 1(A): Alteration in HbA1c percentages in respect to months

years and 20-39 years and were lower compared with subjects older than 40 years (40-65; 65-80 and >80 years; p<0.001, p<0.001 and p=0.003, respectively). The mean HbA1c levels in those aged 40-65 and 65-80 were also higher than in subjects aged over 80. HbA1c levels between sex and age subgroups are presented in Table 1.

The mean 25(OH)D levels of all participants was 28.2±15.5 ng/mL. No significant difference was obtained between the mean levels 25(OH)D of men and women (27.5±15.9 ng/mL, 27.4±20.2 ng/mL, respectively) (Table 1). When the vitamin D levels were evaluated across different seasons, all groups' highest vitamin D levels were in summer. The lowest was in winter with a difference of 6.5 ng/mL (30.98±12.7 vs. 24.48±17.8 ng/mL, p<0.001). The mean 25(OH)D levels were also higher in summer compared with autumn and spring (29.8±17.9 ng/mL, 26.9±21.0 ng/mL, respectively; p<0.001, for both) (Table 2). The monthly alterations in 25(OH)D levels demonstrated a peak in August (34% increase) through January to August, and tended to decline following October. Significant differences were found between vitamin D levels of January and July/August (p<0.001 for both), and also between December and August, and September (p<0.001, for both) (Figure 1B). Average monthly 25(OH)D levels are presented in Figure 1(B).

When we compared 25(OH)D levels according to the age groups, the groups aged 6-19 and 20-39 years were the lowest among all age groups. The mean 25(OH)D concentrations of individuals in these groups were significantly lower than those aged 0-5 years, 40-65 years, and 65-80 years (p<0.001 and p<0.001) (Figure 2B) (Table 1).

The prevalence of vitamin D deficiency for all participants was 42.4%: 41.4% for men and 44.5% for women.

Factors associated with the risk of 25(OH)D deficiency and the risk of prediabetes and diabetes mellitus were evaluated using multiple logistic regression models. Age, sex, and some months were associated with increased

Table 2: Mean HbA1c and 25(OH) vitamin D levels and average temperatures for Istanbul in different seasons

Seasons	Mean temperature (°C)	HbA1c (%)	25(OH) Vitamin D (ng/mL)
Spring	15.1	6.61±1.9	26.9±21.0[c]
Summer	25.7	6.66±1.9[a,b]	30.98±12.7[a]
Autumn	18.5	6.52±1.7	29.8±17.9[a]
Winter	6.98	6.53±1.9	24.5±17.8

[a]: p<0.001: in comparison with winter, [b]: p<0.01: in comparison with autumn, [c]: p<0.001: in comparison with summer

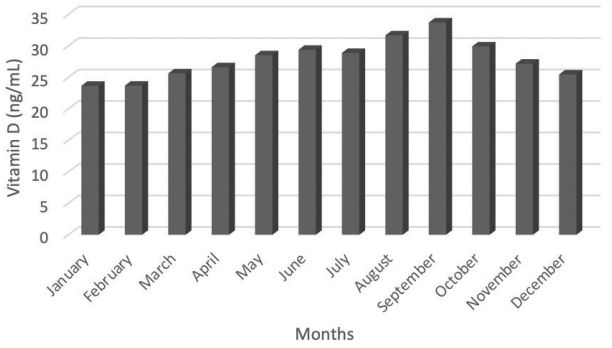

Figure 1(B): Alteration in 25(OH)vitamin D concentrations in respect to months

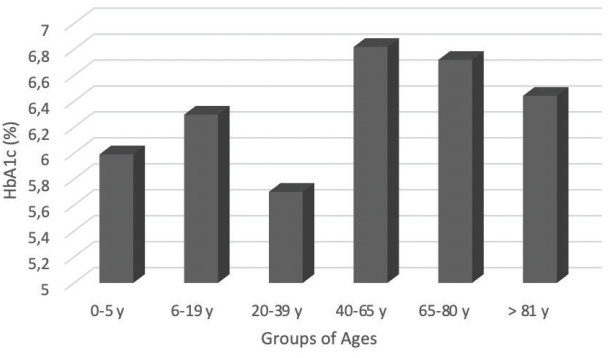

Figure 2(A): Alteration in HbA1c percentages in respect to age groups

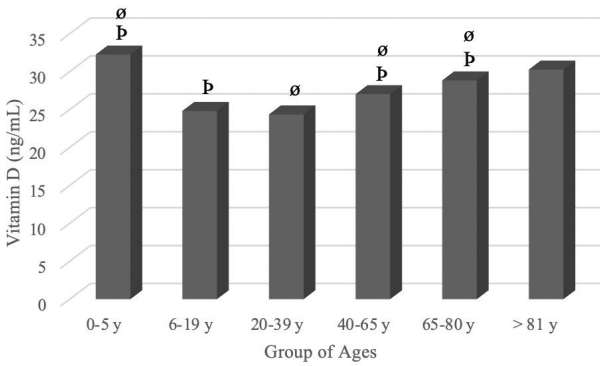

Figure 2(B): Alteration in 25(OH)vitamin D concentrations in respect to age groups

Þ: Differences between group 6-19y to 0-5y, 40-65y and 65-80y; p<0.001

ø: Differences between group 20-30y to 0-5y, 40-65y and 65-80y; p<0.001

HbA1c concentration (the results were not shared). In women, an increase in HbA1c levels was associated with a higher risk in the prevalence of diabetes than in men (p=0.002 in women, p=0.021 in men). The months April and May seemed to be associated with an increase in HbA1c levels (p=0.005 and p=0.002, respectively).

Age, certain months (February, March), and Ramadan (Jun 6 to July 4, 2016) were determined as associated with vitamin D deficiency (p<0.001, p=0.025, p=0.009, and p=0.004, respectively). Also, an association was found between seasonal temperature change and 25(OH)D levels (r=0.184, p<0.001).

DISCUSSION

Studies have shown that in both hemispheres of the world, HbA1c levels decrease when the air is warm and increase when the weather is cold. This situation has been associated with an increase in dietary energy and decreased physical activity during the winter months in most of the studies. In fact, in studies conducted in different geographies, it has been observed that the HbA1c values are even lower in the colder winter months (23, 24, 26, 27). However, contrary to expectations, we have seen that HbA1c is 0.3% higher in summer than in winter in Istanbul, where the temperature change over a year is 22.5°C, the coldest month is January, and the hottest month is August. Tseng et al. reported that the temperature differences were around 25°C for East Orange, New Jersey in the United States of America, with a difference in HbA1c values of 0.22%, in accordance with our findings (24). They also suggested that seasonal variations in HbA1c levels were greater where the temperature differences were larger (24). For example, seasonal variation was not observed in a study conducted in a tropical country with no significant temperature differences (30).

A possible explanation of our findings could stem from the presence of Ramadan in the summer months between 2015-2017, which requires fasting from dawn to sunset, and may cause a change of dietary habits and frequency in people who are fasting. Consuming a high calorie diet depending on the long fasting period may cause high glucose and increased HbA1c levels. The loss of circadian rhythm and increased evening cortisol levels have been reported to cause detrimental effects on metabolic regulation during Ramadan (28). According to our data, the peak of HbA1c seen in July can be explained by the fact that the fasting periods for Ramadan were in June for both years and 50% of the HbA1c reflects the average glucose level over the last 30 days in a three-month period (29). Additionally, it is also inevitable that people who fast do less physical activity because of the low energy intake during the whole day.

When we tested the HbA1c levels according to sex and age groups, the HbA1c levels in women were significantly lower than in men. The mean HbA1c levels in subjects aged 40-65 and 65-80 were higher than in subjects aged under 40 years and over 80 years. These findings are consistent with the previous studies' results (15, 26). When the HbA1c levels were controlled with logistic regression models, the months depending on the temperature differences remained associated with increases in HbA1c. Women also had a higher risk than men related to the increased HbA1c values. Aging is another known factor associated with the higher HbA1c-associated increased prevalence of prediabetes and diabetes mellitus. In our cross-sectional population-based study performed in urban and rural parts of Turkey, the prevalence of prediabetes and diabetes was 8.2% and 16.5%, respectively, higher in women than in men. We also found an association between increasing age and the prevalence of diabetes in both sexes (31).

In recent years, several studies have investigated the relation between serum 25(OH)D and glucose homeostasis (32, 33). We obtained the highest vitamin D levels in August and September and the lowest in January and February, with a difference of 6.5 ng/mL between summer and winter, which is consistent with other studies (34-36). The 25(OH)D level of summer was also higher than autumn and spring. Nevertheless, studies show no seasonal differences in vitamin D levels associated with the sunny season (22, 37). When we tested changes in vitamin D levels over the months, we found that the highest vitamin D levels from January to August were in August (a 34% increase from January). They tended to decline following October, presenting significant differences between January with July and August. However, studies have sug-

gested that seasonal temperature changes are not the only reason for alterations in vitamin D concentrations. Aging, body mass index/body fat percentage, calcium intake, genetics, dietary fat content, some diseases, and some medications are other factors that affect levels of 25(OH)D (38, 39).

According to our results, 42.4% of our study group had vitamin D deficiency (41.4% for men and 44.5% for women). Similar or higher deficiency rates were reported by other studies (22, 31, 40). It has been reported that vitamin D deficiency is more prevalent in certain regions of the w orld (41). In the current study, 25(OH)D levels were lower in subjects aged 0-5 years than over 40 years. Heidari et al. also reported higher vitamin D levels for subjects aged over 60 years and lower levels for those younger than 30 years, in accordance with our findings (22). When we controlled the 25(OH)D levels with multivariate logistic regression models, the effect of age, month (February, March), and Ramadan remained significant. According to our data, the lowest vitamin D levels were between January and March. Zho et al. reported a significant effect of seasons on 25(OH)D levels by logistic regression analysis (42). Also, the significant association of temperature in a year with 25(OH)D concentrations supports the importance of a sunny climate in synthesizing vitamin D (41). Several studies have revealed that the generation of 25(OH)D is decreased with aging and skin pigmentation (18, 43, 44). Although the subjects had higher vitamin D levels between 40-65 years than at younger ages, significantly lower vitamin D levels were obtained from subjects above 80 years compared with middle ages. These findings also indicate that the skin's ability to synthesize vitamin D decreases with older age. The fact that the average 25(OH)D levels were not significantly different in both sexes could be due to our study group's characteristics. Our study group consisted of subjects selected from our electronic medical records. Accordingly, we had no information about their dietary habits, diseases such as tuberculosis, diabetes, therapeutic drugs, including vitamin D and/or oral antidiabetics, and the lack of other biochemical parameters supporting vitamin D deficiency or serum glucose levels. Therefore, other potentially confounding factors could not be evaluated, which is the major limitation of our study.

Our study has shown that although many studies concretely reveal the close relationship between changes in air temperature, HbA1c, and 25(OH)D, the changes in physiological and human habits caused by Ramadan can change what is known. However, our study could not determine how many subjects fasted, received vitamin D replacement, had diabetes or a disease that could affect sugar regulation, or used drugs that could cause an increase in blood sugar. Therefore, further studies are needed to investigate the effects of dietary habits, exercise, and chronic diseases on HbA1c and vitamin D levels.

Note: The editor in chief was not involved in the evaluation, peer-review and decision processes of the article, and these processes were carried out by the associate editors.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 29.03.2019, No: 06).

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Author Contributions: Conception/Design of Study- S.G., B.Ö., A.K.Ü., F.Ç.; Data Acquisition- H.H., F.Ç., A.F.K., R.A.; Data Analysis/Interpretation- A.D., S.G., A.K.Ü., F.Ç.; Drafting Manuscript- S.G., A.K.Ü., F.Ç., H.H.; Critical Revision of Manuscript- S.G., B.Ö., A.K.Ü., F.Ç.; Approval and Accountability- F.Ç., H.H., A.F.K., R.A., A.D., A.K.Ü., B.Ö., S.G.

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PULMONARY SARCOIDOSIS WITH BONE INVOLVEMENT MIMICKING METASTATIC CANCER

KEMİK TUTULUMU İLE METASTATİK KANSERİ TAKLİT EDEN PULMONER SARKOİDOZ OLGUSU

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ABSTRACT

Sarcoidosis is a disease of unknown etiology which can affect the lungs and lymph nodes, skin, eyes and other organs and, in rare cases, bones and bone marrow cells. We present a case of a 39-year-old male patient with an iliac bone involvement of sarcoidosis mimicking malignancy. Lymphoma and metastatic cancer proved a challenge to discard in differential diagnosis. He underwent multiple biopsies including tru-cut lung parenchyma and iliac bone biopsies. All of the biopsy specimens were reported as non-necrotising granulomatous inflammation. He underwent corticosteroid treatment. His response to the steroid treatment was insufficient so methotrexate was added to the corticosteroid therapy. Afterwards, clinical and radiological improvement was recorded. We aimed to highlight the utilization of taking new biopsies and histopathological re-evaluations in order to confirm the diagnosis and distinguish a benign disease from a malignant one.

Keywords: Sarcoidosis, differential diagnosis, malignancy

ÖZET

Sarkoidoz, etyolojisi bilinmeyen, özellikle akciğer ile lenf bezlerini, cildi, gözleri ve diğer organları tutabilen; fakat nadiren kemik ve kemik iliğini tutan bir hastalıktır. Otuz dokuz yaşında, iliak kemikte maligniteyi taklit eden lezyonu olan, biyopsi ile sarkoidoz olduğu kanıtlanan bir erkek hastayı sunduk. Lenfoma veya metastatik kanser ayırıcı tanısı yapmak son derece zordu. Hastaya akciğer ve iliak kemikten birçok biyopsi yapıldı. Hepsinin sonucu nonnekrotizan granulomatöz inflamasyon olarak raporlandı. Hastaya kortikosteroid tedavisi başlandı. Steroid tedavisine yeterli yanıt alınamayan hastanın steroid tedavisine metotreksat ilave edildi. Bunun sonrasında klinik ve radyolojik iyileşme kaydedildi. Bu olguda, maligniteyi benign bir hastalıktan ayırt edebilmek ve tanıyı doğrulamak için yeni biyopsilerle ytekrarlanan histopatolojik değerlendirmenin öneminin vurgulanması hedeflendi.

Anahtar Kelimeler: Sarkoidoz, ayırıcı tanı, malignite

INTRODUCTION

Sarcoidosis is a multisystem, chronic disease characterised by non-necrotising granulomatous inflammation of unknown etiology (1). About 90% of patients with sarcoidosis have pulmonary involvement (2). As is well known, sarcoidosis may also affect the skin, eyes, kidney, joints and other organ systems, but bone involvement of sarcoidosis is rare, accounting for only 3-13% of all patients (3). We present a case of a 39-year-old male patient with an iliac bone lesion that mimicked malignancy, yet the confirmed result of the biopsy taken from the lesion proved to be sarcoidosis.

CASE REPORT

A 39-year-old man was admitted to our outpatient clinic who had been suffering from shortness of breath, malaise and fatigue for one month. His past medical history was unremarkable. He was a nonsmoker and had

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been working in a polyvinyl chloride (PVC) pipe factory for 10 years. He had neither fever nor weight loss . Night sweats were absent. A chest computed tomography (CT) was performed after his chest X-ray showed a multifocal reticulonodular pattern in both lung fields and bilateral hilar enlargement (Figure 1). CT revealed multiple, irregular shaped, peribronchovascular nodular lesions, predominantly in the upper lobes in both lung fields (Figure 2). These were initially reported as an infectious disease. Laboratory findings were all within normal limits. Erythrocyte sedimentation rate (ESR) and white blood cell levels were normal. Fiberoptic bronchoscopic evaluation was insignificant. Both lung systems were normal and bronchoalveolar lavage (BAL) fluid was obtained in order to detect infectious disease, predominant cell features and the possibility of malignancy. Tuberculosis smear test, mycobacteria culture, atypical mycobacteria culture and cytology of BAL fluid were within normal limits and there was no sign of infectious disease. Purified protein derivative (PPD) skin test demonstrated no induration and was reported as 0 mm.

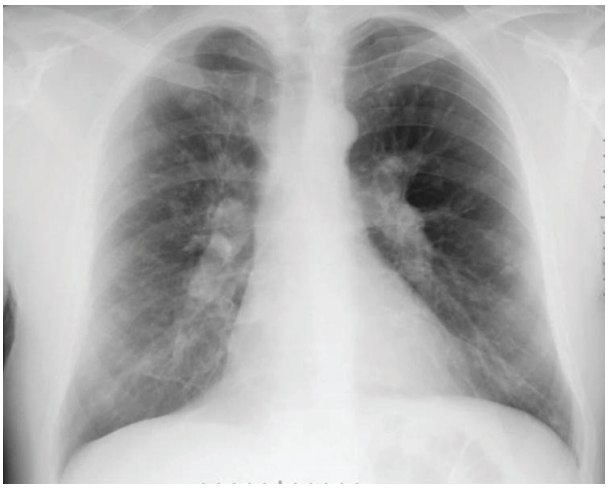

Figure 1: Multifocal reticulonodular pattern in both lung fields and bilateral hilar enlargement

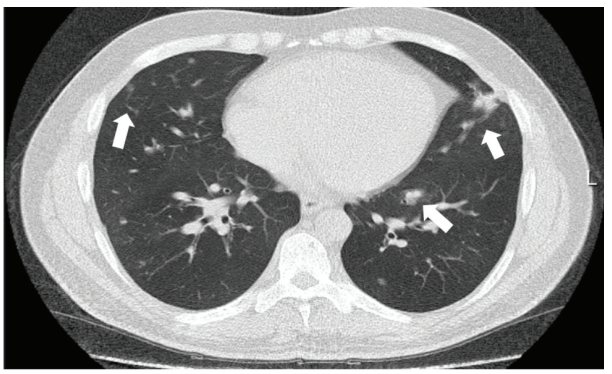

Figure 2: Multiple, irregular shaped, peribronchovascular nodular lesions (organising pneumonia)

Serum calcium, vitamin D levels and angiotensin-converting enzyme (ACE) levels were within normal limits.

Tests performed for syphilis, amoebiasis, brucellosis and also echinococcosis were negative. Thyroid function tests were within normal limits. Tumor markers (Ca 19-9, Ca-125, total and free prostate-specific antigen (PSA), alpha-Fetoprotein (AFP)) and autoantibody profile (antinuclear antibody, antineutrophil cytoplasmic antibody) were examined and all of them were within normal limits, too.

The patient was discussed in our Multidisciplinary Interstitial Lung Disease Council and his illness was classified as organising pneumonia (Figure 2). Methylprednisolone treatment was recommended. Through this therapy the symptoms of the patient improved. At the end of a 1-month- corticosteroid (CS) therapy a chest CT was performed to re-assess the response to therapy and the CT showed the regression of organising pneumonia. Clinical and radiological findings had significantly improved , and a chest CT re-assessment was planned after finishing six months of CS therapy. The patient presented to our clinic for persistent dyspnea after 6 month-corticosteroid treatment. The chest CT showed lymphadenopathies

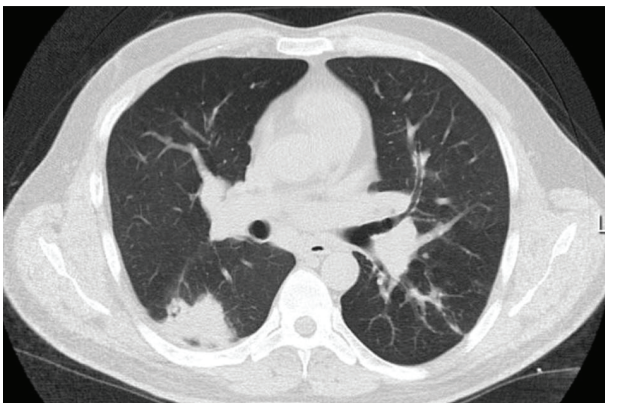

Figure 3a: 4 cm consolidation in the right lower lobe

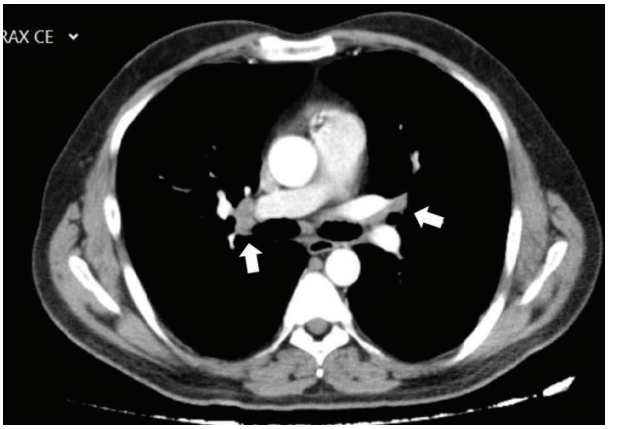

Figure 3b: 2 cm lymphadenopaties in both hilum

of 2 cm in both pulmonary hila and a newly developed peripheral consolidation of 4 cm in diameter that was located in the superior right lower lobe (Figure 3a, 3b). A transthoracic fine needle aspiration biopsy and tru-cut biopsy were taken from this 4 cm lesion in the right lung by interventional radiology and histopathological evaluation was reported as non-caseating, non-necrotising granulomatous inflammation. There was no evidence of a malignant neoplasm, according to the report. Bronchoscopic evaluation was performed again for the second time and both a bronchoalveolar lavage (BAL) fluid specimen and a transbronchial biopsy were obtained. The biopsy result was negative for malignancy. BAL fluid examination indicated that the number of CD4 was 3.75 (61%), the number of CD8 was 1.8 (29.5%) and the ratio of CD4/CD8 was 2. The percentage of lymphocytes in BAL fluid was 84.9% (6.323). The Multidisciplinary Interstitial Lung Disease Council decided to continue CS therapy for 1 month due to the increased lymphocyte percentage in the BAL fluid. At the end of 1 month of CS therapy a repeat chest CT revealed the progression of the lesion in the right lower lobe from a size of 4 cm to 5 cm. Positron emission computed tomography (PET/CT) was ordered to evaluate whether there was any involvement in other parts of the body. PET/CT showed lymphadenopathies of 10 millimeters in diameter in both axillae with a maximum standardized uptake value (mSUV) level of 10, mass lesions of 4 cm in both lung parenchyma with 16 mSUV, and multiple mediastinal and abdominal lymphadenopathies with 15 mSUV and 12 mSUV, respectively. Both the vertebral column and the pelvic bone showed increased metabolic activity with 11.8 mSUV in multifocal areas (Figure 4, 5). An *iliac* bone biopsy was recommended to the patient so as to show whether the diagnosis is lymphoma or primary lung cancer.

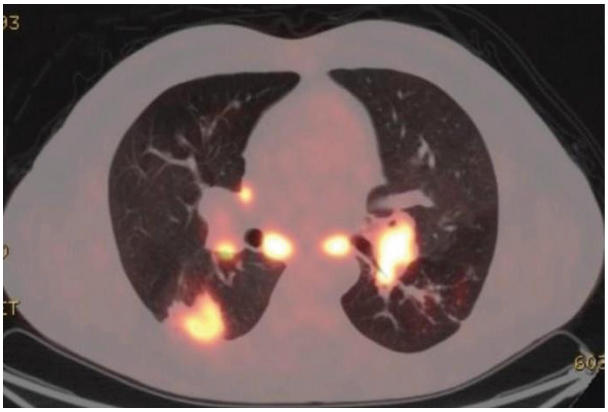

Figure 4: 4-centimeter-mass lesions in both lung parenchyma with 16 mSUV, multiple mediastinal lymphadenopaties with 15 mSUV

The CT guided iliac bone biopsy was obtained from the PET positive lesion in the left iliac bone by interventional radiology. The histopathological evaluation was reported as non-caseating, non-necrotising granulomas once again. The patient was diagnosed with steroid-resistant sarcoidosis. Methotrexate, a disease modifying anti-rheumatic drugs (DMARDs) was added to the steroid treatment. Through this combination therapy, the patient's symptoms and radiological findings gradually re-

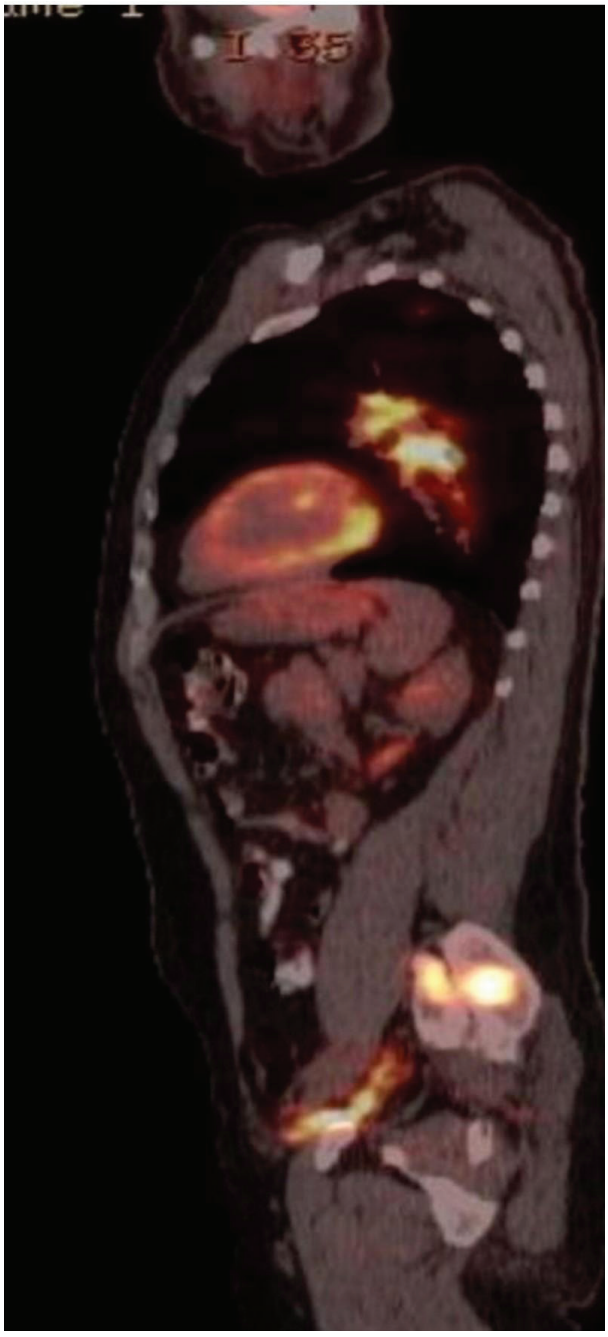

Figure 5: Pelvic bone involvement with 11.8 mSUV

gressed. Eventually, a chest CT was carried out at the end of six months of combination therapy with methotrexate and tapering dose steroid which revealed that all radiological findings (mass-like lesions, lymphadenopathies and consolidations) improved significantly (Figure 6). In addition to this, the CT did not show any novel lesions. The treatment continued by tapering current doses so as to reduce side effects.

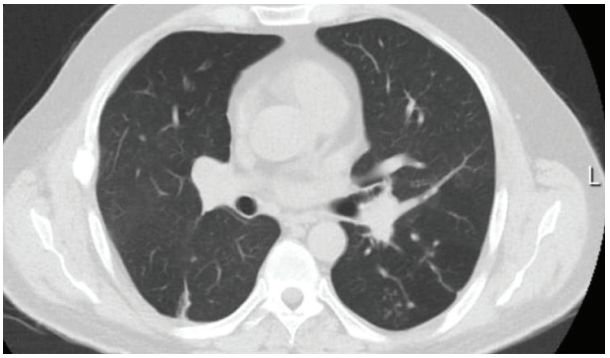

Figure 6: Resolution of mass like lesion and hilar lymphadenopaties

DISCUSSION

Sarcoidosis is a disease, which may affect multiple organ systems. Ninety percent of patients with sarcoidosis have lung involvement, whereas bone and bone marrow involvement is rare and comprises 3-13% of all cases. Non-necrotizing granulomatous inflammation is typical in sarcoidosis (1-3). Since both metastatic cancer and lymphoma can have bone and bone marrow involvement, differential diagnosis is crucial to avoid misdiagnosis. In addition to the well-known clinical and radiological manifestations of sarcoidosis, the diagnosis is also made by showing non-caseating epitheloid cell granulomas in biopsy specimens, thus discarding other granulomatous diseases (4).

Our case is special for two reasons. One is that radiological and laboratory findings are not specific for sarcoidosis with a 4-centimeter mass-like lesion, which normally suggests malignancy. A PPD test was also implemented and there was no induration, so it was recorded as 0 mm which suggests sarcoidosis. On the other hand the ratio of CD4/CD8 more than three in BAL fluid has high specifity for sarcoidosis (5,6), and may be useful. However, this ratio has a low sensitivity for sarcoidosis (7). However, the CD4/CD8 ratio of our patient was two, which is not compatible with sarcoidosis. Serum protein electrophoresis and immunofixation electrophoresis were also measured within normal limits. Serum levels of calcium, vitamin D and ACE were within normal limits. There was no evidence of lymphoproliferative disorders . At this stage the diagnosis was highly challenging, with inadequate evidence. Second, at the beginning of CS treatment, clinical and radiological improvement was remarkable, but after a few months it changed and became resistant to steroid therapy. Our first diagnosis, organised pneumonia, was excluded due to the fact that the response to steroid therapy was unsatisfactory. The patient was discussed in our Multidisciplinary Interstitial Lung Disease Council due to the failure of treatment. This made us re-evaluate the patient once again with biopsies and a PET/CT scan in order to rule out metastasis or lymphoma. The PET/CT scan revealed various high metabolic active lesions whose mSUV level was approximately 15, which suggested a malignant rather than a benign lesion. The pelvic bone indicated 11.8 mSUV level. An iliac bone biopsy was carried out with a high suspicion of malignancy, and a non-necrotising granulomatous inflammation was again reported. After the patient was diagnosed with sarcoidosis with resistance to steroids, we resolved to combine methotrexate with a tapering dose of CS. The response to this combination therapy was extremely good. Both clinical and radiological improvement proved that the diagnosis was sarcoidosis with bone involvement. Bone involvement of sarcoidosis generally has a diminished response to CS treatment, with a poor prognosis, and has a tendency to be multisystemic, as seen in our case (8).

In cases with atypical and obscure elements like this case, clinicians need to take account of differential diagnosis until they make the definitive diagnosis. Additional biopsies and further investigation may be helpful. A multidisciplinary approach may help in managing difficult cases like ours. We recommended our patient to have routine check-ups four times a year so as not to be late in case of malignant transformation.

CONCLUSION

We presented a young male patient who had involvement in various parts of his body, including bones, and therefore was suspected of having a malignancy at first glance.

However, multiple biopsies revealed that the case was in fact sarcoidosis . It is essential to discard malignancy and other causes of granulomas in cases where radiological and laboratory findings are not consistent with sarcoidosis. Bone involvement of sarcoidosis is an uncommon but significant entity due to its mimicking of metastatic cancer.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.Ş., A.H.I.; Data Acquisition- E.Ş.; Data Analysis/Interpretation- A.H.I.; Drafting Manuscript- E.Ş.; Critical Revision of Manuscript- E.Ş., A.H.I.; Approval and Accountability- E.Ş., A.H.I.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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CASE REPORT / OLGU SUNUMU
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A RARE CASE OF AN EXTENSIVE SUBCUTANEOUS NECROSIS IN THE LEFT TEMPORAL REGION CAUSED BY ORANGE OIL INJECTION AND ITS MANAGEMENT

SOL TEMPORAL BÖLGEYE PORTAKAL YAĞI ENJEKSİYONU SONRASI GELİŞEN YAYGIN DERİ ALTI NEKROZU OLGUSUNA YAKLAŞIM

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ABSTRACT

Orange oil (isoparaffin C11-C15) is a solvent material that dissolves tooth filling materials effectively. A 29-year-old male who had extensive subcutaneous left temporal necrosis after an inadvertent orange oil injection is presented. Due to the injection, an immediate oedema and cellulitis formed in the left buccal, zygomatic, and temporal region. After a prompt drainage and antibiotherapy, the patient's complaints were resolved. Due to partial temporal muscle necrosis and fibrosis, the interincisal mouth opening (IIO) was 15 millimeters. With a precise physical medicine and rehabilitation regimen, the IIO became 35 millimeters at the postoperative first year.

Keywords: Drainage, interincisal opening, isoparaffin C11-15, mouth stretching exercise, orange oil

ÖZET

Portakal yağı (izoparafin C11-C15) diş dolgusu maddelerini etkin şekilde çözen bir kimyasal maddedir. Yanlışlıkla portakal yağı enjeksiyonu sonrası sol temporal bölgesinde yaygın nekroz gelişmiş olan yirmi dokuz yaşında bir erkek hasta sunulmuştur. Enjeksiyona bağlı olarak sol yanak, zigoma ve temporal alanlarda aniden ödem ve sellülit gelişmiştir. Erken drenaj ve antibiyoterapi sonrası hastanın şikayetleri gerilemiştir. Kısmi temporal kas nekrozuna ve fibrozisine bağlı olarak kesici dişler arası ağız açıklığı 15 milimetreye düşmüştür. Düzenli bir fiziksel tıp ve rehabilitasyon tedavisi ile bu açıklık ameliyat sonrası birinci yılda 35 milimetreye çıkmıştır.

Anahtar Kelimeler: Drenaj, kesici dişler arası ağız açıklığı, izoparafin C11-C15, ağız germe egzersizleri, portakal yağı

INTRODUCTION

Root canal treatment is a frequently performed dental procedure that treats periapical periodontitis (1). In case of failure, root canal retreatment is the most effective alternative (2). However, previously applied filling materials must be dissolved in order to reach the periapical region in the retreatment phase (3). As a natural essential fat, orange oil (isoparaffin C11-C15) is a solvent material that is able to dissolve the filling material very effectively (4). In this case report, a patient who had extensive subcutaneous left temporal necrosis after an inadvertent injection is presented and a management plan is proposed.

CASE REPORT

The patient was informed about each step of his treatment and he gave consent to all treatments and to the reporting of the case.

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After a left maxillary premolar tooth extraction, a 29 year-old male patient was referred to the Infectious Disease Department by his dentist who detected an immediate oedema and cellulitis formation in the left buccal, zygomatic and temporal region (Figure 1). The dentist reported an inadvertent subcutaneous injection of 5 milliliters of orange oil at the left gingivobuccal sulcus. The patient, who was an active cigarette smoker (10 packages/year), did not have any comorbidities in his medical history, he did not have any antibiotic therapy before tooth extraction, and severe paresthesia and erythema were found in the physical examination. The laboratory tests showed a neutrophilic leukocytosis, and blood culture was negative for any microorganisms before any antibiotics were given. Because of the presence of the necrotic regions, multidrug antibiotherapy with carbapenem, vancomycin, and clindamycin was administered in order to prevent any possible future infection and the patient was transferred to the Plastic Reconstructive and Aesthetic Surgery Department on the fifth day of hospitalization.

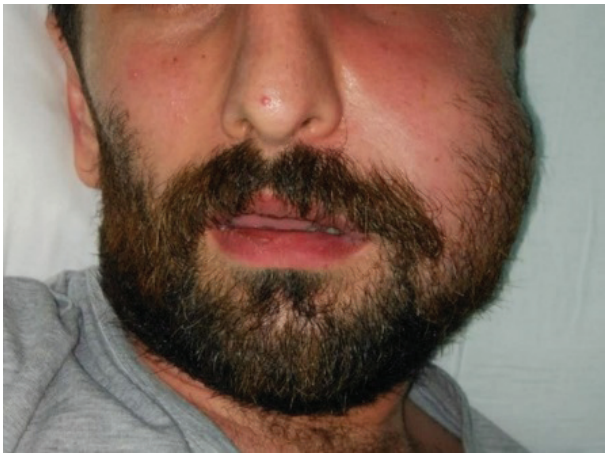

Figure 1: Oedema and cellulitis formation of the left buccal and zygomatic region are seen.

A fluctuation was detected in the left temporal region of the patient and left buccal mucosa was found to be affected by the same fluctuation during the intraoral examination. With a differential diagnosis of necrotic abscess, the patient was taken to the operating theatre promptly and dual drainage was performed. The left buccal region was drained through a transmucosal incision, whereas the left temporal region was drained through a transcutaneous incision. The purulent and necrotic exudate was removed and the total exudate volume was 125 cc. A Penrose drain was inserted in each incision and the patient was transferred to the ward. The patient was hospitalized for a week and manual drainage was continued through the Penrose drains. At the end of the hospitalization, exudative discharge ended and the drains were removed. The microbiologic tests found that the exudate was sterile and the pathological examination demonstrated extensive fat necrosis.

During the follow-up period, the interincisal mouth opening of the patient decreased to 15 millimeters at the first postoperative month (Figure 2). This change was thought to be caused by the pain that the patient had felt in his necrotic left temporal muscle when he tried to open his mouth. The patient was referred to the Physical Medicine and Rehabilitation Department. Mouth opening and active and passive stretching tests were advised and the patient was compliant with this treatment regimen. The patient was examined at the first year after hospitalization and the interincisal mouth opening was found to be 35 millimeters thanks to the precise physical therapy treatment that had been performed (Figure 3). The left tem-

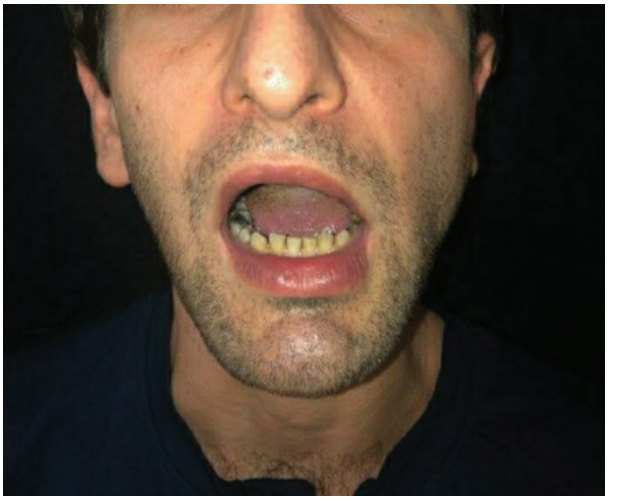

Figure 2: At the first postoperative month, the interincisal mouth opening of the patient decreased to 15 millimeters.

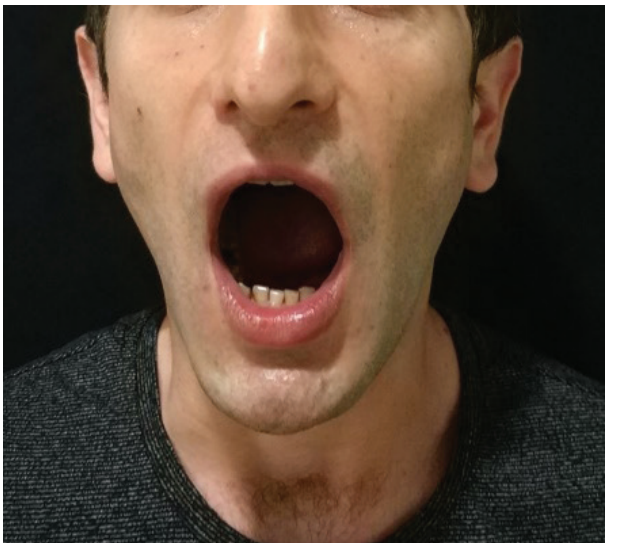

Figure 3: The patient is seen at the first postoperative year. The interincisal mouth opening is restored and no temporal asymmetry is detected.

poral area was symmetric with the right temporal region and there was no need for any reconstructive procedures in order to restore the facial contours of the patient.

DISCUSSION AND CONCLUSION

Inadvertent subcutaneous injection of various solutions may cause an extensive necrosis in patients. Histolytic materials and solutions should be labelled and used with caution to prevent accidental in-vivo applications. Thorough information must be obtained about the physical and chemical properties of the insulting materials and immediate drainage should be performed in order to prevent permanent sequela. The importance of the patient's safety should always be kept in mind and the patient should be informed about the probable complications and other treatment options at the first referral.

Regardless of the etiology, physical medicine and rehabilitation measures should be kept in mind for the injuries and insults that affect the temporomandibular joint. These measures should be utilized as either a primary or an adjunctive treatment in this setting.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.; Data Acquisition- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.; Data Analysis/Interpretation- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.; Drafting Manuscript- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.; Critical Revision of Manuscript- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.; Approval and Accountability- H.Ö.B., B.E.A., E.K., M.S., M.Y., U.E.

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CASE REPORT / OLGU SUNUMU
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A RARE CASE: PRIMARY MUCINOUS CYSTIC NEOPLASM OF THE GALLBLADDER

SAFRA KESESİNİN PRİMER MÜSİNÖZ KİSTİK NEOPLAZMI: NADİR BİR VAKA

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ABSTRACT

Cystic lesions originating from the gallbladder are very rare. Mucinous cystic neoplasms of the liver and biliary system principally occur in the liver, followed by the extrahepatic biliary system. Only a few case reports were reported primarily in the gallbladder.

A 63-year-old woman was admitted to our hospital with complaints of pain in the right upper abdomen, increasing after meals, and abdominal discomfort. A laparoscopic cholecystectomy was performed. Macroscopically, the cyst was localized in the serosa of the fundus. Based on immunohistochemical examination, the cyst was diagnosed as primary mucinous cystic neoplasia of the gallbladder.

Although it is very rare, mucinous cystic neoplasia should be kept in mind when dealing with cystic lesions of the gallbladder. Due to serious associated problems such as the risk of malignant transformation or bile duct obstruction, particularly in larger cysts, a cholecystectomy is mandatory.

Keywords: Cholecystectomy, gallbladder, mucinous cystic neoplasia

ÖZET

Safra kesesinden kaynaklanan kistik lezyonlar oldukça nadirdir. Hepato-bilier sisteminin müsinöz kistik neoplazileri esas olarak karaciğerde meydana gelir ve bunu ekstrahepatik biliyer sistem izler. Primer olarak safra kesesi tutulumu olan sadece birkaç vaka rapor edilmiştir.

Karın sağ üst tarafta yemek sonrası artan karın ağrısı ve dispepsi şikayetleri olan 63 yaşında kadın hasta hastanemize başvurdu. Laparoskopik kolesistektomi yapıldı. Makroskopik olarak kist fundusun serozasında lokalize edildi ve immünohistokimyasal inceleme neticesine göre safra kesesinin primer müsinöz kistik neoplazisi olarak değerlendirildi.

Her ne kadar çok nadir görülse de, safra kesesinin kistik lezyonlarında musinöz kistik neoplaziler mutlaka akılda tutulmalıdır. Böyle hastalarda malign transformasyon ve özellikle daha büyük kistlerde safra kanalı tıkanıklığı gibi riskler nedeniyle kolesistektomi yapılması zorunludur.

Anahtar Kelimeler: Kolesistektomi, safra kesesi, müsinöz kistik neoplazi

INTRODUCTION

Cystic lesions originating from the gallbladder are very rare. Previously these have also been reported as mucinous cystadenoma, hepatobilier cyst adenom, biliary cystadenoma and mucinous cystic neoplasm (MCN) in the literature.

According to the World Health Organization MCN of the liver and biliary system is defined as the cyst–forming epitelial neoplasia, typically shows no contact with the bile ducts and consists of epithelium that produces mucin variably, from cubic to columnar (1). Cystic neoplasms and cystadenocarcinomas originating in the hepatobiliary system most commonly occur in the liver (85% of patients) and are related with 1% of liver cystic lesions and 5% of symptomatic liver cysts (2). MCN originating from the gallbladder is very rare and constitutes only 0.02% of cases originating from the hepatobiliary system (3, 4).

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With this case report, it is aimed to present an unusual case of MCN of the gallbladder and to review the literature.

CASE REPORT

A 63-year-old woman was admitted to our hospital with complaints of pain in the right upper abdomen, increasing after meals, and abdominal discomfort. Also the patient has a past history of ovarian tumor, having an excision 16 years ago. On physical examination the patient was obese and there was atenderness in her right upper abdomen and epigastric region. An ultrasonography of the abdomen revealed that the gallbladder dimensions were normal while the wall thickness were increased and there were calculus approximately 38 mm diameter along with axis. The diameter of the common bile duct and intrahepatic bile ducts were normal. Laboratory findings showed that liver enzymes, bilirubins and alkaline phosphatase were also within the normal limits (Alkaline phosphatase 55 U/L, gamma glutamyl transferase 28 U/L, direct bilirubin 0.45 mg/dL, total bilirubin 0.96 mg/dL). Leukocyte was 8.97 K/μL and C-reactive protein was 1.3 mg/L. A laparoscopic cholecystectomy was performed and the patient was discharged on the first day of the postoperative period without any complications.

Macroscopically, the cyst was localized in the serosa of the fundus, it was 1x1 cm in diameter and demonstrated well-defined margins and contained mucinous fluid in the lumen. The inner surface of the cyst was smooth and there were no septations. In this case, the lesion had a diagnostic challenge and we needed to differentiate it from mucinous tumors of the ovary because of the past history of ovarian tumor excision which took place 16 years ago. Microscopically, the cyst was lined by mucin-producing, tall, columnar, epithelium (Figure 1). Focal areas of the cyst were denuded. Ovarian stroma was not seen. There were no atypia, pleomorphism or increased mitotic activity in the epithelium. The gallbladder histology in our case showed a thickened cyst wall lined by a single layer of tall columnar nonmucin secreting cells, invagination into the underlying subepithelium was also seen. The underlying stroma showed congested blood vessels and dense fibrosis. There was no communication between the cyst and the gallbladder lumen. In immunohistochemical examination, the test for cytokeratin 7 (CK 7) was positive and cytokeratin 20 (CK 20), CDX 2, Wilms Tumor protein1 (WT 1), Paired-box gene 8 *(PAX 8)* was negative.

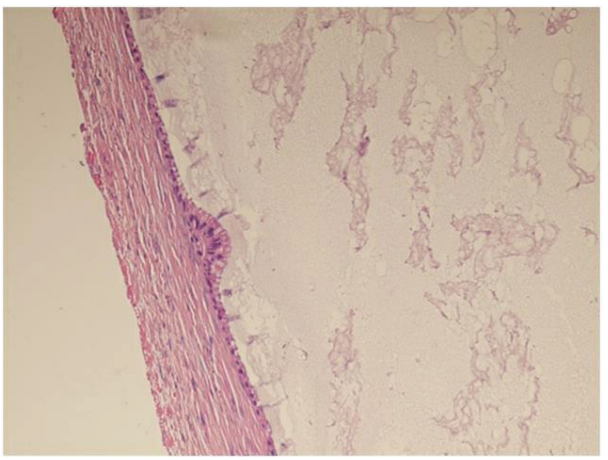

Figure 1: The figure shows the cysts are lined by flat, mucin-producing, cuboidal to columnar epithelium with basally oriented uniform nuclei.

Based on these results, the cyst was diagnosed as MCN. Thus the patient had no need for a secondary treatment.

DISCUSSION

MCN of the liver and biliary system principally occur in the liver, followed by the extrahepatic biliary system (5). Only a few case reports have been reported primarily in the gallbladder. The first case was described by Bishop in 1901, in a 40-year-old woman with a large cystic lesion in the gallbladder (6). Authors reported only 10 cases of MCN in England between 1977 and 2017 (5). A study by Devaney et al. includes 52 MCNs, of which 85% were located in the liver. Only one case (0.02%) was in the gallbladder (1). MCNs mostly occur in women. Devaney et al. also reported that 96% of patients were women (1). Our presented case was also a 63-year-old a female patient.

The etiology of MCN is not exactly understood. One theory focuses on the relation with the congenital anomalies of the biliary tract (2). Although it is known as benign lesions, there are studies reporting the association of the malignancy and MCN as well. It is reported that MCNs have a potential risk for progression to invasive carcinoma with a rate of 30% (6).

The clinical presentation depends on the location and the size of the cyst. Patients may be asymptomatic or obstructive jaundice, abdominal pain, palpable mass, nausea and vomiting may occur. Lesions originating from the cystic duct generally lead to obstructive symptoms (5).The size of the cyst may vary from small to giant cysts reaching 30 cm in size (7). In the presented case, the cyst size was 1 cm in diameter and the cyst was asymptomatic. The patient had only symptoms related to chronic cholecystitis. As in the present case, most MCNs may be incidentally discovered.

Ultrasonography (USG) is more sensitive than computed tomography in diagnosis of the cyst. Features of the internal wall of the cyst such as septation and debris are clearly evaluated by USG (1). Tomography may be useful in defining the anatomical structure, size and spread of the cyst (1). Endoscopic retrograde cholangio pancreatography can be used for therapeutic and/or diagnostic purposes, especially in patients having obstructive icterus. In the present case, the cyst was not seen with imaging methods,

it was found incidentally in macroscopic examination. Due to having no definite imaging criteria, it may be difficult to differentiate from cystadenocarcinoma preoperatively (2). However, nodular septations, or mural nodules, calcifications in the wall or septa may indicate malignancy (8).

Hydatid cysts and congenital cysts should be kept in mind in the differential diagnosis. Hydatid cyst can also be multi loculated, similar to MCNs. However, the wall of the hydatid cyst tends to be thicker and usually contains round or oval-shaped daughter vesicules (7). Serologic tests such as the hydatid cyst hemagglutination test may also help in the differential diagnosis. Lymphangiomas and abscess are the other cystic lesions of the gallbladder. Fine needle aspiration biopsy is not recommended for diagnosis of MCN because of the possibility of spreading into the peritoneal cavity (1).

Multilocular forms of MCN are more common than unilocular ones. The contents of the cyst may be serous, hemorrhagic, mucinous or mixed (1). Cysts are subclassified as pyloric gland, intestinal and biliary type, histologically. MCNs of the gallbladder may histologically be similar to other mucinous cysts arising from anywhere in the body and show mucin production and subepithelial ovarian-type stroma. Microscopically, they are characterized as three different layers. There is a cubic-columnar epithelium layer at the core, a mesenchymal stroma layer resembling ovarian stroma in the middle, and a hyalinized fibrous layer at the exterior (9). Immunhistochemically, estrogen and progesterone receptors may be positive, especially in MCN containing ovarian-like stroma. In addition, cytokeratin can be positive, and smooth muscle actin may also show strong reactivity (9). However, 10 to 15 percent of cystadenomas lack ovarian stroma. In the present case, ovarian stroma was not seen. Because of the patients having anamnesis of mucinous neoplasia of the ovary, immunhistochemical staining was applied to differentiate the MCN of the gallbladder from ovarian origin. Negative staining PAX 8 and WT 1 confirmed that the cyst was not of an ovarian origin.

In the treatment of gallbladder MCNs that have been diagnosed preoperatively and have no suspicion of malignancy, laparoscopic or open cholecystectomy should be performed (1). Since it is difficult to distinguish between malignant and benign lesions before surgery, the definitive diagnosis is made by histopathological examination. Surgeons should avoid perforating the cyst during cholecystectomy. We also applied a laparoscopic cholecystectomy to our patient. Surgical treatment was sufficient in the patient who had no findings suggesting malignancy on histopathological examination.

Although it is very rare, MCN should be kept in mind in treating cystic lesions of the gallbladder. Due to serious associated problems such as having the risk of malignant transformation or bile duct obstruction, particularly in larger cysts, a cholecystectomy is mandatory.

Informed Consent: Written consent was obtained from the participants.

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CASE REPORT / OLGU SUNUMU
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A DESMOID TUMOR IN PREGNANCY MIMICKING SUBCUTANEOUS ENDOMETRIOSIS; A CASE REPORT

GEBELİKTE NADİR GÖRÜLEN VE ENDOMETRİOMA İLE KARIŞABİLEN DESMOİD TÜMÖR OLGU SUNUMU

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ABSTRACT

Desmoid tumors are benign non-metastasizing tumors. However, they may proliferate and infiltrate into adjacent tissues with high recurrence rates. Even though its etiology is unclear, a previous histories of trauma, surgery, pregnancy, use of medication containing estrogen, and having conditions such as familial adenomatous polyposis and Gardner syndrome can be regarded as contributing or risk factors.

Keywords: Pregnancy, desmoid tumor, abdominal subcutaneous endometriosis

ÖZET

Desmoid tümörler metastaz yapmayan benign tümörlerdir fakat etraf dokulara derin infiltrasyon yapabilir ve proliferasyon gösterebilirler, rekürens oranları yüksektir. Etyolojileri net olamamakla birlikte travma, geçirilmiş operasyonlar, gebelik, eksojen östrojen kullanımı, ailesel adenomatöz polipozis ve Gartner sendromu etken olabilmektedir.

Anahtar Kelimeler: Gebelik, desmoid tümör, karın duvarı ciltaltı endometriozis

INTRODUCTION

Desmoid tumors (DTs) are rare, benign tumors. They do not metastasize, however, they may cause serious morbidity and even mortality by infiltrating into adjacent tissues and organs with high recurrence rates. DTs constitute 0.1% of all tumors, and 3.5% of fibrous tissue tumors (1). Even though its etiology is unclear, a previous history of trauma, surgery, pregnancy, use of medication containing estrogen, having conditions such as familial adenomatous polyposis and Gardner syndrome can be regarded as contributing or risk factors (2). Subcutaneous and abdominal masses encountered during cesarean sections must be carefully evaluated and consulted about with a general surgeon and pathologist to avoid a possible need for adjuvant therapy and medicolegal problems.

CASE REPORT

A 32 year old pregnant woman presented with a right periumblical subcutaneous mass. The probable diagnosis was subcutaneous endometriosis because of a history of having previously had a cesarean section. In the first trimester ultrasound scan and thereafter, both ovaries were reported as normal. The patient had felt the mass for the first time at around the 26th week of her pregnancy. At that time, an ultrasound exam revealed a heteroge-

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neous echogenic subcutaneous mass with smooth contours, resembling a subfascial intramuscular endometriosis. A planned cesarean section and concurrent excision of the mass was performed at the 39th week of pregnancy. After closure of the uterine incision, the mass was completely excised to thesurgical tumor-free margin and the primary closure of the defect was achieved. Yellow and brown tissue discoloration was noticed on macroscopic evaluation of the cross-sectioned specimen. The histopathology revealed a benign proliferative, yet infiltrative tumor located in the striated muscle. The tumor was composed of fibroblast fibers with positive margin (Figure 1, 2, 3). No complication was observed postoperatively. Even though the patient was informed about the riskof recurrence, she did not accept adjuvant therapy. No recurrence developed during the 10 years follow-up period. Written permission was taken from the patient for this case presentation.

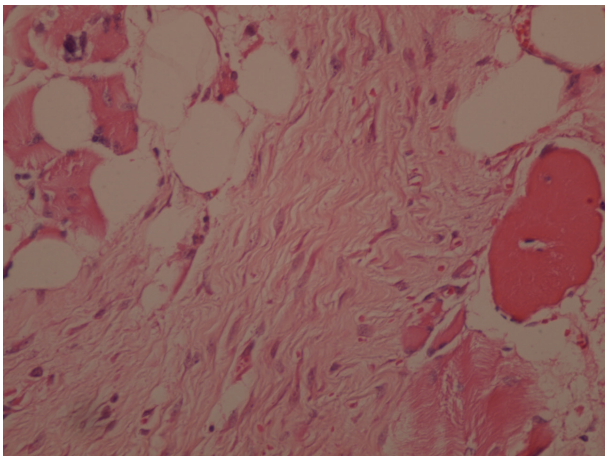

Figure 2: Microscopic closer view

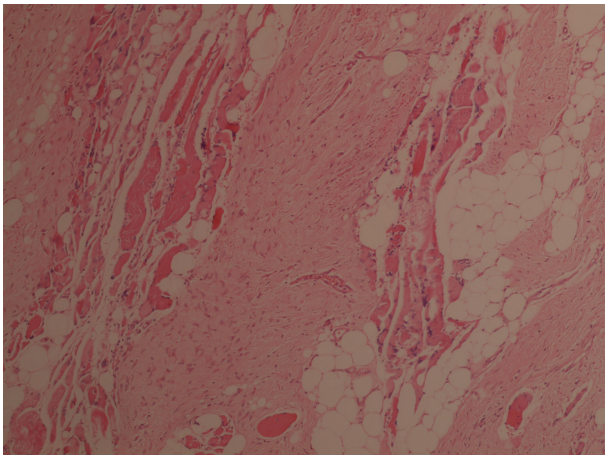

Figure 3: Microscopic view

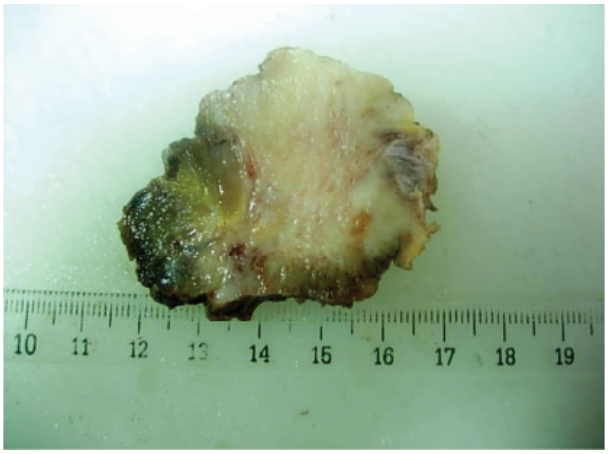

Figure 1: Macroscopic view

DISCUSSION

In the case presented here, the previous cesarean section may be counted as a risk factor and high levels of estrogen owing to pregnancy may be a contributing factor. In Lahat's case series of 16 patients, five of the DTs were diagnosed during or after delivery and two of them had infertility treatments (3). DTs are frequently located in the abdominal wall and abdominal cavity with an incidence of 37-50% and generally develop during pregnancy or postpartum period (1,2,4). In patients with a history of having previously had a cesarean, abdominal wall endometriosis comes first in differential diagnosis (3). The incidence is higher in the reproductive years and tumors generally develop 2 to 5 years following a cesarean operation. Direct inoculation of endometrial cells onto the abdominal fascia and into subcutaneous tissues is believed to be in the etiology of abdominal wall endometriosis. Unlike abdominal wall endometrioses which are located at the superficial parts of the muscle and fascia, DTs are generally located adjacent to musculoaponeurotic tissues. Computerized tomography or magnetic resonance imaging (MRI) maybe helpful in differentiating between DTs andendometriosis.

A universally accepted management protocol for DTs does not exist but complete surgical resection with negative microscopic margins is generally accepted as the gold standard approach. Adjuvant therapies, such as radiotherapy, chemotherapy and antiestrogens (i.e. tamoxiphene) can be administered for the prevention of local recurrence in selected cases (1). Fiore et al. investigated the relationship between DTs in pregnancies and local recurrences. They found that pregnancy related DTs had a better prognosis than the sporadic DT cases and the local recurrence rate decreased after surgical resection (2). In their study on 426 cases with sporadic DTs, Salas et al. found that the microscopic examination of surgical mar-

gins had no effect on prognosis, but age, tumor size and tumor location had significant effects on progression-free survival (5). They also found that an age younger than 37 years, a tumor size smaller than 7 cm and abdominal wall/abdominal cavity locations were good prognostic factors with low postoperative recurrence rates. Conservative management is generally recommended as initial approach only in selected cases with DTs due to high recurrence rates and the local aggressive behavior (5). Similarly, Kumar et al. and Le Roc'h et al. suggested conservative management of DTs in pregnancy, and leaving the surgical excision to the postpartum period (6,7). Removal of assumed subcutaneous endometriosis during a cesarean section is recommended for pathologic confirmation or, as in our case, for finding out the true nature of solid masses. Pathologic diagnosis may also help in deciding the need for possible future adjuvant therapies in selected cases and may provide coverage for medicolegal loopholes.

In summary, DTs in pregnancy are usually located on the abdominal wall or in the abdominal cavity and expectant management strategy during pregnancy is a rational option. In cases with initial tumor size larger than 7 cm or progressive growth verified by appropriate imaging modality, surgical resection is a favorable as primary approach. DTs should be kept in mind among other differential diagnoses when abdominal masses are encountered during or right after pregnancy. Long term followup must be recommended due to high recurrence rates. In cases with smaller tumors (<7 cm), whichare located on the abdominal wall or in the cavity, close followup may be an appropriate option for the management even with positive margins. In the cases of extragenital endometriosis encountered during an operation, carefull evaluation of the abdominal cavity, especially the ovaries, have to be kept in mind. The decision to remove an ovarian endometrioma during surgery mainly depends on the symptomatic severity, stage and the fertility expectancy of the patient.

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