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LETTER TO EDITOR

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Primary Cavernous Hemangioma of the Thyroid Gland

ABSTRACT

Hemangioma of the thyroid gland is a rare diagnosis and less than 35 cases have been previously published in the literature. The pathogenesis is unknown but it is suggested to be a developmental anomaly which is associated with an incapability of angioblastic mesenchyma to form canals. On microscopic examination, it should be distinguished from reactive endothelial hyperplasia, angiosarcoma or hemangiosarcoma. We report a case of a primary thyroid hemangioma, with nonspesific US appearance. Cytologic findings were nondiagnostic and final diagnosis was made at pathologic examination of the hemithyroidectomy specimen.

Keywords: Hemangioma, Cavernous, Thyroid Gland, Histopathology

Tiroid Bezinin Primer Kavernöz Hemanjiyomu

ÖZET

Tiroid bezinin hemanjiyomu nadir bir tanıdır ve literatürde günümüze kadar 35'ten az vaka yayınlanmıştır. Patogenezi tam bilinmemekle birlikte, anjiyoblastik mezenşimin kanal oluşturma yetersizliği ile ilişkili gelişimsel bir anomali olduğu ileri sürülmektedir. Mikroskobisinde, reaktif endotelyal hiperplazi, anjiyosarkom ve hemanjiyosarkomdan ayırt edilmelidir. Biz nonspesifik ultrasonografik görünüme sahip bir primer tiroid kavernöz hemanjiyomu olgusunu sunuyoruz. Sitolojik bulgular tanısal değildi ve kesin tanı spesimeninin patolojik incelemesinde kondu.

Anahtar Kelimeler: Hemanjioma, Kavernöz, Tiroid Bezi, Histopatoloji

Dear Editor,

Hemangioma is a benign neoplasm of blood vessels that can be found in all organ systems. Hemangioma of the thyroid gland is an extremely rare entity and less than 35 cases have been previously published in the literature (1,2). Here, we report a case of a primary thyroid hemangioma (TH), with nonspesific ultrasonographic appearance. Cytologic findings were nondiagnostic and final diagnosis was made at pathologic examination of the hemithyroidectomy specimen.

A 24-year-old euthyroid female presented with a slowly growing neck swelling. She had compression symptoms. She had no history of trauma, fine needle aspiration (FNA) or other surgical procedures. Ultrasonography (USG) revealed a solitary hypoechoic nodule of the right thyroid lobe. Surgery in the form of right hemithyroidectomy was done with a preoperative diagnosis of nodular goiter. On macroscopic examination, most of the right lobe was occupied by a reddish, solid lesion with a diameter of 3.8 cm that proved to be a cavernous TH. Histologically, the tumor was encapsulated with fibrous tissue and contained many cavernous thin walled vessels, some of which were thrombosed (Figure 1a, 1b). The vascular nature of the structures was confirmed by immunohistochemistry: the cells were positive for the endothelial markers CD34, SMA, FactorVIII and negative for TTF-1 (Figure 1c, 1d). No features of malignancy were identified. The definitive diagnosis of a benign and isolated hemangioma of the right thyroid lobe was made.

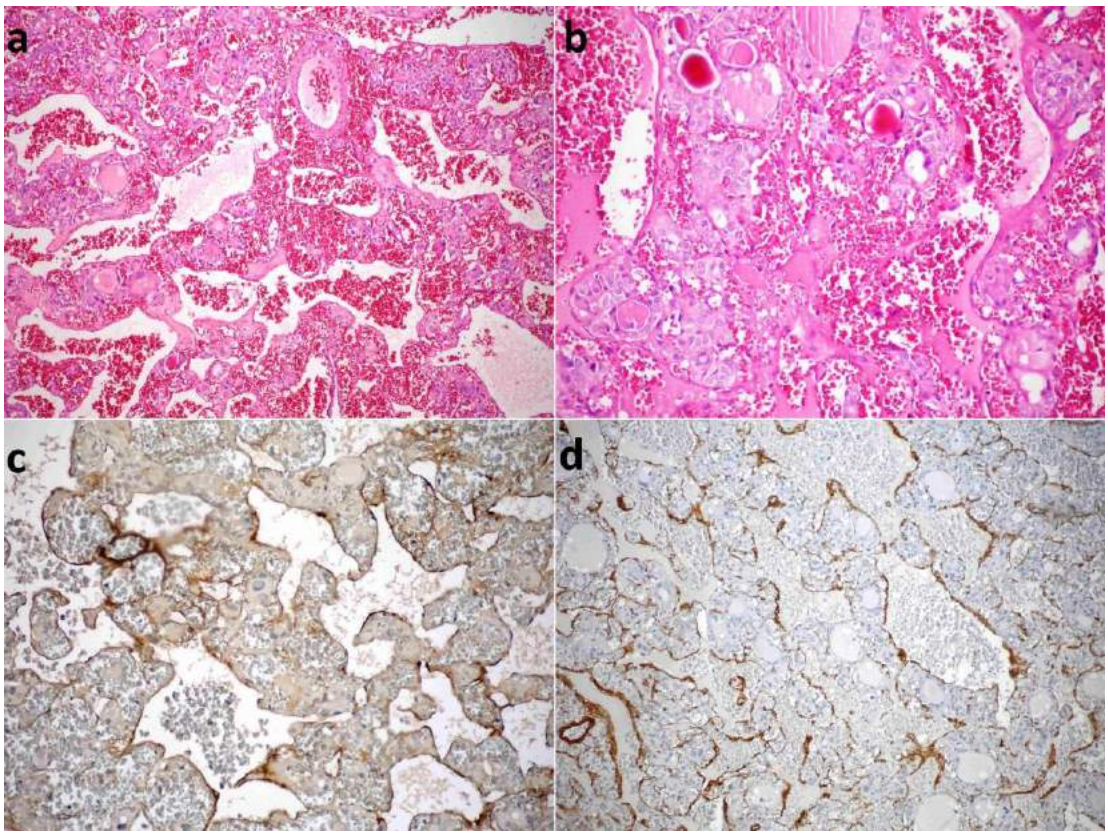

Figure 1: a, b) The tumor contains many cavernous thin-walled vessels, some of which are thrombosed (H&E, x100; H&E, x200). **c)** The cells of vascular structures were positive for the endothelial immunohistochemical markers: FactorVIII related antigene (x200). **d)** SMA expression in endothelial cells (x100).

Secondary hemangiomas have been defined as pseudomalformations, caused by the organization of a hematoma after a trauma or FNA. Primary TH is well-circumscribed capsulated mass with diameters in 20-40 mm that have a predilection for males, and predominantly affects the left lobe of thyroid (3).

The pathogenesis is unknown but it is suggested to be a developmental anomaly which is associated with an incapability of angioblastic mesenchyma to form canals (1). It is difficult to differentiate cavernous hemangiomas of the thyroid gland from other typical thyroid diseases due to the similar pattern they exhibit on USG, computed tomography or FNA cytology. Preoperatively, the diagnosis may be overlooked. Definitive diagnosis is reached only with histopathological examination. THs should be distinguished from reactive endothelial hyperplasia, angiosarcoma or hemangiosarcoma. Hemi/total thyroidectomy is the treatment of choice. An effort should be made to dissect the thyroid without rupture of these lesions in order to minimize blood loss during the operation (4).

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RESEARCH ARTICLE

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The Effectiveness of Favipiravir Treatment in Severe COVID-19 Pneumonia: a Single Centre Experience

ABSTRACT

Objective: The aim of this study was to investigate the efficacy of favipiravir (FVP) in severe COVID-19.

Methods: This is a retrospective study of 142 COVID-19 patients with severe pneumonia signs, who received inpatient treatment between March 15 and May 20, 2020. The patients were divided into two groups according to the use of FVP treatment; group 1 (n = 99) included patients who treated with FVP and group 2 (n = 43) who didn't receive FVP.

Results: Mean age was 66.47 ± 11.89 in group 1, and 68.58 ± 14.78 in group 2. Forty patients (40.4%) in group 1 and 22 (51.2%) in group 2 were treated in the intensive care unit (P > 0.05). The proportion of eosinophil, tendency of increasing thrombocyte counts and eosinophil/neutrophil ratio in FVP group was significantly higher than non-FVP group (p < 0.05). In Group 1, patients had significantly reduced erythroid series, and elevated uric acid levels as side effects of FVP. With respect to complications during hospitalization, there was no significant difference among the groups for mechanical ventilator requirement, acute kidney injury, dialysis requirement and sepsis (P > 0.05). The mortality rates in Group 1 (n = 26 [26.3%]) were lower than those in group 2 (n = 16 [37.2%]), but it was not statistically significant.

Conclusions: While the treatment of COVID-19 pneumonia options were limited during the initial stages of the pandemic, the FVP may be effective in severe cases. To confirm this effect, randomized controlled studies are needed in patients of all disease severities.

Keywords: COVID-19 Treatment, Favipiravir, Laboratory Parameteres, Severe COVID-19

Şiddetli COVID-19 Pnömonisinde Favipiravir Tedavisinin Etkinliği: Tek Merkez Deneyimi

ÖZET

Amaç: Çalışmamızda, şiddetli COVID-19'da favipiravirin (FVP) etkinliğini araştırmak amaçlandı.

Gereç ve Yöntem: 15 Mart - 20 Mayıs 2020 tarihleri arasında yatarak tedavi gören, ağır pnömoni belirtileri olan 142 COVID-19 hastası retrospektif olarak analiz edildi. Hastalar FVP tedavisinin kullanımına göre iki gruba ayrıldı; grup 1 (n = 99) FVP ile tedavi edilen hastaları ve grup 2 FVP tedavisi almayan hastaları içeriyordu.

Bulgular: Grup 1'de ortalama yaş 66,47 ± 11,89, grup 2'de 68,58 ± 14,78 idi. Grup 1'de 40 hasta (% 40,4) ve grup 2'de 22 (% 51,2) yoğun bakım ünitesinde tedavi edildi (P> 0,05) . FVP tedavi grubunda eozinofil düzeyi, trombosit sayısı ve eozinofil / nötrofil oranı FVP tedvisi almayan gruba göre anlamlı olarak yüksek bulundu (p <0.05). Grup 1'de hastalarda FVP'nin yan etkileri olarak eritroid serileri önemli ölçüde azalmış ve ürik asit seviyeleri yükselmiştir. Hastanede yatış sırasındaki komplikasyonlar açısından mekanik ventilatör ihtiyacı, akut böbrek hasarı, diyaliz gereksinimi ve sepsis açısından gruplar arasında anlamlı fark yoktu (P> 0.05). Grup 1'deki mortalite oranları (n = 26 [% 26.3]), grup 2" deki hastalardan (n = 16 [% 37.2]) daha düşüktü, ancak istatistiksel olarak anlamlı değildi.

Sonuç: COVID-19 pnömonisinde tedavi seçenekleri pandeminin ilk aşamalarında sınırlı iken, ciddi vakalarda FVP etkili olabilir. Bu etkiyi doğrulamak için, tüm hastalık şiddetlerindeki hastalarda randomize kontrollü çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: COVID-19 tedavisi, favipiravir, laboratuvar parametreleri, şiddetli COVID-19

INTRODUCTION

While the fight against the novel type coronavirus (COVID-19) pandemic continues, the numbers of COVID-19 related deaths worldwide have exceeded one million cases. The mortality rates are higher in patients with advanced age, males, and presence of more than one comorbidity (1-3). Death rates in the intensive care units can be as high as 26-80% (4-6). To date, no vaccine or WHO-approved antiviral treatment for the new virus is available. Although numerous drugs have been suggested for treatment, their efficacies are still debated (7-9). One of the interesting antivirals suggested for COVID-19 treatment is FVP, which is effective against numerous RNA viruses including the ebola virus (10). FVP was first developed in Japan in 2014, against neuraminidase resistant influenza. It is a prodrug that first enters the infected cells via endocytosis, then transformed into active favipiravir ribofuranosyl phosphate (11). FVP has been shown to demonstrate a more efficient and rapid viral clearance in COVID-19 patients when compared to other antivirals (12). The most reported side effects are abnormal liver function enzymes, diarrhea, and hyperuricemia (13). There is limited information in the literature about the role of FVP in the treatment of COVID-19 pneumonia. We aimed to investigate the efficacy of FVP in patients diagnosed with severe COVID-19 pneumonia, whose symptoms did not improve despite treatment with hydroxychloroquine (HQ), oseltamivir, and azithromycin.

MATERIAL AND METHODS

Study Design and Patient's Population: This is a retrospective study of 142 COVID-19 patients with severe pneumonia signs, who tested positive on nasopharyngeal (NP) swabs and received inpatient treatment between March 15 and May 20, 2020. The present study protocol was conducted in accordance with the Declaration of Helsinki, and after approval of the Ethics Committee of Sakarya University Faculty of Medicine (No:71522473/050.01.04/261).

According to the algorithm constructed by the coronavirus scientific advisory board, set up by the Turkish Ministry of Health, the recommended first step treatment in patients diagnosed with COVID-19 pneumonia consisted of HQ, oseltamivir, and if necessary, azithromycin. FVP, tocilizumab, or convalescent plasma are applied in patients with respiratory failure or tachypnea, and need intubation or transfer to the intensive care unit. During the initial stages of the pandemic, patients had not received FVP due to the unavailability of the drug in Turkey.

The patients were divided into two groups according to the use of FVP treatment; group 1 (n = 99) included patients who treated with FVP and group 2 (n = 43) who didn't receive FVP. Both groups were compared by measurement of the biochemical parameters, including organ dysfunction assessments before and after treatment. Initial treatment prior to FVP, age, sex, comorbid disease status, and length of hospital stay were recorded. Also, the reasons for starting FVP, initiation of treatment in the ward or intensive care, and the data for deceased patients were recorded. Patients in Group 1 had received the following drugs prior to FVP: HQ in 99%, azithromycin in 77.8%, and oseltamivir in 67.8%. In Group 2, all patients had been given HQ, azithromycin and oseltamivir without any FVP treatment. The FVP doses were, 1600 mg twice daily in day 1, 600 mg twice daily in days 2 - 5.

The inclusion criterion was COVID-19 patients with severe pneumonia signs (Presence of pneumonia clinical signs plus one of the following: respiratory rate > 30 breaths/min, severe respiratory distress, or SpO2 < 90% on room air) (14). Patients aged below 18 or above 90 years, had active bacterial infections, elevated liver enzymes, used immunosuppressive medications, and had malignancies were excluded from this study.

Statistical Analysis: Statistical analysis was performed using the IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency. To compare the qualitative data, the chi-square test or Fisher's exact test (when chi-square test assumptions do not hold due to low expected cell counts) was used. The Mann–Whitney U test was used to compare the variables that were not normally distributed. On the other hand, Student's t test was used to compare the variables with normal distribution. The statistically significant two tailed p-value was considered as < 0.05.

RESULTS

Ninety-nine patients in Group 1 (mean age 66.47 ± 11.89) and 43 patients in Group 2 (mean age 68.58 ± 14.78 years) were evaluated. There were 55 males (55.6%) in group 1, and 24 (55.8%) in group 2. Forty patients (40.4%) in group 1 and 22 (51.2%) in group 2 were treated in the intensive care unit ($p > 0.05$). Mean time of hospitalization was 15.03 ± 8.50 days for group 1, and 13.49 ± 3.73 for group 2 ($p > 0.05$). Baseline characteristics and laboratory properties of all patients are presented in Table 1 and 2. Assessment of basal biochemical parameters revealed that patients in Group 1 were more hyponatremic and hypoalbuminemic, whereas Group 2 patients had lower eosinophils and eosinophil to neutrophil ratios ($p < 0.05$). After treatment; Group 1 patients had significantly increased eosinophil counts, reduced erythroid series and elevated uric acid levels ($p < 0.05$) (Table 2). Comparison of the complications during hospitalization, there was no significant difference among the groups for mechanical ventilator requirement, acute kidney injury, sepsis and requirement to renal replacement

therapy (RRT) ($p > 0.05$) (Figure 1). Also, the mortality rates in Group 1 (n = 26 [26.3%]) were lower than those in group 2 (n = 16 [37.2%]), it was not statistically significant.

Table 1. Baseline characteristics of patients according to disease groups

Variables	Group 1 (n=99)	Group 2 (n=43)	p value
Age (year)	66.47±11.89 (37.0-90.0)	68.58±14.78 (28.0-92.0)	0.370*
Sex (M/F) n, (%)	55/44 (55.6/44.4)	24/19 (55.8/44.2)	0.977**
Onset of Symptoms			
Fever	84 (84.8)	34 (79.1)	0.399**
Shortness breathing	81 (81.8)	33 (76.7)	0.490**
Cough	70 (70.7)	36 (83.7)	0.092**
Myalgia	27 (27.3)	12 (27.9)	0.938**
Diarrhea	12 (12.1)	7 (7.0)	0.553***
Sore throat	11 (11.1)	3 (7.0)	0.552***
Anosmia	4 (4.0)	1 (2.3)	0.521***
Comorbid situations (%)			
Hypertension	49 (49.5)	24 (55.8)	0.610**
Diabetes mellitus	27 (27.3)	12 (27.9)	1.000**
Heart disease	16 (16.2)	9 (20.9)	0.656**
COPD	4 (4.0)	5 (11.6)	0.130***
Antihypertensive use (%)			
ACEI	22 (22.2)	7 (16.3)	0.561**
ARB	15 (15.2)	7 (16.3)	1.000**
Smoking (yes/no) (%)	12/87 (12.1/87.9)	5/38 (11.6(88.4)	0.934**
The onset of O2 saturation			
Mean values ± SD	88.04±8.68	90.86±4.02	0.142****
(min.-max.)	(50.00-99.00)	(80.00-95.00)	
O2 recruitment (no) (%)	78 (78.8)	32 (74.4)	0.723**
Torax CT findings			
Unilateral/Bilateral (no) (%)	12/87 (12.1/87.9)	3/40 (7.0/93.0)	0.553***
Hospitalization to ICU (no) (%)	40 (40.4)	22 (52.28)	0.235**
Mean time of hospitalization (days)	15.03±8.50 3.00-46.00	13.49±3.73 6.00-22.00	0.784****
Time of symptoms onset to admission (days)	4.42±2.27 1.0-10.0	4.41±2.66 1.0-10.0	0.759***
The first line treatment was given before FVP treatment (%)			
Hydroxychloroquine	98 (99.0)	43 (100.0)	0.697***
Azathioprine	77 (77.8)	43 (100.0)	**<0.001****
Oseltamivir	68 (68.7)	43 (100.0)	0.794**

*Independent-Samples T test, **Chi Square test, ***Fisher's Exact test, ****Mann-Whitney U tests were used. COPD: Chronic obstructive pulmonary disease, ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin II receptor blocker, CT: Computerized tomography, ICU: İntensive care unit, FVP: Favipiravir

Table 2. Comparative analysis of laboratory values obtained during baseline and discharging of Favipiravir and control groups

Variables	Basal Group-1 mean values ± SD (min.-max.)	Basal Group-2 mean values ± SD (min.-max.)	p value	End of follow up Group-1 mean values ± SD (min.-max.)	End of follow up Group-2 mean values ± SD (min.-max.)	p value
White Blood Cells NV: 5.6-10.2 (K/uL)	8.76±5.64 (2.04-37.00)	8.57±4.56 (3.20-22.00)	0.972*	6.70±2.52 (2.27-14.00(	7.11±1.75 (4.28-9.07)	0.409*
Lymphocyte NV: 0.6-3.4 (K/uL)	1.29±0.98 (0.10-8.48)	1.38±0.69 (0.36-3.70)	0.333*	1.77±0.91 (0.32-4.76)	1.58±0.53 (0.91-2.35)	0.966**
Neutrophil/ Lymphocyte ratio	7.76±8.07 (0.75-38.15)	6.47±8.19 (1.14-44.44)	0.211*	3.01±1.84 (0.83-10.38)	2.85±1.96 (1.01-8.18)	0.488*
Eosinophil NV: 0-0.7 (K/uL)	0.06±0.10 (0.001-0.56)	0.02±0.06 (0.001-0.34)	**<0.001 ***	0.20±0.14 (0.002-0.91)	0.13±0.11 (0.00-0.51)	**0.008***
Eosinophil/neutrophil ratio	0.01±0.02 (0.00-0.19)	0.007±0.16 (0.00-0.008)	**0.002***	0.05±0.04 (0.00-0.22)	0.04±0.05 (0.00-0.23)	0.072*
Red Blood Cells NV: 4.04-6.13 (K/uL)	4.50±0.70 (2.66-6.00)	4.55±0.75 (1.41-5.90)	0.654**	4.09±0.57 (3.02-5.82)	4.22±0.94 (3.20-5.71)	**0.021****
Hemoglobin NV: 12.2-18.1 (gr/dl)	12.61±2.04 (6.20-17.20)	12.92±1.85 (6.70-16.90)	0.398**	11.57±1.48 (8.37-14.50)	12.05±2.32 (9.93-16.00)	**0.003****
Platelet NV: 142-424 (K/uL)	206.07±83.39 (52.60-555.0)	198.25±79.31 (68.2-507.0)	0.446*	272.38±102.24 (41.9-580.0)	270.17±76.02 (196.0-403.0)	0.563**
D-Dimer NV: 0-500 (ug/L)	1942.2±4559.8 (119.0-35100.0)	2291.7±48488 (110.0-29.5)	0.594*	1260.2±1202.3 (158.0-7630.0)	1618.1±1150.9 (320.0-3910.0)	0.599*
Ferritin NV: 21.8-274.6mcg/L	687.30±1237.0 (9.19-9587.0)	491.51±539.6 (46.0-2069.0)	0.780*	359.16±298.87 (23.8-1261.0)	417.00±326.51 (144.0-972.0)	0.942*
Serum creatinine NV: 0.67-1.17 mg/ml	1.15±1.41 (0.20-10.00)	1.15±0.86 (0.35-4.58)	0.632*	1.21±1.50 (0.40-10.00)	0.76±0.71 (0.28-2.16)	0.344*
Uric acid NV: 3.5-7.2 (mg/ml)	4.87±1.67 (2.3-10.9)	5.55±2.29 (3.20-13.0)	0.196*	5.68 2.36 (2.20-12.00	5.40±2.86 (2.20-10.10)	**0.006***
Sodium NV: 135-145 mEq/L	136.72±5.40 (124.0-165.0)	137.98±3.45 (130.0-147.0)	**0.032***	136.74±2.96 (128.0-142.0)	134.66±4.50 (128.0-141.0)	0.678*
Serum albumin NV: 35-52 (gr/L)	30.84±4.80 (17.0-44.0)	32.54±4.22 (22.20-40.20)	**0.019***	32.30±5.20 (21.40-44.90)	31.53±4.64 (27.30-38.70)	0.174**
Lactate dehydrogenase NV: 0-248 (U/L)	349.22±144.02 134.00-855.00	307.58±96.63 (137.0-591.0)	0.215*	285.72±90.53 (147.0-506.0)	252.00±65.78 (147.0-344.0)	0.156**
C-Reactive Protein NV: 0-5 (mg/L)	79.69 67.22 (2.14-286.00)	74.79 74.62 (3.55-298.0)	0.635*	22.89±23.56 (3.02-106.00)	39.15±47.05 (3.60-102.00)	0.815*

* Mann-Whitney test and, **Independent-Samples T test were used

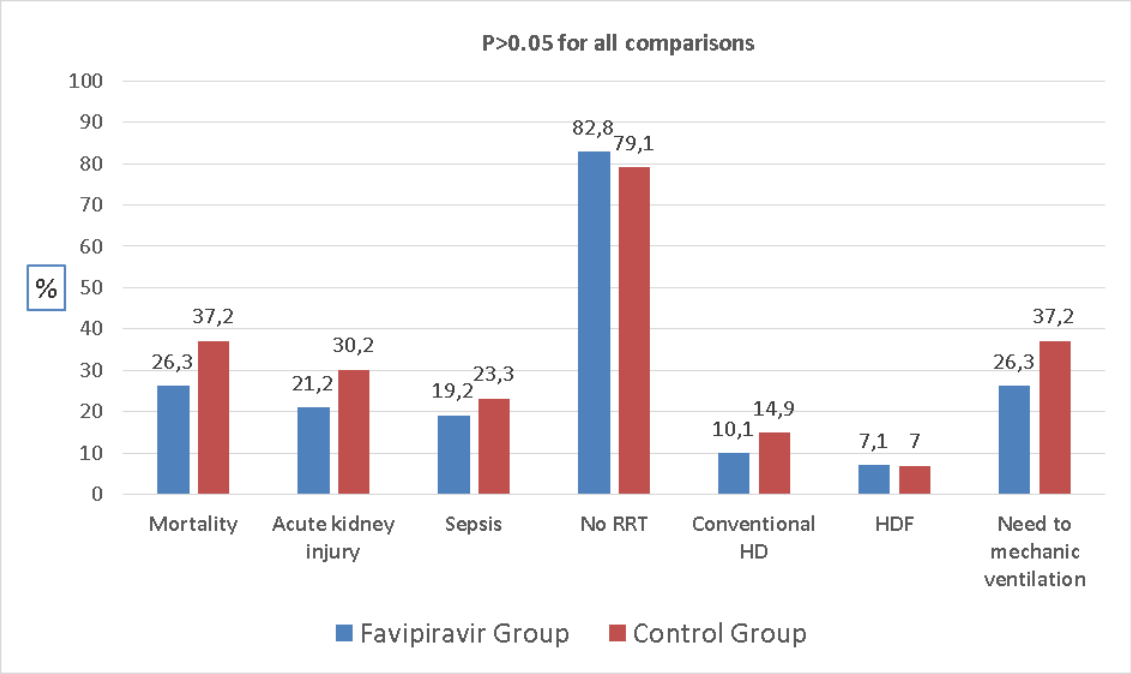

Figure 1. Comparison of two groups in terms of complications occurred during treatment period
RRT: Renal replacement therapy, HD: Hemodialysis, HDF: Hemodiafiltration

DISCUSSION

In our study, we compared FVP with some drugs used at the onset of the COVID-19 pandemic to investigate the efficacy of FVP in severe COVID-19 patients. There are no human studies that investigated the effects of FVP on COVID-19 related mortality in the literature. In the present study, although the mortality rates were lower in the FVP group compared to the Group 2, the difference was not significant ($p > 0.05$). It could be due to a small number of our patients. Because it is being used in the treatment of several RNA virus infections, FVP has been tested on numerous experimental and clinical studies (10,12,14). One non-randomized interventional small study that enrolled 80 non-severe COVID-19 patients investigated the efficacy of FVP treatment and reported a possible increase in viral clearance at day 7 with FVP (12). Our outcomes provide a comprehensive analysis of the demographic features, comorbidities, and laboratory abnormalities that are associated with mortality in COVID19 as in the literature (15,16). The important point was that the present study population included just patients who had severe disease criteria. Because there are no data related to the effect of FVP on mortality of COVID-19 infection, we believe this information is very important. FVP mortality studies were previously reported on non-COVID-19 patients. In a study that investigated the effect of high dose FVP (day 0: 6,000 mg; day 1 to day 9: 2,400 mg/d) against the Ebola virus, 99 patients were randomized by their cycle threshold (Ct) 20-value, and Ct 20 was adjusted to a RNA viral load of 7.7 log10 viral genome copies/ml. Mortality at day 14 of patients in the Ct ≥ 20-group was 20%, whereas mortality in the Ct < 20-group was 91%. These results showed that FVP treatment was highly effective in patients with high Ebola viral load (10). Another study that compared FVP monotherapy against FVP-oseltamivir combination in critically ill influenza patients did not find any significant differences (17).

Severe complications including the requirement to mechanical ventilation, acute kidney failure, sepsis, and RRT requirement were similar in both groups. The treatment protocol in our country was recommended FVP treatment in those patients who do not respond to treatment or who show disease progression, therefore FVP could be initiated only after a mean period of 5.0 ± 3.18 days. There are currently no studies that have investigated the start of FVP treatment in mild-moderate disease or before disease progression.

In our study, the proportion of eosinophil, coagulation parameters, tendency of increasing thrombocyte counts and eosinophil/neutrophil ratio in FVP group was significantly higher than non-FVP group. However, the reduction in erythroid series and hyperuricemia as side effects of FVP were significantly higher than group 2 ($p < 0.05$). Eosinophils constitute only 1-3% of the leukocytes in the circulation, they possess a proinflammatory potential and they appear at various levels in numerous diseases (18-20). A study that investigated eosinopenia as a marker for distinguishing COVID-19 pneumonia from non-COVID showed that it had 74.7% sensitivity, 68.7% specificity, and 67.3% positive predictive value (PPV). When assessed together with high

sensitive CRP, the sensitivity was 67.9%, specificity was 78.2%, and PPV was 72.8% (21). Another study found eosinopenia in 52.9% of COVID-19 patients. The eosinophil counts showed a positive correlation with lymphocyte counts in non-severe and severe patients (r = 0.486 and 0.469, respectively) ($p < 0.001$) (18). Similarly, we found that on the day of discharge the patients with severe disease receiving FVP treatment had improvements in eosinopenia, and eosinophil to neutrophil ratio values.

We recently showed that the using of standardized dose of FVP for five days reduced the erythroid series as side effects in a small study involving 62 COVID-19 positive patients (22). Also, FVP related hyperuricemia was reported previously (23).

In our patients, if FVP treatment had been initiated earlier, maybe more viral clearance could have been attained. An open-label non-randomized control study comparing FVP with lopinavir/ritonavir study in COVID-19 disease found a shorter viral clearance time for the FVP group versus the lopinavir/ritonavir group (median (interquartile range, IQR), 4 (2.5–9) day versus 11 (8–13) day, $p < 0.001$). The FVP group also showed significant improvement in chest computerized tomography compared with the control group, with an improvement rate of 91.43% versus 62.22% ($p = 0.004$). Also, FVP was independently associated with a faster viral clearance (12). However, it is not easy to talk about viral clearance with this small-scale study. Larger randomized controlled studies are needed to prove the antiviral clearance of FVP.

The limitations of the study are lack of patients with mild or moderate severity illness in either group, retrospective nature and, not adding the side effect information to the study data caused by lack of knowledge on side effects of the other drugs. In addition, because the study included patients at the outbreak onset, it was not compared with the results of patients receiving recently proven steroid therapy.

In conclusion, COVID-19 outbreak has been spreading quickly all over the world; while specific vaccine or drugs have not yet been consolidated for the time being. It is a controversial issue, at the beginning of present study, but not now, according to the algorithm determined by the scientific committee, it was deemed appropriate to start FVP treatment only in patients with severe disease criteria. Although we found lower mortality rates in severe patients using FVP, we did not find a significant difference between the two groups. In our opinion, to test the efficacy and reliability of FVP, randomized controlled studies in which the drug is given as a first line treatment to patients with different disease severities are needed.

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RESEARCH ARTICLE

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Analysis of General Surgical Consultations Requested from the Emergency Department in the Period of Pandemic and Non-Pandemic

ABSTRACT

Objective: To investigate the emergency department admissions, the general surgery consultation request, and the necessity of immediate treatment of patients even though the society did not leave the house unless necessary during the pandemic period.

Methods: The files of patients were retrospectively scanned between March-May 2020, which is the Covid 19 pandemic period, and March-May 2019, as a period in which normal social life continues. Age, gender, complaints, comorbid diseases, the necessity of truly emergency surgical treatment as well as the socioeconomic level scale for each individual were recorded and analyzed.

Results: The rate of request for consultation was 2.7% (n = 170) in the pandemic period, and 3.1% (n = 316) in the non-pandemic period. The most common complaints were nausea-abdominal pain-difficulty defecation in both periods. Although individuals without comorbidities are predominant in both periods, the proportion of individuals with comorbid diseases was higher during the pandemic period (p = 0.001). Patients who did not require urgent treatment in both periods were in the majority. In patients requiring urgent treatment, the rate of hospitalized patients was higher during the pandemic period, whereas the rate of operated patients was higher in the non-pandemic period (p = 0.005). The majority of the patients had a low socioeconomic level and a small portion had a moderate socioeconomic level in both periods (p> 0.05).

Conclusions: Although there is a significant decrease in the number of emergency department admissions and the number of general surgical consultations requested during the pandemic period, the fact that more than half of the patients who are consulted do not require emergency surgical treatment still shows the unnecessary use of emergency services.

Keywords: Covid 19, Pandemic, Emergency Department, General Surgery, Consultation

Acil Servisten İstenen Genel Cerrahi Konsültasyonları: Pandemi ve Pandemi Dışı Dönemin Analizi

ÖZET

Amaç: Pandemi döneminde toplumun gerekmedikçe evden çıkmama durumuna rağmen acil servise yapılan başvurular ve istenen genel cerrahi konsültasyonlarının acil tedavi gereksinimi incelemeyi amaçladık.

Gereç ve Yöntem: Acil servisine başvuran ve genel cerrahi konsültasyonu istenen hastaların dosyaları Covid 19 pandemi dönemi olan mart-mayıs 2020 ve normal sosyal hayatın devam ettiği bir zaman dilimi olarak seçilen mart-mayıs 2019 tarihleri arasında retrospektif olarak incelendi. Hastaların yaş, cinsiyet, başvuru şikayeti, mevcut ek hastalıklar, gerçekten acil cerrahi tedavi gerekme durumu ve sosyoekonomik durumları kayıt edilerek analiz edildi.

Bulgular: Konsültasyon isteme oranı pandemi döneminde %2.7 (n=170) iken pandemi dışı dönemde ise %3.1 (n=316) tespit edildi. Hastaların başvuru şikayetleri her iki dönemde de en sık başvuru şikayeti bulantı-karın ağrısı-dışkılama güçlüğü idi. Her iki dönemde de ek hastalığı olmayan bireyler ağırlıklı olmakla birlikte, ek hastalığı olan bireylerin oranı pandemi döneminde daha yüksektir (p=0.001). Acil müdahale gereken hastalarda ise pandemi döneminde yatırılarak tedavi edilen hasta oranı yüksek iken pandemi dışı dönemde ise ameliyat edilen hasta oranı daha yüksek tespit edildi (p=0.005). Her iki dönemde hastaların çoğunluğunun düşük ve az bir kısmının da orta sosyoekonomik düzeye sahip olduğu tespit edildi (p>0.05).

Sonuç: Her ne kadar pandemi döneminde acil servis başvuru sayısı ve istenen genel cerrahi konsültasyon sayısında belirgin bir azalma olsa da konsültasyon istenen hastaların yarısından fazlasının acil cerrahi tedavi gerektirmemesi acil servislerin hala gereksiz kullanımını göstermektedir.

Anahtar Kelimeler: Covid 19, Pandemi, Acil Servis, Genel Cerrahi, Konsültasyon

INTRODUCTION

The crowdedness and unnecessary applications made to the hospitals' emergency department (ED) throughout the world; lead us to investigate the reasons for this conundrum. During the COVID-19 pandemic period, with the 'stay at home 'call made to the community, individuals should apply to the hospitals only if necessary and postpone all other health services to prevent contamination.

Individuals with health problems cannot distinguish when and where to apply to their complaints. A clear example of this situation is seen in the emergency department of the hospitals. There are unnecessary applications to the EDs in every segment of society, and there is more than one reason for it. Patients' applications to EDs are shaped by reasons such as the type of health insurance, increasing life expectancy in the world, accompanying chronic diseases, difficulties in accessing healthcare, and incompatibilities during working hours, thus adding to the ED workload (1).

The COVID-19 disease becoming pandemic, overwhelmed health systems, strangling the global economy, and caused a devastating loss of life (2). Our hospital started serving as a pandemic hospital like all other hospitals during this period with the Ministry of Health's decision. It became an example of how to overcome this burden. Our hospital was ready to support the diagnosis and treatment of Covid-19 patients shortly from the beginning of the outbreak in Turkey. For this purpose, all units were restructured in our hospital. Most of the inpatient services in the hospital, and many healthcare personnel started to provide services for COVID-19 positive patients. The routine health services provided, other than emergency health services, was postponed (3). Emergency physicians lead the initial assessment and coordination of COVID-19 cases and continue the treatment and management of other medical emergencies. During the COVID-19 pandemic management, the emergency organization was restructured to manage other patients who would require emergency medical intervention. In our general surgery unit, elective surgeries have been delayed to provide care to patients with COVID-19. However, some interventions continued, such as trauma surgery, acute abdomen, and emergency endoscopies. This study aims to evaluate the necessity of consultations from ED to general surgery during the COVID-19 pandemic.

MATERIAL AND METHODS

Study Design and Data Collection: This retrospective cross-sectional study was conducted by scanning the files of patients consulted to general surgery by ED between 19 March-19 May 2020 (Pandemic period), where there are social incentives and measures to avoid going out of the house unless it is urgent, and between 19 March-19 May 2019 (non-Pandemic period), which is chosen as a period in which normal social life continues.

Age, gender, complaint, additional diseases, socioeconomic levels (SEL) were recorded and analyzed. Emergency surgical treatment requirements were divided into urgent and non-urgent treatment according to diagnosis. A diagnosis such as an ileus, gastrointestinal system perforations, acute appendicitis, active gastrointestinal system bleedings, ischemia and infarcts due to intraabdominal vascular occlusions, parenchymal organ injuries, perianal abscess, retroperitoneal abscess, acute thrombosed hemorrhoids, cholecystitis, biliary pancreatitis were categorized as general surgery emergencies and included in urgent treatment. Other diagnoses such as non-specific abdominal pain, peptic ulcus activation, and gastroenteritis were included in non-urgent treatment. SEL's of the patients were measured using the socioeconomic level scale. 6-14 points were calculated as low SEL, whereas 15-23 points were medium SEL, and 24-32 points were high SEL.

Primary outcome of our study was the number of consultations, and secondary outcome was the number of urgent/non-urgent diagnosis in the pandemic/non-pandemic periods.

Inclusion/Exclusion Criteria: Regardless of the rationale, all consultations requested from patients were included in the study. Consultations requested from other branches except for ED in the hospital and patients with the missing data were excluded from the study.

Statistical Analysis: IBM SPSS Statistics 22 (SPSS IBM, Turkey) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used when evaluating the study data. Pearson Chi-Square test and Fisher's Exact test were used to comparing qualitative data. The student's t-test was used to compare two groups of normally distributed quantitative variables. Significance was evaluated at the level of $p < 0.05$.

RESULTS

In the study period, a total of 16517 ED applications and 486 (2.9%) general surgery consultations were detected. While 61.8% (n=10209) of the applications were in the non-pandemic period, 38.2% (n = 6308) were in the pandemic period. In the non-pandemic period, the rate of requesting was 3.1% (n = 316), while it was 2.7% (n = 170) in the pandemic period. The ages of the patients ranged between 18 and 90, and the mean was 51.78 ± 18.55 years. The percentage of female patients in the pandemic period was 45.9%, and 48.7% in the non-pandemic period. The percentage of male patients in the pandemic period was 54.1%, and 51.3% in the non-pandemic period. The most common complaint was nausea-abdominal pain-defecation problems, with 68.7% (n = 334). There was no additional disease in 60.1% (n = 292) of the patients. 85.8% (n = 253) of 295 patients whose socioeconomic levels could be measured had low SEL. While 20.8% (n = 101) of the patients who required consultation from general surgery were operated, 20.2% (n = 98) were treated by hospitalization. The demographic and characteristic features of the patients are summarized in Table 1.

Table 1. Demographic and Characteristic Features of Patients

			Period		
		Total (n=486)	**Pandemic (n=170)**	**Non-Pandemic (n=316)**	
		n (%)	**n (%)**	**n (%)**	***P***
Gender	**Female**	232 (47.7)	78 (45.9)	154 (48.7)	[a]***0.548***
	Male	254 (52.3)	92 (54.1)	162 (51.3)	
Age in years	**18-29**	76 (15.6)	26 (15.3)	50 (15.8)	[a]***0.259***
	30-44	108 (22.2)	33 (19.4)	75 (23.7)	
	45-59	111 (22.8)	35 (20.6)	76 (24.1)	
	60-74	131 (27.0)	48 (28.2)	83 (26.3)	
	≥ 75	60 (12.3)	28 (16.5)	32 (10.1)	
	Min-Max (Median)	18-90 (52)	18-89 (55.5)	18-90 (51)	‡***0.071***
	Mean±SD	51.78±18.55	53.85±19.51	50.66±17.94	
•Complaints					
Nausea-abdominal pain- defecation difficulty		334 (68.7)	116 (68.2)	218 (69.0)	[a]***0.865***
Bloody vomiting-bloody stool		72 (14.8)	29 (17.1)	43 (13.6)	[a]***0.307***
Trauma		22 (4.5)	6 (3.5)	16 (5.1)	[a]***0.438***
Redness and tenderness on the skin		59 (12.1)	19 (11.2)	40 (12.7)	[a]***0.633***
Additional disease	**No**	194 (39,9)	50 (29,4)	144 (45.6)	[a]***0.001*****
	Yes	292 (60.1)	120 (70.6)	172 (54.4)	
Additional Diseases*					
Diabetes		41 (8.4)	23 (13.5)	18 (5.7)	[a]***0.003*****
Pulmonary diseases (Astım/ COPD)		14 (2.9)	10 (5.9)	4 (1.3)	[b]***0.008*****
Cardiac diseases (HT, CAD)		83 (17.1)	40 (23.5)	43 (13.6)	[a]***0.006*****
Stroke+ Other neurological diseases		22 (4.5)	7 (4.1)	15 (4.7)	[a]***0.750***
Organ deficiencies (Renal/liver)		31 (6.4)	18 (10.6)	13 (4.1)	[a]***0.005*****
Autoimmune (inflammatory / rheumatological)		35 (7.2)	12 (7.1)	23 (7.3)	[a]***0.929***
Malignancies		107 (22.0)	36 (21.2)	71 (22.5)	[a]***0.743***
Psychiatric diseases		7 (1.4)	6 (3.5)	1 (0.3)	[b]***0.009*****
Other (obesity surgery, hypo-hyperthyroidism)		45 (9.3)	18 (10.6)	27 (8.5)	[a]***0.458***
Treatment	**Surgery**	101 (20.8)	31 (18.2)	70 (22.2)	[a]***0.005*****
	Hospitalization	98 (20.2)	48 (28.2)	50 (15.8)	
	Outpatient	287 (59.1)	91 (53.5)	196 (62.0)	
Urgency	**Urgent**	199 (40.9)	79 (46.5)	120 (38.0)	[a]***0.069***
	Non urgent	287 (59.1)	91 (53.5)	196 (62.0)	
SEL (n=295)	**Low**	253 (85.8)	95 (88.0)	158 (84.5)	[a]***0.411***
	Middle	42 (14.2)	13 (12.0)	29 (15.5)	
	High	0 (0)	0 (0)	0 (0)	

SEL, Socioeconomic level; COPD, Chronic obstructive pulmonary disease; HT, Hypertension; CAD, Coronary artery disease
[a] *Pearson Chi-Square Test*; [b]*Fisher's Exact Test*; [‡]*Student t Test* ; * *Multiple options are marked*, ***p<0.01*

When the emergency treatment requirements of the patients who requested general surgery consultation from the ED were examined, only the emergency treatment requirement of the patients with an autoimmune disease was found to be statistically significantly higher in the pandemic period (Table 2).

Tablo 2. Analysis of Emergency Treatment Needs According to Patients' Additional Diseases

		Pandemic (n=170)	Non-Pandemic (n=316)	
Additional Disease	**Urgency**	**n (%)**	**n (%)**	***p***
Absent	**Urgent**	26 (52.0)	55 (38.2)	***[a]0.088***
	Non urgent	24 (48.0)	89 (61.8)	
Diabetes	**Urgent**	9 (39.1)	10 (55.6)	***[a]0.295***
	Non urgent	14 (60.9)	8 (44.4)	
Pulmonary diseases	**Urgent**	2 (20.0)	1 (25.0)	***[b]1.000***
	Non urgent	8 (80.0)	3 (75.0)	
Cardiac diseases	**Urgent**	15 (37.5)	16 (37.2)	***[a]0.978***
	Non urgent	25 (62.5)	27 (62.8)	
Neurological diseases	**Urgent**	1 (14.3)	5 (33.3)	***[b]0.616***
	Non urgent	6 (85.7)	10 (66.7)	
Organ deficiencies	**Urgent**	4 (22.2)	3 (23.1)	***[b]1.000***
	Non urgent	14 (77.8)	10 (76.9)	
Autoimmune diseases	**Urgent**	9 (75.0)	6 (26.1)	***[a]0.006*****
	Non urgent	3 (25.0)	17 (73.9)	
Malignancies	**Urgent**	18 (50,0)	27 (38,0)	***[a]0.236***
	Non urgent	18 (50.0)	44 (62.0)	
Psychiatric diseases	**Urgent**	3 (50.0)	1 (100)	***[b]1.000***
	Non urgent	3 (50.0)	0 (0)	
Other	**Urgent**	10 (55.6)	12 (44.4)	***[a]0.465***
	Non urgent	8 (44.4)	15 (55.6)	

*[a]Pearson Chi-Square Test; [b]Fisher's Exact Test; **p<0.01*

When the treatment complaints and emergency treatment needs of the patients were examined, the emergency treatment needs of the patients who applied with nausea-abdominal pain and defecation difficulties were found to be significantly higher during the pandemic period (Table 3).

When the age group and SELs of the patients and the urgent treatment requirements were examined, the number of patients who did not require urgent treatment was found to be significantly higher only in the 75-year-old group (Table 4).

Tablo 3. Analysis of Patients' Complaints and Emergency Treatment Needs

		Pandemic (n=170)	Non-Pandemic (n=316)	
Complaints on admission	**Urgency**	**n (%)**	**n (%)**	***p***
Nausea-abdominal pain-defecation difficulty	**Urgent**	61 (52.6)	90 (41.3)	***[a]0.048****
	Non urgent	55 (47.4)	128 (58.7)	
Hematemesis-hematochezia	**Urgent**	5 (17.2)	11 (25.6)	***[a]0.404***
	Non urgent	24 (82.8)	32 (74.4)	
Trauma	**Urgent**	3 (50.0)	3 (18.8)	***[b]0.283***
	Non urgent	3 (50.0)	13 (81.3)	
Redness and tenderness on the skin	**Urgent**	10 (52.6)	16 (40.0)	***[a]0.361***
	Non urgent	9 (47.4)	24 (60.0)	

*[a] Pearson Chi-Square Test; [b]Fisher's Exact Test; *p<0.05*

Tablo 4. Analysis of Patients According to the Age Group, Socioeconomic Level and the Emergency Treatment Needs

Age group in years	**Socioeconomic level**	**Urgency**	**Pandemic (n=170)**	**Non-Pandemic (n=316)**	
			n (%)	**n (%)**	***p***
18-29	**Low**	**Urgent**	12 (80.0)	6 (40.0)	***[a]0.025****
		Non urgent	3 (20.0)	9 (60.0)	
	Middle	**Urgent**	1 (50.0)	2 (50.0)	***[b]1.000***
		Non urgent	1 (50.0)	2 (50.0)	
30-44	**Low**	**Urgent**	10 (41.7)	10 (25.0)	***[a]0.164***
		Non urgent	14 (58.3)	30 (75.0)	
	Middle	**Urgent**	1 (33.3)	5 (62.5)	***[b]0.545***
		Non urgent	2 (66.7)	3 (37.5)	
45-59	**Low**	**Urgent**	14 (73.7)	19 (44.2)	***[a]0,032****
		Non urgent	5 (26,3)	24 (55,8)	
	Middle	**Urgent**	1 (50.0)	1 (16.7)	***[b]0.464***
		Non urgent	1 (50.0)	5 (83.3)	
60-74	**Low**	**Urgent**	14 (58.3)	18 (40.9)	***[a]0.169***
		Non urgent	10 (41.7)	26 (59.1)	
	Middle	**Urgent**	1 (33.3)	2 (28.6)	***[b]1.000***
		Non urgent	2 (66.7)	5 (71.4)	
≥ 75 yaş	**Low**	**Urgent**	0 (0)	8 (50.0)	***[b]0.003****
		Non urgent	13 (100)	8 (50.0)	
	Middle	**Urgent**	2 (66.7)	3 (75.0)	***[b]1.000***
		Non urgent	1 (33.3)	1 (25.0)	

*[a] Pearson Chi-Square Test; [b]Fisher's Exact Test; *p<0.05; **p<0.01*

DISCUSSION

There is a decrease in the number of individuals consulted from the ED to the general surgery in the pandemic period. While the ones who are urgent are expected to increase in the applications; in both periods, the majority consists of applications that do not require urgent treatment. Also, the presence of autoimmune disease in those who need urgent treatment in the pandemic period is more than a non-pandemic period. In performed studies, daily admission rates during the pandemic period were found lower for acute medical conditions, transient to moderate strokes, acute exacerbation of the chronic obstructive pulmonary disease, trauma, hospitalization for cardiovascular events, and also utilization of EDs was reduced (5-9). The government and public health campaign to discourage "over-burdening the healthcare system", public fear, and neglected treatments of chronic disease during the pandemic period may contribute to the overall decrease in the admissions and consultations.

Application complaints, presence of additional diseases, the status of socioeconomic levels, and the distribution of urgent treatment needs do not differ from the period that reflects normal social conditions compared to the pandemic period. Emergency treatment requirements in general surgical consultations are similar in both periods. The reason for obtaining similar data in both periods can be unnecessary ED applications, which continued as a result of increased stress and anxiety during the pandemic period. In performed studies, most of the patients seen in ED by general surgery physicians were taken non-operative diagnosis in both periods, even if the percentage of operative diagnosis raised in the pandemic period. In a study conducted in France, it was stated that a managerial and systematic approach should be adopted to reduce unnecessary applications to EDs (10).

During the pandemic, disease awareness is felt at different levels in each individual, group, or social class as in social life. Fear of getting COVID-19 disease, uncertainty, and similar evaluations are a source of intense anxiety, and support should be provided in this direction (11-13).

Many psychiatric symptoms can be observed in the context of COVID-19; however, increased anxiety in patients with different psychiatric diagnoses may become an important public health problem in this period (14). Considering the consultations in our study, the number of applicants who did not require urgent treatment in individuals with psychiatric diseases increased during the pandemic period. Sanguino et al. (15) pointed out an increase in depressive symptoms, anxiety, and posttraumatic stress disorder, more pronounced in the female gender during the pandemic period. While the applications to EDs during the pandemic period are expected to increase in favor of the female gender, no significant difference was observed between the two genders in our study. We think that this is due to the inclusion of only patients who required general surgery consultation in our study and that the psychiatric emergency may show a different result.

Rapid triage is essential in the operation of EDs. Pagliantini et al. reported that patients who had to be hospitalized for more than 4 hours in the ED increased from 75% to 83%. He emphasized the need for new health planning and the use of preventable health care models in acute exacerbations of chronic disease to reduce applications to the ED (16). Pines et al., in a study conducted in the United States, stated that there is an inequality between the black (African-American?) and non-black patient groups in hospital waiting for durations, a transition to service, and transition to intensive care units (17). It was found that the applications made to the ED in both periods are mostly individuals with low SEL. The fact that patients with higher education and socioeconomic level frequently apply to private health institutions has a great effect on this result (18). Emergency medical service use in low urgency cases was found high in rural areas and individuals over 65 years old (19). In our study, most of the individuals requesting consultation are low, and some are in middle SEL, and there is no individual in high SEL.

To compare the non-pandemic and the COVID-19 pandemic periods, consultations performed only for general surgery in the two months of the previous year were evaluated. The most important limitation of our study is to include only two months as the study period and not to include other emergency cases. A missing data of the patients due to retspective nature of our study was another important limitation.

As a result, EDs are the main building blocks of health services that cannot be neglected, and these units must be used for appropriate urgent reasons, as the name suggests. Although there is a significant decrease in the number of ED admissions and the number of general surgical consultations requested during the pandemic period, the fact that more than half of the patients who are consulted do not require urgent surgical treatment still shows the unnecessary use of EDs. These units that serve a dynamically should be turned into units that provide higher quality service with new, rational approaches.

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RESEARCH ARTICLE

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Relationship of ABO Blood Groups to SARS-COV-2 Infection Causing COVID-19 Disease

ABSTRACT

Objective: We aimed to investigate whether there is a predisposition to COVID-19 with ABO and Rh blood group systems.

Methods: The clinical data of 455 patients with COVID-19 seen between April 17, 2020 and June 30, 2020 at the Necip Fazıl City Hospital were retrospectively analyzed studied. The differences in the ABO blood group distribution between COVID-19 patients and the control group (7844 cases) were analyzed.

Results: The percentage of patients with type O blood in the COVID-19 group was significantly lower than that in the control group (29.7% vs. 35.6%, p = 0.009). The percentage of patients with type A and B blood in the COVID-19 set was higher than in the control group however, there were no significant difference, respectively (44.4% vs. 40.8%, 19.3% vs.16.6%, p = 0.134, p=0.123). Patients with blood group O had a lower risk of COVID-19 than A, B blood group patients (respectively; OR = 0.732, OR=0.594, p = 0.023, p=0.001). The risk of COVID-19 was higher for patients with blood groups A and B than with a blood group O (OR = 1.365, OR= 1.684, p = 0.023, p=0.001). The Rh blood group phenotype was not statistically significant in determining a patient's vulnerability.

Conclusions: The results of the present study suggest that while the blood group O had a low risk an decreased risk for infection with SARS-CoV-2, whereas blood group A and B was associated related with a increased risk, indicating that certain specific ABO blood groups were connected correlated with SARS-CoV-2 susceptibility.

Keywords: COVID-19, SARS-COV-2, Susceptibility, ABO Blood-Group System

ABO Kan Gruplarıyla SARS-COV-2 Enfeksiyonunun Neden Olduğu COVID-19 Hastalığı Arasındaki İlişki

ÖZET

Amaç: Çalışmamızda ABO ve Rh kan grubu sistemleri ile COVİD-19'a yatkınlık olup olmadığı araştırılması hedeflenmiştir.

Gereç ve Yöntem: 17 Nisan – 30 Haziran 2020 arasında Necip Fazıl Şehir Hastanesine başvuran 455 COVID-19 hastası retrospektif olarak çalışılmıştır. COVID-19 hastaları ile 7844 kontrol grubu arasındaki kan grubunun dağılım farklıkları araştırılmıştır.

Bulgular: O kan grubu taşyan COVİD 19 hastalarının oranı kontol grubu hastalarına göre anlamlı derecede düşük bulunmuştur (%29, %35, p = 0.009). A ve B kan grubu taşıyan Covid-19 hastalarının oranı kontrol grubuna göre daha yüksek bulunsa da istatistiksel olarak anlamlı bulunmadı (%44, %19.3, %16.6, p=0.123). COVİD 19 hastalarında O kan grubu taşıyan hastaların A kan grubu taşıyanlara göre daha düşük riskte bulundu (OR=0.732, OR=0.023, p=0.001). Covid -19 riski A ve B grubu taşıyan hastalarda O grubuna kıyasla daha fazla bulundu. (OR = 1.365, OR= 1.684, p = 0.023, p=0.001). Rh kan grup antigenleri hastalıkla istatistiksel olarak anlamlı bulunmadı.

Sonuç: Bu çalışmanın sonuçları; SARS-CoV 2 enfeksiyon riski O kan grubu taşıyan hastalarda daha düşük, A ve B kan grubu taşiyan hastalarda ise daha yüksek olup, ABO kan grubunun SARS-Cov-2 enfeksiyonu ile bağlantili olduğunu işaret etmektedir.

Anahtar Kelimeler: COVID-19, SARS-COV-2, Duyarlılık, ABO Kan Grubu Sistemi

INTRODUCTION

Corona viruses are enveloped viruses containing single stranded, positive–sens (+), non-segmented, ribonucleic acid (RNA) genome, the largest genome among RNA viruses. (1,2) Currently there are seven types of Corona viruse which known to be infected humans.(3) Among them; 229E, HKU1, OC43 and NL63 were not considered as profoundly pathogenic for humans and typically causes mild respiratory diseases. (3,4) Coronaviruses are known to cause outbreaks which occurred as the severe acute respiratory syndrome (SARS-CoV) in China and the Middle East (Middle East respiratory syndrome [MERS-CoV]). (5)

In December 2019, atypical pneumonia of unknown cause emerged from the Huanan Seafood Market that is located in Wuhan, the capital city of Hubei Province in China. Unfortunately, livestock animals were also traded in the market. The virus, causing the pneumonia, was named COVID-19 (Coronavirus Disease-2019) by International Committee on Taxonomy of Viruses (ICTV). [(6)] COVID-19 disease was announced as a pandemic infection by World Health Organization in March 12, 2020. (7)

ABO blood group antigens are a complex of carbohydrate molecules which are existent on the membrane of erythrocytes. It was discovered in the early 19th- century. (8) The genome that regulates ABO blood group antigens, resides on chromosome 9 (9q34). The four major blood groups regulated by the existence or inexistence of the two antigens – A and B – on erythrocyte surface. (9) Previous studies showed the association between the major blood groups and virus infections caused by certain strains. (10)

In endemic areas, Type-O blood group was shown to be asossiated to a high risk of Hepatitis B virus (HBV) infection, while Type-B blood group was related to the lower risk for HBV infection. (11,12) In another study, Type-O blood group was linked to infection caused by Norwalk virus, while Type-B blood group demonstrated resistance to the infection. (13) The P/Gb3 blood group antigen was shown to be correlated to susceptibility to Human Immunodeficiency Virus (HIV) infection. (14) High frequency of Type-A and Type-B blood types among patients diagnosed with influenza (H1A1) in comparison to the individuals with Type-O and Type-AB blood types groups, (15) and the increased susceptibility to influenza A and B in Type-AB blood group were reported previously as well. (16,17) Type-O blood group, on the other hand, was suggested to inhibit the interaction between the spinous protein of SARS-COV and the angiotensin-converting enzyme 2 (ACE2) receptor of host cell, while the inhibitory effect was demonstrated to be dose-dependent. (18) These findings suggest the ABO blood group antigens to be significant factors in the cellular invasion of viruses.

The aim of this study is the investigation of relationship between the major blood group systems considering the ABO and Rh antigens and the rate of COVID-19 diagnosis in a single health center.

MATERIAL AND METHODS

Data Sources: All patients, who were diagnosed with COVID-19 in the period from April 17th, 2020 to June 30th 2020, were enrolled in this retrospective study.

Study Design: A total of 455 patients diagnosed with COVID-19 were included in this study. The patients were allocated to four study groups formed considering the major blood groups: Type-A group, Type-B group, Type-O group, and Type-AB group. The distribution of ABO blood groups among SARS-COV-2 nucleic acid positive patients was recorded and compared with the distribution of blood groups in the control group, which included 7844 patients with determined major blood type and admitted to the center before the onset of the COVID-19 outbreak (i.e. in the period from November 2019 to December 2019). The ABO blood types and demographic features were retrieved from the database of the center.

Laboratory Testing and Data Collection: The diagnosis of COVID-19 was reached by the positive result using the real time reverse transcription polymerase chain reaction (RT-PCR) (Bioeksen, Turkey) in COVID-19 nucleic acid test, applied to nasopharyngeal and oropharyngeal samples. The Rh and ABO blood types were determined using colon agglutination method (Ortho Clinical Diagnostic, USA).

E**thics Statement:** The approval for the study was obtained from the Ethical Committee of Clinical Trials in Kahramanmaras Sutcu Imam University Faculty of Medicine (26.08.2020/20).

Statistical Analysis: All statistical analyses were performed using SPSS 25.0 software. Continuous variables are stated as the mean ± standard deviation, median (minimum - maximum values), while the categorical variables are indicated by numbers and percentages. The difference between categorical variables was evaluated using Chi Square analysis. The association between the blood types and COVID-19 positivity was assessed using Logistic Regression models. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Type-A was the most common whereas Type-AB was the least common blood groups both in the study and control groups. The blood group distribution in the control group was as follows: Type-A in 40.8%, Type-O in 35.6%, Type-B in 16.6%, and Type-AB in 7%. Rh positivity rate was 88.7% while Rh negativity rate was 11.3%. In the study group including PCR positive patients, 44.4% had Type-A blood group, 29.7% had Type-O blood group, 19.3% had Type-B blood group, and 6.6%

had Type-AB blood group in descending order of frequency (Table1).

The mean age was 44.21±19.72 years (Med:41) and 33.88±21.92 (Med:29) in the study group and control group, respectively. The patients in the former group was significantly older than the latter group. The mean age of patients was about ten years higher than uninfected individuals. The age was found as a risk factor in cases of SARS-CoV-2 infection (p=0.0001, OR=1.021) (Table 2). The rate of male patients (8.5%) were significtly high compared to the rate of female patients (3,6%) in the study group. Men were infected more than women by coronavirus among patients. The male gender was found as a risk factor in cases of SARS-CoV-2 infection, which demonstrated a statistically significant difference. (OR= 2.091) (p=0.0001) (Table 2). When the blood groups of the patients with PCR positive of COVID-19 and the normal individuals taken as a control group were compared, the major blood groups were compared between the study and control groups. The rate of Type-O blood group was low in the COVID-19 patients compared to the control group (29.7% vs. 35.6%, p = 0.009). Type-A and Type-B blood groups were more frequent in the COVID-19 patients than the control group. However, the differences were insignificant (p = 0.134 for Type-A blood group, p=0.123 for Type-B blood group). The difference between the study group and control group was minor considering the Rh antigen as well (p= 0.532) (Table 1).

Table 1. Covid-19 with diagnosis PCR (+) blood group distribution and analysis of patients and healthy individuals

		Patients (+) n=455 (%)	**Controls n=7844 (%)**	**X^2**	**P**
Age mean, med, range		44.21±19.72 (Med:41) (0-95)	33.88±21.92 (Med:29) (0-95)	Z= -9.57	0.0001*
Sex	**Male**	271 8.5%	2905 91,5%	92.37	0.0001*
	Female	184 3,6%	4939 96,4%		
A blood Type		202 44.4%	3204 40.8%	2.239	0.134
O blood type		135 29.7%	2793 35.6%	2.369	0.009*
B blood Type		88 19.3%	1300 16.6%	6.644	0.123
AB blood type		30 6.6%	547 7%	0.096	0.756
Rh positive		408 89.7%	6959 88.7%	0.389	0.532
Rh negative		47 10.3%	885 11.3%	0.389	0.532

Based on multiple logistic regression model, the patients with Type-O blood group demonstrated to have a lower risk of acquiring COVID-19 than the patients with Type-A group (OR=0.732, p=0.023) and Type-B blood group (OR=0.594, p=0.001).

The likelihood of COVID-19 positivity was high in patients with Type-A group (OR=1.365, p=0.023) and Type-B blood group (OR=1.684, p=0.001) compared to those patients with Type-0 blood group (Table 2).

DISCUSSION

COVID-19 disease, the latest worldwide viral infection, caused social and economic impacts which appear to continue for several years from the onset of the disease. Male gender, elderliness, and the presence of comorbidities such as hypertension and diabetes mellitus have been reported as the risk factors of the disease so far [19]. The results of the present study were consistent with the established knowledge since the study group was old with significant difference as compared to the uninfected control group and the male patients considerably prevailed in the study group that involved the COVID-19 cases.

Numerous researches have reported the association between the blood groups and various diseases. (10-18,20-27) In other words, each of those researches suggested relative resistance or vulnerability of certain blood groups to several viral infections. Researchers have recently reported a significant relation between the ABO blood types and the rate of SARS-CoV-2 infection as well as the duration of the disease. (26) Regular antibodies of the ABO framework were suggested to hinder the interaction between SARS CoV spike protein and angiotensin converting enzyme 2. (18)

Table 2. Multiple logistic regression models of Covid-19 patients

Reference Category		**Multiple**			
		p	O.R.	95% C.I.for O.R.	
				Lower	Upper
-	age	0.0001*	1.021	1.016	1.025
Female	sex(male)	0.0001*	2.091	1.671	2.617
0	A	0.023*	1.365	1.044	1.786
	B	0.001*	1.684	1.227	2.311
	AB	0.082	1.489	0.951	2.331
Reference Category		Multiple			
		p	O.R.	95% C.I.for O.R.	
				Lower	Upper
-	age	0.0001*	1.021	1.016	1.025
Female	Sex(male)	0.0001*	2.091	1.671	2.617
A	0	0.023*	0.732	0.560	0.958
	B	0.161	1.233	0.920	1.653
	AB	0.695	1.090	0.708	1.679
Reference Category		Multiple			
		p	O.R.	95% C.I.for O.R.	
				Lower	Upper
-	age	0.0001*	1.021	1.016	1.025
Female	Sex(male)	0.0001*	2.091	1.671	2.617
B	0	0.001*	0.594	0.433	0.815
	A	0.161	0.811	0.605	1.087
	AB	0.602	0.884	0.556	1.405
Reference Category		Multiple			
		p	O.R.	95% C.I.for O.R.	
				Lower	Upper
-	age	0.0001*	1.021	1.016	1.025
Female	Sex(male)	0.0001*	2.091	1.671	2.617
AB	0	0.082	0.672	0.429	1.052
	A	0.695	0.917	0.596	1.413
	B	0.602	1.131	0.712	1.798

Although the association between the ABO/RH blood types and vulnerability to various viral infections has previously been studied, the relation between the blood types and SARS-CoV-2 infection has not been established yet.

Guillon et al. reported that the individuals with Type-O group were relatively resistant to infection. The study included a cell model of adhesion to investigate the effect of common antibodies of the ABO framework on the interaction between the S protein and angiotensin converting enzyme 2. The authors mentioned that anti-A antibodies might inhibit the engagement of SARS-CoV antigen and its receptor, which was based on the relative vulnerability of individuals with Type-A blood group to SARS-CoV infection and the relative resistance of individuals with Type-O blood group in the study. (18)

Arac et al. reported the preponderance of Type-A blood group among the COVID-19 patients as compared to the other blood groups, particularly the Type-O group. However, the statistical analysis was mentined to demonstrate no significant difference between the infected patients and healthy individuals in terms of ABO blood groups. Furthermore, the authors suggested a relationship between Rh (D) positivity and the susceptibility to SARS-CoV infection. (25)

Zhao et al. analyzed the rates of ABO blood groups among COVID-19 patients diagnosed by using SARS-CoV-2 test in a multicenter study. The authors mentioned the patients with O histo-blood group are less likely to suffer from a COVID-19 infection. On the other hand, according to the study, individuals with A histo-blood group were identified as high-risk. (27)

The investigation on the prevalance of SARS among healthcare workers, who were exposed to infected patients without protection, reported diminished susceptibility to SARS disease in individuals with Type-O group. It has been reported that severe acute respiratory syndrome coronavirus (SARS-CoV) is less likely to infect people with blood group O compared with other blood groups. (21)

Another research, including the rates of blood groups among SARS-CoV-2 infected patients from 105 countries, suggested increased vulnerability to the infection and severity of COVID-19 disease among individuals with Type-A blood group. Type-B and Type-O blood groups, on the other hand, were found to be more resistant to the SARS-CoV-2 infection and developing less severe COVID-19 diease. (24)

Abdollahi et al. suggested a relationship between the ABO histo-blood phenotypes and vulnerability to the COVID-19 disease as well. A higher rate of infection was mentioned for the patients with the Type-AB blood group, in contrast to the lower rate of infection for the patients with the Type-O blood group. However, the Rh blood phenotypes were reported to display no significant effect on the susceptibility to the disease. (23)

It can be speculated that the decreased susceptibility of individuals with Type-O blood group and the increased susceptibility of individuals with Type-A and Type-B blood groups to COVID-19 could be linked to the presence of natural anti-blood group antibodies, particularly anti-A antibody, in the serum. However, other possible mechanisms, which obviously necessitate further researches to unveil the mystery, may cause those correlations between the blood types and rate of COVID-19 infection.

In the present study, it was determined that the proportion of patients that had COVID-19 with Type-O blood group was significantly less than the proportion of other blood groups among the infected individuals. An insignificant difference was found between the Type-A and Type-B blood groups when compared among the individuals infected with SARS-CoV-2. In all COVID-19 patients diagnosed by PCR positivity, no significant difference could be demonstrated between the Rh groups in terms of frequencies observed in this study. Also, it was found that the COVID-19 affects older individuals to a greater extent so that the mean age of patients was about ten years higher than uninfected individuals. Furthermore, according to our survey, the male gender demonstrated as a risk factor in cases of SARS-CoV-2 infection, too, which demonstrated a statistically significant difference. Our study demonstrated that men were infected more than women by coronavirus so that among patients, (OR= 2.091) (p=0.0001).

This study had some limitations. First, we couldn't reach how many patients followed-up in the intensive care unit and how many patients die due to COVID-19 in our study population. Second, we couldn't add the relationship between the severity of the disease and ABO groups in our study.

CONCLUSION

This study supports the previous observations indicating that individuals with O histo-blood group are less susceptible to SARS-CoV-2 virus infection, unlike the Type-A and Type-B blood groups contribute to the risk of COVID-19 disease. Additionally, our study suggested that age and male sex are a risk factor for the development of COVID-19. The inuence of blood group antigens on the body's immunity still needs further research to better understand the pathophysiology of SARS-COV-2 infection, which could be helpful in patient management and disease control.

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RESEARCH ARTICLE

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Evaluation of the Behavior, Anxiety and Stress of University Students in the New Type of Coronavirus Pandemic

ABSTRACT

Objective: Acute respiratory disease called COVID-19 caused by SARS-CoV-2 caused a pandemic affecting the whole world. Our aim in this study is to evaluate the cleaning behaviors and social behaviors, anxiety and stress situations of university students caused by COVID-19 pandemic.

Methods: At the beginning of Covid-19 pandemic; university students were asked questions about their socio-demographic characteristics, their cleaning and social habits, and their level of stress about being infected for themselves and their family members and also a questionnaire was applied which includes a generalized anxiety disorder scale to measure anxiety levels and Impact of Events Scale (IES) to assessing traumatic stress symptoms.

Results: It was observed that hand washing behavior increased in 62.4% of 463 participants. The mean of the students' generalized anxiety score was determined as 3.86 ± 4.86 and 17.5% (n = 81) of all students had high anxiety. The mean scores of the impact of event scale were 14.23 ± 10.9 and stress points average was found as 5.57 ± 2.9. Students' stress and anxiety affect their cleaning and social behaviors. In addition, when compared between student groups, anxiety, stress, and the IES and subscales were found to be significantly higher in female students and also in students of faculties other than medical students.

Conclusions: The state of anxiety and stress caused by pandemics affecting the whole world on people affects society and human behavior. At the beginning of the Covid 19 pandemic, an increase is observed in university students, especially in handwashing. In addition, other student groups other than medical students and female students are more affected by anxiety, stress and events.

Keywords: Coronavirus, Outbreaks, Student, Anxiety

Üniversite Öğrencilerinde Yeni Tip Corona Virus Pandemisinde Davranış, Kaygı Ve Stress Durumunun Değerlendirmesi

ÖZET

Amaç: SARS-CoV-2'nin neden olduğu COVID-19 olarak adlandırılan akut solunum yolu hastalığı tüm Dünya'yı etkileyen pandemiye neden olmuştur. Bu çalışmadaki amacımız üniversite öğrencilerinin COVID-19 pandemisi nedeniyle oluşan temizlik ve toplumsal davranışlarını, kaygı ve stress durumlarını değerlendirmektir.

Gereç ve Yöntem: Covid-19 pandemisi başlangıcında; üniversite öğrencilerine sosyodemografik özellikler, temizlik ve toplumsal alışkanlıklar, kendisine ve aile üyelerine bulaşmasıyla ilgili endişe düzeyini sorgulayan sorular ve kaygı düzeyleri ölçmek için Yaygın Anksiyete Bozukluğu Ölçeği ve stres durumlarını ölçen Olayların Etkisi Ölçeği'ni içeren anket uygulandı.

Bulgular: 463 katılımcıdan, %62,4'ünde el yıkama davranışının arttığı gözlendi. Öğrencilerin yaygın anksiyete puanı ortalaması 3,86±4,86 ve tüm öğrencilerin %17,5'inde (n=81) yüksek düzeyde anksiyete mevcuttu. Olay etki ölçeği puan ortalamaları 14,23±10,9 ve endişe puanları ortalaması 5,57±2,9. Öğrencilerin anksiyete ve endişeleri temizlik ve toplumsal davranışlarını etkilemektedir. Ayrıca öğrenci grupları arasında karşılaştırıldığında anksiyete, endişe ve olay etki ölçeği ve alt ölçekleri kız öğrencilerde ve tıp fakültesi öğrencilerine göre diğer fakülte öğrencilerinde anlamlı düzeyde daha fazla bulundu.

Sonuç: Tüm Dünya'yı etkileyen pandemilerin insanlar üzerinde yarattığı anksiyete ve endişe durumu, toplumu ve insan davranışlarını etkilemektedir. Covid 19 pandemisinin başlangıcında da üniversite öğrencilerinde özellikle el yıkama davranışında artış gözlenmektedir. Ayrıca tıp öğrencileri dışındaki diğer öğrenci gruplarında ve kız öğrencilerde daha fazla anksiyete, stres ve olaydan etkilenme yaşamaktadırlar.

Anahtar Kelimeler: Coronavirüs, Salgın, Öğrenci, Anksiyete

INTRODUCTION

SARS-CoV-2, which causes the acute respiratory disease called COVID-19, is a new beta-coronavirus. It first emerged on December 31, 2019 as a new acute respiratory infection disease of unknown etiology in Wuhan, Hubei Province, China. On January 30, 2020, the World Health Organization (WHO) declared the disease as an Internationally Important Public Health Emergency and on March 11, 2020 as a pandemic (1-3). The first confirmed COVID-19 case in Turkey was reported on March 11, 2020. With the COVID-19 pandemic, a rapid transformation and adaptation process began in the healthcare system and education system in many regions of the world and in Turkey. In order to prevent the spread of COVID-19 in our country, education was suspended in primary, secondary, and higher education as of March 16, 2020. Education at universities was moved online rather than face-to-face, and changes were made to educational processes in university institutions (4). In addition, life and work plans changed with the outbreak, and long-term home isolation further increased the levels of mental stress and anxiety in most university students (5).

Studies have shown that the number of people reporting mental health and psychosocial problems increases significantly during outbreaks (6). In addition to posing serious threats to the physical health of vulnerable people, emerging infectious diseases and large-scale outbreaks may worsen negative effects on mental health such as anxiety, depression, and other negative emotions, and they may even cause psychological crises (5). In addition, social behaviors of students change during the pandemic process. Studies show that behaviors that prevent infection, such as handwashing, are becoming more common during the pandemic (7,8).

Research shows that public health emergencies creates many psychological effects, such as fear and anxiety, and behavioral changes in university students (9). Our aim in this study is to evaluate the cleaning behaviors and social behaviors, anxiety and stress situations of university students during the coronavirus pandemic.

MATERIAL AND METHODS

A cross-sectional survey was designed to assess students' cleaning and social behavior, stress, and anxiety during the COVID-19 outbreak. The study was approved by the ethics committee of the Pamukkale University Clinical Research Ethics Commission.. It was conducted between March 3, 2020 and April 30, 2020, that is, it started in the period when COVID-19 cases were not yet detected in our country and ended when the cases started to emerge.

A face-to-face questionnaire was conducted. The students participating in the study were asked in the first part, which was six questions, about cleaning and social behaviors, along with sociodemographic features (age, gender, university department), and they were asked to score their level of stress about coronavirus infecting them and their family members between 1 and 10. In the second part, the generalized anxiety disorder scale was used to measure students' anxiety. In the third part, the Impact of Events Scale (IES) was used to measure post-traumatic stress levels.

Generalized Anxiety Disorder Scale: A seven-item, four-point Likert-type generalized anxiety disorder scale was used to evaluate the generalized anxiety disorder developed by Spitzer et al. This scale is a self-report scale with Turkish validity and reliability performed by Konkan et al. In the YAB-7 Turkish form, the most acceptable cutoff value for specificity and sensitivity was determined to be 8. This cutoff value was used in our study (10).

The Impact of Events Scale (IES): The IES is a five-point Likert-type scale composed of 22 items developed by Horowitz, Wilner, and Alvarez (1979) and whose validity and reliability in Turkish was performed by Çorapoğlu, Yargıç, Geyran, Kocabaşoğlu (2006). The scale has three subscales: 'Intrusion', 'Avoidance', and 'Hyperarousal'. The lowest score on the scale is 0, and the highest score is 88. The total score of the scale is calculated by adding up the scores obtained from each item. A high score indicates that the respondent is more affected by a negative life event, and a low score indicates that the respondent is less exposed to a negative life event (11).

RESULTS

The study included 463 students. The mean age of the students was 21.86 ± 1.96. Of the total number of students, 67% were female students. The majority of students (n = 349) stated that they received information about the coronavirus from social media. Table 1 presents the demographic characteristics of students and their sources of information about the coronavirus.

Table 1. Students' demographic characteristics and sources of information about corona virüs

Variables	n/mean	%
Age	21.56±1.96	
Gender		
Female	310	67.0
Male	153	33.0
Students' department		
Medical student	257	55.5
Health student	132	28.5
Other student	74	16.0
İnformation Sources*		
Ministry statements	209	45.1
Ministry websites	69	14.9
WHO	79	17.1
Other websites	144	31.1
Health institutions	110	23.8
Tv /newspaper	251	54.2
Social media/internet	349	75.4
Families and friends	199	43.0

* Multiple options are marked

When students were asked about changes in cleaning and social behaviors after the coronavirus started to be seen in China, it was determined that handwashing behavior increased in 62.4% of students, handshakes decreased in 34.4%, and presence in crowded environments decreased in 33.0%. Table 2 presents the findings of the answers about students' cleaning and social behaviors The mean of the students' generalized anxiety score was determined to be 3.86 ± 4.86, and 17.5% (n = 81) of all students had high anxiety. The mean scores of the IES were 14.23 ± 10.9 and rate of intrusion the subscales was 6,89 ± 4.41. Avoidance was 2.89 ± 3.9 and hyperarousal was 2.89 ± 3.9.

Table 2. Changes in students' cleaning and social habits

	Unchanged		Slightly increased		İncreased a lot	
	n	%	n	%	n	%
Washing hands	174	37.6	227	49.0	62	13.4
Buying cleaning supplies	361	78.0	89	19.2	13	2.8
Avoiding contact with people	195	42.1	183	39.5	85	18.4
	Unchanged		Slightly decreased		Decreased a lot	
	n	%	n	%	n	%
Handshake	304	65.7	148	32.0	11	2.4
Being in crowded environments	310	67.0	147	31.7	6	1.3
Frequency of purchasing products from China	278	60.0	158	34.1	27	5.8

When student groups were compared, anxiety, stress, the impact of the event, intrusion, and hyperarousal were statistically significantly lower in medical students than other health students and students from other departments (p = 0.044; p = 0.002; p = 0.001; p = 0,0001; p = 0,0001 respectively). When compared in terms of gender, anxiety, stress, the impact of the event, and subscale scores were significantly higher in girls than in boys (p = 0,0001; p = 0,0001; p = 0,0001; p = 0,0001; p = 0,0001; p = 0,0001, respectively), (Table 3).

Table 3. Assessment of students' anxiety, impact of the event and stress scores

	Anxiety score	İmpact of event score	Intrusion score	Avoidance score	Hyperarousal score	Stress score
Gender						
Female	4.42±5.06	15.29±10.47	4.85±4.45	7.13±4.15	3.27±4.03	6.05± 2.89
Male	2.73±4.22	12.09±11.63	3.54±4.71	6.39±4.84	2.13±3.58	4.58±2.96
Statistics[a]	*z=-4.278*	*z=-4.503*	*z=-2.408*	*z=-4.408*	*z=-4.210 p=*	*z=-4.952*
P	*p= 0.0001*	*p= 0.0001*	*p= 0.16*	*p= 0.0001*	*0.0001*	*p= 0.0001*
Students' department						
Medical student	3.43±4.51	12.94±10.39	3.84±4.59	6.64±4.25	2.44±3.55	5.19±2.88
Health student	4.44±5.43	14.99±11.55	4.92±4.64	6.87±4.76	3.14±4.03	5.75±3.12
Other student	4.32±4.91	17.38±11.19	5.53±4.10	7.76±4.10	4.05±4.68	6.54±2.90
Statistics[b]	*6.259*	*13.777*	*21.048*	*5.086*	*15.497*	*12.16*
P	*P=0.044*	*P=0.001*	*P=0.0001*	*P=0.079*	*P=0.0001*	*P=0.002*

[a] Significance of difference determined using Mann Whithey U
[b] Significance of difference determined using Kruskal walls

Table 4 shows the relationship between the cleaning and social behaviors of the students and anxiety, stress, and the impact of the event. It is observed that as students' anxiety, stress, and being affected by the event increase, handwashing behavior, cleaning equipment and avoiding contact with others increases and handshaking behavior decreases. In addition, anxiety, stress, and the impact of the event show a positive relationship with each other.

DISCUSSION

In this study, which began before COVID-19 cases were seen in Turkey and ended after a few cases emerged, high levels of anxiety were observed in 17.5% of university students. In studies conducted with university students in different parts of China, anxiety rates were found to be 7.7%–24.9% (5, 12). In an anxiety evaluation of nursing students in Israel, which was performed in the third week of the quarantine process, moderate anxiety was found in 42.8% and severe anxiety in 13.1% of the students (13). Anxiety was more common in female students and in students studying discplines other than medicine. In studies conducted during the outbreaks of MERS-CoV and SARS, which have similar transmission pathways, it was reported that more anxiety was observed in female students in both health and non-health areas (14-16). It was observed that high levels of anxiety were caused by the stress students experienced over whether they or

Table 4 The relationship between students' behavior, anxiety, stress and impact of event

		(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
Anxiety (A)	*r*									
	p									
Stress (B)	*r*	.308								
	p	.000								
impact of event (C)	*r*	.548	.394							
	p	.000	.000							
Intrusion (D)	*r*	.499	.428	.833						
	p	.000	.000	.000						
Avoidance (E)	*r*	.312	.202	.808	.462					
	p	.000	.000	.000	.000					
Hyperarousal (F)	*r*	.588	.419	.781	.688	.425				
	p	.000	.000	.000	.000	.000				
Washing hands (G)	*r*	.169	.297	.317	.360	.135	.337			
	p	.000	.000	.000	.000	.004	.000			
Handshake (H)	*r*	-.126	-.166	-.196	-.202	-.076	-.220	-.321		
	p	.007	.000	.000	.000	.103	.000	.000		
Cleaning materials (I)	*r*	.204	.175	.262	.266	.141	.263	.372	-.268	
	p	.000	.000	.000	.000	.002	.000	.000	.000	
Avoiding contact with people (K)	*r*	.173	.245	.273	.278	.159	.259	.264	-.074	.168
	p	.000	.000	.000	.000	.001	.000	.000	.114	.000

their families might be infected. In studies evaluating students' anxiety levels during the COVID-19 pandemic, student anxiety was shown to be related to their place of residence, the source of parental income, whether they lived with parents, and whether a relative or acquaintance was infected with COVID-19 (12).

Students commonly experience stress during these outbreaks. In a study on medical stuents carried out during the MERS-CoV epidemic in Saudi Arabia, it was stated that, although medical students have a high estimated total stress level, medical students are less stressed than nursing and non-medical university students (14, 15, 17). Similarly, this study showed that students focusing on areas other than nursing and healthcare had higher stress scores than medical students. Studies have shown that medical students have a more positive attitude, as they have more information about infectious diseases that cause epidemics (18, 19). We postulate hat this situation explains why the stress levels of non-medical students are higher than those of medical students.

In our study, an increase in cleaning and social-avoidance behaviors, especially handwashing, was observed among students. A study conducted among Korean university students during the H1N1 pandemic showed an increase in handwashing compared to the year prior (20). In a study conducted by Aker et al., 86.7% of university students saw frequent handwashing as the most important method of protection against COVID-19 (21). In another study, the majority of students stated that the first line of defense against the COVID-19 pandemic was regular handwashing, social-isolation strategies, and advanced personal-hygiene measures (22). In addition, as anxiety levels and the impact of the event increased, an increase was observed in cleaning and social behavior adjustment In the study conducted by Al-Rabiaah et al., the stress level scores and anxiety scores correlated significantly with each other, and both the stress level scores and anxiety scores were associated with social avoidance and a change of cleaning habits (14). Similarly, another study showed that there were significant changes in healthcare workers' compliance with hand hygiene and universal precautions (for example, wearing masks and gloves) during the COVID-19 pandemic, and there was a decrease in social visits, handshake habits, and the use of public facilities (23). Consequently, anxiety over and fear of pandemic situations can affect the behavior of individuals. Anxiety over becoming infected with COVID-19, given the evident global effects, may prompt individuals to adopt preventative behaviors (24, 25).

It was observed that our student group used the internet and social media as sources of information about the COVID-19 pandemic. Studies in the literature indicate that participants report using various media types to gain information about the COVID-19 pandemic; the most-used media types are social media and the internet, followed by television and newspapers (16, 18, 26). This shows that the young population is comfortable accesing these media types, and it is possible to communicate with young people through social media and the internet to change social behaviors to prevent outbreaks.

Limitations

As far as we know, this study began investigating the effects of anxiety and stress on university students before COVID-19 was detected

in Turkey. However, our research is limited by its cross-sectional structure and lacks longitudinal tracking. Finally, the research reachs only students within our university, and its voluntary nature may have led to selection bias. The participants also may not represent the entire population.

CONCLUSION

Anxiety and stress caused by infectious diseases and pandemics surrounding the world affect human behavior and society. This study started when there were no cases in our country yet and ended in the days when the cases started to appear. The results of the study are important in terms of showing the anxiety and stress it creates on people even at this point. In order to guide social behavior and take necessary precautions in the management of pandemic diseases, it is necessary to carry out such studies with more working groups during various periods of this pandemic.

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RESEARCH ARTICLE

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Evaluation of Online Anatomy Education Given in Medicine and Dentistry Faculties of Universities During Covid-19 Pandemic with Student

ABSTRACT

Objective: We are experiencing the effects of Covid-19 pandemic as the whole world. All educational facilities have been negatively affected within this period. In this study, the aim was to evaluate online Anatomy education during Covid-19 pandemic with students' feedbacks and it was questioned whether it would be efficient to use online education more actively in the following years.

Methods: A total of 1127 first and second year students from Dentistry Faculty and Medicine Faculty of Düzce, Karabük and İnönü Universities were included in the study. The survey prepared in "Google Forms" was sent online to students via "WhatsApp" application. Descriptive statistical analyses were used in data.

Results: According to analysis results, it was found that the students agreed on the content and efficiency of online anatomy education, not having learning difficulties, the layout of the lessons, the importance of the lesson, the necessity for their profession, the importance of visual tools, they are worried about not being able to do face-to-face lessons, and anatomy theoretical courses shouldn't be taught online when the pandemics is over. It was found that medicine faculty students placed more importance on anatomy education in terms of professional aspects.

Conclusions: As a result, we believe that the online Anatomy education students receive is important in terms of their professional development. However, we believe that it won't be possible for online Anatomy education to replace face-to-face education. This study will be a resource for studies to be conducted in medicine and health sciences fields in terms of online education.

Keywords: Anatomy, Online Education, Covid-19 Pandemic

Covid-19 Pandemisi Sürecinde Üniversitelerin Tıp ve Diş Hekimliği Fakültelerinde Verilen Online Anatomi Eğitiminin Öğrenci Geri Bildirimleri ile Değerlendirilmesi

ÖZET

Amaç: Dünya olarak Covid-19 pandemisinin etkilerini yaşamaktayız. Bu süreçte tüm eğitim faaliyetleri olumsuz etkilendi. Bu çalışmada Covid-19 pandemisi sürecinde online Anatomi eğitiminin öğrenci geri bildirimleri ile değerlendirilmesi amaçlandı ve online eğitimin sonraki yıllarda daha aktif kullanılmasının verimli olup olmayacağı sorgulandı.

Gereç ve Yöntem: Çalışmaya Düzce, Karabük ve İnönü Üniversitesi'nin Diş Hekimliği ve Tıp Fakültesi Dönem I ve II öğrencilerinden toplam 1127 öğrenci dahil edildi. Öğrencilere "WhatsApp" uygulaması üzerinden "Google Forms"da hazırlanan anket online olarak gönderildi. Verilerde tanımlayıcı istatistiksel analizler kullanıldı.

Bulgular: Analiz sonuçlarına göre öğrencilerin verilen online anatomi eğitimin içeri, verimliliği, öğrenme zorluğu çekmedikleri, derslerin düzeni, dersin önemi, meslekleri için gerekliliği, görsel unsurların önemi, dersi yüz yüze ders yapamamaktan dolayı endişeli oldukları ve pandemi bittikten sonra anatomi teorik derslerinin online olarak yapılmasını istemedikleri konularında hemfikir oldukları görüldü. Tıp fakültesi öğrencilerinin mesleki açıdan anatomi eğitimine daha çok önem verdikleri gözlendi.

Sonuç: Sonuç olarak öğrencilerinin aldığı online anatomi derslerinin mesleki gelişimleri için önemli olduğu kanısındayız. Ancak yine de online eğitimin yüz yüze örgün eğitim sisteminin yerini almasının söz konusu olmayacağını düşünmekteyiz. Bu çalışma online eğitimler açısından tıp ve sağlık bilimleri alanlarında yapılacak çalışmalar için bir kaynak oluşturacaktır.

Anahtar Kelimeler: Anatomi, Online Eğitim, Covid-19 Pandemisi

INTRODUCTION

Covid-19 pandemic affected all aspects of life negatively in the world and after the healthcare field; the biggest negative effects were experienced in the field of education. In this context, this is the first time that education has been affected on such as global scale and after the pandemic with the suspension of physical education in schools of all countries in the world, a transition was made to online education by necessity. For the whole world, online education is now the only resort, not the last resort (1). Countries quickly started to close this compulsory gap in education with different distance education platforms and tried to overcome the negative effects of the process on education with minimal damage. The effects of this process on the learning process of medicine and dentistry faculty students can be very serious (2).

In medicine faculties in our country, pre-graduation medical education is generally mostly classical and in the first three years theoretical courses are carried out in lecture halls, while in the second three years applied and theoretical education is conducted in faculty hospitals. Seeking alternatives to classical medicine education, especially with new technological devices that increase visuality are becoming more and more common around the world (3). This process has also been mentioned in our country recently. Anatomy is one of the disciplines in which visuality is very important and in this process of change, anatomist can have a leading role, they can switch to the fast-moving education model with modern approaches, flexible and innovative attitudes (4). Students play a vital role in medical education. At every stage of education, it is necessary to ensure student participation in the determination of objectives and curricula, faculty management and evaluation of the education and results (5, 6).

Dentistry education consists of a stressful and complicated process of education. The aim of dentistry faculties is to prepare students for the profession theoretically and practically. In dentistry faculties in our country, theoretical and clinical practices are generally carried out together (7, 8). Everyday education and technological innovations are becoming intertwined. Lectures through online systems and using three dimensional education materials in the field of education are just two simple examples (9).

Anatomy is a discipline which is not a stranger to this development. 3D digital materials produced for Anatomy education, online Anatomy atlases and technological devices such as hololens started to be used in some universities in our country. The aim of this study was to evaluate online Anatomy education given in medicine and dentistry faculties of universities during Covid-19 pandemic with students' feedbacks and it was questioned whether this compulsory online education could be a preliminary to technological innovations which are considered to be used more actively in the following years.

MATERIAL AND METHODS

In order to conduct this study, first of all permission was taken from "Scientific Research Studies on COVID-19" in the Scientific Research Platform of Republic of Turkey Ministry of Health. After İnönü University non-interventional Ethics Committee Approval (Code: 2020/817) was taken, the study was conducted on Düzce University, Karabük University and İnönü University first and second year students. The survey prepared in "Google Forms" was sent online to students via "WhatsApp" application.

In the survey text, after the information was given, the students were asked to participate voluntarily. In order to increase the reliability of feedbacks, the students who filled in the survey were told in the text that they did not have to write their names and student numbers. In this way, they were able to express their ideas more openly without being under pressure. The questions used were designed as multiple choice questions and open-ended questions were used as little as possible. Two data collection instruments were used in the study. The first one is "student information form". This form includes multiple choice and open-ended questions including students' age, gender, year of study and their views on online Anatomy education. In multiple choice questions were asked such as the importance of taking their opinions for the anatomy course, whether the interrupted anatomy course worries them, which source they use most in their laboratory studies and which resource they cannot use in online education process can affect their education more. The second data collection instrument was "Evaluation of the Online Anatomy Education Received during Covid-19 Pandemic". As a result of related literature review, the categories to be evaluated were organized as the evaluation of online Anatomy courses. In open-ended questions were asked about online anatomy educations duration, competence, visual tools, resources, comprehensibility of Latin terms, physical conditions of online education environment, importance of anatomy education in professional life. In the questions in the second part, the expressions strongly disagree, disagree, undecided, agree, strongly agree were included (10).

In power analysis, it was calculated that when α:0,05 1-β(power): 0,80, at least 191 subjects had to be included for average change in the feedbacks and recommendations of medicine and dentistry faculty first and second year students about online Anatomy education to be 4 units.

Statistical Analysis: Descriptive statistics were used in the analyses and SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA) for Windows package program was used for analysis. Average±standard deviations (ave±sd) and minimum-maximum (min-max) values of students' ages were given.

RESULTS

Descriptive statistics were used in the analysis of this study. Descriptive statistical analysis results were shown in the tables.

A total of 1127 first and second year Medicine Faculty and Dentistry Faculty students from Düzce University, Karabük University and İnönü University participated in the study. A total of 280 students, 111

(39.6%) male and 169 (60.4%) female, from Dentistry Faculties participated in the study. A total of 847 students, 366 (43.2%) male, 481 (56.8%) female, from Medicine Faculties participated in the study. Average±standard deviations (ave±sd) and minimum-maximum (min-max) values of the individuals who participated in the study are given in Table 1.

Table 2 shows the questions in the first part of the survey and the answers of students to these questions.

Table 1. Average±standard deviation (min-max) values of students

Faculty	Male		Female	
	ave±sd	min-max	ave±sd	min-max
Dentistry Faculty	20.2±1.3	18-25	20±0.9	18-24
Medicine Faculty	20.1±1.4	17-25	19.8±1.2	(17-28)

Table 2. Answers given to the questions in the first part of the survey

Survey Questions (Part I)	Dentistry Faculty	Medicine Faculty
What do you think about getting the opinions of students related with the teaching of anatomy course?		
Not necessary	7.9%	4.1%
Necessary	92.1%	95.9%
Does the fact that your formal anatomy education is interrupted due to Covid-19 pandemic worry you in terms of your internship period?		
No, I am not worried	45%	45%
Yes, I am worried	55%	55%
Which of the following did you benefit more in your laboratory studies during you formal education?		
Working with my friends	27.5%	20.8%
Working in the laboratory with models	45.4%	52.7%
Working alone with atlas/lecture notes	21.1%	20.3%
Working with cadaver	6 %	6.2%
Which of the following do you think affected your anatomy education process during your online education?		
Not being able to provide a formal education environment at home	38.6%	30.9%
Not being able to work with models in the laboratory	41.4%	48.3%
Not being able to examine the information learned on cadaver	14.6%	14.4%
Not being able to ask the instructor the question you want during online courses	5.4%	6.4%

Table 3 and Table 4 show the questions asked to the students in the second data collection part of the survey. The highest rated answers given by the students to the survey questions are shown in tables. The tables were grouped in two sections as the answers that were agreed and disagreed for the students of dentistry and medicine faculty according to the highest rated answers given to survey questions.

According to analysis results in Table 3, it can be seen that dentistry and medicine students agree upon the content and efficiency of online education given during the pandemic. However, according to survey results, students stated that they did not want Anatomy theoretical courses to be taught online after pandemic was over.

Table 3. Student opinions agreed upon according to the highest rated answers given to questions in the second part of the survey

Survey Questions (Part II)	Dentistry Faculty	Medicine Faculty
The layout of the online curriculum was sufficient for anatomy learning	Disagree (27.5%)	Disagree (32.2%)
I did not have difficulty about learning anatomy with online education	Disagree (25.4%)	Disagree (27.7%)
Visual tools increasing the sufficiency of online course are used	Agree (32.5%)	Agree (39%)
In online anatomy course, visuality is more important than face-to-face education	Agree (31.4%)	Agree (27.9%)
The information I learned in online anatomy course aroused professional interest	Agree (34.3%)	Agree (33.1%)
I consider online anatomy course necessary for my profession	Agree (44.3%)	Agree (46.4%)
I would choose online anatomy course if it was elective	Agree (30.4%)	Agree (28.6%)
I attend online anatomy course with my own will	Agree (42.1%)	Agree (46.8%)
I would attend online anatomy course even if it was not compulsory	Agree (39.3%)	Agree (44%)
Courses should be followed on time to be successful in online anatomy course	Agree (36.4%)	Agree (44.2%)
Sources about online anatomy course were sufficient	Undecided (31.4%)	Undecided (33.8%)
It is important that I prepare for the course to be successful in online anatomy course	Agree (56.4%)	Agree (49.4%)
The physical conditions (internet connection, speed, sound level, interactive facility, etc) of the environment in which I attended online courses were suitable for learning	Agree (32.9%)	Agree (38.3%)
The physical conditions (internet connection, speed, sound level, interactive facility, etc) of the environment in which the instructor taught online courses were suitable	Agree (46.4%)	Agree (46%)
Online anatomy course is taught in relation to clinical courses	Agree (40%)	Agree (40.6%)
Latin terms I learn in online anatomy course facilitate learning subjects in other courses	Agree (49.6%)	Agree (52.5%)
In online anatomy theoretical courses, instructors explain Latin terms by giving their meaning	Agree (47.5%)	Agree (41.9%)
Interruption of formal anatomy courses worries me	Agree (29.6%)	Agree (32%)
I think that anatomy theoretical courses should be taught online when the pandemics is over	Strongly disagree (33.9%)	Strongly disagree (36%)

When analysis results in Table 4 are examined, it was seen that dentistry and medicine faculty students disagreed about some answers. In the light of the answers given in Table 4, we think that medicine faculty students give more importance to Anatomy education professionally and understand the place of anatomy better in their professional life.

Table 4. Student opinions disagreed upon according to the highest rated answers given to survey questions

Survey Questions (Part II)	Dentistry Faculty	Medicine Faculty
Course hours of online curriculum are sufficient for learning anatomy	Agree (33.2%)	Undecided (28%)
Positive changes occurred in my views about the profession after taking the online anatomy course	Agree (31.1%)	Undecided (34.6%)
I can reach the instructors about subjects not understood in online courses	Agree (39.3%)	Undecided (30.6%)
I find the online anatomy education professionally sufficient	Agree (33.6%)	Undecided (28.7%)
I would like to take again if there are online anatomy courses in internship training	Undecided (32.9%)	Agree (35.9%)

DISCUSSION

The aim of this study was to evaluate online Anatomy courses with the feedbacks of medicine and dentistry faculty students who received distance education during Covid-19 pandemic. As a result of the study, online education given to medicine and dentistry faculty students during the pandemic was found to be sufficient by the students. In the light of the questions that medicine faculty and dentistry faculty students disagreed on, it was found that medicine faculty students placed more importance on Anatomy education.

Anatomy is a course which is considered as the foundation stone of medical education and it has been used in medical education since 1200s and visuality is at the forefront in Anatomy course. Each health professional who touches the patient should have sufficient information about anatomy. Anatomy information is also important for completing medical examination, making a diagnosis and communicating correctly with colleagues (11). In a study conducted in the USA, it was reported that 80.000 preventable deaths that occurred in hospitals in one year were due to lack of anatomical information (12). This shows the importance of the quality of Anatomy education given in medicine and dentistry faculties. We believe that high rates of "agree" responses given by medicine faculty and dentistry faculty students to the questions 'The information I learned in online Anatomy course aroused professional interest', 'I consider online Anatomy course necessary for my profession', 'I would choose only Anatomy course if it was elective', 'I attend online Anatomy course with my own will, 'I would attend online Anatomy course even if it was not compulsory' show the importance of Anatomy education for the students.

With the measures taken in Turkey, the country met Covid-19 later than European countries. After it was understood that the effects of pandemic would increase in our country, practices put into effect in other countries were evaluated and it was announced that 2020 spring term would be carried out completely with distance education in higher education (13).

Using distance education is reported to be one of the indicators of adaptation of countries, institutions and even individuals to the age they are in and the technology achieved (14). Despite this, the fact that laboratory and clinical practices have an important place in medicine and dentistry faculties cannot be ignored. In many countries, it is reported that the interrupted medical education causes anxiety in students (15). In our study, the result that 55% of the dentistry faculty and medicine faculty students were worried about the time of their internship is remarkable.

In literature review conducted, it has been reported that cadaver dissection training is more efficient than the best 3D training and that digital learning in Anatomy can be used only as an additional material to formal education (16). Magee (17) reported that increasing practical applications made Anatomy courses more interesting. According to Aziz et al. (18), Anatomy education based on cadaver is a prerequisite for an education that provides the best use of medical information. Adding dissection in this facilitates and accelerates students' gaining professional skills. In addition to giving scientific education, dissection provides showing an honest, ethical and humanistic approach to the patient (18, 19).

In our study, 45.4% of dentistry faculty students and 52.7% of medicine faculty students stated that "they got more benefit from practical Anatomy practices in which they worked with models in the laboratory". This situation can be compensated relatively with rich visual resources to be used in online courses. It can be seen that medicine and dentistry faculty students reported visual elements being used more frequently in online Anatomy courses and they agreed that frequent use of visual elements was more important than formal education. Despite all these, 33.9% of dentistry faculty students and 36% of medicine faculty students responded as 'strongly disagree' to the question 'I think that Anatomy theoretical courses should be taught online when the pandemics is over'.

When a study conducted in 2016 which reported that 78% of the medical faculty students used 'YouTube' social media platform to get Anatomy information (20) was considered, it can be concluded that students can try to compensate Anatomy practices interrupted due to Covid-19 in these platforms and this requires academic and ethical supervision. In our study, we believe that the "undecided" response given by students to the question about finding the resources in Anatomy courses sufficient and "disagree" responses given to the question 'I do not have difficulty in learning Anatomy with online education" show that the students may want to make use of these unsupervised platforms.

CONCLUSION

Covid-19 virus becoming a pandemic in the world affected education and higher education systems in all the countries the pandemic was experienced and it has caused distance education given with the support of nations to replace formal education in order to slow down this pandemic. As a result of our study, while students of the dentistry faculty and medical faculty were concerned about practical courses not being carried out and theoretical courses being taught online, they consider the online anatomy courses they take as professionally important, they will choose anatomy if there is an elective course, and what they learn in the anatomy course has a high level of professional interest. It is seen that they are in a great consensus that they do not want the anatomy education to continue online after the pandemic is over. All these answers shows the importance that they give to anatomy education. It was concluded that although distance education model is a modern education method that can be used 7/24, formal education is preferred by students in sciences which are supported with visual elements and laboratory-dissection practices, like Anatomy.

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Is It Possible To Predict Mortality Using Initial Data Of Adult Patients Hospitalized with COVID-19? A Mortality Prediction Model in the Early Phase of COVID-19

ABSTRACT

Objective: In this study, we aimed to determine the factors that contribute to the early determination of mortality risk in patients hospitalized with COVID-19.

Methods: We included 941 adult inpatients (474 male [50.4%], mean age, 53.5±17.0. The patients were divided into two groups: the discharge group and the death group. Epidemiological data, medical history, underlying comorbidities, laboratory findings, chest computed tomographic scans, real-time reverse transcription polymerase chain reaction detection results, and survival data were obtained with retrospective recordings on admission and follow-up. The statistical relationship between survival data and parameters was analyzed. A mathematical model was created from the data of both groups.

Results: While 863 patients survived, 78 were non-survivors. During the study period, the preliminary case fatality rate of the inpatients was 8.3%. The mean age of the non-survivors was 71.7±11.2 SD (P <0.001). Laboratory findings showed that mortality was high in those with high D-dimer, sodium, lactate dehydrogenase (LDH), troponin, creatine kinase-myocardial band (CK-MB), ferritin, blood lactate, activated partial thromboplastin time, and high blood glucose levels (P <0.05). Furthermore, mortality was high in patients with low albumin, lymphocyte, and platelet levels (P <0.05). The logistic regression model showed that advanced age, hypertension, high D-Dimer (>1000 ng/ml), high C-reactive protein (CRP), CK-MB, and LDH, and low lymphocyte count were associated with poor prognosis.

Conclusions: According to week 1 data of patients with COVID-19, advanced age, hypertension, D-Dimer, CRP, CK-MB, high LDH, and low lymphocyte were associated with poor prognosis. We believe that this model will be useful in predicting patient mortality.

Keywords: COVID-19, Mortality, Predictive Factors

COVID-19 Tanısıyla Hastaneye Yatırılan Yetişkin Hastaların İlk Verilerini Kullanarak Ölüm Oranını Tahmin Etmek Mümkün Müdür? COVID-19'un Erken Evresinde Bir Ölüm Tahmin Modeli

ÖZET

Amaç: Bu çalışmada COVID-19 tanısıyla hastaneye yatırılan hastalarda mortalite riskinin erken dönemde belirlenmesine katkıda bulunan faktörleri belirlemeyi amaçladık.

Gereç ve Yöntem: Hastanede yatan 941 COVID-19 tanılı erişkin hasta (474 erkek [% 50.4], yaş ortalaması 53.5 ± 17 çalışmaya dahil edildi. Hastalar taburcu edilenler ve mortal seyredenler olarak iki gruba ayrıldı. Epidemiyolojik veriler, tıbbi öykü, altta yatan komorbiditeler, laboratuvar sonuçları, akciğer bilgisayarlı tomografi görüntüleri, PCR sonuçları, sağkalım verileri, başvuru ve takipte geriye dönük olarak kaydedildi. Sağkalım verileri ile parametreler arasındaki istatistiksel ilişki incelendi.Her iki grup verilerinden matematiksel bir model oluşturuldu.

Bulgular: 863 hasta hayatta kalırken, 78 hasta mortal seyretti. Çalışma süresi boyunca, yatan hastaların ilk vaka ölüm oranı % 8.3 idi. Mortal grupta hastaların ortalama yaşı 71.7 ± 11.2 SD idi (P <0.001). Laboratuvar bulgularında, D-Dimer, sodyum, laktat dehidrojenaz (LDH), troponin, kreatin kinaz-miyokardiyal bant (CK-MB), ferritin, kan laktat, aktive parsiyel tromboplastin zamanı ve kan şekeri düzeyleri yüksek olanlarda ölüm oranının yüksek olduğu tespit edilmiştir (P <0.05). Ayrıca; albümin, lenfosit ve trombosit düzeyi düşük hastalarda da mortalite yükse saptandı (P <0.05). Lojistik regresyon modeli, ileri yaş, hipertansiyon, yüksek D-Dimer (> 1000 ng / ml), yüksek C-reaktif protein (CRP), CK-MB ve LDH ve düşük lenfosit sayısının kötü prognozla ilişkili olduğunu gösterdi.

Sonuç: COVID-19 hastalarının 1. hafta verilerine göre ileri yaş, hipertansiyon, yüksek D-Dimer, CRP, CK-MB, LDH ve düşük lenfosit kötü prognozla ilişkilendirildi. Bu modelin hasta ölümlerini tahmin etmede faydalı olacağına inanıyoruz.

Anahtar Kelimeler: COVID-19, mortalite, prediktif faktörler

INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak, first detected in Wuhan, China in December 2019, has become a pandemic with millions of cases and thousands of deaths reported (1).The case fatality rate (CFR) is calculated by dividing the number of deaths from an illness/disease by the number of people diagnosed with the disease. CFR is used to identify outbreak measures and evaluate the success of treatment. Mortality in patients with COVID-19 varies across countries, regions, and hospitals. For example, while mortality at the age of 30–39 is 0.3%, it is 1.3% at 50–59 years, 2.5% at 60–69 years, and 9.7% at 70–79 years (2-4). Hence, the chances of survival are related to the country's health system and medical facilities, as well as age-related. Recent studies reported a 3.2% CFR, ranging between 2% and 4% with strong heterogeneity between studies (5), and it differs between mortality rates calculated at the beginning of an epidemic. In the later stages of the epidemic, the CFR varies with the deaths that occur in patients presumed to survive (6).

Many factors or a combination of factors affect mortality and morbidity. Various factors associated with poor prognosis in COVID-19 have been identified. Advanced age, male gender, hypertension, high blood clotting factors, fibrin degradation products, D-dimer, and acute phase reactants were the main defined factors. Identifying these factors is extremely crucial for developing measures needed in new outbreaks. Could the serious course of patients with COVID-19 be predicted by considering the clinical and laboratory findings at the beginning of the disease? This study aims to analyze the initial data of patients hospitalized with COVID-19 and to reveal the model that can rapidly identify those with serious disease risk.

MATERIALS AND METHODS

Hospital and Patients: Sakarya city is a province in Turkey, located in the Eastern Marmara Region, with approximately 1 million inhabitants and gained the status of Metropolitan Municipality. This study was conducted in Sakarya University Training and Research Hospital, which is the only tertiary health care service hospital in Sakarya city. This hospital was commissioned by the Ministry of Health as a reference hospital to serve patients with COVID-19 and had served the most patients in the city during this pandemic. We included 941 adult inpatients (474 male [50.4%], 467 female [49.6%]; mean age, 53.5 ± 17.0 SD [median 54]).

Case Definition: The Republic of Turkey Ministry of Health Covidien-19 met the case definition criteria and verified that a polymerase chain reaction (PCR) test (nasal swab or farineal [nasopharyngeal] sample in the presence of severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] RNA), which enrolled patients. Treatment and care were given to patients in line with this guide.

PCR Samples: Oro/nasopharyngeal samples were collected by Dacron-flocked swabs, using a single swab. First, the swab was inserted into the oropharynx and then into the nasopharynx. Oropharyngeal swabs were collected by inserting the swab into the posterior oropharynx and swabbed for 2–3 seconds. The swab was then inserted through the nostril using a rotating movement until it reaches the nasopharynx and the sample was obtained by gently rotating the swab for 2–3 seconds. The swab was placed into a 5-ml tube containing 2 ml viral transport medium.

After the samples were accepted in the microbiology laboratory, they were taken to level 3 biosafe negative pressure laboratory. Bio-Speedy® Viral Nucleic Acid Isolation Kit for the isolation of total nucleic acid from samples (bioeks, Turkey) was used. Reverse transcription (RT)-PCR Bio-Speedy® COVID-19 RT-qPCR Detection Kit (bioeks, Turkey) was used. Isolation, PCR amplification, and evaluation of the results were carried out in line with the recommendations of the manufacturer.

Treatment: Hydroxychloroquine (HDC) was given to hospitalized patients for five days, and favipiravir was given to patients who did not respond to HDC treatment for five days. Patients who did not respond to favipiravir were evaluated for plasma treatment or intensive care monitoring or both. Enoxaparin was given to all patients with high D-dimer and ferritin or at risk for coagulability.

Serious Disease Criteria: Patients with the severe course were followed up in the intensive care unit. The disease was considered severe if a patient had any of the following:

- respiratory distress and/or respiratory rate >30 minutes
- oxygen saturation <93%
- resting and partial arterial oxygen pressure/inspiratory oxygen fraction ratio of ≤300 mmHg
- respiratory failure requiring mechanical ventilation
- shock
- other organ failures requiring ICU treatment

Laboratory Tests: Blood samples were obtained within 24 hours of admission to the hospital. All measurements were made within 3 hours after the blood was sent to the laboratory.

Discharge Criteria: For a hospitalized patient, no fever in the last 48 hours or disappearing respiratory symptoms or negative test result for the SARS-CoV-2 RT-PCR test at a 24-hour interval after the treatment were evaluated as discharge criteria.

Data Collection: The data were collected by authors O.K. and E.G. at the infectious diseases clinic. Epidemiological data, medical history,

underlying comorbidities, admission, laboratory findings, chest computed tomographic scans, real-time RT-PCR detection results, and survival data were obtained from the retrospective records of patients. These data were examined by two doctors from the public health department.

Statistical Methods: In descriptive statistics, frequencies and percentages were determined. Chi-square test of independence and Fisher's exact test was calculated, comparing the frequency of independent variables in survivors and non-survivors. A p-value <0.05 was considered statistically significant. Phi and Cramer's V values were used in effect size calculations.

A multivariate binary logistic regression model was used to determine the independent effects on the association between mortality and independent variables, estimating odds ratio, and confidence interval (CI, 95%). The variables with statistically significant associations ($P < 0.05$) with mortality were kept in the final model.

Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the model were calculated with 95% CI. When calculating CI for sensitivity, specificity, and accuracy, Clopper–Pearson CI were used and for CI of the predictive values, the standard logit CI stated in Mercaldo et al. 2007 publications were based.

The receiver operating characteristic (ROC) curve was plotted for the regression model and the area under the curve (AUC) was calculated. Analyses were performed using Statistical Package for the Social Sciences version 20.0 (IBM SPSS Statistics; Armonk, NY, USA).

Approvals: This research was carried out with permission from the local hospital management and the Turkish Ministry of Health. All data have been approved by the local ethics committee. In this retrospective cohort type study, the first-week data of adult patients diagnosed with COVID-19 who were hospitalized between 21 March and 30 April 2020 in Sakarya University Training and Research Hospital were included. An ethics committee approval from Sakarya University Medical Faculty was provided for this study (approval number: 71522473/050.01.04./310).

RESULTS

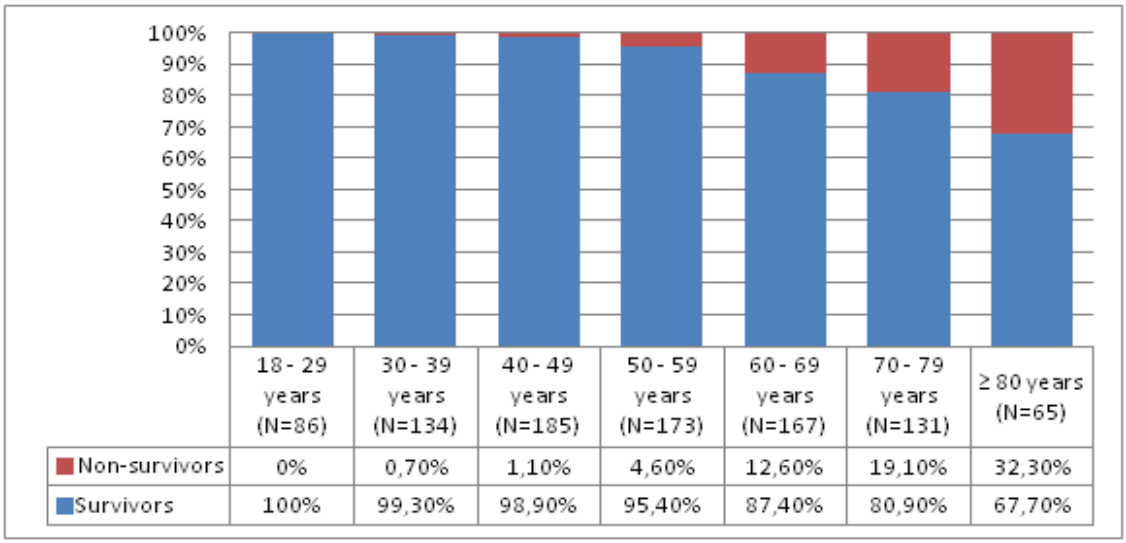
While 863 patients survived, 78 were non-survivors. During the study period, the preliminary CFR of the inpatients was 8.3%. However, the age range at which most deaths were detected was >70 years old. The number of cases >80 years old was small (Figure 1). The mean age of the survivors was 51.9 ± 16.5 SD (median, 51) and the mean age of the non-survivors was 71.7 ± 11.2 SD (median, 72) ($P < 0.001$). The demographic, clinical history, and laboratory findings of the patients are summarized in Table 1.

Table 1. Comparisons of demographics, clinical history and laboratory findings of Covid-19 inpatients in Sakarya University Training and Research Hospital (SUTRH).

Parameters	**Survivors**	**Non-survivors**	**Statistic**
	N (%)	N (%)	Test score *p* value Effect size
Gender			X^2 (1, N = 941) = 1.24
Female	433 (92.7)	34 (7.3)	p = 0.265
Male	430 (90.7)	44 (9.3)	*Phi* = 0.036
Age group			X^2 (1, N = 941) = 80.38
< 60 years of age	567 (98.1)	11 (1.9)	p < 0.001
≥ 60 years of age	296 (81.5)	67 (18.5)	*Phi* = 0.292
Having at least one underlying disease			X^2 (1, N = 941) = 101.52
No	693 (96.8)	23 (3.2)	p < 0.001
Yes	170 (75.6)	55 (24.4)	*Phi* = 0.328
Hypertension			X^2 (1, N = 941) = 32.33
No	775 (93.6)	53 (6.4)	p < 0.001
Yes	88 (77.9)	25 (22.1)	*Phi* = 0.185
Diabetes Mellitus			X^2 (1, N = 941) = 150.27
No	797 (95.7)	36 (4.3)	p < 0.001
Yes	66 (61.1)	42 (38.9)	*Phi* = 0.400
Chronic Renal Disease			N = 941
No	851 (92.0)	74 (8.0)	p = 0.037*
Yes	12 (75.0)	4 (25.0)	*Phi* = 0.080
Chronic obstructive pulmoner disease or Asthma			X^2 (1, N = 941) = 0.20 p = 0.652
No	818 (91.8)	73 (8.2)	*Phi* = 0.015

Yes	45 (90.0)	5 (10.0)	
D-dimer			X^2 (1, N = 940) = 155.99
< 1000 ng/mL	647 (99.2)	5 (0.8)	p < 0.001
≥ 1000 ng/mL	216 (75.0)	72 (25.0)	*Phi* = 0.407
CRP			X^2 (1, N = 918) = 207.73
< 100 mg/L	689 (99.0)	7 (1.0)	p < 0.001
≥ 100 mg/L	151 (68.0)	71 (32.0)	*Phi* = 0.476
Troponin			X^2 (1, N = 320) = 60.67
< 50 ng/mL	223 (86.8)	34 (13.2)	p < 0.*001*
≥ 50 ng/mL	26 (41.3)	37 (58.7)	*Phi* = 0.435
CK-MB			X^2 (1, N = 885) = 97.68
< 25 ng/mL	710 (96.1)	29 (3.9)	p < 0.*001*
≥ 25 ng/mL	105 (71.9)	41 (28.1)	*Phi* = 0.332
Albumin			X^2 (1, N = 684) = 200.70
≥ 30 g/L	508 (98.6)	7 (1.4)	p < 0.001
< 30 g/L	100 (59.2)	69 (40.8)	*Phi* = 0.542
Creatinine			X^2 (1, N = 920) = 162.01
< 1.5 mg/dL	799 (95.6)	37 (4.4)	p < 0.*001*
≥ 1.5 mg/dL	47 (56.0)	37 (44.0)	*Phi* = 0.420
Sodium			X^2 (1, N = 736) = 7.83
< 140 mmol/L	437 (92.0)	38 (8.0)	p = 0.005
≥ 140 mmol/L	223 (85.4)	38 (14.6)	*Phi* = 0.103
Ferritin			X^2 (1, N = 892) = 125.61
< 1000 µg/l	711 (96.7)	24 (3.3)	p < 0.001
≥ 1000 µg/l	110 (70.1)	47 (29.9)	*Phi* = 0.375
LDH			X^2 (1, N = 887) = 125.61
< 500 U/L	725 (96.7)	25 (3.3)	p < *0.001*
≥ 500 U/L	85 (62.0)	52 (38.0)	*Phi* = 0.444
Fibrinogen			N = 260
< 200 mg/dL	5 (83.3)	1 (16.7)	p = 0.505*
≥ 200 mg/dL	187 (73.6)	67 (26.4)	*Phi* = 0.033
Blood Lactate			
< 2 mmol/L	256 (92.1)	22 (7.9)	X^2 (2, N = 568) = 6.74
2 – 3.9 mmol/L	225 (86.5)	35 (13.5)	p = 0.034
≥ 4 mmol/L	24 (80.0)	6 (20.0)	*Cramer's V* = 0.109
Active partial thromboplastin time			X^2 (1, N = 695) = 87.70
< 33.5 sec	596 (92.4)	49 (7.6)	p < 0.001
≥ 33.5 sec	25 (50.0)	25 (50.0)	*Phi* = 0.355
Glucose			X^2 (1, N = 754) = 117.09
< 200 mg/dL	613 (94.7)	34 (5.3)	p < 0.001
≥ 200 mg/dL	65 (60.7)	42 (39.3)	*Phi* = 0.394
Hematuria			X^2 (1, N = 634) = 0.09
< 3 RBC in urine	337 (95.2)	17 (4.8)	p = 0.757
≥ 3 RBC in urine	268 (95.7)	12 (4.3)	*Phi* = 0.012
Proteinuria			X^2 (1, N = 636) = 31.82
Negative	442 (98.2)	8 (1.8)	p < 0.001
Positive	163 (87.6)	23 (12.4)	*Phi* = 0.224
Lymphocyte Count in CBC			
≥ 1000 /mm^3	569 (99.0)	6 (1.0)	X^2 (1, N = 898) = 163.81
800 – 999 /mm^3	104 (92.9)	8 (7.1)	p < 0.001
< 800 /mm^3	148 (70.1)	63 (29.9)	*Cramer's V* = 0.427
Platelet Count in CBC			
≥ 150,000/mm^3	584 (93.4)	41 (6.6)	X^2 (1, N = 898) = 19.22
100,000 – 149,999/mm^3	220 (88.4)	29 (11.6)	p < 0.001
< 100,000/mm^3	17 (70.8)	7 (29.2)	*Cramer's V* = 0.146

**Fisher's Exact Test*



	18 - 29 years (N=86)	30 - 39 years (N=134)	40 - 49 years (N=185)	50 - 59 years (N=173)	60 - 69 years (N=167)	70 - 79 years (N=131)	≥ 80 years (N=65)
Non-survivors	0%	0,70%	1,10%	4,60%	12,60%	19,10%	32,30%
Survivors	100%	99,30%	98,90%	95,40%	87,40%	80,90%	67,70%

Figure 1. Death prevalence of study group according to age

Using one-way statistical analysis, >60 years of age, hypertension, chronic renal failure, and diabetes mellitus were associated with poor prognosis (P < 0.05). In laboratory findings, D-dimer, sodium, lactate dehydrogenase (LDH), troponin, creatinin kinase myocardial band (CK-MB), ferritin, blood lactate level, high blood glucose, and presence of blood and protein in urine were associated with mortality (P < 0.05). In addition, low albumin, lymphocyte, and platelet levels were associated with mortality (P < 0.05) (Table 1).

According to the data obtained using the logistic regression model, advanced age, hypertension, high d-dimer, elevated C-reactive protein (CRP), CK-MB, and LDH, and lymphocyte deficiency were associated with poor prognosis (Table 2). The ROC curve of the model created for the prediction of mortality outcome in Covid-19 patients (Figure 2).

In one-way statistical analysis; over the age of 60, hypertension, chronic renal failure and diabetes mellitus showed poor prognosis (P <0.05). In laboratory findings; D-dimer, sodium, LDH, troponin, CK-MB, ferritin, blood lactate level, high blood glucose, presence of blood and protein in urine were associated with mortality (P <0.05). In addition, low albumin, lymphocyte and platelet levels were associated with mortality (P <0.05) (Table 1).

According to the data obtained using the logistic regression model; advanced age, presence of hypertension, high d-dimer, elevated CRP, creatinin kinase myocardial band (CK-MB) and LDH, lymphocyte deficiency were associated with poor prognosis (Table 2).

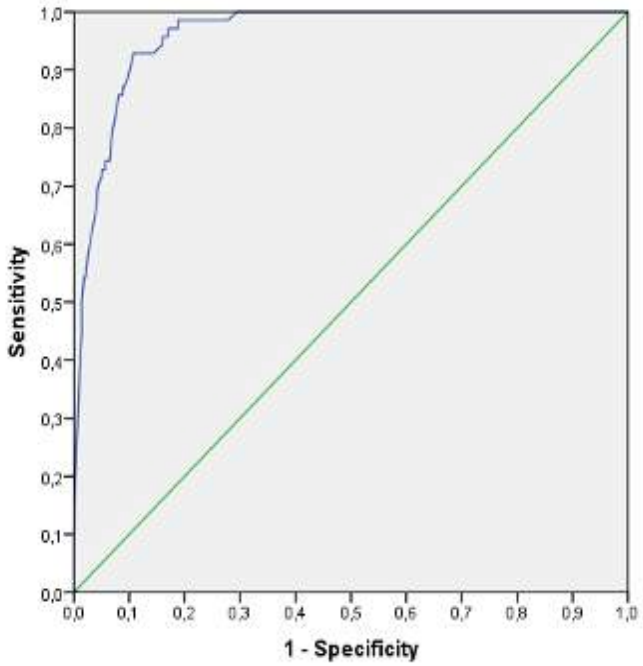


Figure 2. The ROC curve of the model created for the prediction of mortality outcome in Covid-19 patients

Table 2. Multivariate binary logistic regression model predicting likelihood of mortality of Covid-19 inpatients in Sakarya University Training and Research Hospital (SUTRH) based on age group, hypertension history, lymphocyte count, D-dimer, CRP, LDH and CK-MB levels.

Parameters	*B*	SE	Wald	*df*	*p*	Odds Ratio	95 % Confidence Interval for Odds Ratio
Age group < 60 years of age (0*) ≥ 60 years of age (1)	0.96	0.44	4.80	1	0.028	2.62	1.11 – 6.18
Hypertension No (0) Yes (1)	1.29	0.42	9.36	1	0.002	3.63	1.59 – 8.29
D-dimer < 1000 ng/mL (0) ≥ 1000 ng/mL (1)	2.05	0.57	13.15	1	< 0.001	7.77	2.57 – 23.53
CRP < 100 mg/L (0) ≥ 100 mg/L (1)	1.95	0.51	14.66	1	< 0.001	7.05	2.59 – 19.16
CK-MB < 25 ng/mL (0) ≥ 25 ng/mL (1)	1.36	0.37	13.61	1	< 0.001	3.88	1.89 – 7.96
LDH < 500 U/L (0) ≥ 500 U/L (1)	1.07	0.39	7.27	1	0.007	2.91	1.34 – 6.31
Lymphocyte Count ≥ 800 /mm^3 (0) < 800 /mm^3 (1)	0.84	0.41	4.19	1	0.041	1.00 2.31	1.04 – 5.13
Constant	- 7.17	0.72	98.55	1	< 0.001	0.001	

** 0 = Reference Group*

The model X^2 (7, N = 831) = 258.27 p = < 0.001 indicates that the model is significant. For goodness of fit of the model Hosmer Lemeshow test was conducted and p value was found to be 0.86. It suggests the model is a good fit to the data. Nagelkerke R^2 was found to be 0.61. The model can predict 61% of variance in mortality outcome. The predicted correction percentage increased from 91.6 % to 94.5 % with the model. Sensitivity was 54.3% (95% CI 41.9 – 66.3 %), specificity was 98.2% (95% CI 96.9 – 98.9 %), positive predictive value was 73.1% (95% CI 60.8 – 82.6 %), negative predictive value was 95.9% (95% CI 94.8 – 96.8 %), and accuracy was 94.5 (95% CI 92.7 – 95.9 %).

Regression Model Formula = (Age group × 0.961) + (Hypertension × 1.29) + (D-dimer × 2.05) + (CRP × 1.953) + (CKMB × 1.355) + (LDH × 1.067) + (Lymphocyte count × 0.836) - 7.165. The area under the ROC curve was 0.960 (95% CI 0.945 to 0.975), which is an outstanding level of discrimination (7). When these seven parameters (age, hypertension, D-dimer, CRP, CKMB, LDH, Lymphocyte count) in the regression model are given 1 and 0 according to the intervals determined, the total result obtained from the formula is below 0, when the patient is close to death, below 0. those who survived were more likely to survive (Table 2).

DISCUSSION

In this study, we examined the data of 941 patients who were hospitalized and monitored. The severity criteria were investigated according to the initial laboratory and clinical findings of the patients with COVID-19. We compared the data of 77 patients (8.1%) with mortality to those with no mortality. According to the data obtained using the logistic regression model, >60 years old, hypertension, high d-dimer, CRP, CK-MB, and LDH, and low lymphocyte count were associated with poor prognosis.

Furthermore, advanced age (>60 years old) and accompanying comorbidity were associated with poor prognosis. In many different studies,

these factors have already been identified as risk factors for serious illness (8). The mortality rate in patients >60 years of age was higher compared to younger patients. Here, it was found that deaths were concentrated especially in the older population, which could be attributed to the weakened immune systems in the older population to fight off viral infections. Furthermore, patients >60 years old had the highest risk of severe disease and death. As of May 2020, Turkey was among the 10 most-affected countries with COVID-19. However, the mortality rate in Turkey (2.2%) was lower compared to other countries. In this case, it is also effective that the hospital bed capacity is not exceeded and the age structure of our population is relatively younger. While 65+ population was created 9% of the population in Turkey, this ratio is around 20% in many other countries. In some countries, the median age is >80 years. Thus, it would be more accurate to compare age-specific speeds (9,10).

Comorbidity increases with increasing age. Patients with underlying conditions, such as diabetes, cardiovascular disease, hypertension, chronic lung disease, and cancer, have been identified as risk factors for COVID-19. The SARS-CoV-2 binds to the human receptor angiotensin-converting enzyme 2 (ACE2) proteins and enters the target cell. ACE2 protein is found on the surface of many cells. Most importantly, the receptor of ACE2 increases with increasing age (11). Typically, hypertension increases the amount of ACE2 receptors (12). However, most of the severe cases of COVID-19 have been associated with overactivity of the immune system.

COVID-19 is a systemic disease that affects both the bleeding and coagulation system. According to our findings, the mortality rate in patients with lymphopenia is 2.31 times higher. A meta-analysis conducted showed that a lymphocyte count of less than 1.5×10^9/L was the criteria for severe disease and adverse prognosis (13). In this study, a lymphocyte count <800/mm^3 was found to be associated with poor prognosis. Why is the disease more serious in those with lymphopenia? There are some hypotheses on this subject. First, the virus can directly infect lymphocytes, causing immune-mediated lymphocyte death (14). Second, some studies have confirmed that tumor necrosis factor α, interleukin (IL)-6, IL-1, and other proinflammatory cytokines can induce lymphocytopenia (15). According to these findings, lymphopenia should be followed closely from the beginning of the disease.

Based on our analysis, patients with severe COVID-19 have high blood lactic acid levels. Lactic acid can suppress the proliferation of lymphocytes. In addition, the findings of our study suggest that high lactic acid was a risk factor for severe disease (16). In patients with severe COVID-19, elevated lactic acid levels may trigger lymphopenia. However, the number of research on this subject is limited. We believe that further research is needed to examine the relationships between high lactic acid levels and lymphopenia (17). A study reported that lactic acid produced from tumor cells have shown to block T cell production (18).

We found that high D-dimer was associated with the severity of COVID-19 infection. D-dimer is a plasmin-derived fibrin degradation product. Severe COVID-19 infection causes extensive damage to the endothelium. Furthermore, endothelial damage triggers the coagulation cascade. Studies have shown that high D-dimer is associated with mortality in COVID-19. COVID-19 was associated with hemostatic abnormalities, and significantly higher D-dimer levels have been reported in severe cases (19,20). A severe pro-inflammatory response in COVID-19 viremia and this lack of control causes dysfunction of the endothelial cells in the same case. This causes excess thrombin release and D-dimer elevation (21). In addition, postmortem lung organ dissection of critically ill patients with serious COVID-19 showed occlusion and micro thrombosis of small pulmonary vessels (21,22). Considering the hematological clinical findings in COVID-19, we believe that one of the most important issues is coagulopathy, thrombosis, and anticoagulation management. Reportedly, many patients with severe course experienced significant thrombosis (23,24). Some studies and the results obtained in this study suggested that the risk of embolic events was high for these patients and that prophylactic thrombosis-relieving drugs (e.g., enoxaparin) should be used early in these patients.

CRP is a nonspecific marker of infection, inflammation, and tissue damage. In this study, we found that patients with severe prognosis had high CRP (>100 mg/L) values in the early days. This is related to the disease-related inflammatory reaction and destruction of tissues. Studies have shown that a high CRP level is due to lung damage and poor prognosis (25). Our findings showed that CRP can be used as a marker in monitoring the activation of the disease. However, it should be remembered that the half-life of CRP is approximately 18 hours (26). A study in China indicated that CRP could be a valuable marker to estimate the probability of serious progression of COVID-19 (27).

In this study, LDH was associated with severe disease. As previously mentioned, according to our values on day 1, we found a worse course in patients with high LDH levels. LDH has different isomers (LDH-1 in the heart, red blood cells, and brain; LDH-2 in the reticuloendothelial system; LDH-3 in the lungs; LDH-4 in the kidneys and pancreas; and LDH-5 in the liver and striated muscle). Therefore, we believe that high LDH is especially related to widespread damage to the muscles, including the lungs, erythrocytes, and

heart. Many studies reported that high LDH indicated a poor prognosis (28,29).

We analyzed the items found meaningful in this research with modeling. When data from this research were calculated with statistical modeling, we obtained a regression model formula. According to this formula, by applying certain coefficients to the variables, such as age group, history of hypertension, D-dimer, CRP, CK-MB, LDH, and lymphocyte count, the disease will show a severe course compared to this formula and a severe clinical picture when <0. This is not expected. Furthermore, according to the data obtained within the first 7 days after hospitalization, we believe that the application of this formula will help determine the number of patients who need intensive care and those who need specialized treatments (e.g., plasma treatment). According to the ROC curve of this formula, we predicted a value of 0.960 curves, which showed a clear distinction.

Although the sensitivity of this model is low, its specificity is quite good and will, therefore, enable successful prediction of cases that may not be severe. However, we believe that the model we obtained should be validated by evaluating the studies to be conducted in different COVID-19 groups. In addition, we believe that the study design will be planned prospectively (longitudinal) and will establish stronger causal relationships.

As a result, in this study, the data of the first week of approximately 1000 confirmed COVID-19 patients were examined and age, hypertension, d-dimer, CRP, CK-MB, LDH elevation, and low lymphocytes were associated with serious prognosis. We believe that the modeling we have developed for these patients will be beneficial for possible pandemics.

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RESEARCH ARTICLE

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Evaluation of the Effects of Rapamycin Treatment on Antioxidant Enzyme Changes and AgNOR in Testicular Torsion

ABSTRACT

Objective: Testicle torsion/detorsion cause ischemia. Rapamycin has immune suppressive and antioxidant defense mechanisms. Nucleolar-organizing regions (NORs) are loops of ribosomal DNA.

Methods: To evaluate mean AgNOR number and total AgNOR area/total nuclear area (TAA/TNA) ratio and the relation between these proteins and rapamycin in Torsion/Detorsion process of testes. The six groups as control, sham, early and late torsion-detorsion (ETD&LTD) groups, and early and late rapamycin treatment groups (ETD+R&LTD+R) were included. The TAA/TNA and mean AgNOR number of testes cells and biochemical analysis of GPx, SOD and TBARS activities were detected.

Results: Significant differences were detected among the groups for mean AgNOR number and TAA/TNA($p<0.05$). For both mean AgNOR number and TAA/TNA, significant differences were found between control and ETD, between control and ETD+R, between control and LTD, between control and LTD+R. Also, statistically significant relation between both of mean AgNOR numbers and TAA/TNA of testes cells and all of antioxidant enzymes (SOD, TBARS and GPX) were detected ($p<0.05$).

Conclusions: We may obtaine information about the levels and duration of testes injury considering the levels of these proteins. Thus it can be said that, these proteins may be used the developing of the new and more effective therapeutic approaches to prevent the negative effects of the T/D injury.

Keywords: Testes Torsion, NOR, AgNOR, rDNA, Rapamycin

Testis Torsiyonunda Rapamisin Tedavisinin Antioksidan Enzim Değişiklikleri ve AgNOR Üzerindeki Etkilerinin Değerlendirilmesi

ÖZET

Amaç: Testis torsiyonu/detorsiyonu iskemiye neden olur. Rapamisin, immün baskılayıcı ve antioksidan savunma mekanizmalarına sahiptir. Nükleolar düzenleyen bölgeler (NOR'ler) ribozomal DNA'nın döngüleridir.

Gereç ve Yöntem: Testislerin Torsiyon/Detorsiyon işleminde ortalama AgNOR sayısı ve toplam AgNOR alanı/toplam nükleer alan (TAA/TNA) oranını ve bu proteinler ile rapamisin arasındaki ilişkiyi değerlendirmek. Kontrol, sahte, erken ve geç torsiyon-detorsiyon (ETD & LTD) grupları ve erken ve geç rapamisin tedavi grupları (ETD+R & LTD+R) olmak üzere altı grup dahil edildi. TAA/TNA ve testis hücrelerinin ortalama AgNOR sayısı ile GPx, SOD ve TBARS aktivitelerinin biyokimyasal analizi tespit edildi.

Bulgular: Gruplar arasında ortalama AgNOR sayısı ve TAA/TNA açısından önemli farklılıklar tespit edildi ($p<0.05$). Hem ortalama AgNOR sayısı hem de TAA/TNA için, kontrol ile ETD arasında, kontrol ile ETD+R arasında, kontrol ile LTD arasında, kontrol ile LTD+R arasında önemli farklılıklar bulundu. Ayrıca testis hücrelerinin ortalama AgNOR sayıları ile TAA/TNA ile tüm antioksidan enzimler (SOD, TBARS ve GPX) arasında istatistiksel olarak anlamlı ilişki saptandı ($p<0.05$).

Sonuç: Bu proteinlerin seviyelerine göre testis hasarının seviyeleri ve süresi hakkında bilgi edinebiliriz. Bu nedenle, bu proteinlerin, T/D hasarının olumsuz etkilerini önlemek için yeni ve daha etkili terapötik yaklaşımların geliştirilmesinde kullanılabileceği söylenebilir.

Anahtar Kelimeler: Testis Torsiyonu, NOR, AgNOR, rDNA, Rapamisin

INTRODUCTION

Rotation of the testicle with its own pedicle disrupts the blood flow, resulting in ischemia, and if not corrected immediately, it may result in organ loss (1). Despite the restoration of oxygen supply and nutrition, a new handicap emerges: ischemia reperfusion injury, which if not managed well can result in infertility (2,3). I/R injury causes anoxia in particular, resulting in large amounts of ROS, proinflammatory cytokines, lipid peroxidation and cell adhesion molecules, followed by necrosis caused by activation of the apoptosis pathway, leading to more severe ischemic tissue damage (4). Proinflammatory neutrophil infiltration and ROS formation happen in the early stage of I/R injury, are important in the pathogenesis of said injury, and in the early control of the reperfusion phase to reduce its damage (5,6).

For a long time, detailed research efforts have sought to find effective tactics and agents to protect the testicle from I-R damage or to minimize that damage. However, to date, no method has been able to successfully apply in clinical practice (7,8). Rapamycin, on the other hand, continues its adventure as an antifungal drug and continues as an immunosuppressive agent and the objections about this identity are increasing. Are rapamycin and its analogues immunosuppressive or immunomodulatory drugs? Theoretical and practical applications suggest that rapamycin may be effective against hypertension, atherosclerosis and hyper-coagulation diseases (such as myocardial infarction and stroke), cancer, osteoporosis, autoimmune disorders, diabetes, macular degeneration, Alzheimer's and Parkinson's diseases and even obesity (9). Rapamycin, a TOR antagonist, has also been proven to prevent aging (10). Could it show these effects in preventing I/R damage through Nucleolar regulating regions (NOR) proteins? Is there a relationship between the TOR pathway and NOR, ribosomal DNA (rDNA) rings that make up the functional subunits of the nucleolus and are copied into ribosomal RNA?

Nucleolar-organizing regions (NORs) are loops of ribosomal DNA (rDNA) that functional subunits of the nucleolus and are transcribed into ribosomal RNA which becoming a part of mature ribosomes. Some of these are argyrophilic features and stained with silver. Various studies about the significance of the interphase AgNOR quantity in different cells were done (11-30). To our knowledge, any study about the evaluation of mean AgNOR number and total AgNOR area/total nuclear area (TAA/TNA) ratio and the relation between AgNOR protein amounts and rapamycin was conducted on Torsion/Detorsion of testes. So, we performed the current study.

MATERIAL AND METHODS

Experimental Design and Animal Groups: This study was carried out with the approval of Duzce University Local Animal Experiments Ethics Committee (2020/11/1). 90-day-old adult male albino Wistar rats (n=30) weighing approximately 275-325 g were used in this study. The rats were kept in plastic cages under a 12-hour light and 12-hour dark cycle and under temperature-controlled place (21-22 °C). They were fed with standard rodent diet and filtered drinking water at the Experimental Animals Application and Research Center of Duzce University. The experimental animals were divided into 6 groups of 5 rats each: control, sham, early and late torsion-detorsion (ETD&LTD) groups, and early and late rapamycin treatment groups (ELTD+R&LTD+R). In the control group, no surgical procedure or medication was applied but in the sham surgery group, the right testicle was exposed for a short time with a surgical incision without any rotation and was replaced again. While just surgical procedure was performed in the TD groups, 0.2 mg/kg rapamycin was administered by oral gavage once a day for 3 days in addition to the surgical procedure in the TD+R groups.

In the torsion and treatment groups, the right testis, which was taken out of the body with a midline scrotal incision, was torsioned by turning it 720° clockwise after opening the tunica vaginalis. It was fixed to the scrotum with a 5/0 silk suture to maintain the torsion position (7). After 4 hours of ischemia, the testis was detorsioned by rotating it counterclockwise and brought to its natural position. Rapamycin TD+R groups (RAPAMUNE® Oral Solution 1 mg/ml Pfizer PFE) were administered to rats three times by oral gavage; 30 minutes before and 24 and 48 hours after corrective surgery (detorsion). Sampling was performed at the end of the 3rd day (72nd-hour postop) in the ETD and ETD+R, and on the 10th day (postop 240th-hour) in the LTD and LTD+R groups.

AgNOR Staining: The testes samples of each group fixed individually in a 4% formaldehyde solution, were embedded in paraffin blocks (about dimensions of approximately 1x1x1 cm3) and 4 μm sections were obtained from the paraffin blocks. The tissue sections of testes were deparaffinized in xylene and rehydrated in graded alcohol solutions. After rehydratation, the slides were air-dried at room temperature for 15 min and fixed in absolute methanol for 5 min. Then each slides were silver stained with slight modification of Benn and Perle protocol (31) and Lindner (32). For this purpose, the solution made by mixing one volume of 2% gelatine in 1% aqueous formic acid and two volumes of 50% silver nitrate were dropped on the slides and incubated at 37 0C for fifteen min in the dark. Then the slides were rinsed with bi-distilled water.

Image Analysis of Mean AgNOR Number and Total AgNOR area/total Nuclear Area (TAA/TNA) Ratio: Fifty nuclei for per slides have

been evaluated. Silver stained testes cells of each rats were photographed using a light microscope (Eclipse 80i; Nikon. Tokyo. Japan) via digital camera attachment (Digital Sight DS-Fi1c; Nikon) and evaluated using ImageJ version 1.47t image processing software (33). The mean AgNOR number was detected by counting and the TAA/TNA ratio was detected using ‘‘freehand selection’’ tool for each nucleus.

Biochemical Assays: After decapitation, blood samples were taken from the aortic abdominalis and placed in heparin plastered tubes. Plasma obtained by low speed centrifugation (2000×g, 15 minutes) was stored at –80 °C for biochemical analysis of GPx, SOD and TBARS activities. GPx, SOD and TBARS activities were evaluated with Cayman® Elisa kits without ignoring the manufacturer's instructions.

Statistical Analysis: Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS. Inc. Chicago. Illinois. USA) for Windows 23.0. The comparison of all groups (more than two) was done using the Kruskall-Wallis test. For pairwise comparison of all groups, Mann-Whitney U test was carried out. The polynomial regression were performed for the relationship between the variable. The results were given as n, median, range and mean ± SD. The $p<0.05$ was accepted as statistically significant.

RESULTS

The groups, mean AgNOR Number and TAA/TNA ratio of each group were given in the Table 1. Demonstrastive examples of silver stained NOR (a:Control, b:Sham, c:ETD, d: ETD+R, e: LTD f: LTD+R) for testicular cells were given in the Figure 1.

Table 1. The groups, mean AgNOR Number and TAA/TNA ratio of each group

Groups	Mean AgNOR Number ± SD (n=250)	Mean TAA/TNA ± SD (n=50)	Mean AgNOR number of Group/Median (Range) (n=250)	Mean TAA/TNA of Group/Median (Range) (n=250)	$\chi2$	p
C1	1.276±0.532	0.030±0.012	1.269±0.019/1.276	0.030±0.001/0.298		
C2	1.289±0.493	0.029±0.01	(0.048)	(0.001)		
C3	1.278±0.451	0.029±0.011				
C4	1.241±0.423	0.030±0.01				
C5	1.259±0.431	0.030±0.011				
S1	1.17±0.351	0.030±0.071	1.228±0.065/1.220	0.030±0.001/0.030		
S2	1.22±0.423	0.030±0.016	(0.140)	(0.002)		
S3	1.16±0.331	0.029±0.011				
S4	1.29±0.557	0.030±0.012				
S5	1.3±0.471	0.031±0.01				
ETD1	2.56±1.150	0.110±0.047	2.659±0.214/2.560	0.112±0.002/0.112		
ETD2	2.98±0.949	0.114±0.028	(0.540)	(0.006)	25.258*	0.000*
ETD3	2.765±1.116	0.108±0.040				
ETD4	2.55±0.994	0.112±0.133				
ETD5	2.44±1.191	0.113±0.086				
ETD+R1	1.937±0.982	0.094±0.025	1.789±0.464/1.655	0.080±0.001/0.078		
ETD+R2	2.52±1.147	0.072±0.032	(1.163)	(0.023)		
ETD+R3	1.357±0.638	0.071±0.031				
ETD+R4	1.655±0.637	0.078±0.034				
ETD+R5	1.474±0.684	0.086±0.038				
LTD1	2.08±0.703	0.104±0.038	2.242±0.225/2.100	0.104±0.003/0.105		0.000&
LTD2	2.59±0.891	0.105±0.047	(0.510)	(0.007)		
LTD3	2.35±0.667	0.100±0.031			27.194&	
LTD4	2.09±0.785	0.106±0.033				
LTD5	2.1±0.881	0.107±0.031				
LTD+R1	2±1.125	0.096±0.044	1.868±0.218/1.980	0.089±0.008/0.089		
LTD+R2	1.84±0.866	0.089±0.866	(0.520)	(0.017)		
LTD+R3	1.5±0.707	0.081±0.023				
LTD+R4	2.02±0.589	0.08±0.026				
LTD+R5	1.98±0.589	0.098±0.024				

C: Control; **S:**Sham; **ETD:** early torsion detorsion; ETD**+R:** early torsion detorsion+rapamycin treatment; **LTD:** late torsion detorsion; **LTD+R:**late torsion detorsion+rapamycin treatment; *:For Mean AgNOR Number; &=For TAA/TNA; **TAA/TNA:** Total AgNOR area/total nuclear area

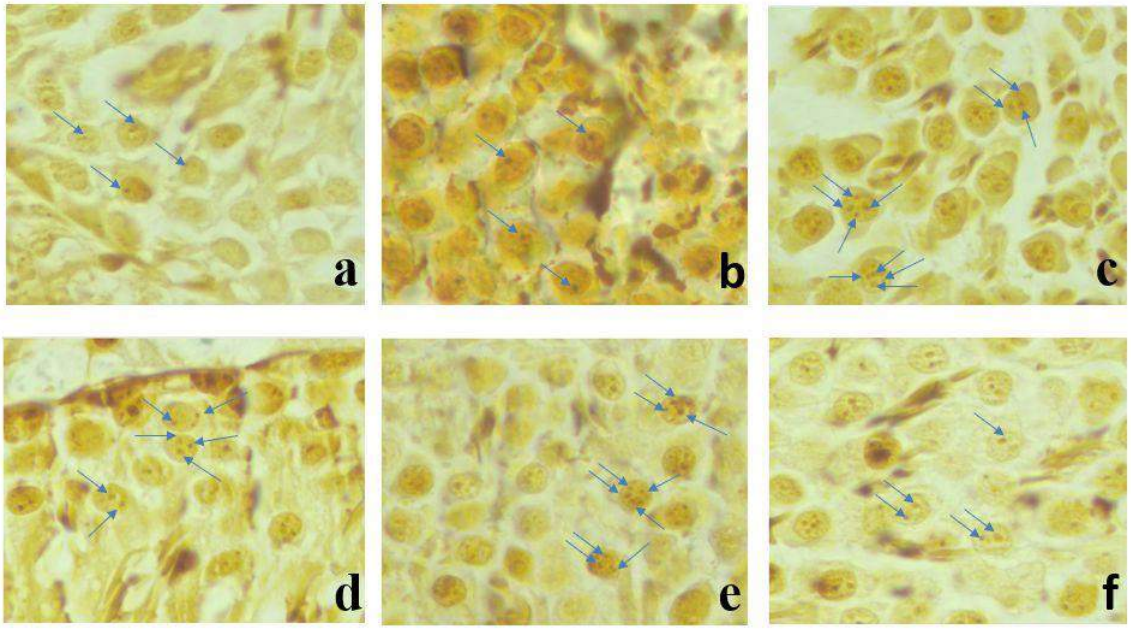

Figure 1. Demonstrative examples of silver stained NOR (**a:**Control, **b:**Sham, **c:**ETD, **d:** ETD+R, **e:** LTD **f:** LTD+R) for testicular cells

When the all groups to be considered, statistically significant differences were detected among the groups for both mean AgNOR number ($\chi 2$=25.258, p=0.000) and TAA/TNA ($\chi 2$=247.194, p=0.000), respectively (Table 1 and Figure 2). In order to understand the causes of these differences from which groups, binary comparison of the groups was performed.

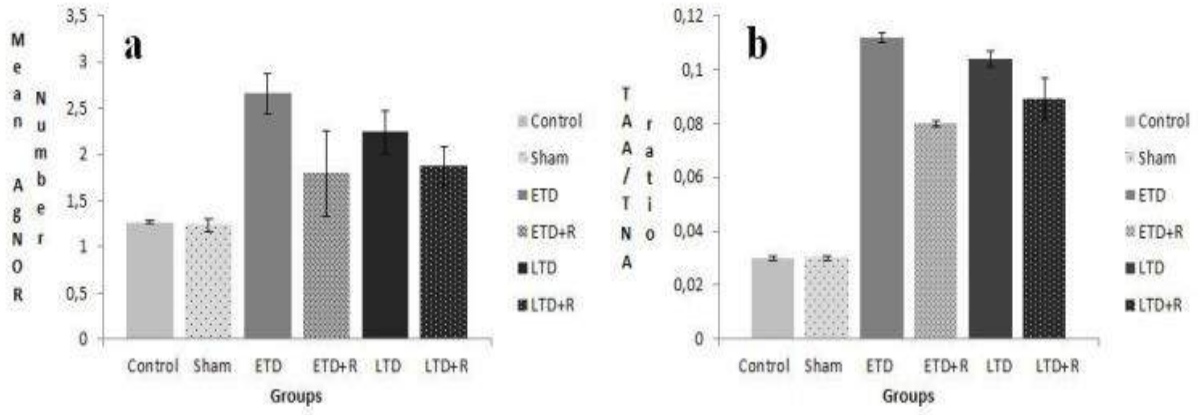

Figure 2. Comparison of mean AgNOR number and TAA/TNA ratio of all groups.

When the two groups are compared in terms of mean AgNOR number, statistically significant differences were found between control and ETD (Z=-2.611, p=0.009), between control and ETD+R (Z=-2.611, p=0.009), between control and LTD (Z=-2.619, p=0.009), between control and LTD+R (Z=-2.619, p=0.009), between sham and ETD (Z=-2.611, p=0.009), between sham and ETD+R (Z=-2.611, p=0.009), between sham and LTD (Z=-2.619, p=0.009), between sham and LTD+R (Z=-2.611, p=0.009), between ETD and ETD+R (Z=-2.402, p=0.016), between ETD and LTD (Z=-1.984, p=0.047), between ETD and LTD+R (Z=-2.611, p=0.009), between LTD and LTD+R (Z=-2.611, p=0.009). But the difference between control and sham (Z=-0.522, p=0.602), between ETD+R and LTD (Z=-1.776, p=0.076), between ETD+R and LTD+R (Z=-0.940, p=0.347), were not significant (Table 2).

Table 2. Double comparison results of the groups for Mean AgNOR Number

Groups	C		S		ETD		ETD+R		LTD		LTD+R	
	Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
C	-	-	0.522	0.602	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**
S	0.522	0.602	-	-	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**
ETD	2.611	**0.009**	2.611	**0.009**	-	-	2.402	**0.016**	1.984	**0.047**	2.611	**0.009**
ETD+R	2.611	**0.009**	2.611	**0.009**	2.402	**0.016**	-	-	1.776	0.076	0.940	0.347
LTD	2.611	**0.009**	2.611	**0.009**	1.984	**0.047**	1.776	0.076	-	-	2.611	**0.009**
LTD+R	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	0.940	0.347	2.611	**0.009**	-	-

C: Control; **S:**Sham; **ETD:**early torsion detorsion; ETD**+R:** early torsion detorsion+rapamycin treatment; **LTD:** late torsion detorsion; **LTD+R:**late torsion detorsion+rapamycin treatment; *:For Mean AgNOR Number

When the two groups are compared in terms of TAA/TNA, the statistically significant differences were found between control and ETD (Z=-2.611, p=0.009), between control and ETD+R (Z=-2.611, p=0.009), between control and LTD (Z=-2.611, p=0.009), between control and LTD+R (Z=-2.619, p=0.009), between sham and ETD (Z=-2.611, p=0.009), between sham and ETD+R (Z=-2.611, p=0.009), between sham and LTD (Z=-2.611, p=0.009), between sham and LTD+R (Z=-2.611, p=0.009), between ETD and ETD+R (Z=-2.611, p=0.009), between ETD and LTD (Z=-2.611, p=0.009), between ETD and LTD+R (Z=-2.611, p=0.009), between ETD+R and LTD (Z=-2.611, p=0.009) and between LTD and LTD+R (Z=-2.1611, p=0.009). Conversely the differences between control and sham (Z=-1.567, p=0.117), between ETD+R and LTD+R (Z=-1.567, p=0.117) were not meaningful for TAA/TNA (Table 3).

Table 3. Double comparison results of the groups for TAA/TNA ratio

Groups	C		S		ETD		ETD+R		LTD		LTD+R	
	Z	P	Z	P	Z	P	Z	P	Z	P	Z	P
C	-	-	1.567	0.117	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	2.619	**0.009**
S	1.567	0.117	-	-	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**
ETD	2.611	**0.009**	2.611	**0.009**	-	-	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**
ETD+R	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	-	-	2.611	**0.009**	1.567	0.117
LTD	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	2.611	**0.009**	-	-	2.611	**0.009**
LTD+R	2.619	**0.009**	2.611	**0.009**	2.611	**0.009**	1.567	0.117	2.611	**0.009**	-	-

C: Control; **S:**Sham; **ETD:** early torsion detorsion; ETD**+R:** early torsion detorsion+rapamycin treatment; **LTD:** late torsion detorsion; **LTD+R:** late torsion detorsion+rapamycin treatment; **TAA/TNA:** Total AgNOR area/total nuclear area

When the antioxidant enzymes to be considered, statistically significant differences were detected among the groups for SOD ($\chi2$=21.124, p=0.001), TBARS ($\chi2$=24.892, p=0.000) and GPX ($\chi2$=26.471, p=0.000), respectively (Table 4).

Table 4. The comparison of antioxidant enzyme levels (SOD, TBARS and GPX) in all groups.

Groups	SOD	Mean±SD/Median (Range) for SOD	TBARS	Mean±SD/Median(Range) for TBARS	GPX	Mean±SD/Median(Range) For GPX	χ2	p
C1	23.15702		13.40782		17.67081			
C2	22.45405	23.573±1.047/23.157	11.30503	12.341±0.919/12.260	15.86335	16.520±1.117/16.433		
C3	25.04380	(2.590)	12.25978	(2.103)	16.43298	(2.608)		
C4	24.23702		13.12782		17.57081			
C5	22.97405		11.60503		15.06335			
S1	27.18595		14.21458		17.89745			
S2	29.34033	27.401±1.607/27.186	13.02455	13.561±0.430/13.525	19.99379	18.163±1.403/17.898		
S3	25.75207	(3.588)	13.42468	(1.190)	16.49068	(3.503)		
S4	25.98595		13.61458		17.30745			
S5	28.74234		13.52455		19.12379			
ETD1	63.570		32.869		45.023			
ETD2	56.786	55.141±6.095/56.786	28.779	28.787±2.702/28.779	44.826	40.973±4.396/42.287	21.124*	0.001*
ETD3	50.375	(15.445)	27.938	(7.513)	37.324	(9.618)		
ETD4	48.125		28.992		35.405			
ETD5	56.846		25.357		42.287		24.592&	0.000&
ETD+R1	49.68595		26.05028		36.18012			
ETD+R2	43.28926	39.221±8.922/42.067	25.58101	23.560±3.768/25.581(8.478)	28.51553	29.168±4.723/28.516	26.471®	0.000®
ETD+R3	42.06694	(23.234)	26.28492		30.44497	(12.932)		
ETD+R4	26.45248		17.80670		23.24857			
ETD+R5	34.61157		21.07486		27.45298			
LTD1	50.035		30.070		40.133			
LTD2	47.020		25.366	24.395±3.622/23.783	31.584	32.989±4.202/31.584		
LTD3	37.621	43.949±5.251/45.681(12.413)	21.984	(9.300)	30.769	(10.704)		
LTD4	39.389		20.770		29.428			
LTD5	45.681		23.783		33.033			
LTD+R1	52.59372		21.23017		20.43478			
LTD+R2	37.14876	36.341±11.478/	18.85642	19.394±1.823/18.856	26.54658	23.169±2.961/22.951		
LTD+R3	29.10992	37.149(30.156)	17.71508	(3.704)	20.12422	(6.422)		
LTD+R4	22.43802		17.74860		22.95093			
LTD+R5	40.41322		21.41899		25.78882			

C: Control; **S:**Sham; **ETD:** early torsion detorsion; **ETD+R:** early torsion detorsion+ rapamycin treatment; **LTD:** late torsion detorsion; **LTD+R:** late torsion detorsion+ rapamycin treatment; **SOD:** Superoxide dismutase; **TBARS:** Thiobarbituric acid reactive substances; **GPX:** Glutathione peroxidase;*:For SOD; &:For TBARS; ®:For GPX

When we performed the polynominal regression analaysis statistically significant relation between mean AgNOR numbers of testes cells and all of antioxidant enzymes (SOD, TBARS and GPX) were detected ($p<0.05$) (Table 5, Figure 3). Also statistically significant relation between TAA/TNA ratio of testes cells and and all of antioxidant enzymes (SOD, TBARS and GPX) were detected ($p<0.05$) (Table 5, Figure 3).

Table 5. Model Summary and Parameter Estimates for AgNOR Numbers, TAA/TNA and antioxidant enzymes

		Model Summery					Parameter Estimates			
Variable	***Equation***	***R^2***	***F***	***df1***	***df2***	***sig***	**Constant**	***b1***	***b2***	***b3***
M-AgNOR-N and SOD	Linear	,216	7,723	1	28	,010	18,918	7,554		
	Logarithmic	,241	8,874	1	28	,006	24,543	14,648		
	Cubic	,272	5,032	2	27	,014	-9,915	40,004	-8,368	,000
M-AgNOR-N and TBARS	Linear	,196	6,842	1	28	,014	11,102	3,281		
	Logarithmic	,228	8,261	1	28	,008	13,470	6,496		
	Cubic	,279	5,212	2	27	,012	-4,909	21,300	-4,647	,000
M-AgNOR-N and GPX	Linear	,254	9,530	1	28	,005	13,496	4,915		
	Logarithmic	,288	11,332	1	28	,002	17,104	9,622		
	Cubic	,353	7,373	2	27	,003	-9,693	31,013	-6,730	,000
TAA/TNA and SOD	Linear	,295	11,727	1	28	,002	21,947	146,794		
	Logarithmic	,321	13,266	1	28	,001	57,756	9,106		
	Cubic	,357	7,492	2	27	,003	13,909	395,219	,000	-16254,235
TAA/TNA and TBARS	Linear	,316	12,945	1	28	,001	12,011	69,242		
	Logarithmic	,396	18,353	1	28	,000	29,752	4,606		
	Cubic	,618	21,862	2	27	,000	-,536	561,584	-3630,342	,000
TAA/TNA and GPX	Linear	,355	15,419	1	28	,001	15,381	96,666		
	Logarithmic	,407	19,179	1	28	,000	39,376	6,148		
	Cubic	,487	12,835	2	27	,000	4,445	525,777	-3164,098	,000

M-AgNOR-N: Mean AgNOR Number; **TAA/TNA:** Total AgNOR area/total nuclear area; **SOD:** Superoxide dismutase; **TBARS:** Thiobarbituric acid reactive substances; **GPX:** Glutathione peroxidase

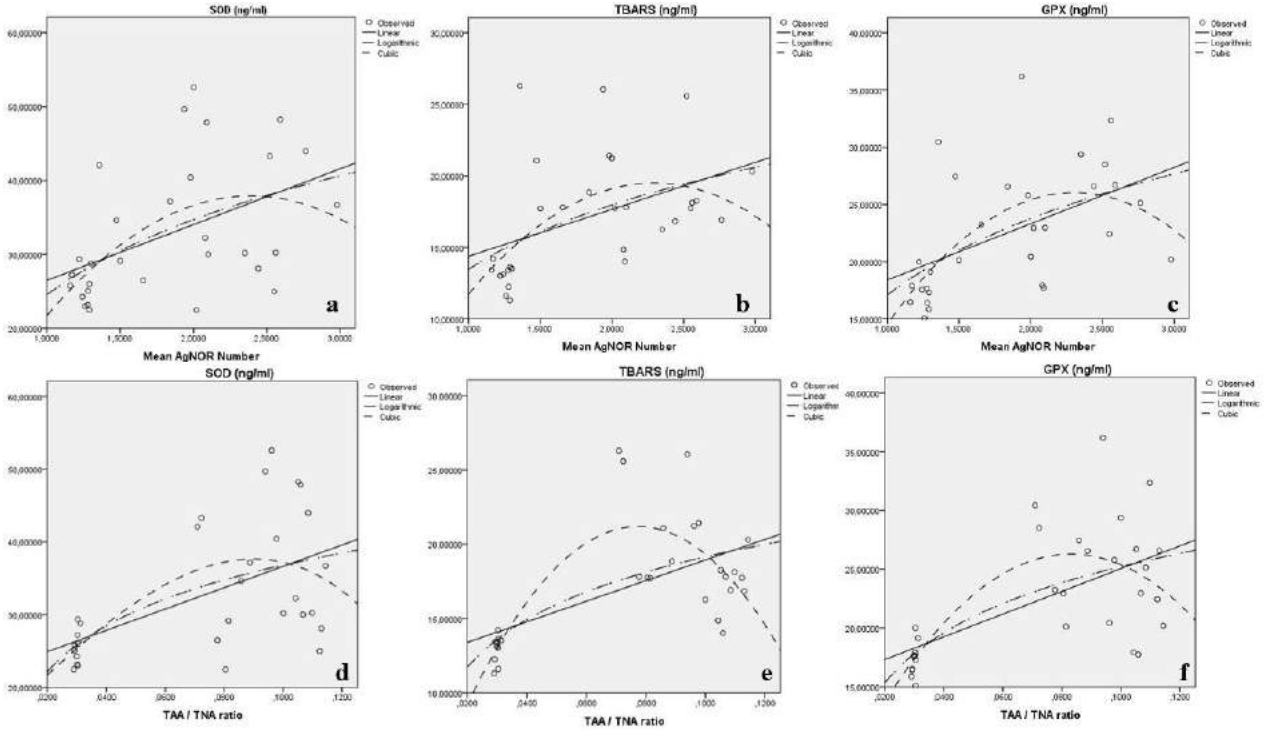

Figure 3. Relation between mean AgNOR numbers of testes cells and SOD (a), between mean AgNOR numbers of testes cells and TBARS (b), and mean AgNOR numbers of testes cells and GPX (c). Relation between mean TAA/TNA ratio of testes cells and SOD (d), between TAA/TNA ratio of testes cells and TBARS (e) and TAA/TNA ratio of testes cells and GPX (f).

DISCUSSION

In case of torsion caused by the rotation of the testicle with its'pedicle, it is a kind of race against time for surgeons dealing with pediatric emergencies to correct before necrosis. If this ischemic condition persists more than a few hours, depending on the degree of torsional and personal characteristics, testicular severely damaged and may even be necessary to remove the affected testicle. However, Ischemia-reperfusion injury (IRI) that occurs after surgical or manual correction is another problem and it is an important cause of fertility dysfunction (34). Thus, preventing or minimizing torsion-induced IRI can improve the long-term results of testicular function. For this purpose, many agents/drugs, one of which is Rapamycin (35), have been studied in the literatüre (7,8).

Rapamycin and its' analogue, everolimus, have been approved by the FDA for use as immunosuppressive in humans, and there have been publications on this subject for decades. At the beginning of the millennium, with an almost 3-fold prolongation of the life span of a mouse strain, rapamycin (called Rapamune or Sirolimus in clinical use) could be used to slow aging in humans and delay the progression of many age-related diseases, which could become a "miracle drug that delays aging today" started writing (36,37). However, it may be more appropriate to evaluate rapamycin in an immunomodulator or anti-inflammatory drug group instead of labeling it as an immunosuppressive agent. The mechanism of slowing down aging of Rapamycin is that it prevents excess, or more accurately, rejuvenates immunity rather than suppressing immunity (38). This means that, on the one hand, it prevents rejection as an immunosuppressor in organ transplant and acts as an immunostimulant on the other hand (39,40), enhancing immunity in cancer patients (41) and the elderly (42,43).

We can obtained the knowledge about the metabolic activities and protein synthesis capacity of the cells using AgNOR staining technique. Various studies are performed on the different cells including human hair root cells (11,12), buccal epithelial cells of down syndrome infants and healthy individuals (13,14), lung cells exposed to carbon monoxide (CO) (15), heart cells exposed to acute and chronic CO intoxication (16,17), femoral and skeletal muscle cells exposed CO gas (18,19), normal, benign and malign thyrocyte cells (20-23), peripheral lymphocytes of patients with chronic obstructive pulmonary disease exacerbation (24), kidney cells exposed to Ischemia/reperfusion (I/R) injury (25), capsaicin exposed human colon adenocarcinoma cells (26,27), rhamnetin exposed ehrlich's ascites carcinoma cells (28) and in oncocytology (29).

We previously reported that the AgNOR proteins amounts increased depending on the CO exposure (cause of hypoxic condition) in lung (15), heart cells (16,17), femoral muscle cells (18,19) and chronic obstructive pulmonary disease (COPD) exacerbation (24). According to our results, statistically significant differences were detected among the groups for both mean AgNOR number and TAA/TNA, respectively. To understand these differences causes from which groups, double comparison was performed. For mean AgNOR number, statistically significant differences were found between control and ETD, between control and ETD+R, between control and LTD, between control and LTD+R, between sham and ETD, between sham and ETD+R, between sham and LTD, between sham and LTD+R, between ETD and LTD. When the TAA/TNA ratio to be considered, the statistically significant differences were found between control and ETD, between control and ETD+R, between control and LTD, between control and LTD+R, between sham and ETD, between sham and ETD+R, between sham and LTD, between sham and LTD+R, between ETD and LTD, between ETD+R and LTD. According to our results, it may be said that the AgNOR proteins amount increase depending on hypoxia injuring caused from torsion/detorsion conditon, too. Considering our results; we think that both AgNOR protein amounts (mean AgNOR number and TAA/TNA ratio) may give information about the levels of the testes injury after T/D process. One of the most known certain reality is the obligation of themselves protection of all living cells to external and internal dangerous agents such T/D injury for keep alive. So, the increasing of these proteins may caused from the continuity of this process or these proteins have functions on the occuring various gene products that protective effect on the regulation of gene expression and/or signaling transduction pathways in the T/D injury. May these proteins be used the developing of the new and more effective therapeutic approaches to prevent the negative effects of the T/D injury? Additional studies including large series are needed to elucidate these topics as more clearly.

A positive correlation between mean AgNOR number and the pCO2 levels in patients with chronic obstructive pulmonary disease (COPD) exacerbation (24), between histopathological injury score and both of mean AgNOR number and TAA/TNA ratio in renal ischemia/reperfusion (I/R) injury (25), between histopathological injury scores and TAA/NA ratio (17), between AgNOR values and both cardiomyopathy and carboxyhemoglobin levels (16), between both the AgNOR values and histopathological scoring methods (15) were reported. In the current study, the level of antioxidant enzymes to be taken into consideration, statistically significant differences were detected among the groups for SOD, TBARS and GPX,

respectively. According to the polynominal regression analaysis, we detected statistically significant relation between antioxidant enzymes (SOD, TBARS and GPX) and both of mean AgNOR numbers and TAA/TNA ratio of testes, too. So it may be said that, we can also obtained information about the antioxidant enzymes and injury levels of testes tissue caused by torsion/detorsion injury.

Rapamycin is the most effective anti-cancer drug known to increase life expectancy in cancer-prone mouse strains (44-47). In fact, it was thought that rapamycin prolonged life expectancy because of cancer prevention. With these properties, how does rapamycin play a role in ischemic events? When determining the dose of rapamycin, it was necessary to consider the short-term effects that limited us. Since the animals were sacrificed after 3 days in the early period groups (groups 3 and 4), drug use was restricted for this period, although it was a safe drug that could be administered for a longer time. While a higher dose range could be given as a dose, the daily routine dose was chosen, whereas recorded fatal cases of acute rapamycin overdose were not detected (48). The LD50 of rapamycin, a measure of drug lethality, could not be measured in rats as it was higher than 2500 mg/kg. A single dose of rapamycin is safe, but sufficient to prolong life and reduce obesity in various rodent models (36,49). Moreover, temporary treatment with rapamycin can be long lasting, prolong life and prevent obesity long after drug withdrawal (50-54). Another restriction in dose selection is the negative effects of rapamycin and its analogs on spermatogenesis. However, research has shown that these effects are temporary and no more important than saving organ life (55). Long-term use and intermittent treatment options will be evaluated as researches conducted to prolong organ life in ischemic events become widespread.

Additionally it was reported that both AgNOR amounts may give information about the most reliable therapeutic dose selection of Curcumin (33), capsaicin (27) and Rhamnetin (28). In our results, statistically significant differences were found between ETD and ETD+R, between ETD and LTD+R, between LTD and LTD+R for mean AgNOR number. For TAA/TNA, statistically significant differences were found between ETD and ETD+R, between ETD and LTD+R and between LTD and LTD+R, too. According to our results, Rapamycin has protective effect for torsion/detorsion injury on testes tissue.

As a results, because the AgNOR proteins amount increase depending on hypoxia injuring caused from torsion/detorsion conditon, we may obtained information about the levels and duration of the testes injury after T/D process considering the levels of these proteins. According to our results, rapamycin has protective effect for torsion/detorsion injury on testes tissue. We can also obtained information about the antioxidant enzymes and injury levels of testes tissue caused by torsion/detorsion injury considering these proteins. So it can be said that, these proteins may be used the developing of the new and more effective therapeutic approaches to prevent the negative effects of the T/D injury.

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RESEARCH ARTICLE

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Evaluation of Nasal Mucociliary Activity in Patients with Familial Mediterranean Fever

ABSTRACT

Objective: The aim of this study was to evaluate nasal airway resistance and nasal mucociliary activity in Familial Mediterranean Fever (FMF) patients using anterior rhinomanometry and saccharin clearance test.

Methods: 30 patients with FMF disease and 30 healthy individuals were included in this prospective, cross-sectional study. Nasal airway resistance was measured via active anterior rhinomanometry and saccharin test was carried out for the measurement of nasal mucociliary clearance (NMC) times of all participants.

Results: Mean values for NMC time in the FMF patient group and control group were found as 14.6 ± 4.7 (range, 8-30) min. and 9.7 ± 2.3 (range, 6-14) min. respectively. Nasal mucociliary clearance time in the FMF patients was significantly prolonged in comparison that of healthy controls ($p < 0.001$). We identified no significant correlation between the disease duration and NMC time ($p=0.921$, $r = 0.019$). When the mean nasal airway resistance values of both groups were compared, there was no significant difference was detected ($p = 0.371$).

Conclusions: The results of our study have revealed that NMC time was longer in patients with FMF than that healthy controls. However, there was no statistically significant correlation between nasal mucociliary clearance time and disease duration. Disruption of NMC increases the risk of infection in the upper and lower respiratory tract. Caution should be exercised against upper and lower respiratory infections in patients with FMF.

Keywords: Familial Mediterranean Fever, Mucociliary Clearance, Rhinomanometry

Ailesel Akdeniz Ateşi Olan Hastalarda Nazal Mukosiliyer Aktivitenin Değerlendirilmesi

ÖZET

Amaç: Bu çalışmanın amacı Ailesel Akdeniz Ateşi (AAA) hastalarında anterior rinomanometre ve sakarin test kullanılarak nazal hava yolu direncini ve nazal mukosiliyer aktiviteyi değerlendirmektir.

Gereç ve Yöntem: Bu prospektif, kesitsel çalışmaya AAA hastalığı olan 30 hasta ve 30 sağlıklı birey dahil edildi. Tüm katılımcılara nazal hava yolu direncini ölçmek için aktif anterior rinomanometre ve nazal mukosiliyer klerens zamanını ölçmek için sakarin test uygulandı.

Bulgular: AAA hasta grubu ve kontrol gruplarında ortalama nazal mukosiliyer klerens zamanı sırasıyla 14.6 ± 4.7 (aralık, 8-30) dk. ve 9,7 ± 2,3 (aralık, 6-14) dk. olarak saptandı. AAA hastalarında nazal mukosiliyer klerens zamanı sağlıklı kontrollere göre anlamlı olarak uzundu ($p <0.001$). Nazal mukosiliyer klerens zamanı ile hastalık süresi arasında istatistiksel olarak anlamlı bir korelasyon tespit etmedik ($p <0.001$, $r=0.019$). Her iki grubun ortalama nazal hava yolu direnci değerleri karşılaştırıldığında, anlamlı bir fark saptanmadı ($p=0.371$).

Sonuç: Çalışmamızın sonuçları, AAA hastalarında nazal mukosiliyer klerens zamanının sağlıklı kontrollere göre daha uzun olduğunu ortaya koymuştur. Bununla birlikte, nazal mukosiliyer klerens süresi ve hastalık süresi arasında istatistiksel olarak anlamlı bir korelasyon yoktu. Nazal mukosiliyer klerensin bozulması, üst ve alt solunum yollarında enfeksiyon riskini artırır. AAA hastalarında üst ve alt solunum yolu enfeksiyonlarına karşı dikkatli olunmalıdır.

Anahtar Kelimeler: Ailesel Akdeniz Ateşi, Mukosiliyer Temizlik, Rinomanometre

INTRODUCTION

Familial Mediterranean Fever (FMF) is an inherited monogenic autoinflammatory disease that occurs worldwide and mostly affecting people living in the Mediterranean region. Its incidence in Turkey has been reported as 0.1% (1). Proinflammatory cytokines particularly IL-1 beta and TNF alpha play a significant role in the pathogenesis of FMF 16 (2). In FMF, a systemic inflammation affecting serosal tissues such as pleura, peritoneum, and sinovium is seen. FMF is characterized by recurrent self-limited attacks of fever associated with peritonitis, arthritis and pleuritis. The diagnosis of FMF is made according to Tel Hashomer criteria based on the clinical findings and can be supported by a genetic analysis (3,4). It has been demonstrated that, subclinical inflammation continues during treatment and even during the attack-free period (5).

Nasal mucociliary clearance (NMC) which is the initial defense system of the respiratory system is defined as the ability of the respiratory mucosa to eliminate inhaled particles and protect mucosal moisture. The time of removal of the inhaled molecules might be used to measure NMC time (6). Saccharin clearance test is an inexpensive, easily applicable and reliable method to measure NMC (6). Mucociliary dysfunction can be primary or secondary as a result of environmental, infectious and inflammatory stimuli that impair normal motility (7,8). Any discomfort in the NMC system can cause upper and lower respiratory tract infections (9). Mucociliary clearance activity can be affected by acute or chronic systemic inflammatory disease (7,8). The hypothesis of the present study is that chronic inflammatory processes in the FMF patients might have affected the nasal mucocilliary activity.

The most common symptom seen in sinonasal diseases is nasal congestion. Rhinomanometry is an easily applicable method that objectively assessment nasal airway patency (10).

The aim of this study was to investigate whether there is a difference between patient with FMF and the healthy controls in terms of nasal airway resistance and nasal mucociliary activity by using anterior rhinomanometry and saccharin clearance test.

MATERIAL AND METHODS

In order to detect a difference between the groups with an anticipated effect size of d=0.75, we needed at least 29 participants in each group (alpha = 0.05 and power = 80%). Therefore, we included 60 patients in this prospective, cross-sectional study, which consisted of 30 FMF patients diagnosed according to Tel-Hashomer criteria and 30 age-matched healthy volunteers without rhinologic diseases.

All otorhinolaryngological physical examinations, including endoscopic examination of both patients and healthy volunteers were performed. Individuals with a history of nasal or paranasal operation, current respiratory tract infection, being smokers, and patients with nasal obstruction due to sinusitis, major septal deviation and nasal polyposis were excluded from the study. All FMF patients were using colchicine and all the measurements were carried out during the attack-free period.

The protocol of this study was approved by the local ethics committee in accordance with the Helsinki declaration (11.04. 2019 2019/105). Written and verbal informed consents were obtained from all participants in the study.

Saccharin test was carried out in order to measure the nasal mucociliary clearance time. Participants were rested for 30 minutes before the test. A sodium saccharin 1 mm in diameter was placed in the medial aspect of the inferior nasal concha when the patient sitting in an upright position. During the time of the saccharin test, the patients were asked to perform swallowing movements at 30-second intervals and to announce the time they felt the sweet taste. The time elapsing until first feeling of a sweet taste was accepted as NMC time.

Nasal airway resistance was objectively assessed by anterior rhinomanometry. In this study, measurement was carried out using SRE 2000 Rhinometer (RhinoMetrics, Lynge, Denmark) device. Three normal breathings were required for each measurement. Left (RL) and right (RR) nasal resistance values were calculated according to Ohm's Law at a pressure of 150 Pa. (11) The formula RL × RR / RL + RR was used to calculate the total nasal airway resistance.

Statistical analysis: Numerical variables were compared using t-test and Mann -Whitney U test for independent groups according to the distribution of data. Dependent t-test for related groups was used to compare right and left nasal measurements. The examination of categorical variables was carried out by Pearson's chi-square analysis. Pairwise relations between numerical variables were analyzed by Pearson's correlation analysis. Data analysis was performed in SPSS 25.0 program and statistical tests were interpreted at $\alpha = 0.05$ significance level.

RESULTS

30 FMF patients (56.7 % female, 43.3 % male, mean age: 28.0 ± 12.4 years) and 30 healthy controls (63.3 % female, 36.7 % male, mean age: 29.2 ± 8.0 years) were included in the study. There was not any significant difference between the FMF patients and the healthy controls in terms of age and gender distribution ($p > 0.05$) (Table 1).

Table 1. Demographic data in FMF group and control groups

		FMF group (n=30)	Control group (n=30)	p
Age (mean±SD; years)[a]		28.0±12.4	29.2±8.0	0.077
Sex[b]	Male	13 (43.3)	11 (36.7)	0.598
	Female	17 (56.7)	19 (63.3)	

[a]Mann Whitney U test [b]Pearson's chi-square test SD = standard deviation FMF: Familial Mediterranean Fever

Mean values for NMC time in the FMF patient group and control group were found as 14.6 ± 4.7 (range, 8-30) min. and 9.7 ± 2.3 (range, 6-14) min. respectively (Table 2). Nasal mucociliary clearance time in the FMF patients was significantly prolonged in comparison that of healthy controls ($p < 0.001$) (Table 2). The average duration of FMF disease was 5.03 (range, 1-20) years. We identified no significant correlation between the disease duration and NMC time ($p=0.921$, $r = 0.019$) (Figure 1).

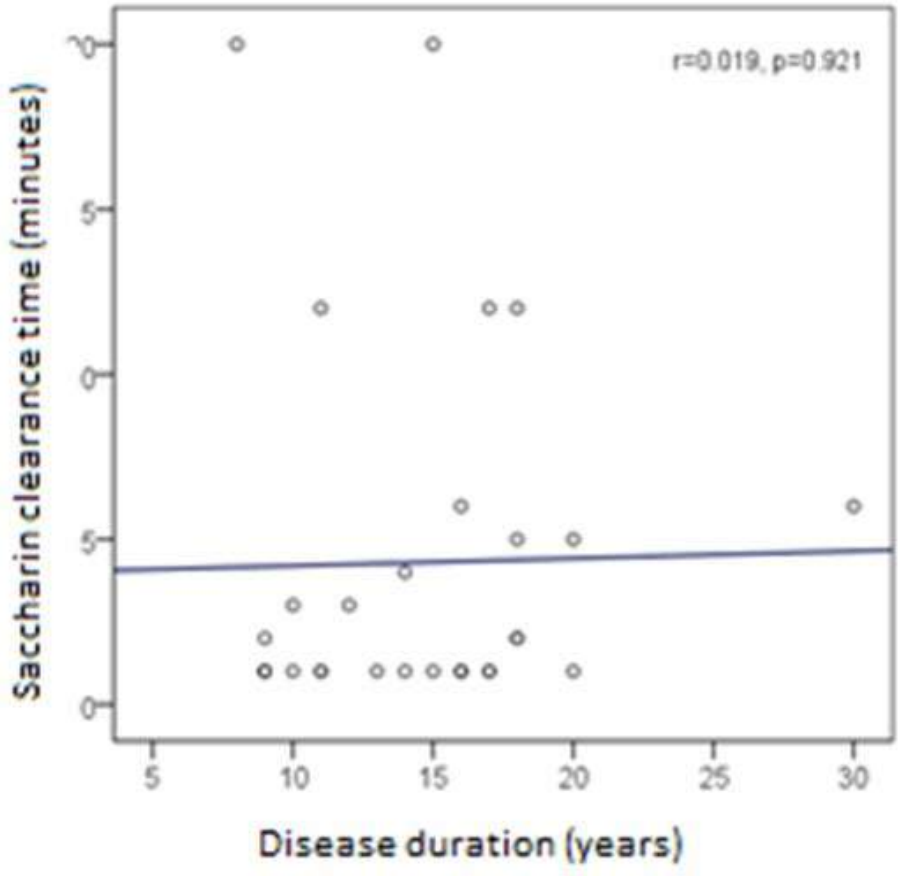

Figure 1. Correlation between the duration of FMF disease and nasal mucociliary clearance time.

The mean total nasal airway resistance in the FMF patient group and control group were found as 0.29 ± 0.11 (range, 0.18-0.60) Pa/mL/s and 0.27 ± 0.08 (range, 0.15-0.45) Pa/mL/s, respectively. (Table 2) When the mean total nasal airway resistance values of both groups were compared, there was no significant difference was detected ($p = 0.371$). (Table 2).

Table 2. Comparison of nasal parameters between FMF group and control group.

	FMF group(n=30)	Control group (n=30)	P[a]- value
Right Nasal airway resistance (Pa*s/ml)	0.65±0.37	0.52±0.15	0.088
Left Nasal airway resistance (Pa*s/ml)	0.66±0.39	0.58±0.19	0.313
Total Nasal airway resistance (Pa*s/ml)	0.29±0.11	0.27±0.08	0.371
Nasal mucociliary clearance time (seconds)	14.6±4.7	9.7±2.3	**<0.001**

[a] Independent samples t-test
Data represent means ± standard deviations Pa*s/ml = Pascal second per milliliter
FMF Familial Mediterranean Fever

DISCUSSION

Our study has presented that nasal mucociliary clearance time is prolonged in the FMF patients with saccharin test results in the attack-free period. However, there was no significant difference was detected in nasal airway resistance. To the best of our knowledge, our study is the first study to investigate the effect of FMF disease on nasal functions or nasal mucociliary activity. Therefore, there is no other study available to be compared with our results.

FMF is an autoinflammatory multisystem disease with heterogeneous clinical symptoms, caused by inherited mutations in MEFV gene, which encodes pyrin (12). A mutated pyrin causes uncontrolled production of interleukin (IL)-1β and IL-18 and consequently aberrant inflammation. These cytokines lead to increased TNF-α and IL-6. Increased serum levels of TNF-α, IL-1β and IL-6 have been demonstrated during FMF attacks and attack-free period (13,14).

Nasal mucociliary clearance system is the primary defense mechanism of the respiratory airway. Proper mucus production and coordinated ciliary activity are required for effective mucociliary clearance. It has been shown in several studies that inflammatory cytokines are effective on NMC (15–17). In their study, Gonzalez et al. (16) have revealed that TNF α, a proinflammatory cytokine, inhibits viscosity-related ciliary activity in human respiratory cultures. Disruption of NMC increases the risk of infection in the upper and lower respiratory tract (9). Hypertonic saline improves mucociliary clearance by reducing mucus viscosity and may reduce the risk of respiratory tract infections in patients with NMC impairment.(18)

In a previous study, it was found that nasal methicillin-resistance bacterial colonization was more frequently seen in FMF patients compared to the healthy population (19). Birik et al. (20) reported that respiratory symptoms and signs were significantly more common during FMF attacks. Recently, Celiksoy et al. (21) stated that severe atopic disease and recurrent respiratory infections are characteristic features of FMF disease. In the present study, it was found that there was longer NMC times in FMC patients compared to healthy controls. When the data were evaluated in the light of the literature respiratory symptoms in FMF patients may be associated with the disruption of the mucociliary clearance system.

The association of FMF with various systemic vasculitic disorders such as Henoch Schönlein Purpura, Behçet's disease and polyarteritis nodosa has previously been reported (22,23). The association between FMF and Behçet's disease has been demonstrated in various studies. It has been suggested that both conditions may have a common etiopathogenetic mechanism (24,25). Ozbay et al. (25) showed that NMC time was prolonged and positively correlated with disease duration in patients of Behçet's disease. In the present study, NMC time was found to be longer in the FMF patients compared to that of healthy controls. However, there was no correlation between nasal mucociliary clearance time and the disease duration.

Patients with nasal pathologies that may affect NMC and nasal airway resistance were excluded from the study and this was one of the strengths of our study. On the other hand, an important limitation of the present study was that one of these histology and electron microscopic examinations of the cilia and mucous structures was not performed. Another limitation of the present study was that our sample size was relatively small. Further studies should be conducted with greater number of patients using various methods to confirm the information obtained in this study, to reveal possible pathophysiological causes and to gather information about treatment approaches.

CONCLUSION

The results of our study have revealed that NMC time was longer in patients with FMF than that healthy controls. Disruption of NMC increases the risk of infection in the upper and lower respiratory tract. Caution should be exercised against upper and lower respiratory infections in patients.

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RESEARCH ARTICLE

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The Relationship between the Nutrition Habits, Body Mass Indexes and Academic Successes of the Health School Students

ABSTRACT

Objective: This research was conducted to examine the relationship between university students' nutritional habits, body mass index and academic achievements.

Methods: The study was conducted with 213 students studying at a school of health in a private university. Socio-demographic characteristics, height and weights and eating habits were asked in the questionnaire applied to students who volunteered with the informed volunteer consent form. The academic success of the students was evaluated on the basis of the end of year weighted grade point average scores.

Results: More than half of the participants (51.2%) were students of the Department of Nutrition and Dietetics. The majority of students (69.5%) are among the normal values according to the Body Mass Index classification. Of the students, 35.7% think that they always eat healthy and 49.3% eat healthy intermittently. The ratio of the students who stated that they skipped main meals was 87.8% and most frequently skipped meal was the lunch for 60.6% of the participants. The ratios of the students who always consume snacks and who consume snacks from time to time were 27.2% and 65.7% respectively. The academic success of the students who consume snacks regularly was significantly higher. It was found that the academic achievement of students who thought that they were eating healthy was higher than that of the others. There was a weak but statistically significant negative correlation between students' body mass index values and academic achievement.

Conclusions: A relationship between students' nutritional habits and academic success was found in our study. In order to raise healthy, productive and successful generations, the importance of nutrition should be emphasized more especially in adolescent period in which life-time basic nutrition habits are adopted. Nutrition trainings and lessons may be useful for this purpose.

Keywords: Nutrition, Body Mass Index, Student, Academic Success

Sağlık Yüksekokulu Öğrencilerinin Beslenme Alışkanlıkları, Beden Kitle İndeksleri ve Akademik Başarıları Arasındaki İlişki

ÖZET

Amaç: Bu araştırma, üniversite öğrencilerinin beslenme alışkanlıkları, beden kitle indeksleri ve akademik başarıları arasındaki ilişkinin incelenmesi amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışma bir üniversitede sağlık yüksekokulunda okuyan 213 öğrenci ile gerçekleştirilmiştir. Bilgilendirilmiş gönüllü olur formu ile gönüllü olan öğrencilere uygulanan ankette sosyodemografik özellikleri, boy ve kilosu, beslenme alışkanlıkları sorulmuştur. Öğrencilerin akademik başarıları ise yılsonu ağırlıklı genel not ortalamaları (AGNO) ile değerlendirilmiştir.

Bulgular: Araştırmaya katılan öğrencilerin yarıdan fazlası (%51,2) Beslenme ve Diyetetik Bölümü öğrencisidir. Öğrencilerin çoğunluğu (%69,5) Beden Kitle İndeksi (BKİ) sınıflandırmasında normal değerler arasındadır. Öğrencilerin % 35,7'si her zaman, %49,3'ü ise bazen sağlıklı beslendiğini düşünmektedir. Öğrencilerin %87,8'i öğün atladığını belirtirken, %60,6'sı en sık öğle yemeğini atladığını belirtmiştir. Öğrencilerin %27,2'si her zaman, %65,7'si bazen ara öğün tüketmektedir. Düzenli olarak ara öğün tüketen öğrencilerin akademik başarıları istatistiksel olarak daha yüksek bulunmuştur ($p<0,05$). Sağlıklı beslendiğini düşünme durumuna göre öğrencilerin akademik başarıları arasında anlamlı farklılık olduğu saptanmıştır. Sağlıklı beslendiğini düşünen öğrencilerin akademik başarılarının bazen sağlıklı beslendiğini düşünen ve sağlıklı beslendiğini düşünmeyen öğrencilere göre anlamlı olarak yüksek olduğu bulunmuştur ($p<0,05$). Öğrencilerin beden kitle indeksleri ile akademik başarıları arasında anlamlı, negatif yönlü zayıf bir ilişki olduğu tespit edilmiştir ($r= -,178$; $p=0,009$).

Sonuç: Öğrencilerin beslenme alışkanlıkları ile akademik başarıları arasında ilişki saptanmıştır. Özellikle gelişimin devam ettiği ve kazanılan alışkanlıkların erişkin yaşlarda devam edebileceği adölesan çağda sağlıklı, üretken ve başarılı nesiller yetiştirmek amacıyla beslenme konusuna daha çok önem verilmelidir. Beslenme eğitimleri ve teşvikler bu amaca faydalı olabilecektir.

Anahtar Kelimeler: Beslenme, Beden Kitle İndeksi, Öğrenci, Akademik Başarı

INTRODUCTION

There are many factors that determine our health. Our lifestyle has an important place among these factors. The most important element of our lifestyle is nutrition. People's healthy living, being productive and successful are related with adequate and balanced nutrition (1). Adequate and balanced nutrition is the intake of all the nutrients necessary for the growth of the body, the renewal and functioning of the tissues in sufficient quantity, in the required amount and the proper use in the body (2). Inadequate and unbalanced nutrition has negative effects on mental development as well as physical development, namely it causes a decrease in learning, intelligence averages and also leads behavioral disorders (3). Poor nutrition also affects the immune system negatively and decreases the body resistance. Nutrition plays an important role on the efficiency of school and business lives (4).

Sociodemographic and geographic features can create differences in nutritional habits. Unbalanced eating habits can be seen more in families with low economic status. In addition, if the family also has poor and unbalanced eating habits independent of the socioeconomic conditions, individuals grown in such families may maintain poor eating habits in the future (5).

Balanced, regular and adequate nutrition is important for healthy growth and development in the adolescent age (6). In addition, increasing aesthetic concerns during adolescence can cause inadequate and imbalanced eating habits. These aesthetic concerns are especially highamong young females (7). Especially during these periods, the frequency of eating out increases due to social activities, staying in dormitories or not being able to prepare their own meals (8). Unhealthy food alternatives such as junk food and fast food cause deficiencies in a healthy and adequate diet. Junk food and fast food do not adequately meet the micronutrients needed by the body (9).

A significant number of students attending higher education live away from their families and this creates difficulties in nutrition, housing, school expenses and health problems. Nutrition is a problem especially for students staying in dormitories (10). In our country, studies about the dietary habits of university students reported that there are serious problems with nutrition such as irregular eating habits and skipping meals, especially the breakfast. (8).

When fed with high-energy but non nutritious foods, obesity and overweight condition may develop along with unnecessary calories. Problems that start in this period can cause adolescents to become obese and overweight adults. Solving these problems is much more difficult in adulthood than in adolescence (11). The body mass index (BMI) is the most common and valid standard height-weight index accepted by the World Health Organization (12). Individuals according to BMI values are classified as weak, normal, overweight and obese. Obese people can also be divided into classes among themselves.

In a research conducted on 1,120 students at Atatürk University, it was found that the nutritional habits and nutritional knowledge levels of the students were largely far from ideal. In this study, a positive correlation was found between students' body mass index and nutritional habit score, monthly personal income (13).

In a study conducted on the first year university students, factors such as nutrition habits, sleep time, awakening time, exercise, mental health status that might affect academic success were examined. Positive effects of breakfast on memory were emphasized (14). In order to achieve better concentration and higher academic success levels breakfast is essential. In a study conducted on 115 adolescents, the students who have regular breakfast habits were found to have more ability to comprehend arithmetic (15). Breakfast is important in terms of concentration, especially in school-age individuals. In addition to this, it is also effective on academic performance since cognitive performance will decrease when breakfast is skipped. Starting the day with breakfast has an effect on short term and long term memory (16). In a study, it was found that breakfast consumption 30 minutes before the exam had positive effects on recall skills (17).

This research was carried out to determine the nutritional habits and body mass indices of university students and to examine the relationship of these characteristics with academic success.

MATERIAL AND METHODS

This descriptive study was carried out with 213 students of the school of health in a private university in Istanbul. The target group was 402. The study group was 213. The participation rate was 53%. Approval from the Ethics committee and institutional permissions were obtained for the implementation of the research (Decision No: 29.11.2016, 55-14; Institution permission date: 11.01.2017).

The study was conducted on a voluntary basis, and a questionnaire was applied to the students after the informed volunteer consent form. In the creation of the questions in the survey form we based on the questions applied in the work of Yilmaz and Ozkan (2007) (18). In the survey form we asked the gender, age, sociodemographic variables, nutritional habits, weight and height. Some variables can be seen in Table 1 and Table 2. The students provided their height and weight measurements and the researchers calculated the index values for each student.

The academic success of the students was determined regarding their weighted Grade Point Average (GPA) scores obtained from Student Affairs Office. The data were analyzed using the

SPSS statistics program. The significance level was taken as $p < 0.05$ for all statistical analyses. Frequency and percentage calculations, one way analysis of variance (ANOVA), student t test and Pearson correlation analysis were used to analyze the data.

RESULTS

Mean age of the participants was 21,69 + 2,77. 84.9% of the students was female. The average body mass index of students was calculated as 21,9 + 3,19. Socio-demographic characteristics of the participants were provided in Table 1.

Table 1. Sociodemographic characteristics of Students

Sociodemographic Variables		**N**	**%**
Department	Nutrition and Dietetics	109	51.2
	Midwifery	20	9.4
	Physiotherapy and Rehabilitation	74	34.7
	Health Institutions Management	10	4.7
BMI (Body Mass Index)	Weak	32	15.0
	Normal	148	69.5
	Overweight	28	13.1
	Obese	5	2.3
Grade	First	30	14.1
	Second	58	27.2
	Third	47	22.1
	Fourth	78	36.6
Last School Graduated	Vocational High School	16	7.5
	High School	28	13.1
	Science/Anatolia/Super High School	137	64.3
	University / College	32	15.0
Where to Stay	With their parents	116	54.5
	At the dormitory	44	20.7
	At home with friends	38	17.8
	Other	15	7.0
Income status	Income less than expense	28	13.1
	Income equal to expense	125	58.7
	Income more than expense	60	28.2
Source of the allowance	Family	154	72.3
	Scholarships or loans	59	27.7
Mother's Educational Background	Not literate	2	.9
	Elementary school graduate	93	43.7
	High school graduate	80	37.6
	College graduate	38	178
Father's Educational Background	Elementary school graduate	53	24.9
	High school graduate	84	39.4
	College graduate	76	35.7
TOTAL		213	100.0

More than half (54.5%) of students said that they have received nutrition related training previously. When asked which meal they cared most about, 41.8% of the students answered breakfast and 41.3% answered dinner. We determined that students attached equal importance to breakfast and dinner. It is seen that 15% of the students do not think that they were eating healthy. 34.7% of them were skipped meals, 53,1% sometimes skipped meals and it was seen that the most skipped meal was lunch (60.6%) (Table 2).

Table 2. Relationship Between Nutritional Habits, Sociodemographic Variables and Body Mass Index

NUTRITIONAL HABITS AND SOCIODEMOGRAPHIC VARIABLES		BODY MASS INDEX			Total
		Weak	Normal	Lightweight and fat	
Get nutrition training	Yes	21	80	15	116
	No	11	68	18	97
Think eating healthy	Yes	12	57	7	76
	Sometimes	17	69	19	105
	No	3	22	7	32
Which meal the most important	Breakfast	14	65	10	89
	Lunch	4	23	7	34
	Dinner	13	59	16	88
	Other	1	1	0	2
Skipping meal	Yes	12	52	10	74
	Sometimes	17	75	21	113
	No	3	21	2	26
Most skipped meal	Breakfast	10	58	16	84
	Lunch	22	90	17	129
Snack consumption	Yes	13	39	6	58
	Sometimes	0	13	2	15
	No	19	96	25	140
Where to Stay	With Family	20	83	13	116
	At the Dormitory	7	26	11	44
	At home with friends	4	27	7	38
	Other	1	12	2	15
Department	Nutrition and Dietetics	19	78	12	109
	Midwifery	2	14	4	20
	Physiotherapy and Rehabilitation	11	51	12	74
	Health Institutions Management	0	5	5	10
Where it meets the allowance	From family	25	108	21	154
	From scholarships or Loans	7	40	12	59
Income status	Income less than expense	3	18	7	28
	Income equal to expense	16	93	16	125
	Income more than expense	13	37	10	60
Mother Education Status	Not literate	0	2	0	2
	Elementary school graduate	9	70	14	93
	High school graduate	17	50	13	80
	College graduate	6	26	6	38
Father Education Status	Elementary school graduate	5	37	11	53
	High school graduate	19	56	9	84
	College graduate	8	55	13	76

When questioned the reasons why you skipped meals, the students were " I could not wake up in the morning (29.6%)"," I did not have an appetite / no craving (29.1%)", "I can not have a preparation (26.3%)," "I'm late for school (26.3%)" answered. When questioned like where you consume the snack food 63.8% of students said that they consume in the home environment, 44.6% in the restaurant / cafe / patisserie and 32.9% in the school / dormitory canteen refectory. It was determined that, 27.2% of students consumed every time but 7.0% consumed sometimes snack foods and 65.7% did not consume snacks. The foods and beverages that students have stated that they consume the most at breakfast were listed as cheese (88.3%), tea (81.2%), egg (76.5%), bagel / bread (67.1%); the foods and beverages that consumed the most in the lunch and dinner meals by them were seen such as meat / chicken / fish / meatball (89.7%), soup (81.2%), salad (76.1%), bread (58.7%), meatless vegetable dishes (55.9%), rice (54.9%), legumes (53.5%), pasta (52.6%),

vegetables with meat (47.9%), milk / yogurt / ayran (65.3%), juice (62.9%) .

When the body mass index is classified as weak, normal, overweight-obese no statistically significant relationship was found in terms of body mass index, nutritional habits, sociodemographic characteristics and foods consumed in meals. The rate of overweight and obese people was 11.0% in Nutrition and Dietetics Department and this rate was 16.2% for the Physical Therapy and Rehabilitation Department, 20.0% for the Midwifery Department and 50% for the Health Institutions Management. When the curricula of the departments are examined, it is noteworthy that there are no courses on nutrition in other departments.

Findings Regarding Relationships Between Body Mass Index and Academic Success

• GPA scores of students with normal weight was found to be significantly higher than those of overweight students (one way analysis of variance, p= 0.034).

• A significant negative correlation was found between students' body mass index values and GPA scores (pearson correlation analysis, r = -. 178; p = 0.009).

Findings Regarding Relationships Between Nutritional Habits and Academic Success

• It has been determined that there is a significant difference between students' academic achievement according to the state of thinking that they are eating healthy. It was found that the GPA scores of the students who thought that they always eat healthy was higher than those of students who thought that they eat healthy sometimes and who thought that they eat unhealthy (one way analysis of variance, $p < 0.05$).

• There is a significant difference between students' GPA scores according to the snack consumption (one way analysis of variance , $p < 0.05$). The GPA scores of students who consume snacks regularly were found significantly higher than those of students who doesn't consume snacks.

• There was no significant relationship between any of the other parameters mentioned in Table 2 and GPA scores.

DISCUSSION

In our study, the relationship between university students' body mass index, sociodemographic factors, eating habits and academic achievements were examined. The average body mass index of students was normal.

In a study conducted on Faculty of Education Sciences students to determine the nutritional consumption status, nutritional habits and nutritional knowledge levels according to the BMI 65.4% students were normal and 0.6% were obese (19). In this study, the low number of obese can be interpreted that obesity was not common yet before 2000s. In another study conducted with adolescents 14.7% of students were overweight (20). In a study conducted with students studying at the Vocational School of Health Services, 69.7% of students' the body weight were found to be normal weight, 18.8% were overweight (21). In another study conducted on 424 students, 15.6% were overweight and 2.4% were obese (22). In the studies published in 2000s, the remarkable situation is the decrease of the weak students and the increase of overweight and obese students.

In another study conducted on 424 students regarding nutritional habits, most of students skipped meals and it was observed that the most important reason for this was the lack of time (22). In another study, the main meal that students skip the most was breakfast and the vast majority of them were overweight, indicating that the students did not eat enough, balanced and regularly (1). In another study, it was found that most of the students skipped the main meal and the most skipped meal was lunch (23). In another study conducted with higher education students, it was found that the most irregular and inadequate food consumed meal was the morning meal and 20.3% of students skipped one of the three meals daily (24). The most common reason for skipping meal is stated as not having time. The reasons stated as the reason for skipping meals are generally related to unplanned life and wrong eating habits (24). While the nutritional habits, the effecting factors and the nutritional status of 557 students studying at the Faculty of Medicine were examined it is found the students who were fed with three main meal a day were 29.6%. The percent of the students who are fed with three main meal plus at least one snack food were 41.3%. It was determined that the main meal that students attach importance to was dinner (25). In a study conducted with university students, the most skipped meal among the students was breakfast (26). In another study 52.48% of the students prefer to have their meals in the restaurant. 27.46 % of students skip the breakfast in the morning. Students showed for unwillingness, lack of time and similar reasons in this regard (27). In another study, it was determined that 61.2 % of students did not have breakfast because they're late to the lesson or did not have time (28). Different from these research results, in our study when asked which meal they cared most about we determined that students attached equal importance to breakfast and dinner. In our study, it was determined that 15% of students did not think that they were eating healthy, 34.7% of them skipped meals always and the most skipped meal was as lunch. As a result, the less important meal for the students is determined as lunch in our study.

In order to take energy and nutrients at the recommended level during the youth period, attention should be paid to the consumption of main and intermediate meals. The number of meals is

important in adequate and balanced nutrition. Skipping meals, especially breakfast, negatively affects school success in children and adolescents, it prepares the ground for inadequate and unbalanced nutrition (29,30).

In a study, it was seen that most of the students had breakfast at the dormitory, and preferred cheese-bread, pastry, bagels and tea (31). In the other study, the most preferred foods by university students for breakfast were cheese-olives, bagel and tea (32). Similar to these findings, in our study the foods and beverages that students stated that they consume the most at breakfast were cheese, tea, egg and bagel / bread.

In a study conducted with the students of the Faculty of Education, it is found 55.6% of students had breakfast in the morning (19). In another study conducted with the students of the Faculty of Health Sciences, 69.7% of students have breakfast every day (33). In a study conducted on 144 students, 16.7% of students never reported having breakfast, and the frequency of those who regularly consume breakfast, dinner and snacks was 32.6%, 75.0% and 23.6% was seen respectively (34). In a study conducted with students at the School of Health, students' regular breakfast, lunch and dinner consumption rates were 72.5%, 88.4% and 98.5% respectively (35). In a study conducted with university students in Nigeria, the most skipped meal was found to be breakfast with a rate of 73% (36).

In our study, a significant negative correlation was found between students' body mass indexes and academic achievement. In a study (Hollar et al., 2010) conducted with 4588 students, assessed the effects of a school-based obesity prevention intervention that included dietary, curricula, and physical activity components on body mass index (BMI) percentiles and academic performance among low income elementary school children. It was observed that more obese students in the intervention than in the control decreased their BMI percentiles. Intervention students had significantly higher math scores (37).

In a study aimed to determine the relationship between the nutritional habits of university students and their academic success it was determined that 66.2% of the students had normal weight and there was a negative correlation between the nutrition habits index scores of the students and their academic success points. It was determined that as the risk level of nutritional habits of students increased, their academic success decreased (38).

In a study it was found that there is a relationship between academic failure and social inequality. Students with lower socio-economic level have lower academic achievement than students with upper socio-economic level (39). While the increase in the education level of the mother and father affects nutrition positively, the increase in the monthly income of the family increases the fast food consumption (40). In our study, no significant relationship was found between the mother or father education level, the students' body mass index and academic success.

In our study, the academic success of students who stated that they consumed snacks was found higher than students who stated that they did not consume or sometimes consumed. It is the right approach to gain the habit of consuming snacks in the daily diet. Snack consumption prevents individuals from eating too much and contributes to the regulation of fasting blood sugar. In a study, it was determined that 91.7% of the participants were consuming snacks (41). Similarly, in another study, it was found that 90.0% of university students have snack habits (42). It is necessary to pay attention to the consumption of snacks in order to get energy and nutrients at the recommended level and for school success in youth (31).

In our study there was no significant relationship between skipping meals and GPA scores. Different from our research results, in a study conducted by Diremler in 2009, it is seen that the average success of students who do not skip meals during the day is higher than those who skip meals (43). In a study conducted on 115 adolescents, it was found that the adolescents who had regular breakfast their abilities to grasp the arithmetic were higher than those who did not have breakfast (15). According to the results of 47 studies that examined the relationship between breakfast consumption and nutritional efficiency, body weight and academic performance in children and adolescents, skipping breakfast is quite common in the USA and Europe (10% - 30%) depending on age group, population and definition. Evidence supports that breakfast consumption can improve cognitive function related to memory, test grades and school attendance (29). In a study conducted on 5200 students in Canada, it was found that socioeconomic factors and diet quality had a significant impact on academic performance. The same study emphasizes the effect of not only breakfast but also food quality on the academic performance (44).

According to the findings obtained in a research conducted with the students studying in the college; 34.0% received nutrition education, 75.5% received this education from the school and 24.5% from the conference-panels; 36% believed that they were eating healthy and 64% did not believe that they were eating healthy (45). In our study, more than half (54.5%) of students said that they have received nutrition related training previously.

CONCLUSION AND SUGGESTIONS

As a result, in our study, it was found that the students with normal body mass index, who were eating healthy and consuming snack meals had higher academic success. A biopsychosocial

approach can be applied to students with high body mass index in the medical centers of universities with a team of medical doctors, nutritionists and dietetics specialists and psychologists. Considering that the habits to be acquired in adolescence and the disturbances that may occur will continue in adulthood, this period is very important. Growing successful, healthy and productive generations in the future is possible with sufficient and healthy nutrition. Healthy eating habits should be gained primarily at home; everyone who gives education to children and adolescents should be aware of this issue. In this context, it is thought that it would be an appropriate approach to place nutrition lessons in the whole education programs of medical schools and health sciences.

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RESEARCH ARTICLE

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Choledocholithiasis without Cholecystolithiasis, After Endoscopic Retrograde Cholangiopancreatography Follow-Up without Cholecystectomy

ABSTRACT

Objective: The widespread use of imaging methods increases the diagnosis and incidence of cholelithiasis. In recent years, management of patients with cholelithiasis has improved significantly owing to significant advances in surgical and endoscopic intervention.

Methods: Between February 2015 and January 2018, the data of patients who underwent ERCP at our institution were collected prospectively and analyzed retrospectively. Forty-four patients included in the study were patients with choledocholithiasis without stones in the GB.

Results: The mean age of 52 subjects (group A) included in the study was 64.83 ± 17.06, of which 23 (44.2%) were <65 years old and 29 (55.8%) were ≥65 years old. Twenty-four (46.2%) women and twenty-eight (53.8%) men were included in the study. Malignancy was suspected in 6 (11.5%) patients and no suspicion of malignancy was confirmed after Endoscopic Retrograde Cholangio-Pancreatography (ERCP) procedure. In group A, 14 patients (26.9%) required a second ERCP, while in group B, the number of patients requiring a second ERCP was 163 (21.3%). The mean length of hospital stay in group A and group B patients was 5.29 ± 3.38 and 6.29 ± 5.39, respectively, and the average cost was 474 $ ± 286 $ and 564 $ ± 664 $, respectively, with no statistical difference between the groups.

Conclusions: In conclusion, we think that in patients with secondary or primary choledochal stones without gallstones and with functional GB might be following up without cholecystectomy after the stone is removed from the bile duct by ERCP.

Keywords: Gallbladder, Laparoscopic Cholecystectomy, Endoscopic Retrograde Cholangiopancreatography

Kolelitiazis Olmadan Gelişen Koledokolitiazis Hastalarının Endoskopik Retrograd Kolanjiopankreatografi Sonrası Kolesistektomisiz Takibi

ÖZET

Amaç: Görüntüleme yöntemlerinin yaygın kullanımı kolelitiyazis tanı ve insidansını artırmaktadır. Son yıllarda, kolelitiazisli hastaların tedavisi, cerrahi ve endoskopik müdahaledeki gelişmeler nedeniyle önemli ölçüde iyileşmiştir.

Gereç ve Yöntem: Şubat 2015 - Ocak 2018 tarihleri arasında kurumumuzda ERCP uygulanan hastaların verileri prospektif olarak toplandı ve geriye dönük olarak analiz edildi. Çalışmaya dahil edilen kırk dört hasta, Safra kese'de (SK) taşsız koledokolitiazis hastalarıdır.

Bulgular: Çalışmaya dahil edilen 52 kişinin (grup A) ortalama yaşı 64,83 ± 17,06 idi, bunların 23'ü (% 44,2) <65 yaşında ve 29'u (% 55,8) ≥65 yaşında idi. Çalışmaya 24 (% 46,2) kadın ve yirmi sekiz (% 53,8) erkek dahil edildi. 6 (% 11,5) hastada malignite şüphesi vardı ve Endoskopik Retrograd Kolanjiyo-Pankreatografi (ERCP) prosedürü sonrasında malignite şüphesi doğrulanmadı. Grup A'da 14 hasta (% 26,9) ikinci bir ERCP'ye ihtiyaç duyarken, grup B'de ikinci bir ERCP gerektiren hasta sayısı 163 (% 21,3) idi. Grup A ve grup B hastalarında ortalama hastanede kalış süresi sırasıyla 5,29 ± 3,38 ve 6,29 ± 5,39, ortalama maliyet sırasıyla 474 $ ± 286 $ ve 564 $ ± 664 $ idi ve gruplar arasında istatistiksel fark yoktu.

Sonuç: Sonuç olarak, safra taşı olmayan sekonder veya birincil koledok taşı olan hastalarda fonksiyonel SK olan hastalarda taş ERCP ile safra kanalından çıkarıldıktan sonra kolesistektomi yapılmadan takip edilebileceğini düşünüyoruz.

Anahtar Kelimeler: Safra Kesesi, Laparoskopik Kolesistektomi, Endoskopik Retrograd Kolanjiyopankreatografi

INTRODUCTION

The widespread use of imaging methods increases the diagnosis and incidence of cholelithiasis. In recent years, management of patients with cholelithiasis has improved significantly owing to significant advances in surgical and endoscopic intervention. In contrast, there are some questions that require answers in the management of this common disease. Choledocholithiasis occurs in approximately 20% of patients with cholelithiasis (1). Most stones are formed in the gallbladder (GB) and then migrate to the common bile duct, causing biliary obstruction. Primary choledocholithiasis refers to stones directly formed in the bile tree wheras secondary choledocholithiasis refers to stones originating from the GB and migrating to the common bile duct (2). It is estimated that 20% of patients with choleithiasis have choledocholithiasis and half of the cases are asymptomatic. Biliary colic precedes complications in 90% of cases. The natural course of choledocholithiasis is unpredictable and not well defined. Untreated bile duct stone obstruction usually causes secondary biliary cirrhosis after about 5 years (4). The aim of this study is to investigate whether cholelithiasis or recurrent choledocholithiasis develops during routine follow-up of patients who underwent Endoscopic Retrograde Cholangio-Pancreatography (ERCP) for choledocholithiasis without gallstones. At the same time, the aim is also to contribute to the management of cholelithiasis and choledocholithiasis with short and long term follow-up results of our study by accepting that cases in our study are primary or secondary choledocholithiasis.

Choledocholithiasis without cholelithiasis

Nowadays, it is obvious that the gold standard in the treatment of cholelithiasis is Laparoscopic Cholecystectomy (LC). On the other hand, in the literature, Gallbladder-sparing surgery (GSS) has been started to apply in GB stones. In their study of 65 patients with the diagnosis of cholelithiasis, Tan Y et al. performed minimally invasive surgery with GB preservation; and for 61 of them, GB was perfectly preserved without complication. In the other four cases, laparoscopic cholecystectomy was performed because of tiny stones obstructing the cystic duct. According to this study, it has been shown that GB can maintain its function and can effectively avoid various complications of cholecystectomy. They also reported that gall bladder function was not affected during the follow-up of patients and the rate of stone recurrence was very low (5). In that study, they didn't mention the patients with acalculous cholecystitis, existed due to trauma, burn, cardiopulmonary resuscitation and staying in intensive care unit. In our study, cases of acalculous cholecystitis were excluded, and we presented the patients who were admitted to our clinic without gallstone and with jaundice or cholangitis due to choledocholithiasis. Also in our study, we investigated whether short and long-term follow-up results may be a source of light for some new ideas after ERCP procedure applied to patients with choledochal stones without gallstones. It was also quite difficult to prove whether the common bile duct stones were primary or secondary in our study. However, secondary choledocholithiasis may be considered if there is a ultrasonography (USG)-proven stone before the diagnosis of choledocholithiasis. On the other hand, there is another possibility that there was only one stone in the GB and then this stone migrated to the common bile duct. In our study, none of the patients had a diagnosis of cholelithiasis in their USG, applied before ERCP. The colors of biliary stones are also important to assess whether the common bile duct stones are primary or secondary. Epidemiologically, primary and secondary bile duct stones are highly variable. In Western societies, most bile duct stones are secondary and prevalence increases with age. Primary bile duct stones are more common in Asia. Primary stones are associated with bacterial contamination of common bile duct due to biliary enteric anastomoses, sphincterotomy, stents, instrumentation and portal bacteremia. Periampullary diverticula provide a suitable site for bacterial proliferation because of subsequent backflow to the bile duct (6). Cholesterol is the main component in about 80% of GB stones, and 10% of these stones are made up of pure cholesterol. Pigment stones, by definition, contain less than 25% cholesterol and the main component is calcium bilirubinate (7). In the presence of stones in the common bile duct, even if there is no stone in the GB, this is an indication for ERCP. Diagnosis of choledocholithiasis is not always easy and clinical evaluation and biochemical tests are often not sufficient to make a definitive diagnosis. Several methods are available to evaluate patients with choledocholithiasis, including laboratory tests, ultrasound, computed tomography scans (CT), and magnetic resonance cholangiopancreatography (MRCP) (8). Imaging tests, especially abdominal ultrasound, are routinely used to confirm the diagnosis. Ultrasound has the best sensitivity and specificity to evaluate patients with suspected GB stones (9). Liver function tests (LFT) can be used to estimate common bile duct stones. Elevated serum bilirubin level and alkaline phosphatase level typically show biliary obstruction; however, they are not very sensitive or specific for common bile duct stones. Elevated GGT level has been suggested to be the most sensitive and specific indicator for choledocholithiasis. In this study, we summarized the literature and discussed the factors that determine the appropriate treatment method. In the literature, the number of studies about GSS has

increased and interest for this topic has been rising. In our study, patients were followed up without cholecystectomy after common bile duct stone removal. Therefore, our study also addresses GSS, a new trend in the current literature. However, it has been accepted that the treatment of cholecystolithiasis is surgical and LC is the gold standard. Our aim in this article is to evaluate the treatment results of patients with choledocholithiasis without cholecystolithiasis and to review the current literature and make recommendations for such patients' management.

MATERIAL AND METHODS

Between February 2015 and January 2018, the data of patients who underwent ERCP at our institution were collected prospectively and analyzed retrospectively. Forty-four patients included in the study were patients with choledocholithiasis without stones in the GB. Transabdominal USG was used to determine the absence of stones in the GB of these patients. All patients planned to undergo ERCP (Figure 1) were diagnosed as choledocholithiasis by MRCP In patients with acalculous cholecystitis (intensive care unit patients, burn patients) and with cholecystectomy, stones that were formed in the first two years (secondary bile duct stones) and stones formed after 2 years (primary bile duct stones) were not included in this study. Patients with choledocholithiasis without cholelithiasis were identified as "group A" and patients who underwent ERCP for other reasons were identified as "group B". Demographic findings, length of hospital stay, cost, and LFT findings were evaluated among the groups. In the short and long-term follow-up after ERCP in group A, patients were evaluated whether there was a stone formation in GB and there was an acute cholecystitis attack, by using USG.

Written informed consent for the procedure and use of data was obtained from each patient in accordance with the Declaration of Helsinki. This study was approved by the institutional review board in the institution.

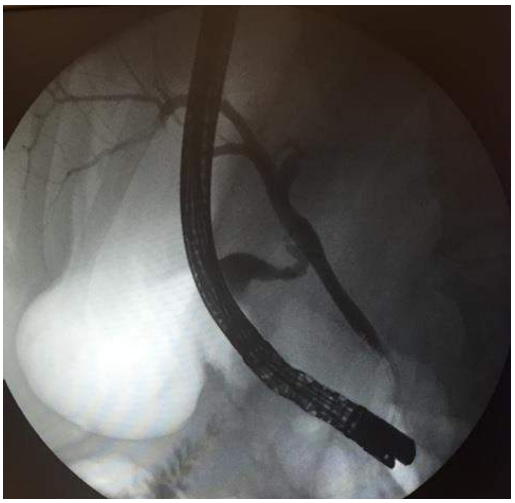

Figure 1. Bile ducts and gallbladder were visualized on cholangiography**.**

Statistical Analysis: The normality of distribution of continuous variables was tested by Shaphiro Wilk test. Mann Whitney u test was used to compare 2 independent group for non-normal data and Kruskal Wallis and Dunn multiple comparison test were applied for three group comparisons.

Chi-square test was applied to investigate relationship between 2 categorical variables. Statistical analysis was performed with SPSS for Windows version 24.0 and a P value < 0.05 was accepted as statistically significant.

RESULTS

The mean age of 52 subjects (group A) included in the study was 64.83 ± 17.06, of which 23 (44.2%) were <65 years old and 29 (55.8%) were ≥65 years old. Twenty-four (46.2%) women and twenty-eight (53.8%) men were included in the study. Malignancy was suspected in 6 (11.5%) patients and no suspicion of malignancy was confirmed after ERCP procedure. In group A, 14 patients (26.9%) required a second ERCP, while in group B, the number of patients requiring a second ERCP was 163 (21.3%) (Table1).

Table 1. Demographic findings and procedures

		Stone-free gall bladder and ERCP		
		Yes (n=52) Choledocholithiasis without cholelithiasis	**No (n=760) Choledocholithiasis with cholelithiasis(and other cause for ERCP)**	**P**
Age	<65	23 (44,2)	399 (52,5)	0.248
	>=65	29 (55,8)	361 (47,5)	
Gender	M	28 (53,8)	345 (45,2)	0.224
	F	24 (46,2)	419 (54,8)	
Suspected Malignancy	Yes	6 (11,5)	26 (3.4)	0.003
Bile Leakage	Yes	1 (1,9)	8 (1)	0.558
2. Ercp Needs	Yes	14 (26,9)	163 (21,3)	0.344
Status	PD	52 (100)	760 (99,5)	0.601
Stent Insertion	Yes	9 (17,3)	155 (20,3)	0.604
Sphincterotomy	Yes	49 (94,2)	719 (94,1)	0.971
Sclerotherapy	Yes	1 (1,9)	18 (2,4)	0.841

*Significant at 0.05 level. Chi-square test.

ERCP, Endoscopic Retrograde Cholangiopancreatography. PD, Patient discharged(PD)

Difficult ERCP was due to the difficulty of selective cannulation of choledochus. Precut sphincterotomy was performed in these patients and the procedure was performed successfully in the second ERCP (Figure 2). The mean length of hospital stay in group A and group B patients was 5.29 ± 3.38 and 6.29 ± 5.39, respectively, and the average cost was 474 $ ± 286 $ and 564 $ ± 664 $, respectively, with no statistical difference between the groups.

Figure 2. Choledochal stone was removed after endoscopic sphincterotomy

The presence of suspicion of malignancy was significantly higher in patients with stone-free gallbladder, who had undergone ERCP (P = 0.003). The mean age was significantly higher in group A patients than group B (P = 0.001). In addition, liver function tests were significantly lower in group A, while WBC was higher (Table 2). Long-term results were followed up but 10 patients could not be reached, and two elderly patients died due to another cause. One patient underwent ERCP 12 months later due to recurrent stone formation in the common bile duct. In two patients, biliary sludge was detected in gallbladder on control abdomen USG, and after medical treatment and diet, there was no sludge in gallbladder. Laparoscopic cholecystectomy was performed in two patients because of gallbladder stone formation, and in two patients because of acute cholecystitis which developed in early period. In the routine outpatient clinic visits of the remaining 34 patients (4-18 months of follow-up), no stones were detected in the gallbladder and routine outpatient control was recommended as the patients had no complaints.

Table 2. Hospitalization, cost and liver function tests

Stone-free gall bladder and ERCP

Variables	Yes (n=52) Choledocholithiasis without cholelithiasis	No (n=760) Choledocholithiasis with cholelithiasis(and other cause for ERCP)	P
Age	64.83 ± 17.06	61.45 ± 19.03	0.001*
Hospital Stay	5.29 ± 3.38	6.29 ± 5.39	0.936
Cost	2008.78 ± 1213,13	2389.05 ± 2812,26	0.779
ERCP Number	1.17 ± 0.38	1.18 ± 0.53	0.078
MN White blood cells	6.42 ± 2.51	6.42 ± 2.34	0.002*
MX White blood cells	12.5 ± 9.88	12.25 ± 7.09	0.484
MN GGT	192.55 ± 165,46	197.53 ± 201,22	0.005*
MXGGT	310.29 ± 232,79	369.89 ± 338,36	0.020*
MN Total bilirubin	0.74 ± 0,9	0.88 ± 1.57	0.091
MX Total bilirubin	3.25 ± 3.41	3.41 ± 3.69	0.153
MN ALT	56.27 ± 59.77	62.79 ± 69.95	0.001*
MX ALT	199.63 ± 267,78	210.03 ± 240,31	0.010*
MN AST	35.85 ± 19.88	41.21 ± 42.96	0.031*
MX AST	202.69 ± 322,35	189.85 ± 226,59	0.150
MN Amylase	76.23 ± 118,28	62.2 ± 79.79	0.354
MX Amylase	351.08 ± 493,26	488.49 ± 691,77	0.502

*Significant at 0.05 level. Mann whitney u test.
MN, The minimum value during hospitalization. MX, The maximum value during hospitalization. *GGT*, Gamma-glutamyl transferase. *ALT*, *A*lanine aminotransferase. *AST* Aspartate aminotransferase. ERCP, Endoscopic Retrograde Cholangiopancreatography.

DISCUSSION

The presence of stones in the bile duct without stones in the GB requires the primary or secondary distinction of these stones. The actual numbers of such patients are unknown and there are not enough publications in the literature. It is difficult to determine the primary or secondary distinction of stone in patients with choledocholithiasis without cholelithiasis, but it is partially possible with some methods. As weak evidence, it is possible to have an idea about distinction via looking for presence of

stones in the GB in USG of the patient in the last 3 months, and via evaluating the visual color and composition of gallstones in private laboratories. In our study, all patients were evaluated by transabdominal ultrasonography before ERCP and it was shown that there were no stones in the GB.

USG is very sensitive in determining the presence of stones in the GB. In the presence of gallstones, visualization of GB wall thickening using ultrasound has a positive predictive value of 95% for the diagnosis of acute cholecystitis (10). Another opinion questions whether the results are related to the experience of radiologists. In a prospective study conducted by Grancharov et all, it was found that correct primary diagnosing of uncomplicated gallstones via using ultrasound by experienced and novice radiologists were found to be similar. Based on this, they reported that their level of expertise was good (11).

Endoscopic ultrasonography (EUS) is considered to be a superior method compared to transabdominal ultrasonography used for imaging of GB lesions because devices with lower ultrasound frequency can provide high resolution images of small lesions (12). Since this facility is not available in our hospital, in our study, all patients were evaluated by US and MRCP before the ERCP procedure and it was shown that there was stones in biliary tract and no stones in GB. In the cases included in our study, the fact that the GB was not evaluated by EUS can be considered as a criticism.

There are two questions that should be answered when patients have stones both in the GB and bile duct at the same time; (1) what is the best method for clearing the bile duct; and (2) what should be done with the GB? In the United States and Europe, 75% of bile duct stones are cholesterol stones. If these stones are of bile origin, it is reported that GB dysfunction persists even if stones are removed. Approximately 15% of patients with cholecystolithiasis have simultaneous choledocholithiasis whereas 95% of patients with choledocholithiasis are associated with cholecystolithiasis (14). In our study, 4 % of the patients who underwent ERCP had no stones in the GB. Patients, who did not have any complaints after ERCP procedure, were discharged with the suggestion of outpatient control 6-8 weeks later. In the treatment of cholecystolithiasis and choledocholithiasis, the traditional method is firstly ERCP and then cholecystectomy 6-8 week later. Currently, early cholecystectomy (<72 hours) may be preferred in the treatment of acute cholecystitis due to its advantages such as less complications (especially biliary tract injury) and shorter hospital stay (15). In the case of cholelithiasis and choledocholithiasis, there are some studies suggesting ERCP and laparoscopic cholecystectomy even in the same session (16, 17). In our study, there was also a dilemma about the GB. After eradication of choledochal stones with ERCP, these patients were taken to the outpatient clinic. In the first month follow-up visits after ERCP procedure, the patients did not have any complaints about GB and control USG revealed no GB stones, so follow-up decision was taken. On further consideration, it is difficult to say that the treatment is complete without a procedure involving the GB if the stone in the common bile duct is primary. If the stone is secondary, which means that it migrated from GB to the bile duct, it is debatable how safe it is to follow-up only. Some studies have shown that the formation of cholesterol stones in the GB is due to postprandial GB emptying. Therefore, if we accept some of the common bile duct stones as secondary bile duct stones in our study, the decision to follow-up is open for discussion; therefore a criticism can be made in our study on this subject. The distinction between primary and secondary choledocholithiasis is of practical importance as it requires different treatment. In a study to determine this difference in the literature, microbiological and biochemical analyses were performed on the bile of two patient groups (27 patients with GB-derived stones and 5 patients with primary main bile duct stones). Although it did not make sense for patients with primary choledocholithiasis, the incidence of duodenal diverticulosis was "p=0.15" and the incidence of E.coli-positive culture in bile was "p <0.001" (18). Besides, the color of the stones can give information about the formation mechanism of the stones. It has been reported that black and brown pigment stones have different pathogenic mechanisms and bacterial infection is important only in the formation of brown pigment (19). Secondary stones are composed of cholesterol whereas primary stones are composed of pigmented bile acids. In our study, microbiological and biochemical analyses of stones extracted from choledochus with ERCP were not performed and therefore it may be subject to criticism.

Cholecystectomy is the treatment method of GB stones. However, recent studies have focused on the use of GSS in patients with cholelithiasis, which has become a topic of interest in the literature. In study Tan YY et al. (20) involving 61 patients, only GB stone was removed by preserving the GB with minimally invasive surgery and the success rate was 93.8%. After the operation, the patients were taken to clinical and US monitoring every 6 months. Total stone recurrence rate was 4.92% over a mean follow-up of 26 months (range 6-40). In addition, it was reported that GB function was not affected and stone recurrence rate was very low (20).

GSS is an important field of application of minimally invasive endoscopic surgery in the treatment of biliary diseases, thus this practice provides a complementary approach to the treatment of cholelithiasis (21). Based on an increasing number of GSS applications, some authors have reported that this option is a viable technique for cholelithiasis patients in selected cases with normal GB function, and randomized controlled trials are required to be accepted (22).

In a study of Tian MG et al., retrograde biliary drainage was concluded to be an optimal method for the preservation of GB during surgical treatment of primary bile duct stones. After surgical removal of primary bile duct stones, preserved intact GB did not develop new gallstones (23). When we review the

literature, we think that it is possible to follow up without cholecystectomy when GSS is performed in patients who have secondary or primary common bile duct stones and have functional GB.

In conclusion, we think that in patients with secondary or primary choledochal stones without gallstones and with functional GB might be follow up without cholecystectomy after the stone is removed from the bile duct by ERCP. In cases where cholecystitis attacks do not develop in the follow-up of these patients, they can be followed without cholecystectomy.

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RESEARCH ARTICLE

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The Systemic Cell Apoptotic-Based Neutrophil–Lymphocyte Ratio: Experience in Children Diagnosed with ADHD and Autism Spectrum Disorder

ABSTRACT

Objective: In recent years, the rate of Neutrophil/Lymphocyte ratio (NLR) has been shown to be a marker of systemic inflammation that associated with prognosis in many diseases like malignancies and chronic inflammatory diseases. Based on previous studies, there is not any finding about NLR and cellular morphological mechanism studied together in inflamation-related diseases; attention deficit hyperactivity disorder(ADHD) and autism spectrum disorders(ASD). We assessed the effect and association of these parameters on ethiopathogenesis of neurodevelopmental disorders.

Methods: 30 healthy and 30 each patients who were diagnosed with ADHD and ASD were evaluated at psychiatry department in tertiary hospital. The hemogram profile were analyzed and NLR parameter was statistically evaluated among groups. However, apoptotic stage of cells were staining with 2 different methods. Apoptotic mechanism of ADHD, ASD and control group were comparably displayed.

Results: NLR values in patients diagnosed with ADHD and ASD were significantly higher compare to control; lymphocyte count was found significantly lower level in patient groups. Apoptotic morphology becomes evident as degree of disease increment.

Conclusions: This parameter can be used as an easily applicable method is estimated to be risk for psychiatric diseases. The positive association of NLR with apoptotic imaging indicates a marker of cellular degradation with neurodegenerative disorders.

Keywords: Hemogram Profile, ASD, ADHD, Apoptotic Tunnel Variation, NLR

Otizm Spektrum Bozukluğu ile Dikkat Eksikliği Hiperaktivite Bozukluğu Olan Çocuklarda Apoptotik Hücre Tabanlı Sistemik Nötrofil-Lenfosit Oranı

ÖZET

Amaç: Son yıllarda, Nötrofil / Lenfosit oranının (NLR) sistemik inflamasyonun bir belirteci olduğu gösterilmiştir ve maligniteler ve kronik inflamatuar hastalıklar gibi birçok hastalıkta prognoz ile ilişkilidir. Önceki çalışmalara dayanarak, NLR ve hücresel morfolojik mekanizmanın inflamasyonla ilişkili hastalıklarda dikkat eksikliği hiperaktivite bozukluğu (DHB) ve otizm spektrum bozuklukları (ASD)'nın birlikte çalışıldığı bir bulgu yoktur. Araştırmamızda bu parametrelerin nörogelişimsel bozukluklar etiyopatogenezine etkisini ve ilişkisini değerlendirdik.

Gereç ve Yöntem: DHB ve ASD tanısı alan 30 sağlıklı ve 30 hasta, üçüncü basamak bir hastanede psikiyatri kliniğinde değerlendirildi. Tüm gruplarda hemogram profiline bakılarak NLR sayısı istatistiksel olarak karşılaştırıldı. Ayrıca hücrelerin apoptotik evresini belirlemek üzere 2 farklı yöntemle boyandı. DHB, ASD ve kontrol grubunun apoptotik mekanizması karşılaştırılabilir şekilde görüntülendi.

Bulgular: DHB ve ASD tanısı alan hastalarda NLR değerleri kontrole kıyasla anlamlı derecede yüksekti; lenfosit sayısı hasta gruplarında anlamlı olarak düşük bulundu. Apoptotik morfoloji, hastalık artışı derecesi ile korele olarak farklılık gösterdi.

Sonuç: Bu parametre, psikiyatrik hastalıklara yakalanma riski olan durumlarda, erken tanıda kolay uygulanabilir bir yöntem olarak kullanılabilir. Nötrofil/Lenfosit oranının apoptotik görüntüleme ile pozitif ilişkisi, nörodejeneratif patolojilerde hücresel bozulmanın bir belirtecini gösterir.

Anahtar Kelimeler: Hemogram Profili, ASD, ADHD, Apoptotik Tunel Değişimi, NLR

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder which was diagnosed mostly on childhood and characterized by a persistent pattern of inattention, hyperactivity and impulsivity that leads to functional impairment in social and academic life (1). Recent systematic reviews report the prevalence of ADHD in worldwide as 5.29% (2). High heritability rates for ADHD were shown in genetic studies approximately 75% (3,4) but also gene-environment interaction plays an important role for emergence on clinical presentation of ADHD (5). Autism Spectrum Disorders (ASD) are also neurodevelopmental disorders characterized by impairments in social interaction and communication, and the presence of restrictive and repetitive behaviors that begin in early childhood (6). ASD as well as ADHD are both highly heritable neurodevelopmental disorders, commonly 70-80% the phenotypic variance of each disorder may be explained by genetic factors (3,7,8). However genetic factors seem to be very important for both ADHD and ASD, environmental factors contribute or exacerbate clinical manifestations so as to both disorders (9). Recent studies show high levels of comorbidity and symptom overlap between ASD and ADHD that might refer a common genetic and environmental pathway for these two neurodevelopmental disorders (10).

Both animal and clinical studies have reported a major role as an immune system alterations in the etiology of ASD. Studies show mostly complex interaction between genetic/environmental factors and the immune system such as; increased levels of pro-inflammatory cytokines and autoantibody production, high incidence of familial autoimmunity, maternal immune dysfunction, abnormal levels of immunological markers, cytokine/chemokine imbalance (11). Also there are some findings that immune dysregulation and free radical-mediated neuronal damage may play an important role in the immunopathogenesis of ADHD (12,13).

Neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) are cheap and easily calculated indexes correlating with the prognosis of systemic inflammatory diseases. Especially useful in inflammatory, cardiovascular and cancer diseases have been discovered (14). NLR analysis has been suggested as a clinical marker of systemic inflammation (15,16). This ratio has been investigated in various psychiatric disorders such as schizophrenia, bipolar disorder, and depression (17,18,19). So far, no study has been investigated NLR in patients with both ASD and ADHD.

The apoptosis mechanism deterioration may lead to pathological consequences such as neoplasia, viral infections, autoimmune diseases, AIDS. Apart from genetic control, extracellular factors (hormones, cytokines, chemical, physical and viral agents) are also involved in the initiation of apoptosis. Neutrophil apoptosis is essential for the recovery of acute inflammatory response in genetic diseases that constitute working position, to provide immunity and to prevent inappropriate tissue destruction (20). Histopathological studies have shown that the development of these cell-mediated diseases is characterized by the alteration of lymphocyte cell morphology, especially for inflammatory factors (21).

In this study, we aimed to investigate the relationship between NLR from whole blood count parameters and histopathologically changes of cellular apoptotic activity which lead the prognosis these disease analyzing with two different methods in ADHD and ASD diseases. The main goal is to demonstrate that full blood count parameters change markedly with number and quality during inflammation and this was stimulated by disruption of a single cell.

MATERIAL AND METHODS

Patient Material: This research evaluated the predictive value of Neutrophil/lymphocyte ratio on children diagnose with ADHD and ASD. 30 each children with ADHD and ASD children managed at Duzce University Hospital, between December 2016 and August 2017. Also 30 healthy children without any psychiatric history were included as control group. The ethics committee approved the study for ethics. Histological and biochemical analyzes were performed according to taking blood in different tubes considering the criteria of inclusion and exclusion at patients. Heparine and EDTA tubes were clasified and stocked at -20oC for later measurement.

Measurement of Blood Cell: The leukocyte count and neutrophil percentage were measured by an automated hematology analyzer (Coulter® LH 780 Hematology Analyzer, Beckman Coulter Inc., Brea, CA, USA). The upper limits of the reference interval for lympocyte counts were 10.0 – 48.0% of Leukocyte level 3.8 – 10.0 $x10^3/\mu L$. The calculation of the NLR (Neutrophil/Lympocyte ratio) may provide a sensitive parameter to differentiate complicated ADHD and Autism predisposing factors. Lymphocyte and Neutrophil counts were determined after hemogram measurement.

Tunnel Assay / Staining Method: The blood samples of Autism, ADHD and healthy experimental group were spread thinly over the lamellae. Prepared blood spreads were waited for drying at room temperature and then fixed with methanol for 3 minutes. After the fixation process, the lamellas left to dry in the room heat. Two different techniques with Tunnel painting and Giemsa painting procedures were applied to compare apoptotic changes.

Apoptosis staining was performed using Millipore ApopTag Plus Peroxidase. In Situ Apoptosis Detection Kit S7101 (Lot: 2325062). The tunnel slides were first incubated at 75°C for 40 minutes. Lames held in Xylene 3 times for 10 minutes. Laminates from the xylene were passed through 96%, 80% and 70% ethyl alcohols, respectively. Washed twice for 5 minutes in PBS. Slides were incubated for 15 minutes at 370C in Proteinase-K solution diluted to 20 µg / ml in PBS. Waited in the dark for 5 minutes in 0.3% H2O2 solution. The slides were equilibrated in buffer for 10 minutes as 130 microliters per cm2/ml. The sections at 370C were incubated with TdT enzyme for 1 hour in the presence of 110 microliters per cm2. The sections were agitated in the stop buffer for 15 seconds and left for 10 minutes. DAB chromogen was applied for 6-7 min. Antifouling was done for 7 to 8 minutes with 1% methylene green. The lamella was closed using Entellan.

Giemsa Assay / Staining Method: The lamellas in the study group were fixed with methanol for 3 minutes. Then the blood spots were dried in the room heat. Giemsa stock solution was diluted to 1/10 ratio and laid to cover the slides and applied to the slides for 30 minutes. The blood springs were thoroughly washed in distilled water and covered with lamellae using intellect.

Cell Morphology Detection: The staining methods were applied on an Olympus CX41 microscope where the Zeiss AxioCam ICc5 camera was attached. In order to detect changes in the morphology of cells belonging to control, ADHD and autism groups, cell images were obtained using the Zen 2 Lite program. Images were obtained by scanning the whole area with the Zen 2 Lite program.

Statistical Analysis: Statistical analysis was done with SigmaStat software version 2.03 (Jandel Scientific). Microscopic immunohistochemical analysis was done by Oxiron and photomicrographs were captured by Carl Zeiss AxioCam MRc5 camera. Quantitative data are presented as mean, SE, min and max. Statistical significance of difference between control, ADHD and autism group was determined by Kruskall–Wallis test Test and Mann–Whitney U tests was used for pairwise comparison of groups, respectively. The $P < 0.05$ was considered significant.

RESULTS

The average age of the pediatric patients included in the study was calculated and all criteria has designed for inclusion and exclusion requirements. Neutrophil/lymphocyte ratios for ADHD¸autism and control groups included in this research according to determined criteria were evaluated by Kruskal-Wallis test. There was a statistical difference (49,33 ± 2) between groups ($P < 0.001$) when they were evaluated in triplicate shown as Table 1.

Table 1. Statistical differences between groups duplicate comparations

NLR*	C /ADHD	C /ASD	ADHD/ASD
Z value	-5,994	-3,379	-5,491
P	,000	,001	,000

Paired comparisons were made according to the Mann-Whitney Test and it was found among the Control-ADHD groups (17,00 ± 5,99); between control and autism group (248.00 ± 3.37); between ADHD and autism (67.00 ± 5.49) values were determined. There was a statistically significant difference ($P < 0.001$) between the mean ratios obtained in comparative groups (Figure 1).

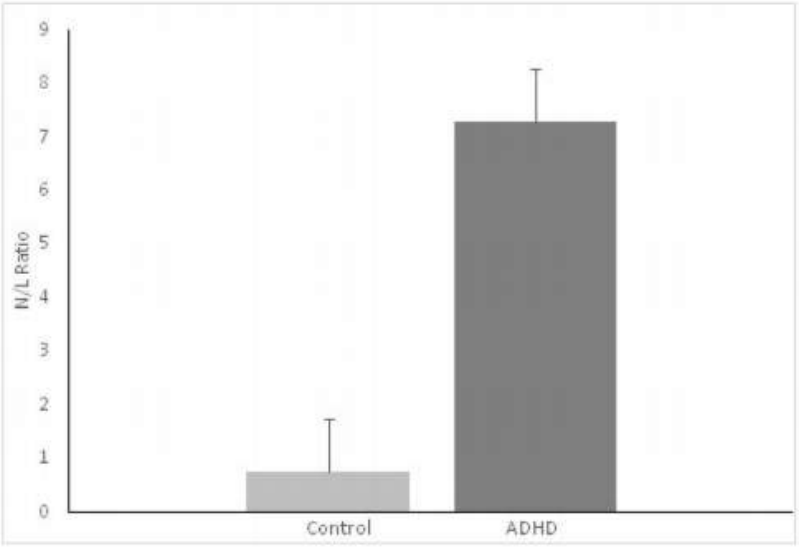

Figure 1. The variaton of Neutrophil/Lymphocyte ratio according to hemogram profile in ADHD patient group

When the hemogram analysis results of all groups were examined, it was aimed to determine the relationship between neutrophil/lymphocyte ratios and cellular apoptotic mechanism. Based of this hypothesis, datas of each group of datas were explained more detaily. The mean NLR in control group was 0.756 ± 0.50, while the min and max values ranged from 0.004 to 2.91, respectively. The mean NLR in ADHD group was 7,281 ± 4,98, the min and max value ranges are; 1,72 and 19,50; In the autism group, mean NLR was 1,662 ± 0,37, min and max values have been identified among 0,18 to 6,32, variably (Figure 2).

Significant difference was observed in the control, ADHD and ASD groups with reference to the obtained data. Moreover, this significant difference is related to the change of the cellular mechanism in the binary and triple comparisons of experimental groups (Figure 3).

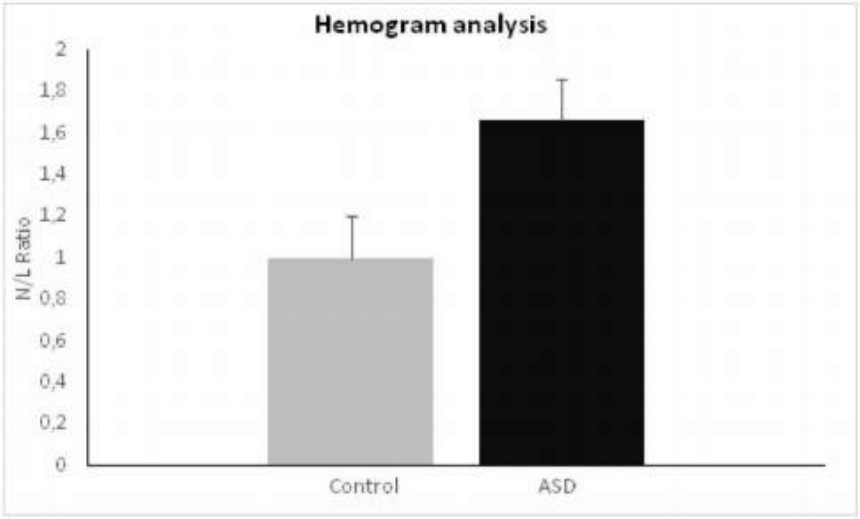

Figure 2. The variaton of Neutrophil/Lymphocyte ratio according to hemogram profile in Autism patient group

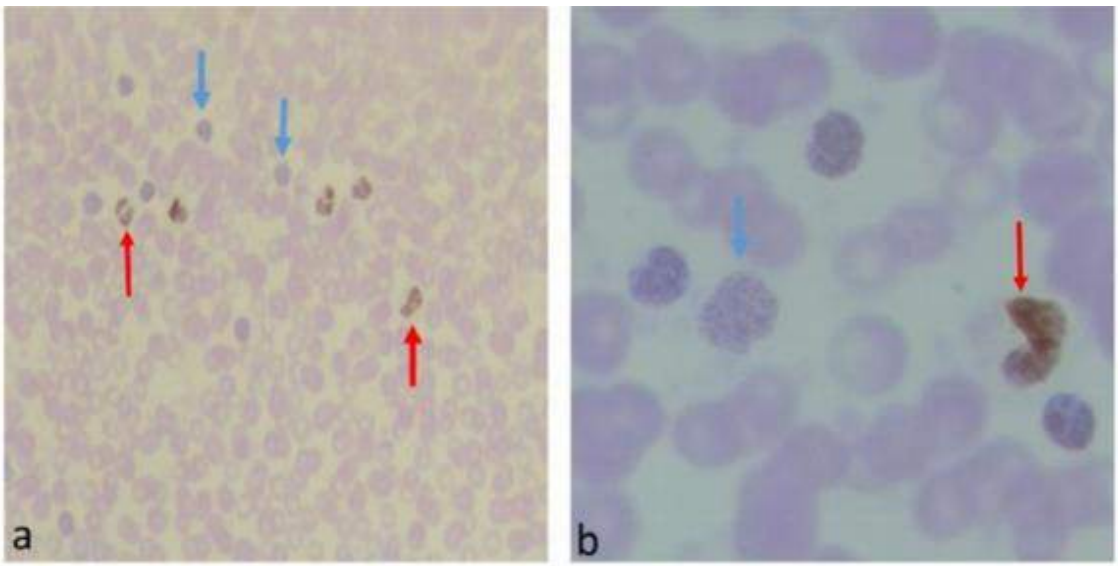

Figure 3. Cell morphological results by different (by Tunnel) staining [3a. Apoptotic leukocyte (red arrows) and normal leukocyte (blue arrows) (x40), 3b. Apoptotic leukocyte (red arrow) and normal leukocyte(blue arrow) (x100)]

The nuclei of apoptotic cells appear to be brown by Tunnel method. After the blood stains with Giemsa, cells which have chromatin condensation on the edge of the nucleus membrane and vacuolization in the nucleus were evaluated as apoptotic cells (20) (Figure 4, Figure 5).

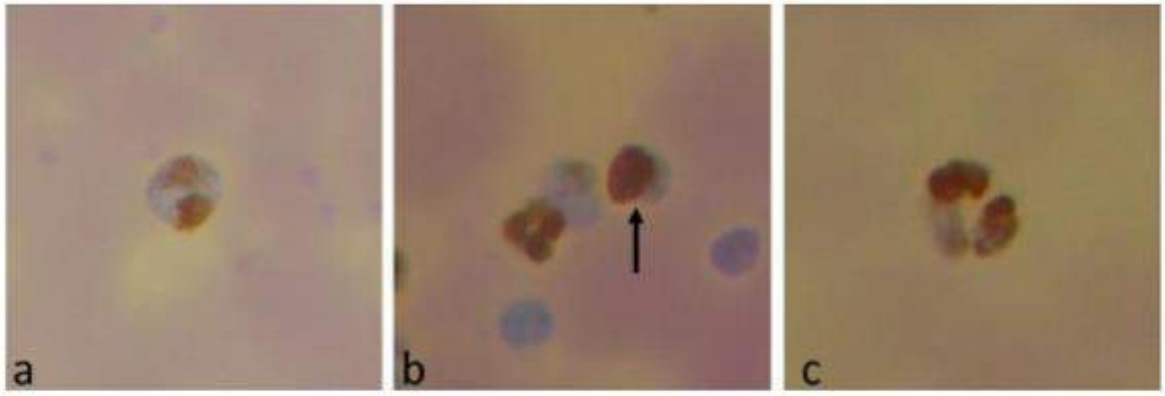

Figure 4. Apoptotic alteration of Leukocytes in Autism Group (Tunnel Method) [4a. Apoptotic eosinophil. 4b. Apoptotic lymphocyte (black arrow). 4c. Apoptotic neutrophil (x100)]

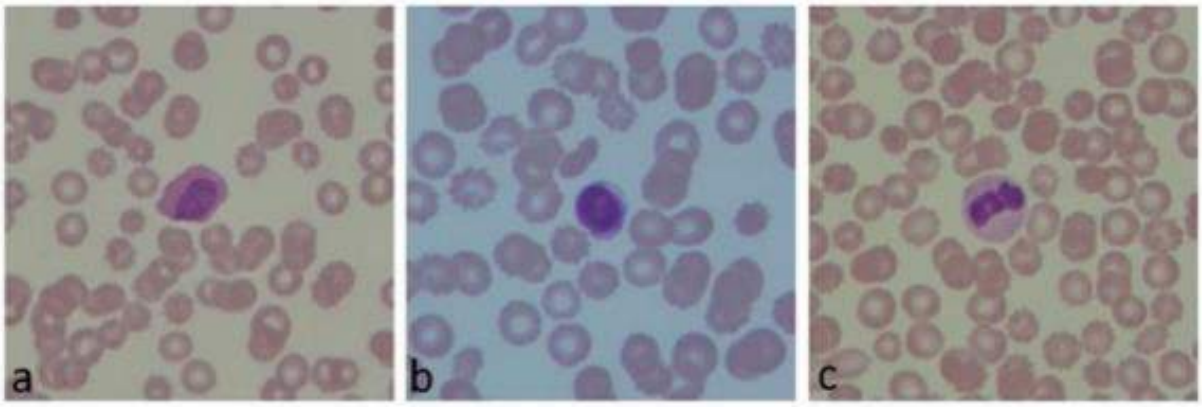

Figure 5. Apoptotic alteration of Leukocytes in Autism Group (Giemsa Method) [5a. Apoptotic eosinophil. 5b. Apoptotic lymphocyte 5c. Apoptotic neutrophil (x100)]

When the 3 major histologic experimental groups (healthy control, ADHD, autism) to be taken into consideration, we found a significant association between elevated N/L ratio corresponding increasement apoptotic sight in ADHD and autism.

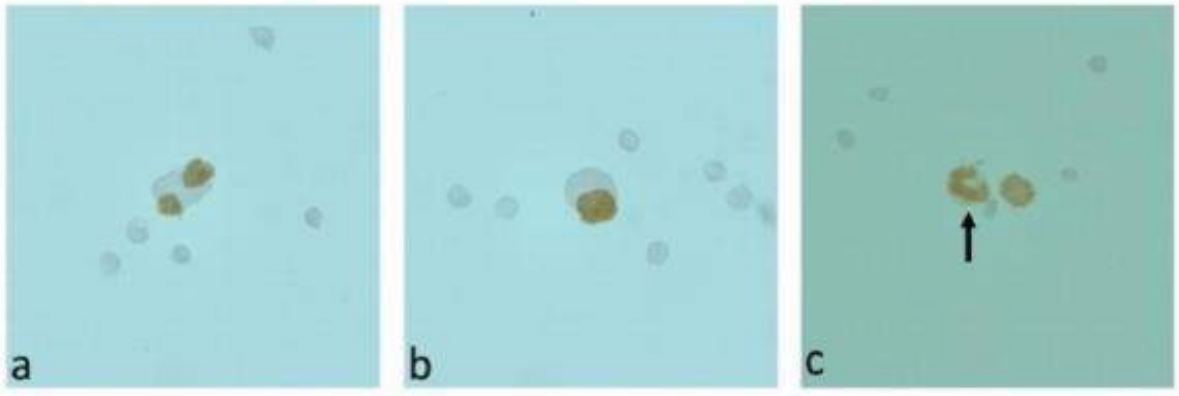

Figure 6. Apoptotic alteration of Leukocytes in ADHD Group (Tunnel Method) [6a. Apoptotic eosinophil. 6b. Apoptotic lymphocyte. 6c. Apoptotic neutrophil (black arrow) (x100)]

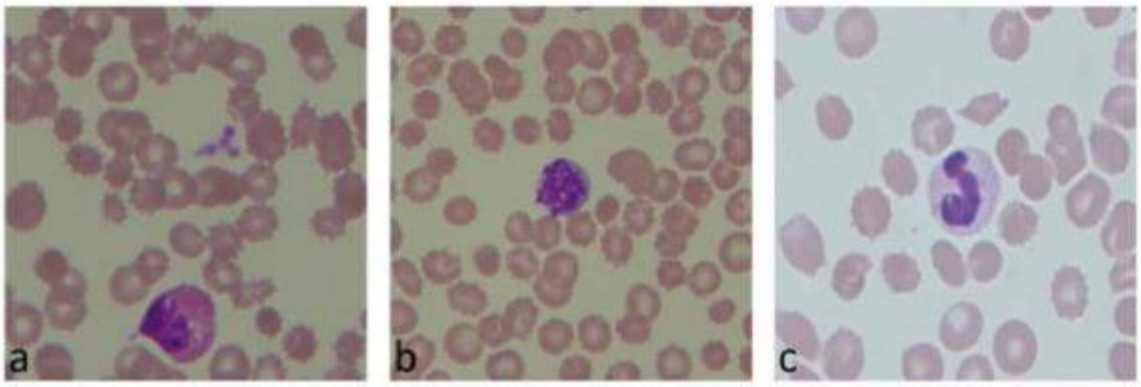

Figure 7. Apoptotic alteration of Leukocytes in ADHD Group (Giemsa Method) [7a.Apoptotic eosinophil.7b.Apoptotic lymphocyte. 7c.Apoptotic neutrophil (x100)]

Apoptotic appearance of blood-shaped components correlated with an increasement in NLR. These datas show that the degradation of cellular structure is effective in initiation and prognosis of the ADHD or ASD.

DISCUSSION

Apoptosis is perceived as a positive repair mechanism in some conditions; despite is used as a prognostic factor for diagnosis of disease. When apoptosis is inhibited in some situations, there is no explanation for the neutrophil status although it may cause an increase or prolongation of the inflammatory response. Another approach is that the neutrophils are followed by alternative cell death pathways leading to necrosis, in which an increase in the inflammatory response is encountered (22).

In the study conducted by Misso et al, apoptosis-induced neutrophils showed that intracellular glutathione concentration decreased, neutrophil apoptosis accelerated by increasing H2O2 production by exogenous glutathione (23) Turkmen et al. have found that NLR has a better association with inflammatory cytokines such as IL-6 and TNF-alpha in renal disease patients. Also NL Ratio has been shown to be associated with progression and metastasis of gastric cancer, non-small-cell lung cancer, ovarian cancer, intrahepatic cholangiocarcinoma, hepatocellular carcinoma, pancreatic cancer, CRC and nasopharyngeal cancer. They also found a significant association between an elevated N/L ratio and tumour size and the presence of histopathological tumour necrosis (24,25,26). Studies conducted by Rodriquez and colleagues showed a significant association between disease severity and acute phase markers and NLR levels in patients with romatoid artritis (27,28).

Another research on hepatocellular carcinoma, the mean survival time was 14 months with NLR values above 3, while the NLR value was reported as 26 months in below 3. In patients with non-small cell lung cancer, a strong association was found between the NLR values above 5 and the presence of advanced disease. Meta-analysis of 26 studies on primary liver tumors suggests that high NLR values are indicative of poor prognosis and vascular invasion of the tumor (29).

Lymphocytes levels are at the highest in the neonatal period, this proportion decreases deliberately up to 18 years. For Aydın et al, the lowest levels of NLR values were detected in the 0-1 age group. Datas observed that the NLR values continued to increase until the age of 20, then entered a plateau period, beside the NLR values tended to rise again after the age of 60 years. Also it was observed that there was a significant difference between male and female genders in all age groups after 30 years of age, when there was no significant difference between sexes in terms of NLR values between 10 and 30 years of age (30,31). The patients group includes 90 male whose age range was 8.2±2.8 in the current study. However, there is still no consensus on the range of the normal values of NLR in different age groups and different genders.

In the study, the tunnel assay in neutrophil and lymphocytes changes for patient group was statistically significant compare to control group cell morphology. The slowing down of apoptosis which the main key to cancer, the immune system and embryonic development, breaks the body's resistance to infection and inflammation. This formation indicates that neutrophile ratio variated with genetic diseases (32,33,34). At current study there are significant differences between the groups correlated with previous ones. Neutrophile/Lymphocyte ratio is consistent with cell apoptosis view between groups by Tunnel and Giemsa protocols in our study. The increasement in number of apoptotic cells indicates that the cell cycle genetic mechanism has changed significantly in ADHD and Autism patient group. On the other hand, we were compared 2 staining methods, Tunnel can be used more selectively during evaluate apoptosis while Giemsa was tested as more easier and quicker protocol than Tunnel (20, 35).

In autism literature, both prenatal and postnatal immune dysregulation which may lead to

neurological dysfunctions have been suggested (35,36). An altered immune cell ratio, decreased level of T lymphocytes, higher levels of monocytes as an indicator of chronic inflammation and decreased lymphocyte/monocyte ratio have been found in previous studies (37).

Latest researches indicate that the neutrophil lymphocyte ratio can also be used to calculate morbidity and mortality. This rate is regarded as a sign of lymphocyte proliferate function and activation. If NLR rates changes high level, that have been admissible to an independent risk and poor prognostic factor for many systemic disorders like acute myocardial infarction, renal artery stenosis, cancer, diabetes mellitus, hypertension and hyperlipidemia. In a study conducted by Tural et al, alterations in NLR from complete blood cell parameters in autism were investigated. They suggested increased levels of NLR and also a positive correlation of NLR with autistic symptom severity were found in ASD patients. NLR increase has been also reported in various psychiatric disorders associated with inflammation, such as schizophrenia,bipolar disorder and major depressive disorder (36,38).

CONCLUSION: Full blood count is simple, inexpensive but contains important follow-up parameters for many diseases. It is estimated that NLR values will be measured and NLR reference interval can be lightable for clinicians will form a source for other clinical work to be done.

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RESEARCH ARTICLE

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Effects of Preemptive Single Dose Sustained Release Non-Steroidal Anti-Inflammatory Drugs on Postoperative Complications Following Third Molar Surgery

ABSTRACT

Objective: The aim of this study was to compare the effectiveness of two preemptive sustained-release non-steroidal anti-inflammatory drugs in terms of pain, edema and trismus following third molar surgery.

Methods: Overall, 30 patients with double-sided vertically positioned impacted third molars were included in this study. The study was randomized and double-blinded. 30 minutes before the surgery, patients were given sustained-release (SR) dexketoprofen trometamol 75 mg and following the surgery the drug administration continued postoperatively for 1 week, once a day. 2 weeks later, the same procedure was applied for the counterside impacted third molar with 75 mg. sustained-release (SR) diclofenac sodium. The pain was assessed postoperatively by VAS (Visual Analogue Scale) levels at the 6th, 8th, 12th, 24th and 48th hours and on the 3rd, 5th, and 7th days. Edema and trismus measurements were evaluated on the postoperative 2nd and 7th days.

Results: There was a statistically significant difference between the groups in VAS levels at 6th, 8th, 12th, 24th, and 48th hours, and on the 3rd and 5th days ($P < 0.01$). Dexketoprofen trometamol SR group had lower VAS levels than diclofenac sodium SR group. There was no statistically significant difference between the groups in terms of mean trismus and edema measurement on the 2nd and 7th days ($P < 0.05$).

Conclusions: Dexketoprofen trometamol SR and diclofenac sodium SR are similarly effective for the reduction of edema and trismus following impacted third molar surgery; however, dexketoprofen trometamol SR is found to be more efficient in reducing pain.

Keywords: Third Molar, Postoperative Complications, Sustained-Release Preparations, Nonsteroidal Anti-Inflammatory Agents

Preemptif Tek Doz Sürekli Salınımlı Non-steroid Antienflamatuar İlaçların Üçüncü Molar Cerrahisi Sonrası Postoperatif Komplikasyonlar Üzerine Etkileri

ÖZET

Amaç: Bu çalışmanın amacı, üçüncü molar cerrahisini takiben iki preemptif sürekli salınımlı nonsteroidal antienflamatuar ajanın ağrı, ödem ve trismus açısından etkinliğini karşılaştırmaktır.

Gereç ve Yöntem: Çift taraflı vertical pozisyonda gömülü üçüncü azı dişleri olan 30 hasta çalışmaya dahil edildi. Çalışma randomize ve çift kör olarak planlandı. Ameliyattan 30 dakika önce hastalara 75 mg sürekli salınımlı (SR) deksketoprofen trometamol verildi ve ameliyat sonrası ilaç uygulaması günde bir kez olmak üzere 1 hafta boyunca devam etti. 2 hafta sonra, 75 mg sürekli salınımlı (SR) diklofenak sodyum ile diğer gömülü üçüncü molar için aynı prosedür uygulandı. Ağrı postoperatif 6., 8., 12., 24. ve 48. saatlerde ve 3., 5. ve 7. günlerde VAS (Görsel Analog Skala) ile değerlendirildi. Ödem ve trismus ölçümleri postoperatif 2. ve 7. günlerde gerçekleştirildi.

Bulgular: 6., 8., 12., 24. ve 48. saatlerde ve 3. ve 5. günlerde VAS düzeylerinde gruplar arasında istatistiksel olarak anlamlı bir fark bulundu ($p < 0.01$). Deksketoprofen trometamol SR grubunun VAS düzeyleri, diklofenak sodyum SR grubundan daha düşüktü. Gruplar arasında 2. ve 7. günlerde trismus ve ödem ölçüm ortalamaları açısından istatistiksel olarak anlamlı fark yoktu ($p < 0.05$).

Sonuç: Deksketoprofen trometamol SR ve diklofenak sodyum SR, gömülü üçüncü molar cerrahisini takiben ödem ve trismusun azaltılması için benzer şekilde etkilidir; bununla birlikte, deksketoprofen trometamol SR'nin ağrıyı azaltmada daha etkili olduğu bulunmuştur.

Anahtar Kelimeler: Üçüncü Azı Dişi, Postoperatif Komplikasyonlar, Sürekli-Salınımlı Preparatlar, Steroid Olmayan Antienflamatuar Ajanlar

INTRODUCTION

Due to several reasons such as adjacent teeth position, mucosal thichkness or high bone density, third molars can remain impacted in the mandibula (1). According to studies the prevalence of third molar impaction is 20%–30% of the population and this makes mandibular third molar surgeries one of the most frequent procedure in oral and maxillofacial surgery (2).

Despite the atraumatic techniques, third molar removal surgeries can generate an inflammatory reaction which may cause trismus, pain and edema in the maxillofacial region (3). Postoperative complication reduction increases the quality of life, especially in the first three days following surgery (4). A great number of articles and methods are introduced for managing instant inflammatory response associated with the surgery. They involve using drain for edema elemination, the use of medications such as corticosteroids, analgesics, antihistaminics and antibiotics or nerve stimulation for pain reduction (5,6).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are prescribed to patients in order to avoid postoperative inflammatory symptoms such as edema, pain, trismus and infection. They are mostly used for their capacity of anti-inflammation, antipyretic and analgesic effets in routine practice (7). Dexketoprofen trometamol is a NSAIDs and inhibits COX-1 and COX-2 for its well known anti inflammatory and analgesic effects. By means of its lipophilic properties, it is rapidly absorbed and its activity starts in a very short time (8). Diclofenac is a derivative of benzene acetic acid and prescribed for pain and edema since 1974 (9). It is available in two different salt formulations; diclofenac sodium and diclofenac potassium. Diclofenac sodium is a well-known NSAID with anti-inflammatory, analgesic and antipyretic activities. Many studies showed efficacy of diclofenac as compared to other NSAIDs in management of inflammatory findings following dental surgical procedures (10,11).

Long lasting anti-inflammatory effects needs continuous medication, independent of the strategy selected. Besides, patients' cooperation might decrease considerably when a drug needs to be administrated more than once a day, which is a risk for postoperative complications. Sustained release (SR) of the medications; meaning release of the medications in small quantites over a prolonged period of time, is an effective approache for managing post surgical complications (12).

Recent developments in postoperative pain management have revealed the concept about preemptive medication. The essential prediction of this concept is that, if NSAIDs are adminestered to patient before the onset of surgery, postoperative comorbidities might be prevented or reduced (13).

The objective of this study is to compare the preemptive administration of diclofenac sodium SR and dexketoprofen Trometamol SR on postoperative management of trismus, pain and edema following surgical removal of mandibular third molars.

MATERIAL AND METHODS

This study received approval from Ethics Commitee (Date: 25.01.2017 Decision Number:1/8) and was conducted in accordance with the guidelines on the Helsinki Decleration on Human Rights. The experimental part was performed at Dentistry Faculty, Oral and Maxillofacial Surgery Department. 30 patients with double sided vertically positioned fully impacted third molars without any sign of infection, with absence of smoking and alcohol consumption and absence of hypersensitivity reaction to NSAIDs and aspirin were included. The exclusion criteria of this study were the absence in show up controls on postoperative days, incomplete filling of VAS (Visual Analogue Scale), prolonged surgery time for more than 25 minutes, pregnancy, lactation, NSAIDs or antibiotic intake within the last 3 weeks before surgery and alveolitis development. Detailed information was provided to all patients and patients gave consent for inclusion in the study via a consent form.

In present study, all the surgeries were performed by the same surgeon. 60 mandibular impacted third molars which matched the inclusion criterias were surgically extracted from 30 patients. Following n. alveolaris inferior and n. buccalis blocks, buccal mucoperiosteal flap was elevated through a sulcular and a horizontal incision. The bone tissue was osteotomized with steel rond and fissure burs under physiological saline irrigation (0.9 %) and the third molar was extracted by means of bein elevators and forceps. All the surgeries were performed under 2 cartridges of 40 mg/ml Articaine hydrochloride with 0.0012 mg/ml epinephrine hyrochloride (Ultracain DS forte-Aventis İlaç Sanayi Tic., Türkiye). Following the extraction, the mucoperiosteal flap was repositioned primarily and sutured with 3-0 silk sutures. During all operations, both bone osteotomy time and total surgery time were recorded. Sutures were removed at the end of the 7th postoperative day. After the soft tissue healing was observed, an appointment was made for the other mandibular impacted third molar operation 15 days later.

The patients were randomly divided into two groups (according to the medications given) by the assistant physician and the surgical nurse. Patients were given dexketoprofen trometamol 75 mg sustained-release (Dexfull SR 75 mg tablet, Neutec Drugs, Turkey) in tablet form in order to start 30 minutes before the first operation and continue postoperatively for 7 days, once a day and at the same time every day. Before the second operation, patients were given diclofenac sodium 75 mg sustained-release (Voltaren SR 75 mg tablet, Novartis Health, Food and Agricultural Products,

Turkey) in tablet form for starting 30 minutes before the second operation and continue postoperatively for 7 days, once a day and at the same time every day. Antibiotics (625 mg amoxicillin + clavulanic acid, Augmentin, Glaxo Smith Kline Drug, Turkey) and 0.2% chlorhexidine gluconate (Klorhex Mouthwash, 200 mL, Drogsan Drug Industries, Turkey) were also given to all patients following the surgery.

Measurement of Pain Intensity: For every patient the VAS score of 100 mm was recorded by a questionnaire at the 6th,8th,12th,24th and 48th hours and on the 3rd, 5th and 7 days following the operation. The first pain assessment for establishing the effectiveness of the preemptive analgesics was evaluated at the 6th hour as the alleviation of local anesthesics effects and the maximum levels of postoperative pain observed between 6-8 hours.

Measurement of Trismus – Mouth Openning Capacity: Digital calipers were used for measuring (in mm) the mouth opening capacity pre and postoperatively on 2nd and 7th days. The incisal edges of maxillary and mandibullary 1 st incisal teeth were used as reference points before and after the surgery. The measurements were done 3 times repeatedly and mean values were recorded.

Measurements of Edema: Soft tissue measurements were obained in order to evaluate swelling preoperatively and on the postoperative 2nd and 7th days using the method of Üstün et al. (14). Points on the face were used for measurement of swelling: eye cantus – angulus mandibula, tragus – corner of mouth, and tragus – pogonion. The distances between these points were marked, measured, and recorded.

Statistical Analysis: For the statistical analyses, Minitab 17 (Minitab Inc., State College, PA, USA) was used for the findings obtained in the study were evaluated. Shapiro Wilks test was run in order to test the normality distribution of the parameters. Student's t-test was used for between-group comparison of normally distributed parameters and the Mann-Whitney U test was used for between-group comparison of non-normally distributed parameters. Variance analysis was carried out for repeated measures in within-group comparisons. Significance value level was accepted as $p<0.05$ and $p<0.001$.

RESULTS

The study was conducted on 30 patients, 19 (63.3%) female and 11 (36.7%) male, aged between 18-33. The mean age of the patients was 23.73 ± 3.85. There was no statistically significant difference between the groups in terms of total operation time and bone osteotomy time ($P > 0.05$) (Table 1).

Table 1. Evaluation of total surgery times and bone osteotomy times

	Dexketoprofen Trometamol SR Group	Diclofenac Sodium SR Group	p
	Mean±SS	Mean±SS	
Total surgery time (min)	15.44±2.39	15.07±2.00	0,596
Bone osteotomy time (min)	2.05±0.81	2.18±0.82	0,667

Mann Whitney U test

A statistically significant difference was found between the groups in terms of VAS levels at the 6th, 8th, 12th, 24th and 48th hours and on the 3rd and 5th days ($P < 0.05$, $P < 0.01$). Pain levels of Dexketoprofen Trometamol SR group were statistically significantly lower than the Diclofenac Sodium SR group (Table 2).

In the evaluations of the VAS levels within the group, statistical differences were detected on the 6th, 8th, 12th, 24th, and 48th hours, and on the 3rd, 5th, and 7th days ($P < 0.01$). In both groups, the maximum pain level was determined at the 6th hour postoperatively (Table 2).

Table 2. Postoperative pain intensity values measured by VAS (Visual analogue scale)

VAS (mm)	Dexketoprofen Trometamol SR Group	Diclofenac Sodium SR Group	p
	Mean±SS	Mean±SS	
6th hour	43.33±16.88	50.67±16.39	0.001
8th hour	43.33±16.26	49.00±17.29	0.03
12th hour	35.67±15.47	43.67±14.50	0.001
24th hour	25.33±14.08	36.67±14.93	0.000*
48th hour	15.67±12.51	26.33±13.51	0.000*
3rd day	11.00±9.95	18.67±11.37	0.002
5th day	6.67±8.02	12.67±11.12	0.008
7th day	1.33±3.45	2,00±4.06	0.489
p	0.000*	0.000*	

Student t test *p<0.001

There was no statistically significant difference between the groups in terms of mean trismus values on the 2nd and 7th days (P > 0.05) (Table 3).

There was a statistically significant difference between the mean trismus values in the preoperative, 2nd, and 7th days within the group (P= 0.000; P < 0.01). The decreases seen in the 2nd and 7th-day measurements compared to the mean trismus values in the preoperative period are statistically significant (P < 0.01). The increase in trismus values on the 7th day compared to the 2nd day is statistically significant (P < 0.01) (Table 3).

Table 3. Evaluation of the groups in terms of trismus measurements

Trismus	**Dexketoprofen Trometamol SR Group**	**Diclofenac Sodium SR Group**	**[1]p**
	Mean±SS	**Mean±SS**	
Preop	42.36±4.23	42.36±4.23	-
2nd day	33.50±4.79	33.16±4.41	0.430
7th day	40.93±4.11	40.30±4.15	0.487
[2]p			
Preop-2nd day [2]p	0.000*	0.000*	
Preop-7th day [2]p	0.000*	0.000*	
2nd day-7th day [2]p	0.000*	0.000*	

[1]Student t test *[2]Analysis of variance in repetitive measurements* *p<0.01

There was no statistically significant difference between the groups mean edema values between the eye cantus-angulus mandible, tragus-pogonion and tragus-corner of mouth anatomical points on the 2nd and 7th days (P > 0.05) (Table 4). In the intra-group evaluations, the increases observed on the 2nd day compared to the preoperative period of the eye cantus-angulus mandible, tragus-pogonion and tragus-corner of mouth measurement average in both groups were statistically significant (P < 0.01). A decrease observed in the edema values between the eye cantus-angulus mandible, tragus-pogonion and tragus-corner of mouth anatomical points on the 7th day compared to 2nd day was statistically significant (P < 0.01; P < 0.05). There was no significant difference between the preoperative and 7th day edema measurements in both groups (P < 0.05) (Table 4).

Table 4: Postoperative swelling measurements in milimetres (eye cantus-angulus mandibula, tragus pogonion, tragus-corner of mouth) preoperatively, on the 2nd and 7th days

Eye cantus/angulus mandible	**Dexketoprofen Trometamol SR Group**	**Diclofenac Sodium SR Group**	**p**
	Mean±SS	**Mean±SS**	
Preop	101.50±4.79	101.50±4.79	-
2nd day	105.33±5.16	106.20±5.09	0.572
7th day	102.00±4.70	102.43±4.86	0.759
[1]p			
Preop-2nd day [1]p	0.0009*	0.0001*	
Preop-7th day [1]p	0.566	0.340	
2nd day-7th day [1]p	0.0025*	0.0014*	
Tragus/pogonion			
Preop	144.46±7.76	144.46±7.76	-
2nd day	149.20±7.99	149.63±8.62	0.634
7th day	145.16±7.86	145.36±8.11	0.940
p			
Preop-2nd day [1]p	0.002*	0.0014*	
Preop-7th day [1]p	0.583	0.577	
2nd day-7th day [1]p	0.0084*	0.0048*	
Tragus/corner of mouth			
Preop	111.80±5.10	111.80±5.10	-
2nd day	115.60±5.83	116.53±5.96	0.573
7th day	112.13±5.30	112.60±5.46	0.694
[1]p			
Preop-2nd day [1]p	0.0088*	0.002*	
Preop-7th day [1]p	0.812	0.552	
2nd day-7th day [1]p	0.016	0.0103	

Mann Whitney U test *p<0.01

DISCUSSION

Impacted third molar surgeries and management of postoperative complications are crucial for the patient's quality of life. These complications such as edema, pain, trismus can be resolved with NSAIDs and these medications are usually prescribed inorder to compete with these symptoms (15). In the present study, the efficacies of sustained- release forms of dexketoprofen trometamol and diclofenac sodium on edema, pain and trismus following third molar surgery were evaluated. Third molar surgery is accepted as a convenient and highly presumable procedure for investigating the preemptive effects of different drugs, especially NSAIDs (14).

Despite the existence of numerous reports in the literature about the use of drugs in the prevention of postoperative edema, trismus and pain following impacted third molar surgery (14,15), to the best of our knowledge; this is a preliminary study comparing the preemptive effects of two very commonly used drugs in their sustained-release forms, diclofenac sodium, and dexketoprofen trometamol. Although this surgery is very common, a consensus hasn't been created about the optimal type, method of administration, timing, and the dose of the drugs in order to us for the prevention of postoperative complications.

Postoperative complication occurence have been found to be related to extended surgical time (16). In the pesent study, there was no significant difference between the operation and bone osteotomy times in all groups. All the surgeries are performed under 20 minutes.

Following a third molar surgery, patients' inflammatory symptoms are usually the greatest during the first 2 days, accompanying the most intense pain experience on the surgery day (17). In the present study, the evaluation of pain was performed on the 6th,8th,12th,24th and 48th hours following the operation. 3rd, 5th and 7th days were chosen for evaluating the late findings of pain perception following the operation. The results show that in all groups patients indicated their highest VAS scores at 6 hours postoperatively. Since the effect of local anesthesia would pass approximately at 4 hours following administration, this finding was considered as normal.

Trismus and edema are commonly reported complication following third molar surgery. In the literature, measurements of interincisal distance pre and postoperatively are frequently preferred for the evaluation of trismus, as in this study (18). When evaluating trismus, no statistically significant difference was found among the 2 groups. In both groups the trismus on the 7th day was found to be lesser than the 2 nd day as expected.

According to the literature, some investigators preferred preemptive analgesics administration for ameliorating anesthesia effects for a tardy postoperative pain development (19). Preemptive analgesia also provides analgesia before the onset of painful stimulus, leading to a complete or partial block of nociception. The most frequently used method to prevent pain is "preemptive analgesia" that prevents the mechanisms caused by pain before they appear. In the present study, preemptive sustained-release NSAIDs are preferred. The authors of this study believe that the preemptive application of analgesics has a positive outcome on the perception of pain.

The oral and parenteral route is preferred for preemptive use in different surgical branches such as oral and maxillofacial surgery, orthopedic surgery, gynecological surgery, thoracic surgery for postoperative analgesia (20). In the present study, Diclofenac sodium SR and Dexketoprofen trometamol SR were used as oral tablets for preemtive pain control.

The effects of diclofenac sodium were found to be better than tramadol and ketorolac in managing postoperative pain, however less efective than piroxicam, nimesulide and tenoxicam (21). Akbulut et al. (22) examined the efficacy of NSAIDs on pain, trismus and edema on 42 patients. The study concluded that edema on postoperative 2nd day was significantly lowest with diclofenac as compared to others. In the present study, the analgesic efficacy of dexketoprofen trometamol has been shown to be superior to diclofenac sodium. However, in terms of trismus and edema evaluation, there was no difference in efficacy between dexketoprofen trometamol and diclofenac sodium.

Eroğlu et al. (23) evaluated the effectiveness of preoperative dexketoprofen and paracetamol administration in impacted third molar surgery and found that dexketoprofen provides better analgesia. Kesimci et al. (24) evaluated the efficacy of preoperative dexketoprofen, paracetamol, and placebo in lumbar surgery patients. The authors reported that dexketoprofen reduced the use of additional analgesics compared to paracetamol and placebo. In the present study, the effects of dexketoprofen trometamol and diclofenac sodium, which were started preemptively for pain control in impacted third molar surgery, and continued postoperatively, were evaluated. It has been reported that dexketoprofen trometamol is more effective on pain than diclofenac sodium, but complications such as edema and trismus are equally effective.

Nunamaker et al. (25) stated that analgesics, which are sustained-released and used as a single dose, provide sufficient analgesia, increase drug use compliance, and facilitate the postoperative period. In the present study, single dose sustained-release NSAIDs were preferred in order to facilitate the postoperative period, to prevent using too much medication to protect the gastrointestinal system,

and to ensure patient-drug use compliance optimally.

Seymour et al. (26) created an incisional acute minor pain model on laboratory animals. They compared the normal analgesic usage protocol with the sustained-release analgesic usage protocol. As a result of the study, SR formulation has a longer duration of action, causes less stress in animals, and has single-dose ease of use. In the present study, the effectiveness of SR forms of dexketoprofen trometamol and diclofenac sodium were evaluated in postoperative complication management. It has been determined that SR form is effective in the management of postoperative complications in two drugs and it can be used instead of routine 2x1 or 3x1 analgesic regimen due to its ease of use.

Decreasing the number of analgesics consumed after surgical operations reduce the incidence of side effects and increase patient comfort (27). For this reason, in the study, SR forms of diclofenac sodium and dexketoprofen trometamol used as a single dose and sustaining long-term activation throughout the day were used.

CONCLUSION

Despite the widespread literature search, no head-to-head comparative studies were found between SR forms of dexketoprofen trometamol and diclofenac sodium. The present study is one of the rare studies on the effectiveness of SR form NSAIDs in the prevention of postoperative complications after surgical procedure. Therefore, it is difficult to compare the present results with published studies. In conclusion, dexketoprofen trometamol SR found to be more effective in pain relief than diclofenac sodium SR however, equally effective on edema and trismus.

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RESEARCH ARTICLE

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Frequency of Hereditary Prothrombotic Risk Factors in Patients with Down Syndrome

ABSTRACT

Objective: Down Syndrome (DS) is defined as chromosome 21 trisomy and associated with cardiovascular system diseases. We aimed to study inherited thrombophilia genes (*MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII, APOB, ITGB3, FVHR2, FGB, PAI-1* and *ACE*) in patients with DS.

Methods: A total of 53 patients with DS (32 male and 21 female) were included in the study. Demographical, laboratory and clinical features of cases were recorded. 12-lead Electrocardiogram (ECG), transthoracic echocardiography and the inherited thrombophilia genes were evaluated.

Results: The clinical and developmental defect findings of the patients were high. The 39.6% of patients had both heterozygous *MTHFR C677T* and heterozygous *MTHFR A1298C* carriers, the 18.9% of patients had homozygous *MTHFR A1298C* carriers, the 17% of patients had heterozygous *Factor V Leiden G1691A* carriers, the 43.4% of patients had 4G/4G carriers, the 34% of patients had 4G/5G variation carriers for *PAI*, the 22.7% of patients had heterozygous *FactorXIII* carriers, the 49.1% of patients had ins/del carriers and the 37.7% of patients had del/del variation carriers for *ACE*. All patients had at least one of the homozygous and/or compound heterozygous variation for the inherited thrombophilia.

Conclusions: The patients with DS have a high risk for thrombosis-related cardiovascular system diseases. It may be said that the average life expectancy of individuals with DS may be increased by precautions (related to medical, social, lifestyle, etc.) to reduce complications associated with hereditary thrombophilia.

Keywords: Down Syndrome, Inherited Thrombophilia, Prothrombotic Risk Factors, Cardiovascular Diseases

Down Sendromlu Hastalarda Kalıtsal Protrombotik Risk Faktörlerinin Sıklığı

ÖZET

Amaç: Down Sendromu (DS), kromozom 21 trizomisi olarak tanımlanır ve kardiyovasküler sistem hastalıkları ile ilişkilidir. Biz DS'li hastalarda kalıtsal trombofili genlerini (*MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII, APOB, ITGB3, FVHR2, FGB, PAI-1* ve *ACE*) incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya toplam 53 DS'lu hasta (32 erkek ve 21 kadın) dahil edildi. Olguların demografik, laboratuvar ve klinik özellikleri kaydedildi. 12 derivasyonlu Elektrokardiyogram (EKG), transtorasik ekokardiyografi ve kalıtsal trombofili genleri değerlendirildi.

Bulgular: Hastaların klinik ve gelişimsel kusur bulguları yüksekti. Hastaların % 39,6'sı hem heterozigot *MTHFR C677T* hem de heterozigot *MTHFR A1298C* taşıyıcısı, hastaların % 18.9'u homozigot *MTHFR A1298C* taşıyıcısı, hastaların % 17'si heterozigot *Faktör V Leiden G1691A* taşıyıcısı, hastaların % 43,4'ü 4G / 4G taşıyıcısı, hastaların % 34'ü *PAI* için 4G / 5G varyasyon taşıyıcısı, hastaların % 22,7'si *heterozigot Factor XIII* taşıyıcısı, hastaların% 49,1'i ins / del taşıyıcısı ve hastaların% 37,7'si *ACE* için del / del varyasyon taşıyıcısı idi. Tüm hastalarda, kalıtsal trombofili için homozigot ve / veya bileşik heterozigot varyasyonlardan en az biri vardı.

Sonuç: DS'lu hastalar tromboz ilişkili kardiyovasküler sistem hastalıkları açısından yüksek risk taşımaktadır. Kalıtsal trombofili ile ilişkili komplikasyonları azaltmak için alınacak önlemlerle (tıbbi, sosyal, yaşam tarzı vb. ile ilgili) DS'lu bireylerin ortalama yaşam beklentisinin artırılabileceği söylenebilir.

Anahtar Kelimeler: Down Sendromu, Kalıtsal Trombofili, Protrombotik Risk Faktörleri, Kardiyovasküler Hastalıklar

INTRODUCTION

Down Syndrome (DS) is defined as chromosome 21 trisomy and occurs when the chromosome 21 does not separate during egg or sperm development. The incidence is approximately 1 in 700 live births. Although the ratio varies according to the mother's age, it is more common especially in births over 45 years of age.

Since many organs and systems are affected simultaneously in DS patients, phenotypic features are variable. DS phenotype often consists of dysmorphic facial features (flat nasal bridge, small chin, slanted eye, smallmouth and large tongue), muscle hypotonia, short stature, congenital heart diseases and cognitive disorders.

The average life expectancy of individuals with DS, thanks to advanced modern medical facilities and social support, in developed countries is 55 years. Congenital heart diseases occur in 40 to 60 % of individuals with DS and this situation is the main reason for morbidity and mortality, particularly in the first 2 years. Among congenital heart diseases, ventricular septal defect (VSD), atrioventricular septal defects (AVSD), atrial septal defect (ASD), and patent ductus arteriosus (PDA) are frequently reported in DS (1,2). In a large study comparing the DS group with the non-DS group, the prevalence of cardiac arrhythmia, pulmonary hypertension, diabetes, congenital heart disease and the frequency of cerebrovascular events were reported to be higher in the DS group (3). Also in other studies, vascular disorders such as artery occlusion, cerebral venous sinus thrombosis (CVST) have been associated with DS (4-7). Cardiovascular system diseases and thromboembolic events developing on this basis suggest that there may be a tendency for hypercoagulation or thromboembolism simultaneously. Therefore, we aimed to study a number of genes that have the potential to increase the tendency to thrombosis in individuals with DS. These genes (*MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII(V34L), APOB, ITGB3, FVHR2, FGB, PAI-1* and *ACE*) have been associated with cardiovascular system diseases such as deep vein thrombosis (DVT), pulmonary embolism, myocardial infarction, stroke, and congenital heart disorder (8-15).

MATERIAL AND METHODS

A total of 53 patients with DS (32 male and 21 female) were included in the current study. Demographical, laboratory and clinical features of cases were recorded (Table 1).

Children with Down syndrome who gave informed consent to participate the study and whose inherited thrombophilia factors genes analysis were performed (including *MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII (V34L), APOB, ITGB3, FVHR2, FGB, ACE*) were included in the study.

Table 1. Clinical and developmental defects findings of patients with DS

	Mean ± SD / n;%
Mean age	5.164±4.428
Mean length	96.955±29.293
Mean weight	22.056±21.482
Mean birth weight	2955.283±397.122
Delay in holding their head	22 (79.2%)
Delay in unsupported sitting	43 (81.1%)
Delay in walking	41 (77.4%)
Hypotonia	28 (52.8 %)
Mild developmental delay	20 (37.7%)
Moderate developmental delay	23 (43.4)
Severe developmental delay	7 (13.2%)
System anomalies	44 (64.1%)
Hypothyroidism	27 (51%)
Hyperthyroidism	3 (5.7)
Hearing problem	12 (22.6%)
Vision problem	7 (13.2%)
Convulsion history	2 (3.8%)
Cardiac operation history	8 (15.1%)

n: Number of patients **SD:** Standard deviation

12-lead Electrocardiogram (ECG) was done for each cases at rest. Also, all of the patients in the study were evaluated with transthoracic echocardiography (Siemens Acuson SC 2000). Transthoracic two dimensional (2D) guided, color Doppler echocardiogram, and continuous wave Doppler were performed with suitable probes according to age. Cardiac anatomy, ventricular function and valve competence were assessed using standardized projections and measurements were performed according to the recommendations of the American Society of Echocardiography (16). Additionally, inherited thrombophilia factors including *MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII(V34L), APOB, ITGB3, FVHR2, FGB, PAI-1* and *ACE* genes were evaluated. The study protocol was certified by the local Ethics Committee (2018/220).

Statistical Analysis: Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA). Quantitative variables are expressed as mean±standard deviation, numbers and percentages. The descriptive statistic was carried out.

RESULTS

The clinical and developmental defect findings of the patients were given in Table 1. Echocardiographic and electrocardiographic findings of patients were given in Table 2.

When we analyzed hereditary thrombophilic factors in patients included in the study, we found a remarkably high rate especially in some parameters.

Table 2. Echocardiographic and electrocardiographic findings

	(n;%)
Atrial septal defect (ASD)	13 (24.5%)
Ventricular septal defect (VSD)	9 (17%)
Patent foramen ovale (PFO)	5 (9.4%)
Patent ductus arteriosus (PDA)	3 (5.7%)
Aortic regurgitation (AR)	3 (5.7%) / 2 of them had mild AR and 1 of them had moderate AR
Mitral regurgitation (MR)	6 (11.4%) / 4 of them had mild MR, 1 of them had moderate MR, and 1 of them had severe MR
Tricuspid regurgitation (TR)	16 (30.3%) / 11 of them had mild TR, 5 of them had moderate TR
Pulmonary regurgitation (PR)	2 (3.8%) (Mild)
Pulmonary hypertension (PH)	14 (26.4%) / 2 of them had stage I (PAP between 25 and 40mmHg), 11 of them had stage II (PAP between 41 and 55mmHg) and 1 of them had stage III (PAP >55mmHg).
Electrocardiography	14 of cases (26.4%) had incomplete right bundle branch block

n: Number of patients, PAP: Pulmonary arterial pressure

Twenty one patients (39.6%) had heterozygous *MTHFR C677T* carriers, twenty one patients (39.6%) had heterozygous *MTHFR A1298C* carriers, ten patients (18.9%) had homozygous *MTHFR A1298C* carriers, nine patients (17%) had heterozygous *Factor V Leiden G1691A* carriers, twenty-three patients (43.4%) had 4G/4G carriers, eighteen patients (34%) had 4G/5G variation carriers for *PAI*, twelve patients (22.7%) had heterozygous *FactorXIII(V34L)* carriers, twenty-six patients (49.1%) had ins/del carriers and twenty patients (37.7%) had del/del variation carriers for *ACE*.

All patients had at least one of the homozygous and/or compound heterozygous variations for the inherited thrombophilia. The frequency of inherited thrombophilia factors was given in Table 3.

Table 3. Frequency of inherited thrombophilia factors

Gene	**Homozygous (n;%)**	**Heterozygous (n;%)**
MTHFR A1298C	10(18.9%)	21(39.6%)
Factor II *G20210A*	-	1(1.9%)
Factor V Leiden *G1691A*	-	9(17%)
Factor V Cambridge *G1091C*	-	3(5.7%)
MTHFR C677T	1(1.9%)	21(39.6%)
FactorXIII(V34L)	1(1.9%)	12(22.7%)
ITGB	-	6(11.3%)
FGB	-	2(3.8%)
APOB	-	-
FVHR2	-	-
PAI	23(43.4%) for 4G/4G	18(34%) for 4G/5G
ACE	20(37.7%)for del/del	26(49.1%) for ins/del

n: Number of patients ***MTHFR*:** methylenetetrahydrofolate reductase ***ITGB:*** Integrin beta-1 ***FGB:*** β-fibrinogen gene ***APOB:*** Apolipoprotein B ***FVHR2:*** Factor V HR2 ***ACE1:***Angiotensin Converting Enzyme Inhibitors **PAI:**Plasminogen Activator Inhibitor-1

DISCUSSION

To the best of our knowledge, there are no studies evaluating the risk of cardiovascular system disease in DS patients by studying a large number of genes. In this study, we examined patients with DS in terms of genes related to cardiovascular system diseases.

According to our results, considering all patients included in the study, the carrier rate for ins/del and del/del variation for *ACE*, heterozygous *MTHFR A1298C*, *homozygous MTHFR A1298C*, heterozygous *MTHFR C677T*, 4G/4G and 4G/5G variation for *PAI*, heterozygous *FactorXIII*, heterozygous *Factor V Leiden G1691A* attract attention.

In the literature, *ACE* I/D genotype (del/del), homozygous *MTHFR (A1298C)* polymorphism and *factor V Leiden (G1691A)* heterozygous were detected in a hereditary thrombophilia evaluation due to diffuse bilateral lower extremity vein thrombosis in a patient with down syndrome in the pediatric age group (17). In a study with non-DS patients, 144 patients with ischemic stroke and 62 myocardial infarction (MI) or peripheral arterial occlusive disease (PAOD) were compared for the prevalence of prothrombotic gene polymorphism. In particular, we to be taken into consideration the polymorphism prevalence of *ACE, MTHFR, PAI-1, Factor V Leiden* genes in patients with DS by

comparing the rates in this study. According to this study *ACE* (ins / del) ratio was 50.7% in the stroke group, while it was 46.8% in MI/PAOD group, *ACE* (del/del) ratio was 19.4% in stroke group, while it was 33.9% in MI/PAOD group (14). In our study, in which we have 53 patients with DS, *ACE* ins / del and del / del variation carrier rates were 49.1% and 37.7%, respectively.

The *MTHFR* gene has two common mutations, C677T and A1298C. Both mutations have been proven to increase the level of homocysteine by decreasing *MTHFR* enzyme activity. In a meta-analysis, C677T and A1298C mutations were associated with high plasma homocysteine levels in cerebral vascular events, DVT, MI and PE (18). In another metaanalysis study evaluating the risk of DS children with maternal *MTHFR* polymorphism (677 C-T) resulted in *MTHFR* 677 C-T as an important risk factor for the birth of DS (19). In outabler study, both heterozygous *MTHFR* C677T and A1298C mutation rates were 39.6%. Although the ratio of heterozygous *MTHFR* C677T in DS patients is similar to the stroke (41.5%) group, it is higher than the MI/PAOD (33.9%) group (14).

PAI-1 gene 4G / 4G and 4G / 5G polymorphism types have been found to be associated with an increased risk of coronary artery disease, MI and ischemic stroke (20-22). In the stroke and MI / PAOD groups, heterozygous *PAI* rates were 49.3% and 38.7%, while homozygous *PAI* rates were 30.3% and 35.5% (14). In our study, the rate of 4G / 4G and 4G / 5G variation carrier for *PAI* was 43.4% and 34%, respectively.

It has been mentioned in different studies that there may be a relationship between *Factor V* gene polymorphism and venous thromboembolism (23,24). Heterozygous *Factor V Leiden* G1691A ratios in the Stroke and MI / PAOD groups were 17.6% and 12.9% (14). We found the ratio of heterozygous *Factor V Leiden* G1691A to 17% in the DS patient group.

In addition to the relationship between *ITGB3* polymorphism and acute coronary syndrome and atherosclerosis, the relationship between *FXIIIB* and cardioembolic ischemic stroke has been demonstrated by different studies (25,26). Both mutations were seen significantly higher in our study. Also, we could not detect *APOB* or *FVHR2* gene mutation in DS patients.

The main limitation of the study was lower number of patients with DS (53 individuals) and the comparasion was performed with control groups of the previous studies. But it is important that the current study was first study in the literature.

Compared to the literature (27), the incidence of congenital heart disease was lower since the average age of the patients included in our study was higher during the evaluation. This situation may be caused by a significant part of congenital heart diseases can recover spontaneously in early childhood.

CONCLUSION

To the best of our knowledge our current study is the first that performed a broad number of inherited thrombophilia including *MTHFR A1298C, MTHFR C677T, Factor II G20210A, Factor V Leiden G1691A, Factor V Cambridge G1091C, Factor XIII(V34L), APOB, ITGB3, FVHR2, FGB, PAI-1* and *ACE* genes in patients with DS. According to our results, the patients with DS have increased cardiovascular system diseases related with thrombosis. May the short life expectancy of patients with DS may be caused by increased inherited thrombophilia risk although the advanced modern medical facilities and social support? When the precautions (related with medical, social, lifestyle etc.) to be developed that decrease the inherited thrombophilia risks, the average life expectancy of individuals with DS may be increased? To obtain more certain knowledge about the current topic, additional studies should be performed.

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RESEARCH ARTICLE

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The Level of Knowledge and Awareness of Male University Personnels about Adult Cancers and Cancer Screening

ABSTRACT

Objective: Cancer incidence rates have been increasing for both genders. The first aim was to determine the knowledge level of men about the types of cancers on adults. The second aim was to assess the level of knowledge of them on cancer screening programs.

Methods: This cross-sectional study was conducted at Karabük University in 2020, with 312 males who worked as academicians, administrative personnel and contract-labor. A questionnaire on sociodemographic characteristics and the knowledge of male employees about cancer and cancer screenings was applied to the participants by interviewing in the university.

Results: The mean age of male participants was 40 years. Of the participants, 39.7% knew that lung cancer is the most common type of cancer among men while 80.4% knew that breast cancer is the most common type of cancer among women. Of the participants, 42.9% heard of the Center of Early Detection and Screening of Cancer (CEDSC) and only 12.5% had a cancer screening test. The participants' answers to the question "Which cancer screening tests are made in CEDSC?" were breast cancer (55.8%), cervical cancer (35.6%), colorectal cancer (31.1%) and prostate cancer (26.0%). Mammography was the most well-known among screening tests (51.9%). The level of knowledge on colon, cervical and prostate cancer screening tests increased with age ($p<0.05$).

Conclusions: The level of knowledge about cancer and cancer screenings of male participants was mostly moderate. It might be possible to increase the level of knowledge on cancer and cancer screening of men by preparing educative and informative programs.

Keywords: Awareness, Cancer, Cancer Screening, Level of Knowledge, Male

Erkek Üniversite Personelinin Erişkin Kanserler ve Kanser Taraması Hakkındaki Bilgi ve Farkındalık Düzeyi

ÖZET

Amaç: Kanser insidans oranları her iki cinsiyette de artmaktadır. Bu çalışmanın ilk amacı, erkeklerin erişkinleri etkileyen kanserler hakkındaki bilgi düzeyini belirlemektir. İkinci amacı ise erkeklerin kanser tarama programları hakkındaki bilgi düzeylerini değerlendirmektir.

Gereç ve Yöntem: Kesitsel tipte olan bu araştırma 2020 yılında Karabük Üniversitesinde yapılmış olup, akademik, idari ve sözleşmeli olarak çalışan 312 erkek çalışma kapsamına alındı. Erkek çalışanlar ile üniversite ortamında görüşülerek; sosyo–demografik özellikler, kanser ve kanser taramalarına yönelik bilgi durumlarını sorgulayan anket uygulandı.

Bulgular: Çalışmamıza dahil edilen erkeklerin yaş ortalaması 40±8.81 (min-max; 19-64) yıl idi. Katılımcıların %39.7'si erkeklerde en sık görülen kanser türünün akciğer kanseri, %80.4'ü kadınlardaki en sık kanser türünün meme kanseri olduğunu bilmişlerdi. Katılımcıların %42.9'u Kanser Erken Teşhis ve Eğitim Merkezini (KETEM) duymuş ve sadece %12.5'ı kanser tarama testi yaptırmıştı. ''KETEM' de hangi kanserler taranır?'' sorusuna katılımcıların; %55.8'i meme, %35.6'sı serviks, %31.1'i kolorektal ve %26.0'ı prostat kanseri cevabını vermiştir. Mamografi tarama testleri içerisinde en iyi bilinendi (%51.9). Kolon, serviks ve prostat kanser tarama testleri üzerine bilgi seviyesinin yaşla birlikte arttığı görüldü ($p<0.05$).

Sonuç: Çalışmamıza katılan erkeklerin kanser ve kanser taramalarına ilişkin bilgi seviyeleri genel olarak orta düzeydedir. Eğitici ve bilgilendirici programlar hazırlanarak kanser ve kanser taraması hakkında erkeklerin bilgi düzeylerinin artırılması mümkün olabilir.

Anahtar Kelimeler: Farkındalık, Kanser, Kanser Taraması, Bilgi Düzeyleri, Erkek

INTRODUCTION

Cancer which is characterized by uncontrolled cell proliferation is among the diseases that can be prevented and treated with early diagnosis and treatment strategies (1,2). The incidence rates of cancer may vary according to countries or even different settlements of countries with the influence of heredity and environmental risk factors. Another risk factor is age and it is predicted that all types of cancer increase as individuals get older (3). Additionally, the other risk factors for cancer are male sex, obesity, insufficient physical activity, smoking and alcohol intake, malnutrition and unbalanced nutrition, exposure to carcinogens, having untreated infections, diseases related to reproductive health, ionized radiation and having a low level of income (4,5,6).

Considering that 18.1 million people were diagnosed with cancer and 9.6 of them lost their lives in 2018, it is concluded that globally, one of approximately six deaths is because of cancer (7). In the same year, 210.537 new cancer cases were reported in Turkey and 116.710 of them lost their lives according to the data. Additionally, 56.5% of new cancer cases and 64% of those who lost their lives were males (8).

The World Health Organization (WHO) has ranked the most common types of cancer among men as lung, prostate and colorectal cancers, and among women as breast, colorectal and lung cancers. Considering the distribution of deaths due to cancer, lung, liver and gastric cancers are among the top three in men while the ranking is breast, lung and colorectal cancers in women. According to the 2018 data of Turkey, the most common three types of cancer among men are lung, prostate and colorectal cancers while the ranking is breast, thyroid and colorectal cancers in women. Lung cancer is in the first place in mortalities due to cancer both among men and women (7,8,9).

It is possible that there are people with inaccurate beliefs, perceptions and fears about cancer in the society, and the number of these individuals varies based on their education levels. Cancer Screening Centers have been established all around the world to educate people about the types of cancer and screening programs for raising the awareness level of society about cancer. The early diagnosis and screening methods for breast, colon, cervical cancers and prostate have been provided free of charge by Center of Early Detection and Screening of Cancer (CEDSC) since 1996 in Turkey (10,11).

Although many studies on the types of cancer have been conducted with women, the studies that included men mostly focused on colorectal and prostate cancers. There was no comprehensive study that measured the level of knowledge of male individuals on the types of cancer affecting both men and women and cancer screening methods and simultaneously assessed these, in the literature. Thus, the aim of this study was to determine the level of knowledge of men who worked at a university about the types of cancer affecting both men and women and screening programs..

MATERIAL AND METHODS

This cross-sectional study was conducted on male employees who worked as academicians, administrative personnel and contract laborers of Karabuk University between June-August 2020. There are 1272 male employees of whom 735 are academicians, 331 are administrative personnel and 206 are contract laborers, at Karabuk University. The minimum number of people who we must reach was calculated as 296 in the sample size analysis at the confidence interval of 95% and power of 80%. In total, 338 individuals were reached, but 26 individuals who left the questionnaire incomplete were excluded from the study, and the data of 312 participants were analyzed.

Data Collection: Data were collected using a questionnaire of 31 questions that was prepared by the researchers. The first section of the questionnaire consisted of 12 questions about the sociodemographic variables of the individuals such as age, sex, marital status and economic status. The second section of the questionnaire consisted of 23 questions that aimed to assess the knowledge attitudes and behaviors about cancers such as the most common types of cancer among men and women, what to do for early diagnosis, what is done to prevent cancer and screening methods. While there were yes/no questions and multiple-choice questions, there was one matching question. The researcher visited all institutions in the university and informed the personnel about the study, and the verbal consent of the participants was obtained. The questionnaire was implemented on men who agreed to participate in the study through the face-to-face interview method following the social distancing rule. Necessary approvals were obtained from the Karabuk University deanships, and the ethical approval (2020/215) was obtained from the clinical research ethics committee for the study.

Statistical Analyses: The data were analyzed on computer using the SPSS 22.0 package program. Categorical data were presented as frequency and percentage distribution. The following classifications were made for the participants; being aged 40 years old and older, being married or single, having an educational level of 12 years and more, having a family history of cancer or not. The Pearson chi-square test and multiple logistic regression analysis were compared with various dependent variables. The significance level was $p< 0.05$.

RESULTS

The study included 312 men including 26.9 % academicians, 50.0 % administrative personnel, and 23.1% contract laborers. The mean age of the participants was found to be 40±8.81 (min-max; 19-64) years. Of them, 73.4% were married, 68.9% were university graduates, 22.4% had a good financial status, and 59.9% lived in the city center. Of them, 34% still smoked cigarettes. Of the participants, 4.2% stated that they were diagnosed with cancer and 27.6% stated that one of their relatives was diagnosed with cancer. The demographic characteristics of the participants are presented in Table 1.

Table 1. Demographic characteristics of the participants

Characteristics	n	%
Age(years)		
40 years old and younger	168	53.8
Older than 40 years	144	46.2
Marital status		
Married	229	73.4
Single	69	22.1
Divorced	14	4.5
Economic status		
Good	77	22.4
Moderate	205	65.7
Poor	37	11.9
Educational level		
Primary-middle school	35	11.2
High School	62	19.9
University	215	68.9
Your duty at the university		
Academician	84	26.9
Administrative personnel	156	50.0
Contract labor	72	23.1
Employment status of your spouse		
Yes	110	35.3
No	202	64.7
Place of residence		
City	187	59.9
District	103	33.0
Village/town	22	7.1
State of smoking		
Yes	106	34.0
No	107	34.3
Quitted	99	31.7
Have you been diagnosed with cancer?		
Yes	13	4.2
No	299	95.8
Family history of cancer		
Yes	86	27.6
No	226	72.4

The rate of those who knew that lung cancer is the most common among men was 39.7% while the rate of those who knew that breast cancer is the most common among women was 80.4% . When the most common type of cancer that causes death in men and women was questioned, 65.1% answered as lung cancer in men and 53.2% answered as breast cancer in women. Of the participants, 42.9% knew about CEDSC. The most well-known cancer screenings performed in the CEDSC unit were breast cancer (55.8%) followed by cervical cancer (35.6%), colon cancer (31.1%) and prostate cancer (26%). The most well-known screening test by the participants was mammography (51.9%) while the least-known was the prostate-specific antigen (PSA) (22.1%). The rate of those who heard of the Human papillomavirus (HPV) was 13.8%. Of the participants, 12.5% had a cancer screening before and 25.0% had a relative who had a cancer screening before. When the participants were asked about the risk factors for cancer, they answered as stress and sadness (78.5%), cigarette (77.2%), genetic factors (68.9%) and alcohol (61.5%). Of the participants, 67.6% stated that they get information about cancer from the internet and 67.0% stated that they want to be educated in detail. (Table 2).

There were no differences between age groups, marital status, educational level and family history of cancer and having had a cancer screening and having knowledge of mammography ($p>0.05$). The rates of knowledge of men who were 40 years old and older about fecal occult blood test (FOBT), colonoscopy, papsmear and prostate specific antigen (PSA) was higher ($p<0.05$). Additionally, there was a significant correlation between the state of knowing FOBT and colonoscopy of the participants who got more than 12 years of education ($p<0.05$). (Table 3). The rate of knowing mammography, colonoscopy, FOBT and PSA was higher among academic personnels than other employees ($p <0.05$).

DISCUSSION

In this study, it was found that the level of knowledge of men about cancer and cancer screenings was moderate, but the recognition of cancer screening tests was parallel to age and educational level. Although the demographic distribution of cancer cases, the society's perception of risk, and diversity and availability of screening programs vary according to countries, it has been for a long time that lung cancer is the most common type of cancer among men and breast cancer is the most common type of cancer among women (4,5,7). Therefore, many countries conduct regular studies to determine the knowledge, attitudes and behaviors of individuals about cancer in the early diagnosis of these two types of cancer.

It has been known for a long time that lung cancer is the most common type of cancer in men while breast cancer is the most common type of cancer in women (12). Of men who participated in this study, 39.7% knew that lung cancer is the most common type of cancer among men while 80.4%

Table 2. Knowledge and behavior of participants about cancers and cancer screening

Questions	n	%
What is the most common cancer among men?		
Lung	124	39.7
Prostate	143	45.8
Colon	31	9.9
Gastric	14	4.5
Which cancer is the most common cause of death among men?		
Lung		
Prostate	203	65.1
Colon	45	14.4
Gastric	47	15.1
What is the most common cancer among women?	17	5.4
Lung		
Breast	25	8.0
Cervical	251	80.4
Gastric	31	9.9
Which cancer is the most common cause of death among women?	5	1.6
Lung		
Breast	76	24.4
Cervical	166	53.2
Gastric	50	16.0
	20	6.4
Have you heard of the CEDSC unit?		
Yes	134	42.9
No	178	57.1
Which cancer screenings are performed in CEDSC?		
Breast cancer	174	55.8
Cervical cancer	111	35.6
Colon cancer	97	31.1
Prostate cancer	81	26.0
Which early cancer screening tests do you know?		
Mammography (breast cancer)	162	51.9
Smear test (cervical cancer)	81	26.0
FOBT (colon cancer)	102	32.7
Colonoscopy (colon cancer)	113	36.2
PSA (prostate cancer)	69	22.1
Have you heard of the HPV vaccine?		
Yes	43	13.8
No	269	86.2
What are the risk factors for cancer?		
Cigarette	241	77.2
Alcohol	192	61.5
Stress and sadness	245	78.5
Genetic factors	215	68.9
Nutritional habits	172	55.1
Other	27	8.7
Have you had a cancer screening before?		
Yes	39	12.5
No	273	87.5
Do you have a relative who had a cancer screening?		
Yes	78	25.0
No	234	75.0
Where did you get information about cancer?		
Healthcare personnel	108	34.6
Tv/radio	162	51.9
Internet	211	67.6
Book/newspaper/magazine	106	34.0
Do you want to get detailed information about cancers?		
Yes	209	67.0
No	103	33.0

Table 3. Comparison between some independent variables and knowledge of cancer screening tests

Independent Variables	n	Performing cancer screening		Chi-square test		Multiple Logistic Analysis	
		n	%	χ^2	p	OR 95 % CI	p
Age							
Younger than 40 years old	168	15	8.9			1	
40 years old and older	144	24	16.7	4.25	0.039	1.62 (0.98-2.65)	0.056
Marital status							
Married	229	29	12.7			1	
Single	83	10	12.0	0.02	0.884	1.32 (0.76-2.31)	0.318
Education							
12 years and less	97	10	10.3			1	
12 years and more	215	29	13.5	0.61	0.432	1.03 (0.62-1.70)	0.891
Family history of cancer							
Yes	86	11	12.8			1	
No	226	28	12.4	0.009	0.932	0.80 (0.48-1.33)	0.401
Knowing FOBT							
Age							
Younger than 40 years old	168	40	23.8			1	
40 years old and older	144	62	43.1	13.05	<0.001	2.27 (1.32-3.89)	0.003
Marital status							
Married	229	86	37.6			1	
Single	83	16	19.3	9.24	0.002	0.58 (0.30-1.13)	0.111
Education							
12 years and less	97	24	24.7			1	
12 years and more	215	78	36.3	4.04	0.044	1.97 (1.11-3.49)	0.020
Family history of cancer							
No	226	73	32.3			1	
Yes	86	29	33.7	0.57	0.811	1,162 (0.66-2.02)	0.598
Knowing Colonoscopy							
Age							
Younger than 40 years old	168	47	28.0			1	
40 years old and older	144	66	45.8	10.70	0.001	2.08 (1.23-3.51)	0.006
Marital status							
Married	229	94	41.0			1	
Single	83	19	22.9	8.69	0.003	0.605(0.32-1.13)	0.117
Education							
12 years and less	97	26	26.8			1	
More than 12 years	215	87	40.5	5.40	0.020	2.09 (1.20-3.65)	0.009
Family history of cancer							
No	226	81	35.8			1	
Yes	86	32	37.2	0.05	0.822	1.17(0.68-2.01)	0.57
Knowing Pap smear							
Age							
Younger than 40 years old	168	21	12.5			1	
40 years old and older	144	60	41.7	34.31	<0.001	6.45 (3.32-12.51)	<0.001
Marital status							
Married	229	63	27.5			1	
Single	83	18	21.7	1.07	0.300	1.79 (0.86-3.74)	0.118
Education							
12 years and less	97	27	27.8			1	
More than 12 years	215	54	25.1	0.25	0.612	1.17 (0.65-2.13)	0.586
Family history of cancer							
No	226	60	26.5			1	
Yes	86	21	21.4	0.14	0.701	0.77 (0.2-1.439	0.421
Knowing Mammography							
Age							
Younger than 40 years old	168	80	47.6			1	
40 years old and older	144	82	56.9	2.70	0.100	1.62 (0.98-2.65)	0.056
Marital status							
Married	229	118	51.5			1	
Single	83	44	53.0	0.05	0,817	1.32 (0.76-2.31)	0.318
Education							
12 years and less	97	51	52.6			1	
More than 12 years	215	111	51.6	0.02	0.877	1.03 (0.62-1.70)	0.891
Family history of cancer							
No	226	120	53.1			1	
Yes	86	42	48.8	0.45	0,501	0.80 (0.48-1.33)	0.401
Knowing PSA							
Age							
Younger than 40 years old	168	28	16.7			1	
40 years old and older	144	41	28.5	6.27	0.012	1.87 (1.03-3.39)	0.040
Marital status							
Married	229	57	24.9			1	
Single	83	12	14.5	3.85	0.050	0.69 (0.32-1.45)	0.329
Education							
12 years and less	97	18	18.6			1	
More than 12 years	215	51	23.7	1.03	0.309	1.47 (0.78-2.75)	0.225
Family history of cancer							
No	226	50	22.1			1	
Yes	86	19	22.1	0.001	0.995	1.03 (0.56-1.92)	0.902

stated that breast cancer is the most common type of cancer among women. The reason why men who participated in this study had such a high rate of knowledge about the fact that the most common type of cancer in women is breast cancer might be that awareness studies and public service announcements on cancer in Turkey focus on the type of cancers experienced by women.

Considering the studies that assessed the individuals' knowledge level of cancer mortality in the literature, they had similar results. Bray et al., found that lung cancer was a primary cause of death. They also found that the mortality of lung cancer was the highest among men while the mortality of breast cancer was the highest among women (12). In a study conducted in the USA, it was reported that the most common type of cancer among women was breast cancer and the most common cause of death was lung cancer (13). Haydaroğlu et al. examined the records on cancer between 1992 and 2017 and found that the type of cancer with the highest mortality rate was lung cancer in men and breast cancer in women (14). In line with the results of the abovementioned studies, 65.1% of the participants in this study knew that lung cancer has a high mortality rate among men and 53.2% knew that breast cancer has a high mortality rate among women.

According to the 2000 data of the Global Cancer Observatory (GLOBOCAN), 1.2 million people got affected by lung cancer and 1.05 million people got affected by breast cancer while the 2018 data of the same research group showed that the number of people who got affected from lung cancer increased to 2.1 million and the number of people who got affected from breast cancer increased to 2.09 million (15,16). Although the incidence frequency of cancer is increasing day by day both in Turkey and the world, it has been detected that individuals' participation in cancer screening programs is insufficient. In the study by White et al. (17) that examined the cancer screening tendencies between 2000 and 2015, it was found that colorectal cancer screenings increased, breast cancer screening did not change and cervical cancer screening tended to decrease. Tekpınar et al., found that only 23.4% of the participants had a cancer screening in their study on cancer screenings (18). Erdem et al. reported that 60.6% of the participants had never heard of CEDSC while 32.8% knew about the types of cancer screened in CEDSC (19). Thus, it was found that 42.9% of men knew about CEDSC in this study. Additionally, the rates of participating in cancer screening programs of themselves and their relatives were low (12.5% and 25.0%, respectively). The participants' answers to the question "Which cancer screenings are performed in CEDSC?" were breast cancer (55.8%) followed by cervical cancer (35.6%), colon cancer (31.1%) and prostate cancer (26.6%). The results of this study and the abovementioned studies indicate that the level of awareness of individuals about cancer and cancer screenings is low.

The role of cancer screening tests is undeniably important in the early diagnosis of cancer and decreasing cancer-related morbidity and mortality. Therefore, screening tests specific to types of cancer have been developed. Mammography and breast self-examination are commonly used screening methods in breast examination while the papsmear test is used in cervical cancer, PSA is used in prostate cancer, and FOBT and colonoscopy are used in colorectal cancers. It was determined that the participants in this study had knowledge of mammography the most among the cancer screening tests (51.9%). On the other hand, it was observed that the participants did not have sufficient knowledge of tests used as screening methods such as smear test, colonoscopy and PSA. These results have shown that the level of knowledge of the participants in this study on breast cancer is high while their level of knowledge on colon, cervical and prostate cancers is relatively low. On the other hand, it was revealed in this study that assessed the effect of age on the level of knowledge on cancer that the level of knowledge on colon, cervical and prostate cancer screening tests was higher in older ages. Considering that the frequency to apply to a health institution increases with age, it can be concluded that aging might affect the level of knowledge of individuals about cancer. Moreover, the present study revealed that academic personnels know better about cancer screening tests. Thereby it can be thought that studying as academician and following current innovations may increase awareness about cancers and its screening tests.

Tobacco, overweight, UV radiation, alcohol, insufficient physical activity and infections are the main preventable risk factors for cancer (4,6,20). Thus, public knowledge of risk factors may play an important role in preventing cancer. In the study by Poudel et al. (21) conducted in Nepal, it was found that the participants knew that cigarettes, alcohol intake, obesity and physical inactivity are the main risk factors for cancer (92%, 82.3%, 58.6% and 51.3%, respectively). In the study by Erdem et al., the participants regarded cigarette (78.5%), anxiety and sadness (71.2%), genetic factors (61.5%), alcohol (58.4%) and diet style (47.2%) as the risk factors for cancer (19). Similar to these studies, 77.2% of men in the present study knew that cigarettes might cause cancer while 61.5% knew that alcohol might cause cancer.

The first limitation of this study is that no study examined the knowledge of men about the types of cancer that affect both men and women and cancer screenings in the literature. Therefore, the results of this study were only be compared to a few studies. Another limitation is that this study included men who only worked at the university; thus, the results cannot be generalized to society.

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RESEARCH ARTICLE

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Diagnostic Value of Platelet Mass Index, Plt/Mpv Ratio and Other Hemogram Parameters in Covid-19 Patients Who Presented to Emergency Department

ABSTRACT

Objective: The objective of this study was to compare platelet mass index, platelets count/mean platelet volume and other hemogram parameters between COVID-19 patients and a control group and to determine the parameters that are significant in discrimination between COVID-19 and healthy control patients without COVID 19.

Methods: Data of a total of 80 patients who presented to the emergency department of our hospital with the symptoms suggesting COVID-19 with a polymerase chain reaction (PCR) positive result and 80 healthy controls with a PCR (-) test results were retrospectively analyzed. Patients' laboratory parameters including white blood cells (WBC), neutrophils count, lymphocytes count, monocytes count, neutrophils to lymphocytes ratio (NLR), lymphocytes to monocytes ratio (LMR), platelets (PLT), platelets to lymphocytes ratio (PLR), mean platelet volume (MPV), red blood cell distribution width (RDW), platelet mass index (PMI), red blood cell distribution width/ mean platelet volume (RDW/MPV) and platelet count/ mean platelet volume (PLT/MPV) ratios were analyzed. At the same time a receiver operating characteristic (ROC) analysis was performed for determining laboratory indicators in distinguishing control and COVID-19 positive cases from each other.

Results: In our stuy, WBC, lymphocytes, monocytes, neutrophils, LMR, platelets, PMI and PLT/MPV levels decreased, while PLR and MPV values increased in COVID-19 patients. According to ROC analysis, among the parameters examined in terms of making discrimination between COVID-19 and contol groups, LMR, PLR, PMI and PLT/MPV parameters had significant areas under curve. The best cut-off points of the parameter were found as <2.91 for LMR, 117.95 for PLR, 2167.65 for PMI and <25.13 for PLT/MPV.

Conclusions: Our study revealed decreased WBC, lymphocytes, monocytes, neutrophils, LMR, platelets, PMI and PLT/MPV levels and elevated PLR and MPV values in COVID-19 patients. We believe that these parameters can be helpful in follow-up of the prognosis of COVID-19 patients and distinguishing these patients from healthy persons without COVID-19.

Keywords: COVID-19, Neutrophils, Lymphocytes, Platelets

Trombosit Kitle İndeksi, Plt/Mpv Oranı ve Diğer Hemogram Parametrelerinin Acil Servise Başvuran Covıd-19 Hatalarında Tanısal Değeri

ÖZET

Amaç: Bu çalışmanın amacı, trombosit kitle indeksi, trombosit sayısı / ortalama trombosit hacmi oranı ve diğer hemogram parametrelerini koronavirüs (COVID-19) hastaları ve kontrol grubu arasında kıyaslamak ve COVID-19 hastaları ile COVID-19 olmayan sağlıklı kontrol hastaları ayırımının yapılmasında anlamlı olan parametrelerin saptanmasıdır.

Gereç ve Yöntem: Hastanemizin acil servisine COVID-19'u düşündüren semptomlarla başvuran ve polimeraz zincir reaksiyonu (PCR) pozitif olan 80 hasta ile PCR (-) olan 80 kontrol birey retrospektif olarak incelenmiştir. Hastaların beyaz kan hücresi (WBC), nötrofil sayısı, lenfosit sayısı, monosit sayısı, trombosit sayısı, nötrofil/lenfosit oranı NLR), lenfosit/monosit oranı (LMR), trombosit sayısı, trombosit/lenfosit oranı (PLR), ortalama trombosit hacmi (MPV), kırmızı hücre dağılım genişliği (RDW), trombosit kitle indeksi (PMI), kırmızı kan hücresi dağılım genişliği / ortalama trombosit hacmi (RDW / MPV) ve trombosit sayısı/ ortalama trombosit hacmi (PLT / MPV) oranları analiz edilmiştir. Aynı zamanda kontrol ve COVID-19'u ayırmada anlamlı parametrelerin belirlenmesi için Receiver Operating Characteristic (ROC) analizi yapıldı.

Bulgular: Çalışmamızda WBC, lenfosit, monosit nötrofil, LMR, trombosit, PMI ve PLT/MPV değerleri COVID-19 hastalarında düşmüş, PLR ve MPV değerleri ise yükselmiştir. LMR, PLR, PMI ve PLT/MPV'nin eğri altında kalan alanları anlamlıdır. İncelenen parametreler için en iyi cut-off noktaları; LMR için <2.91, PLR için 117.95, PMI için 2167.65 ve PLT/MPV için <25.13'tür.

Sonuç: Çalışmamızda COVID-19 hastalarında WBC, lenfosit, monosit, nötrofil, trombosit, PMI ve PLT/MPV düzeylerinin azaldığı, PLR ve MPV değerlerinin ise azaldığı saptanmıştır. Bu parametreler COVID-19 hastalarının prognozunun izlenmesinde ve bu hastaların, COVID-19 bulunmayan sağlıklıkişilerden ayırt edilmesinde yardımcı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: COVID-19, nötrofil, lenfosit, platelet

INTRODUCTION

At the end of December 2019, pneumonia cases of unknown origin have been increasingly seen in Wuhan province of Hubei state in China. The disease was then declared to be caused by a novel coronavirus named Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease was rapidly spread throughout mainland China and then all over the world (1). According to the current data, the early Coronavirus Disease 2019 (COVID-19) cases were associated with a seafood market in Wuhan where wild animals are sold (2). The World Health Organization (WHO) termed this new disease as COVID-19 and declared it as a pandemic on March 12, 2020(3). Today, COVID-19 is continuing to affect all the world as well as Turkey and to cause many deaths and morbidities. As of 03/10/2020, according to the daily report of WHO, there were over 34 million cases and more than 1,000,000 deaths worldwide (4). On the other hand, according to the Turkish Ministry of Health, there were 321,512 cases and 8,325 deaths in Turkey as of 02 October (5).

At present, unfortunately we are not in a position to effectively treat COVID-19, because today no specific antiviral drugs have yet been developed and approved to treat human CoV infections (6-8). Nevertheless, vaccination programs have been launched in several countries at the time of this study. In the literature, first publications about COVID-19 have been predominantly published by Chinese authors (9-15). According to the first studies on COVID-19, the main symptoms of presentation were fever, cough and shortness of breath (16). However as the disease progressed, new symptoms and involvement of cardiac, gastrointestinal and neurologic systems have been increasingly reported.

Although the standard method of diagnosis is polymerase chain reaction (PCR) test, laboratory markers are among the most important indicators for the confirmation of diagnosis of COVID-19. Because the disease has a dynamic process leading to new and unexpected conditions every passing day, laboratory findings are extremely important to evaluate the evolution of COVID-19 and to guide treatment interventions (17). It has been reported that some blood parameters significantly decrease, while the others significantly increase during progression of COVID-19 (18). The laboratory findings, which are observed to change in infectious processes including COVID-19 are widely varied. Several hemogram parameters have been studied so far in COVID-19. However, to our knowledge there is no study investigating diagnostic value of platelet mass index (PMI) and platelets count to mean platelet volume (PLT/MPV) parameters in COVID-19. Therefore, the objective of this study was to compare PMI, PLT/MPV and other laboratory parameters between COVID-19 patients and a control group and to determine the parameters that are significant in discrimination between COVID-19 and healthy control patients without COVID 19.

MATERIAL AND METHODS

In this study, data of a total of 80 patients who presented to the emergency department (ED) of our hospital with the symptoms suggesting COVID-19, confirmed with the diagnosis of COVID-19 through PCR (+) test, but had no lung involvement on chest computed tomography (CT) and, who were advised outpatient treatment and quarantine between 15/05/2020 and 15/08/2020 and 80 healthy control patients with a PCR (-) test results who had suspected contact history, but had no any discomfort as the control group were retrospectively analyzed. Accordingly, a total of 80 patients who presented to the ED with symptoms such as fever, dry cough, dyspnea, sore throat and fatigue that suggest COVID-19 and had PCR (+) test result were assigned to PCR (+) group or COVID-19 group (COVID-19 group), and 80 control subject who presented to the ED only with a suspected contact history and had PCR (-) test result to PCR (-) (control group). Patients with complete data were included in the study. Patients with missing data, malignancy, receiving chemotherapy or using steroids were excluded from the study.

Data of the patients were obtained from the hospital medical records and retrospectively analyzed. The diagnosis of COVID-19 pneumonia was confirmed according to the case definition established by WHO with positive PCR test results through naso-pharingeal swab samples (19).

Patients' demographic characteristics such as age and gender, clinical symptoms and laboratory parameters including white blood cells (WBC), neutrophils count, lymphocytes count, monocytes count, neutrophils to lymphocytes ratio (NLR), lymphocytes to monocytes ratio (LMR), platelets, platelets to lymphocytes ratio (PLR), mean platelet volume (MPV), red blood cell distribution width (RDW), platelet mass index (PMI), RDW/MPV and PLT/MPV ratios were measured and analyzed. All laboratory parameters were studied with automated laboratory methods using commercial kits in line with the instruction of the manufacturer.

At the same time a receiver operating characteristic (ROC) curve analysis was performed for determining laboratory indicators in distinguishing control and COVID-19 positive cases from each other. Accordingly the optimal cut-off points to predict COVID-19 and sensitivity, specificity, positive predictive value and negative predictive value were also calculated.

Ethical Considerations: Ethical consent was obtained from the Harran University Clinical Research Ethics Committee for the study (Date:17/08/2020 decision number:

HRU/20.14.13). Since this study included retrospective evaluations, informed consent was not deemed necessary. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis: Data obtained in this study was statistically analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 (IBM Corporation, Armonk, NY, USA) statistical software. Normality of the data was analyzed using Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and median interquartile range (IQR) when appropriate. Whereas categorical data were expressed as numbers and percentages. Differences between the Control and COVID-19 groups were compared using Student's t test, while Mann-Whitney U test was used in the comparison of continuous variables that did not meet parametric test assumption. Categorical variables were analyzed with Continuity corrected Chi-square ($\chi 2$) test. The optimal cut-off points of laboratory measurements to determine the existence of COVID-19 were investigated in ROC analyses as giving the maximum sum of sensitivity and specificity for the significant test. Sensitivity, specificity, positive, and negative predicted values with 95% confidence interval for each significant laboratory measurement to discriminate the patients with COVID-19 were also calculated. P<0.05 values considered statistically significant.

RESULTS

In our study, PCR (+) group consisted of 80 patients and PCR (-) group 80 control subjects. The mean age was 46.9±17.2 years in PCR (+) group and 45.9±9.3 years in PCR (-) group. No statistically significantly significant difference was found between both groups in terms of ahe (p=0.681). Of patients in PCR (+) group, 53 (66.3%) were male and 27 (33.7%) female, while of subjects in the control group, 58 (72.35%) were male and 22 (27.5%) female. No significant difference was observed between the two group in terms of gender distribution (p=0.493).

When the studies laboratory parameters were evaluated; WBC was significantly lower in the PCR (+) group compared to PCR (-) group (p<0.001). Similarly neutrophils count was significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). Lymphocytes count was statistically significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). Monocytes count also was significantly lower in PCR (+) group compared to PCR (-) group (p<0.001).

LMR ratio was statistically significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). Again, platelets count was significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). On the other hand, PLR ratio was statistically significantly higher in PCR (+) group compared to PCR (-) group (p<0.001). Similarly MPV value was statistically significantly higher in PCR (+) group compared to PCR (-) group (p=0.025). PMI ratio was significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). Again, PLT/MPV ratio was statistically significantly lower in PCR (+) group compared to PCR (-) group (p<0.001). No statistically significant difference was found between the two groups in terms of the other studied parameters. Demographic features and laboratory findings of the groups are given in Table 1.

Table 1. The comparisons of demographic characteristics and laboratory measures

	Control (n=80)	**COVID-19 (n=80)**	**P-value**
Age (years) *	45.9±9.3	46.9±17.2	0.681
Male factor	58 (72.5%)	53 (66.3%)	0.493
Female	22 (27.5%)	27 (33.7%)	
WBC **	9.71 (8.52-11.14)	5.62 (4.64-6.79)	**<0.001**
Neutrophil **	5.46 (4.62-6.93)	3.44 (2.37-4.67)	**<0.001**
Lymphocyte **	3.01 (2.32-3.78)	1.59 (1.13-2.11)	**<0.001**
Monocyte *	0.73±0.24	0.55±0.26	**<0.001**
NLR **	1.74 (1.28-2.59)	2.08 (1.36-3.46)	0.143
LMR **	4.26 (3.31-5.36)	3.23 (2.35-4.98)	**<0.001**
Platelet **	269.5 (240.5-305.0)	210.0 (177.5-226.2)	**<0.001**
PLR **	89.0 (73.4-113.6)	131.1 (105.3-186.8)	**<0.001**
MPV *	10.1±1.07	10.4±0.74	**0.025**
RDW **	13.0 (12.3-13.7)	13.0 (12.3-13.7)	0.852
PMI **	2749.8 (2339.0-3103.1)	2135.1 (1909.6-2695.7)	**<0.001¶**
RDW/MPV	1.27 (1.18-1.43)	1.25 (1.17-1.35)	0.224¶
PLT/MPV	27.4 (22.6-31.6)	20.8 (16.6-25.7)	**<0.001¶**

* Data were expressed as mean ± SD, ** Descriptive statistics were shown as median (25^{th} – 75^{th}) percentiles, † Student's t test, ‡ Continuity corrected χ^2 test, ¶ Mann Whitney U test.

WBC: white blood cell, NLR: neutrophils to lymphocytes ratio, LMR: lymphocytes to monocytes ratio, PLR: platelets to lymphocytes ratio, MPV: mean platelet volume, RDW: red cell distribution width, PMI: platelet mass index, PLT: platelet count

A ROC analysis was performed to determine laboratory values that are significant in distinction between PCR (+) and PCR (-). Accordingly, among the parameters examined in terms of making discrimination between COVID-19 and contol groups, LMR, PLR, PMI and PLT/MPV parameters had significant areas under curve (for all p<0.001) (Table 2).

Table 2. The ROC analysis results of laboratory indicators in distinguishing healthy controls and COVID-19 (+) patients from each other

	AUC	95% CI	P-value
NLR	0.567	0.478-0.656	0.143
LMR	0.664	0.579-0.749	**<0.001**
PLR	0.757	0.682-0.831	**<0.001**
PMI	0.715	0.634-0.795	**<0.001**
RDW/MPV	0.556	0.466-0.645	0.224
PLT/MPV	0.737	0.659-0.814	**<0.001**

AUC: Area under the ROC curve, CI: Confidence interval.

The best cut-off points of the parameters that were significant in distinction between PCR (+) and PCR (-) were found as <2.91 for LMR, 117.95 for PLR, 2167.65 for PMI and <25.13 for PLT/MPV. Sensitivity, specificity, positive predictive value and negative predictive value of these four parameters in making discrimination between PCR (+) and PCR (-) at 95% confidence interval are shown in Table 3 and Figure 1.

Table 3. The optimal cut-off points for statistically significant laboratory measurements according to the ROC analysis and diagnostic performances and 95% CI levels in distinguishing controls and COVID-19 positives from each other

	LMR	PLR	PMI	PLT/MPV
Cut-off point	<2.91	>117.95	<2167.65	<25.13
Sensitivity	43.8 (32.9-54.6)	65.0 (54.5-75.4)	52.5 (41.6-63.4)	71.3 (61.3-81.2)
Specificity	92.5 (86.7-98.3)	80.0 (71.2-88.8)	87.5 (80.2-94.5)	67.5 (57.2-77.8)
PPV	85.4 (74.5-96.2)	76.5 (66.4-86.5)	80.8 (70.1-91.5)	68.7 (58.7-78.6)
NPV	62.2 (53.5-70.9)	69.6 (60.2-79.0)	64.8 (55.8-73.8)	70.1 (59.9-80.3)

PPV: Positive predictive value, NPV: Negative predictive value.

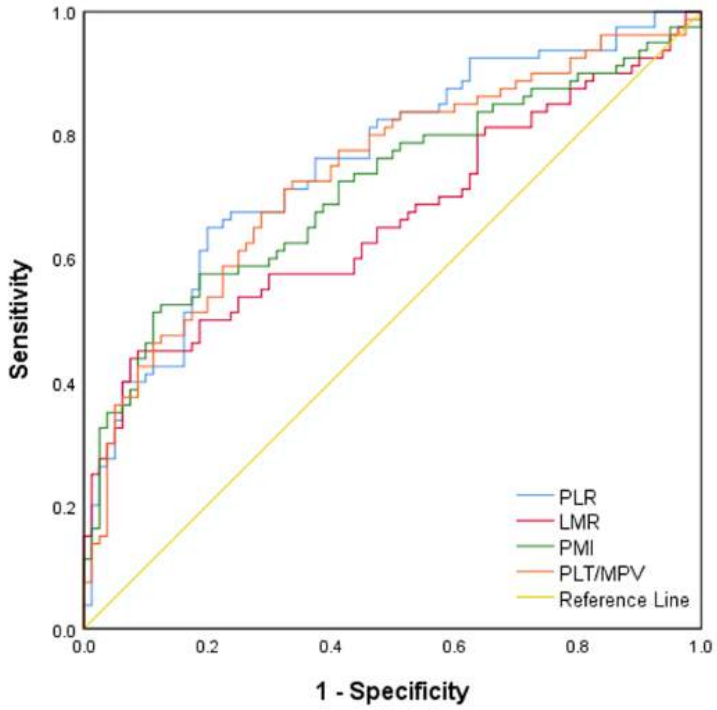

Figure 1. Sensitivity, specificity, positive predictive value and negative predictive value of the parameters that were significant as a result of ROC analysis.

DISCUSSION

Real-Time Reverse Transcription Chain Reaction (RT-PCR) remains the gold standard for the diagnosis and management of COVID-19, although it takes time and the prevalence of false negative results is high (20,21). Several hemogram parameters including white blood cell (WBC), neutrophils count, lymphocytes count and platelets count have been reported to change in COVID-19 patients (10,21,22). Accumulating evidence from studies that will be conducted on this issue will help a rapid diagnosis process and provide contribution to the management.

In the present study where we investigated various blood parameters in COVID-19 and control group; WBC, neutrophils, lymphocytes, monocytes, lymphocytes/monocytes, platelets, PMI and PLT/MPV levels were significantly lower in COVID-19 group compared to the control group. On the other hand, platelet/lymphocytes and MPV values were significantly higher in COVID-19 group.

Although blood picture differs in COVID-19 patients, the most common laboratory findings include normal/low lymphocytes count, unbalanced coagulation, and elevated levels of C-reactive protein (CRP), lactate dehydrogenase, aminotransferase and ferritin. In a study by Guan et al. with 1099 patients, the most common findings was reported as lymphocytopenia by 82.1% (12). Zhou et al. found a correlation between low basal lymphocytes level and poor prognosis in 191 patients (23).

Lymphocyte plays an important role in maintaining hemostasis, an inflammatory response throughout the body and decreased lymphocytes count may cause reduction in immunity. Lymphocytopenia is common in acute infections and is of paramount importance in COVID-19 infections (24). Studies from China and the USA reported low lymphocytes count and coagulatşon disorder in fatal COVID-19 patients (23-25). In a study from Rome, Italy lymphocyte count was found to be low in 60% and limited in 32% of COVID-19 patients (26). In our study, lymphocytes count was one of the parameters lower in COVID-19) patients compared to the control group ($p<0.001$).

In the present study, platelets count was significantly lower in COVID-19 patients compared to the control subjects. Therefore COVID-19 patients are more likely to have lymphopenia and thrombocytopenia. In a publication from Hong Kong lymphopenia was reported in 98% and thrombocytosis in 49% of COVID-19 patients (27).

In a study by Qin et al., NLR ratio was found to be higher in patients with severe COVID-19 than in those with mild disease (28). In our study no significant difference was found between PCR (+) and PCR (-) groups in terms of NLR. However, in the study by Qin et al., all patients included in the study had severe or mild COVID-19 disease. We could conclude that even if NLR can not distinguish COVID-19 and non-COVID-19, at least it has a prognostic value in determining severity of the disease.

In our study, neutrophils count was significantly lower in the PCR (+) group compared to PCR (-) group. Evidence in the literature suggests that neutrophil count increases as the disease progresses. Increased neutrophils count cause a risk for the development of acute respiratory distress syndrome (ARDS) during disease and death (25). In a meta-analysis, it was found with multivariate analysis that PLR ratio is an independent predictor of prolonged hospitalization in a period where platelets peak. It was proposed that high PLR ratio may indicate a more prominent cytokines storm as a result of increased platelet activation (29).

WBC value was found to be significantly lower in COVID-19 patients compared to healthy individuals (30). Similarly, in our study WBC was statistically significantly lower in the PCR (+) group compared to PCR (-) group. Again ın a study by Pan et al., MPV value was significantly increased in COVID-19 patients. Consistently with the literature, in our study MPV value was significantly higher in PCR (+) group compared to PCR (-) group. In the study by Pan et al., monocytes count was significantly lower in COVID-19 patients compared to the healthy controls (30). Similarly in the present study monocytes count was significantly lower in PCR (+) group compared to PCR (-) group.

In our study, we performed ROC analysis in order to determine blood parameters that will make distinction between COVID-19 group and control group. According to the results of this analysis, among the studied parameters areas under curve values of LMR, PLR, PMI and PLT/MPV parameters were found to be significant. Sensitivity, specificity, positive predictive value and negative predictive value of these four parameters in discrimination of COVID-19 and non-COVID-19, and the best cut-off points for these parameters are given in table 3.

Sensitivity and specificity ratios that result from these cut-off values may be influenced by the number of patients included in the study and by demographic features of the patient population. The use of these values we especially determined for PMI and PLT/MPV in clinic: a cut-off value with a high specificity provides a comfortable observation before further investigations and early consultation in suspected COVID-19 patients who presented to ED with complaints such as fever, cough and dyspnea; on the other hand, a test result above a cut-off value of high sensitivity can predict further investigation and early consultation for the patient.

Studies in the literature on this issue have analyzed a wide spectrum of blood parameters, biochemical parameters and enzymes. However, to our knowledge, there is still no study in the literature to investigate PMI and PLT/MPV parameters in terms of COVID-19 disease.

Study Limitations: First, this study has a retrospective design. In addition, it was conducted in a single center. On the other hand, the number of participants was relatively high. Perhaps we could analyze other parameters at the same time. However, given the abundance of parameters that could be studied, the study would be more complex to interpret and healthy conclusions would not be drawn. Therefore many studies have focused on different parameters. The most commonly studied parameters are lymphocytes, neutrophils, platelets, monocytes and WBC. We believe that the results we obtained from this study will be guiding for further studies to be conducted on this issue.

CONCLUSION

Discharge of COVID-19 patients should not be considered an endpoint for monitoring and precautionary measures. The way to full recovery may be long for COVID-19 patients and especially critical patients. In addition, the possibility of re-infection should be evaluated in patients who recovered from the disease. Regular control visits are necessary for monitoring possible changes in blood parameters and biochemical parameters and to assess potential complications in future. For this purpose blood parameters could provide practical methods. In our stuy, WBC, lymphocytes, monocytes, neutrophils, LMR, platelets, PMI and PLT/MPV levels decreased, while PLR and MPV values increased in COVID-19 patients. Our findings should be supported by further multicenter comprehensive studies.

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RESEARCH ARTICLE

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Change in Forensic Cases Admitted to Emergency Department during COVID-19 Pandemic

ABSTRACT

Objective: This study aimed to determine the change in forensic cases admitted to an emergency department after the COVID-19 pandemic, due to its adverse effect on all people around the world in various ways.

Methods: This study was conducted retrospectively in a single center. The sample size included 1645 forensic cases over the age of 18 years admitted to an emergency department between 1 January 2019 and 31 December 2020. Ethical approval before the study was obtained from a Local Clinical Research Ethics Committee. The period between 11 March 2020 and 31 December 2020 was considered the COVID-19 pandemic (DP), while the pre-DP period was named as BP. The distribution rates of forensic cases in BP versus DP were compared.

Results: Both traffic and work accidents were higher in males than females, whereas suicide attempts were higher in females than males ($p<0.001$). Suicide attempts by both women (BP: 14.9%, DP: 17.4%) and men (BP: 3.7%, DP: 4.9%) showed an increase for DP ($p< 0.01$). Work accidents were lower in women (BP: 22.3%, DP: 13.4%) and men (BP: 22.5%, DP: 21.1%) in DP than BP ($p<0.01$).

Conclusions: The COVID-19 pandemic has increased suicide rates in both women and men but reduced work accident rates. Suicide rates were higher in women than men. The COVID-19 pandemic has negatively affected people in many ways, including forensic, employment, economic, and psychological aspects.

Keywords: COVID-19, Forensic Case, Suicide Rates, Work Accident, Traffic Accident

Acil Servise Başvuran Adli Vakaların COVID-19 Pandemisindeki Değişimi

ÖZET

Amaç: Bu çalışmada, tüm dünyada insanların tamamını birçok yönden olumsuz etkileyen COVID-19 pandemisinin acil servise başvuran adli vakaların pandemi öncesine göre gösterdiği değişimi saptamak amaçlanmıştır.

Gereç ve Yöntem: Bu çalışma tek merkezli retrospektif olarak yapıldı. Çalışmaya 01 Ocak 2019- 31 Aralık 2020 acil servise başvuran 18 yaş üzerindeki 1645 adli vaka dâhil edildi. Çalışma için öncesinde Lokal Klinik Araştırmalar Etik Kurulu'ndan etik onay alındı. 11 Mart 2020- 31 Aralık 2020 tarihleri arası COVID-19 pandemisi (DP), önceki tarihler ise pandemi öncesi (BP) olarak kabul edildi. BP ve DP dönemlerinde adli vakaların dağılım oranları karşılaştırılmıştır.

Bulgular: Trafik kazaları ve iş kazaları erkek cinsiyette kadınlara göre yüksek saptandı, buna karşılık kadınlarda intihar girişimleri erkeklerden yüksek saptandı ($p<0,001$). Hem kadınlarda (BP: %14,9, DP: %17,4) hem de erkeklerde (BP: %3,7, DP: %4,9) intihar girişimlerinin artış gösterdiği saptandı ($p<0,01$). Pandemi sırasında, pandemi öncesiyle karşılaştırıldığında kadınlarda (BP: %22,3, DP: %13,4) ve erkeklerde (BP: %22,5, DP: %21,1) iş kazaları düşük saptandı ($p<0,01$).

Sonuç: COVID-19 pandemisi hem kadınlarda hem de erkeklerde intihar oranlarını arttırmış, iş kazası oranlarını ise azaltmıştır. Kadınlardaki intihar oranları erkeklerden yüksek bulunmuştur. COVID-19 pandemisi insanları adli, istihdam, ekonomik ve psikolojik birçok yönden olumsuz etkilemiştir.

Anahtar Kelimeler: COVID-19, Adli Vaka, İntihar Oranları, İş Kazası, Trafik Kazası

INTRODUCTION

Forensic cases are usually first evaluated in emergency departments and constitute a significant part of emergency department admissions. As the first evaluation place of forensic cases, emergency departments have an important place in the detection, diagnosis, and treatment (1). The recognition of forensic cases, the preparation of complete forensic reports for these cases and reporting to forensic institutions are among the responsibilities of emergency physicians. For this reason, not only forensic cases but also their tendencies and epidemiology should be well known and managed by emergency department physicians (2).

The forensic cases evaluated with the emergency departments result from many reasons such as assaults (e.g., blunt injury, sharp object injuries, and gunshot wounds), traffic accidents (e.g., vehicle accident and pedestrian injury), suicide attempts (e.g., drug use, hanging, and falling from height), poisoning (e.g., drugs, alcohol or narcotic, carbon monoxide, snake bites, and scorpion stings), electric shocks, sharp object injuries, severe burns, and work accidents. Their distributions, admission times, and frequencies of these cases in emergency departments may differ in age and gender (3). The social, economic, psychological, physical, and mental states of people can affect the circumstances of a forensic case. Moreover, the frequencies, admission times and epidemiological distributions of forensic cases may differ in events (e.g., disaster, pandemic, and crisis) that concern a large portion of the society (4).

COVID-19 which emerged in Wuhan, Hubei, China in December 2019 (5) and spread all over the world, was declared as a pandemic by the World Health Organization (WHO) in March 2020. The COVID-19 pandemic has caused multidimensional (health, lifestyle, employment, economic, psychological, sociological) negative effects on people in the whole world (6). We think that these negative effects changed the type and distribution of forensic cases admitted to the emergency department. This study aimed to determine the change in forensic cases admitted to the emergency department before and during the COVID-19 pandemic.

MATERIAL AND METHODS

Study Population: This study included 1645 patients over the age of 18 years diagnosed as forensic cases between 1 January 2019 and 31 December 2020 in Ordu University Hospital Emergency Department with an annual emergency admission of approximately 150,000. No additional sample was selected for the study, and the entire population was included as a sample.

Data Collection: For the study, age, gender, diagnosis (traffic accident, assault, work accident, suicide attempt, electric shock, carbon monoxide (CO) poisoning, snake bite, scorpion sting), admission date of the patients were retrospectively collected through the computerized database of the hospital. The period between 11 March 2020 and 31 December 2020 was defined as the COVID-19 pandemic period (DP) (7). The period between 1 January 2019 and 11March 2020 was defined as before the COVID-19 pandemic period (BP).

Ethics Consideration: Ethical approval before the study was obtained from the Local Clinical Research Ethics Committee of Ordu University with the 7 January 2021 dated and 2021/06 numbered decision. Since the study was designed retrospectively, the informed consent forms of the patients were waived. The study was conducted in accordance with the Helsinki Declaration.

Statistical Analysis: All data analyses were conducted using SPSS v26 (IBM Inc., Chicago, IL, USA). Student's t-test was used to assess differences in age between the two groups. Prior to the test, the data were tested for normality using the Kolmogorov-Smirnov test and for homogeneity of variance using Levene's test. Chi-square test was used to compare the frequency of diagnoses between BP and DP. All comparisons were two-tailed, and a p-value less than 5% was considered statistically significant.

RESULTS

Out of the 1645 patients, 59.7% (n = 982) were male, while 40.3% (n = 663) were female. The mean age of the patients was 38.4 ± 15.9. In addition, 65.6% (n = 1079) and 34.4% (n = 566) of the forensic cases were admitted in BP and DP, respectively. The mean age of the forensic cases admitted in BP and DP were 38.4 ± 16.0 and 38.3 ± 15.7, respectively (p = 0.868).

The mean age of women was 38.9 ± 15.8, and the mean age of men was 37.7 ± 16.0 (p=0.141) (Table 1).

Table 1. The mean ages.

Variable		n	%	Age (Mean±SD)	*p*
COVID-19 Pandemic	Before	1079	65.6	38.4±16.0	*0.868*
	During	566	34.4	38.3±15.7	
Gender	Female	663	40.3	38.9±15.8	*0.141*
	Male	982	59.7	37.7±16.0	
Total		1645	100.0	38.4±15.9	

SD: Standard deviation
Student t-test

Considering the diagnosis rates by gender (Table 2), traffic accidents were 41.9% (n = 278) and 57.0% (n = 560) in women and men, respectively, and ranked in the first place for both genders. However, traffic accidents were higher in men than women (p<0.001). Work accidents were 19.6% (n = 130) and 22.0% (n = 216) in women and men, respectively, and ranked in the second place for both genders. Work accidents were higher in men than in women (p<0.001). Suicide attempts by women and men were estimated at 15.7% (n = 104) and 4.2% (n = 41), respectively. Suicide attempts by women ranked in the third place and were higher than those by men (p<0.001). Assaults were in the 3rd rank for the males and higher in females (14.0%, n = 93) than males (10.7%, n = 105) (p<0.001). Snake bites, scorpion stings and CO poisoning were higher in females than males (p<0.001). Electric shock was higher in men than women (p<0.001) (Table 2).

Table 2. Forensic case diagnoses.

Diagnosis	Gender				Total	
	Female		Male			
	n	%	n	%	n	%
Scorpion sting	8	1.2	4	0.4	12	0.7
Assault	93	14.0	105	10.7	198	12.0
Electric shock	3	0.5	10	1.0	13	0.8
Suicide attempt	104	15.7	41	4.2	145	8.8
Work accident	130	19.6	216	22.0	346	21.0
CO poisoning	38	5.7	33	3.4	71	4.3
Traffic accident	278	41.9	560	57.0	838	50.9
Snake bite	9	1.4	13	1.3	22	1.3
Total	663	100.0	982	100.0	1645	100.0
p	***0.000****					

*Chi-square test with Likelihood ratio *:<0.001*

Suicide attempts by both women (BP: 14.9%, DP: 17.4%) and men (BP: 3.7%, DP: 4.9%) showed an increase for DP (p <0.01) (Table 3). Work accidents were lower in women (BP: 22.3%, DP: 13.4%) and men (BP: 22.5%, DP: 21.1%) in DP than BP(p<0.01).

Table 3. Forensic case diagnoses before and during the COVID-19 pandemic.

			COVID-19 Pandemic				Total		*p*
			Before		During				
			n	%	n	%	n	%	
Female	Diagnosis	Scorpion sting	4	0.9	4	2.0	8	1.2	***0.002****
		Assault	65	14.1	28	13.9	93	14.0	
		Electric shock	0	0.0	3	1.5	3	0.5	
		Suicide attempt	69	14.9	35	17.4	104	15.7	
		Work accident	103	22.3	27	13.4	130	19.6	
		CO poisoning	32	6.9	6	3.0	38	5.7	
		Traffic accident	185	40.0	93	46.3	278	41.9	
		Snake bite	4	0.9	5	2.5	9	1.4	
	Total		462	100.0	201	100.0	663	100.0	
Male	Diagnosis	Scorpion sting	4	0.6	0	0	4	0.4	***0.005****
		Assault	56	9.1	49	13.4	105	10.7	
		Electric shock	10	1.6	0	0	10	1.0	
		Suicide attempt	23	3.7	18	4.9	41	4.2	
		Work accident	139	22.5	77	21.1	216	22.0	
		CO poisoning	23	3.7	10	2.7	33	3.4	
		Traffic accident	356	57.7	204	55.9	560	57.0	
		Snake bite	6	1.0	7	1.9	13	1.3	
	Total		617	100.0	365	100.0	982	100.0	
Total	Diagnosis	Scorpion sting	8	0.7	4	0.7	12	0,7	***0.041****
		Assault	121	11.2	77	13.6	198	12.0	
		Electric shock	10	0.9	3	0.5	13	0.8	
		Suicide attempt	92	8.5	53	9.4	145	8.8	
		Work accident	242	22.4	104	18.4	346	21.0	
		CO poisoning	55	5.1	16	2.8	71	4.3	
		Traffic accident	541	50.1	297	52.5	838	50.9	
		Snake bite	10	0.9	12	2.1	22	1.3	
	Total		1079	100.0	566	100.0	1645	100.0	

Chi-square test with Likelihood ratio *: <0.05; **:<0.01

Among women, the proportion of forensic cases involving traffic accidents was higher in the DP period (BP: 40.0%, DP:46.3%, p<0.01). However, traffic accidents involving men decreased in DP (BP: 57.7%, DP:55.9%, p<0.01). In DP, the number of assaults decreased in women (BP: 14.1%, DP:13.9%, p <0.01) and increased in men (BP: 9.1%, DP:13.4%, p< 0.01). CO poisoning decreased in both women (BP: 6.9%, DP:3.0%) and men (BP: 3.7%, DP: 2.7%) in DP (p<0.01) (Table 3).

DISCUSSION

We detected no significant change in the mean age between DP and BP. Similar to our results, Bilgin et al. (8) found the mean age for traffic accidents in 2011 to be 31.7 ± 17.7. In our study, we did not find a significant difference between the mean ages of women and men. Korkmaz et al. (9) reported the mean ages of women (32.77 ± 20.10) and men (32.24 ± 16.61) similar to our study. The reason for this may be because forensic events such as traffic accidents and work accidents occurred more in the young-middle age group, who are more active in social and business life.

Kukul Güven et al. (10) detected that 76.8% of the patients involved in forensic cases in 2006 were male. Also, Levent et al. (3) found that most of the forensic cases admitted to the emergency department in 2013 were men. Similarly, we found that males (59.7%) were in the majority of the forensic cases. This may be attributed to the fact that women in Turkey do not take part in business and social life as actively as do men.

Bilgin et al. [8] determined that most traffic accidents (68.6%) involved men. Similarly, we found that traffic accidents involved more men than women. Traffic and work accidents ranked in the first and second places for both women and men in our study. Similarly, Levent et al. (3) reported the traffic and work accidents as the first and second causes that ended up in the emergency department. Unlike our findings, Demircan et al. (11) in 2008 stated that traffic accidents, assaults, suicide attempts, and CO poisoning were the first four causes for 13823 forensic cases evaluated in six years. In our study, suicide attempts and assaults were the third and fourth causes for women, while the opposite was true for males. The difference may arise from the fact that the old records of work accidents were not kept as well as they are now in Turkey. Differently from our results, Karanfil et al.(12) pointed out that assaults, sexual assaults, and traffic accidents were the most important causes, respectively, from forensic department. The difference may result from the cases frequently examined by the emergency department versus the forensic department. In a study performed on children, the most common forensic cases were found as poisoning and suicide attempts, unlike adults (13).

In our study, suicide attempts were higher in women than men. This may be due to women's less participation in economic enterprises, inadequate professional and social supports for them, and their inadequate economic independence (2, 9, 11). Although snake bites, scorpion stings and CO poisoning were higher in females than males in our study, there was no significant difference in numbers except for scorpion stings. The higher traffic accidents and work accidents among men than women may explain the higher incidences of snake bites, scorpion stings and CO poisoning among women. Also, since the university hospital where our study was conducted provides services to a rural population, this result may be observed.

Given the changes in diagnoses between BP and DP in our study, the most striking results were the increased percent of suicide attempts and the decreased percent of work accidents in both women and men. This may be caused by many reasons such as lockdown processes, death of loved ones, economic difficulties, unemployment, and the increased stress and anxiety levels caused by the pandemic (6, 14, 15).

In our study, there was an increase in suicide attempts by both female and male in DP. Leske et al. (16) observed no significant change for 443 suspected suicide cases between BP and DP. Ammerman et al. (17) stated that the COVID-19 pandemic increased suicidal thoughts and tendencies among adults. Chiba et al. (18) reported that suicide cases rose by 38.5% during the lockdown period. However, more additional studies are needed to detect the change in suicide attempt in DP (6, 14–18).

On the other hand, Chiba et al. (18) detected that pedestrian hits and motorcycle and bicycle accidents decreased during the lockdown but there was no significant change in motor vehicle accidents. We observed a decrease in traffic accidents by men and an increase in traffic accidents by women in DP. The decreased traffic accidents by men may be related to the lockdowns, while the increased traffic accidents by women was parallel to the remarkable decrease in their work accidents. However, an examination of the sub-types of traffic accidents could not be performed due to the lack of available data. Therefore, it is not known which types of traffic accidents are related to the increase and the decrease.

In our study, the percent of assaults decreased with women. Domestic violence cases and assaults were reported to have increased in DP (15, 18, 19). Chiba et al. (18) reported an increase in knife injuries and gunshot injuries in DP. However, we found the decreased incidences of assaults in women. This may be because the emergency department where the study was conducted served rural areas where people live in larger families.

We detected decreased CO poisoning in DP. The incidences of CO poisoning admitted to the emergency department were caused mostly by chimney failures. This decrease appeared to coincide with the spring and summer months of DP.

Study Limitations: This study has some limitations. First, the study was designed as a retrospective study and conducted in a single center. Since the study was conducted retrospectively, forensic case diagnoses could not be collected clearly with sub-diagnoses. Traffic accidents could not be analyzed with sub-diagnoses such as motor vehicle accidents, motorcycle and bicycle accidents, and pedestrian hits. In addition, assaults could not be analyzed with sub-diagnoses such as gunshot injuries, sexual assaults, injuries with sharp objects, and injuries with blunt objects. Finally, the time intervals considered to be before and during the pandemic could not be kept equal. For this reason, the data were compared proportionally, not numerically.

CONCLUSION

We observed a significant change in the forensic cases admitted to the emergency department between BP and DP. Suicide attempts by both women and men increased. Work accidents by both women and men decreased. The COVID-19 pandemic negatively affects people due to not only the disease but also the other factors such as judicial, unemployment, economic, social, and psychological factors. Given few or no studies about the change in the forensic cases before and during the emergence of the pandemic, our study contributes to the related literature. There is an urgent need for larger and multi-center studies to examine the effects of the COVID-19 pandemic on the forensic cases and their multifaceted effects on individuals, through sub-diagnoses.

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RESEARCH ARTICLE

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Evaluation of Plasma Ghrelin, Omentin–1 Levels and Insulin Resistance in Patients With Obstructive Sleep Apnea Syndrome

ABSTRACT

Objective: Available studies support the occurrence of a bidirectional association between obstructive sleep apnea syndrome (OSAS) and cardiovascular disorders. In this study, we aimed to evaluate the plasma ghrelin, omentin–1 levels, insulin resistance (IR) in patients with OSAS and its cardiovascular consequences.

Methods: This study was performed on 150 individuals who applied to the sleep laboratory with complaints such as snoring and sleep-breathing pause. Polysomnographic (PSG) evaluation was applied to every patient. Seventy five individuals with Apnea-Hypopnea Index (AHI) ≥5 were diagnosed as OSAS and seventy five individuals with AHI<5 were included as the without OSAS.

Results: The median omentin-1 level was 59.0 ng/mL in the OSAS group and 105.0 ng/mL in the without OSAS (p<0.001). The median ghrelin level in the OSAS group was 229.0 pg/ml and 180.0 pg/ml in the without OSAS group (p<0.001). The prevalence of OSAS was 2.667 times higher in males than females (OR=2.667). HOMA-IR scores were not different between OSAS and without OSAS groups (p=0.218). Patients with OSAS had higher BMI, neck circumference, and median ESS values compared to those of without OSAS group (p<0.001). In obese, the risk of OSAS was found 3.058 times higher (OR= 3.058) (p<0.001).

Conclusions: Median omentin-1 level was lower in the OSAS group than the without OSAS group, whereas median ghrelin level was higher in the OSAS and obese individuals. Due to the high prevalence of OSAS in hypertensive and obese individuals, effective screening, diagnosis, and treatment of OSAS are required to reduce cardiovascular risk.

Keywords: Obstructive Sleep Apnea Syndrome, Ghrelin, Omentin-1

Obstrüktif Uyku Apne Sendromlu Hastalarda Plazma Ghrelin, Omentin-1 Düzeyleri ve İnsülin Dirençlerinin Değerlendirilmesi

ÖZET

Amaç: Mevcut çalışmalar, obstrüktif uyku apne sendromu (OUAS) ile kardiyovasküler bozukluklar arasında çift yönlü bir ilişki olmasını desteklemektedir. Bu çalışmada OUAS'lı hastalarda plazma ghrelin, omentin-1 düzeyleri, insülin direnci (IR) ve kardiyovasküler sonuçları değerlendirildi.

Gereç ve Yöntem: Bu çalışma uyku laboratuvarına horlama ve uykuda solunum duraklaması gibi şikayetlerle başvuran 150 kişi üzerinde gerçekleştirildi. Her hastaya polisomnografik (PSG) değerlendirme uygulandı. Apne-Hipopne İndeksi (AHI) ≥5 olan yetmiş beş hastaya OUAS tanısı konuldu ve AHI <5 olan yetmiş beş hasta OUAS olmayanlar olarak alındı.

Bulgular: Ortanca omentin-1 seviyesi OUAS grubunda 59,0 ng/mL ve OUAS olmayanlarda 105,0 ng/mL idi (p<0,001). OUAS'larda ortanca ghrelin seviyesi 229,0 pg/ml, OUAS olmayanlarda 180,0 pg/ml idi (p<0,001). OUAS prevalansı erkeklerde kadınlardan 2,667 kat daha fazla idi (OR=2,667). OUAS ile HOMA-IR skorları arasında bir fark bulunmadı (p=0,218). OUAS'lı hastalar OUAS olmayanlara göre daha yüksek BMI, boyun çevresi ve ortanca ESS değerlerine sahipti (p<0,001). Obezlerde OUAS riski 3,058 kat daha fazla bulundu (OR=3,058) (p<0,001).

Sonuç: OUAS olanlarda omentin-1 ortanca değerleri OUAS olmayanlardan daha düşük iken, OUAS ve obez bireylerde ortanca ghrelin düzeyi daha yüksekti. Hipertansif ve obez bireylerde OUAS prevalansının yüksek olmasından dolayı, kardiyovasküler riski azaltmak için bunlarda OUAS'ın etkili taraması, tanı ve tedavisi gereklidir.

Anahtar Kelimeler: Obstrüktif Uyku Apne Sendromu, Ghrelin, Omentin-1, Kardiyovasküler Risk

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a disease characterized by recurrent complete or partial obstructions in the upper respiratory tract during sleep (1). Dyslipidemia, hypertension, type 2 diabetes mellitus, cardiovascular and metabolic abnormalities are common in OSAS patients. OSAS and its cardiovascular consequences have been widely explored in observational and prospective studies. Most evidence verifies the positive relationship between OSAS and hypertension, coronary artery disease, atrial fibrillation, stroke and heart failure.

In non-obese OSAS patients/even in mild forms of sleep apnea, it has been shown to be associated with insulin resistance. OSAS was associated with significantly higher odds of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) after adjusting for age, gender and body mass index (BMI). OSAS-related factors that may contribute to metabolic dysregulation include increased sympathetic activity, due to sleep fragmentation, intermittent hypoxia and proinflammatory cytokine production (2-4). Causative mechanisms relating sleep problems to adverse health outcomes include reciprocal changes in circulating levels of leptin and ghrelin (5). These will increase appetite and calorie intake, reduce energy consumption, facilitate the development of obesity and increasing cardiovascular risk (6). Ghrelin is known as an endocrine pathway in the control of feeding behavior and energy balance. Ghrelin is a 28 amino acid hormone is secreted by many tissues, but its main source is the gastric mucosa. Active form of ghrelin is acylated ghrelin with some metabolic actions like stimulation the appetite, increase the secretion of growth hormone, decrease insulin secretion from the pancreas, reducing in energy consumption by the body and effects on growth and peripheral metabolism especially of fats and carbohydrates (7).

Visceral adipose tissue acts as an endocrine organ and secretes various adipocytokines one of them is omentin (8). Omentin increases insulin-mediated glucose uptake by adipocytes; omentin-1 plays an important role the regulating insulin and has beneficial effects on IR (9). Decreased levels of omentin-1 are also associated with insulin resistance, type 2 diabetes mellitus, coronary artery disease, arterial stiffness, carotid plaque or in other words correlated inversely with the metabolic syndrome (10,11).

In this study, we aimed to evaluate the plasma ghrelin, omentin–1 levels, insulin resistance (IR) in patients with OSAS and its cardiovascular consequences.

MATERIAL AND METHODS

Study Design, Setting, and Population: This cross-sectional analytical typed study was conducted on adult subjects with and without OSAS who were admitted to the Sleep Disorder Clinic, Department of Pulmonary Diseases, Meram Medical Faculty, Necmettin Erbakan University between April 2014 and December 2014. This study was conducted in 150 adults over the age of 18 who applied to the sleep laboratory with complaints such as snoring and sleep-breathing pause that their partners witnessed, excessive daytime sleepiness, fatigue and headache. The patients were admitted randomly according to the order of application to the sleep laboratory on the specified dates. Polysomnographic (PSG) evaluation was applied to every patient who applies to the sleep laboratory. Seventy five individuals with Apnea-Hypopnea Index (AHI) ≥ 5 were diagnosed as the OSAS group and 75 individuals with AHI<5 were included as the without OSAS group.

Ethical Approval: Ethical approval for the study was obtained from the Ethics Committee of Meram Faculty of Medicine, Konya Necmettin Erbakan University (approval number: 2014/44). The participants were informed about the study and their written and verbal consent was obtained according to the principles of Helsinki Declaration.

Sampling Selection: The prevalence of OSAS has been reported approximately as 3% (10). As the number of subjects in the target population for our investigation was unknown, the number of subjects who should be included in the investigation was calculated using the formula $n=t2.p.q/d^2$.

According to the AHI values, the participants were enrolled in the study, of which 75 were OSAS groups and 75 were without OSAS groups.

Data Collection: Sociodemographic characteristics, comorbid diseases, history of medication use, and smoking status were determined using a patient data form prepared previously according to the literature. All participants were administered the Epworth Sleepiness Scale (ESS) and then underwent standard overnight polysomnography (PSG) in the sleep laboratory.

Exclusion Criteria: Exclusion criteria were heart failure, chronic obstructive pulmonary disease, received a systemic steroid or hormone replacement treatment, hepatic or renal failure, anatomical anomalies affecting the respiratory tract, diabetes mellitus, parenchymal lung disease, active malignancy, those who had received medical and/or surgical treatment for a sleep disorder, and those who did not provide written consent to participate in the study.

Anthropometric Measurements: Height, weight, and neck circumference were measured. Neck circumference was measured at the level of the superior border of the cricothyroid membrane. Body mass index (BMI) was calculated by dividing

body weight in kilograms by the square of body height in meters and expressed as kg/m2. The subjects were classified as normal (BMI: 18.5–24.9), overweight (BMI: 25.0–29.9), and obese (BMI ≥ 30.0) (11).

Polysomnographic Evaluation (PSG): Standard overnight PSG was performed to every patient by using a digital PSG system (Somnoscreen plus, Somnomedics GmbH, Randersacker, Germany) in the sleep laboratory. Channels (C1A2, C2A1, O1A2, O2A1, F3A2, F4A1) electroencephalography, two-channels (right and left) electrooculography, and submental electromyography (EMG) probes were placed on the patients for the sleep evaluation. Nasal airflow was recorded by placing an oronasal flowmeter and thermistor into the nose, while thoracic and abdominal motion was recorded after inserting thoracoabdominal effort sensors. Additionally, hemoglobin oxygen saturation and heart beat rate were monitored using pulse oxymetry. Leg movement was recorded using an EMG sensor placed on the anterior tibialis muscle of one leg. Sleep stage and respiration events were scored manually in accordance with AASM scoring criteria (12).

A decrease in airflow by ≥ 90% according to ≥ 10 sec basal values with effort to continue breathing was accepted as obstructive apnea. Decreased airflow by ≥30% for ≥10 sec accompanied by ≥3% oxygen desaturation or arousal from sleep was evaluated as hypopnea. The OSAS diagnosis was established according to the symptom evaluation and results of the sleep tests together.

Apnea-Hypopnea Index: The AHI was calculated by dividing the total number of apnea and hypopnea episodes to sleep duration (per hour). Seventy five individuals with Apnea-Hypopnea Index (AHI) ≥5 were diagnosed as OSAS and seventy five individuals with AHI<5 were included as the without OSAS group. OSAS severity was classified based on AHI values, such that patients with AHI of 5–15, 16–30, and >30 were classified as mild, moderate, and severe OSAS, respectively (12,13).

Epworth Sleepiness Scale (ESS): The ESS is a simple and reliable test used to evaluate daytime sleepiness in adults. This test is composed of eight questions that query the likelihood of falling asleep when the subject is excessively tired. The answers given for each question are scored on a scale of 0–3 and a final score is obtained. Scores >10 are considered daytime sleepiness (14).

Laboratory Evaluation: The blood samples were obtained after PSG. Fasting blood glucose (FBG) and insulin levels were measured immediately after an overnight fast. Plasma samples to test the other parameters were stored at –80°C until testing. Omentin-1 and ghrelin levels were measured using enzyme-linked immunosorbent assays. The homeostatic model assessment of insulin resistance (HOMA-IR) value was calculated using the formula: HOMA-IR = FBG (mg/dL)×plasma insulin (μU/mL)/405. The threshold value for IR was >2.7.

Statistical Analysis: SPSS for Windows 20.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Descriptive statistics for continuous variables were given in terms of average and standard deviation, and descriptive statistics for categorical data were given in terms of frequency and percentage. The Kolmogorov–Smirnov test was used to compare quantitative data to a normal distribution. Since the data do not show to normal distribution; for statistical analysis of quantitative data, Mann Whitney U test was used in paired groups and Kruskall Wallis test was used in triple groups. Chi-square test was used to compare categorical data. Pearson correlation analysis was used for correlations between parameters. Correlation coefficients (r) of 0.00-0.24 were evaluated as weak relationships, 0.25-0.49 as moderate, 0.50-0.74 as strong, and 0.75-1.00 as very strong. Univariate and multivariate linear regression analysis was performed to determine the independent risk factors of dependent variable AHI. The results were evaluated with 95% confidence intervals and a significance level of $p<0.05$. .

RESULTS

This study was conducted with 150 adult subjects comprised of 75 patients with OSAS and 75 subjects without OSAS. Of the OSAS participants; 16.7% (n = 25) were mild, 16.0% (n = 24) were moderate, and 17.3% (n = 26) were severe OSAS based on the AHI. Mean age of the OSAS group was 44.17±11.5 years and that of the without OSAS group was 34.1±12.2 years ($p<0.001$). If a median value of 38 years was the cut-off, the frequency of OSAS in patients aged ≥ 38 years was 70.0% (n=56) whereas that of those aged <38 years was 27.1% (n=19). The incidence of OSAS at ≥38 years was 6.263 times higher than that of patients < 38 years. [OR= 6.263; 95% CI, 3.075–12.758)].

The OSAS group was comprised of 48 males (61.5%) and 27 females (38.5%). The without OSAS group was comprised of 30 (38.5%) males and 45 (62.5%) females (Figure 1).

The prevalence of OSAS was 2.667 times greater in males than females [OR= 2.667; 95% CI, (1.378–5.160)] ($p=0.003$). No significant relation was found between smoking status and any parameters we examined including sleep parameters ($p>0.05$). OSAS was detected in 64 (92.8%) of the 69 subjects who received ESS ≥10 points, and five (7.2%) subjects did not have OSAS ($p<0.001$). Sociodemograhic characteristics of the participants were shown Table 1.

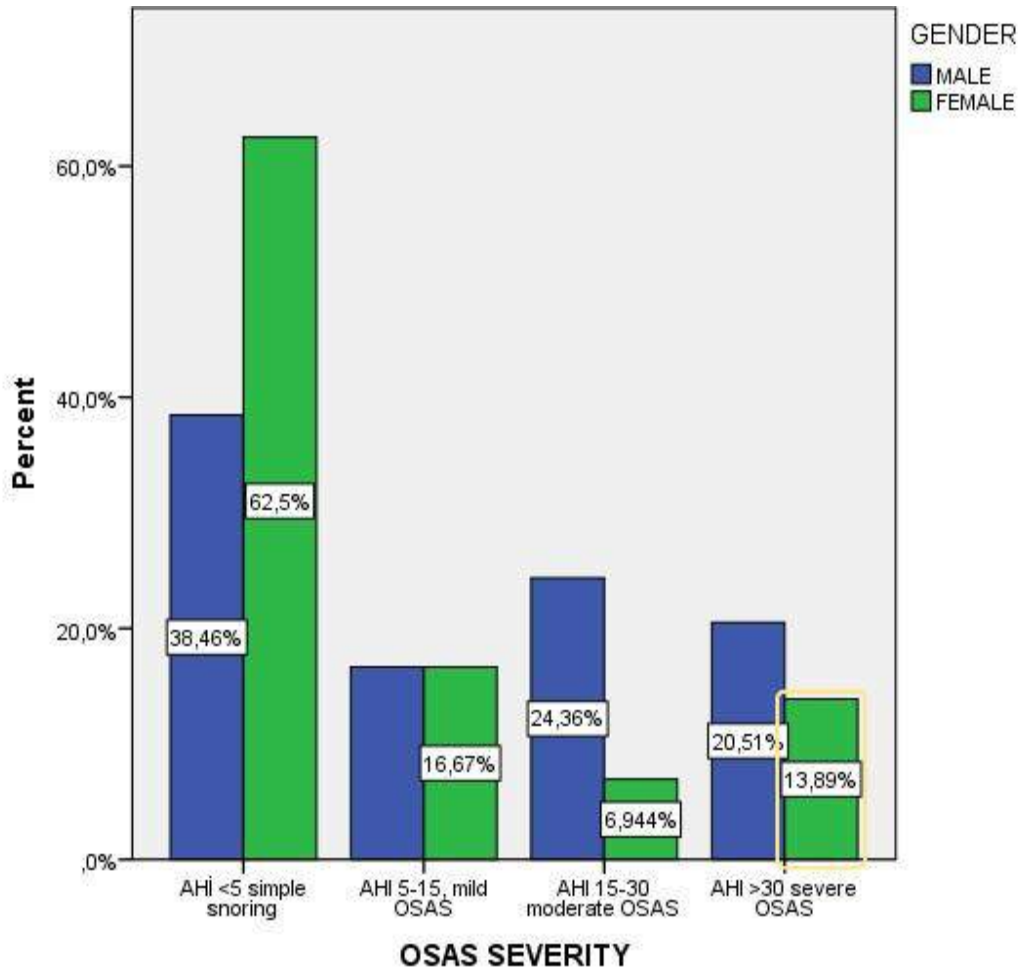

Figure 1. Relationship between OSAS severity and gender

Table 1. Sociodemograhic characteristics of the participants

	With OSAS*		Without OSAS				
	n	%	n	%	Total	χ^2	*p*
Age							
≥38 years	56	70.0	24	30.0	80	27.429	**<0.001**
<38 years	19	27.1	51	72.9	70		
Gender							
Female	27	37.5	45	62.5	72	8.654	**0.003**
Male	48	61.5	30	38.5	78		
Marital status							
Married	62	58.5	44	41.5	106	9.295	**0.002**
Non-married	13	29.5	31	70.5	44		
Smoking status							
Smokers	30	53.6	26	46.4	56		
Non-smokers	45	47.9	49	52.1	54	0.456	0.500
Education status							
≤Primary school educated	18	60.0	12	40.0	30		
Secondary school educated	12	66.7	6	33.3	18	0.276	0.871
University-educated	27	60.0	18	40.0	45		
Epworth SS							
≥10 points	64	92.8	5	7.2	69	93.425	**<0.001**
< 10 points	11	13.6	70	86.4	81		

OSAS* Obstructive sleep apnea syndrome

Of the patients with OSAS, 14 (30.4%), 27 (50.0%), and 34 (68.0%) were normal weight, overweight, and obese, respectively, according to their BMI. Of the subjects in the without OSAS group; 32 (69.6%), 27 (50.0%), and 16 (32.0%) were normal weight, overweight, and obese,

respectively. In obese, the risk of OSAS was found 3.058 times higher than those who were not obese [OR= 3.058; 95% CI, (1.495–12.758)] (p<0.001).

The median omentin-1 level was found to be 59.0 (2-1163) ng/mL in the OSAS group and 105.0 (7-1464) ng/mL in the without OSAS group. There was a significant difference between the groups (p<0.001). The median value of ghrelin was 229.0 (62-5385) pg/ml in the OSAS group and 180.0 (31-3806) pg/ml in the without OSAS group. There was a significant difference between the groups (p<0.001). Patients with OSAS were older, and had higher BMI, neck circumference, and median ESS values but lower blood pressure compared to those of the without OSAS group (p<0.001) (Table 2).

Table 2. Comparison of some parameters in the two groups

	With OSAS	**Without OSAS**		
	Median (min-max)	**Median (min-max)**	**Z**	***p****
Age (year)	47.0 (23-78)	32.0 (19-70)	-5.397	**<0.001**
Systolic BP(mmHg)	130 (90-170)	130 (120-140)	-2.001	**0.045**
Diastolic BP(mmHg)	80 (50-100)	80 (60-90)	-3.947	**<0.001**
BMI (kg/m ²)	29.4(19.3-46.5)	25.9(18.6-45.0)	-4.031	**<0.001**
Neck (cm)	38.0 (33-46)	37.0 (34-45)	-5.568	**<0.001**
Epworth SS	14 (3-24)	3 (0-15)	-9.816	**<0.001**
FBG (mg/dl)	95.0 (74-194)	91.0 (70-136)	-2.782	**0.005**
Insulin (µ / ml)	8.1 (0.9-95)	8.2(1.6-55.3)	-0.718	0.473
Omentin (ng/ml)	59.0 (2-1163)	105.0 (7-1464)	-5.071	**0.001**
Ghrelin (pg/ml)	229.0 (62-5385)	180.0 (31-3806)	-3.724	**0.001**
HOMA-IR	2.1(0.2-32.1)	1.8(0.4-15.9)	-1.231	0.218
NREM	2.3 (0-28.5)	3.9 (0-20.0)	-2.779	**0.005**
REM	3(0-20)	6.0 (0-21.4)	-0.382	0.703
Min. O_2 sat.	83(50-91)	89 (78-94)	-8.433	**0.001**
Average O_2 sat.	90(68-95)	93 (18-98)	-4.347	**0.001**
≤ 90 O_2 sat.	23.9(0-99.8)	2.1(0-9.1)	-8.131	**0.001**

***** Mann–Whitney *U*-test

Gender had no effect on blood pressure, FBG, insulin, omentin-1, ghrelin, HOMA-IR, or BMI values. Median age and neck circumference measurements were significantly higher in males than females (p=0.025 and p< 0.001, respectively).

Median AHI and ESS values were significantly higher in males compared to females (p=0.002, p=0.006, respectively). Non rapid eye movement sleep (NREM) was significantly longer in females than males (p=0.040). AHI, oxygen saturation ≤ 90%, and median ESS values increased significantly as BMI increased and minimum oxygen saturation decreased. Significant differences in NREM and REM sleep duration were observed between the groups.

BMI increased with age and, consequently, neck circumference, diastolic blood pressure, FBG, insulin, and HOMA-IR values increased significantly as BMI increased (Table 3). Correlation between Omentin-1, Ghrelin and some parameters in OSAS patients was shown in Table 4.

Table 3. The effects of body mass index (BMI) on some parameters

	Normal weight BMI:18.5–24.9kg/m²	**Overweight BMI: 25.0–29.9 kg/m²**	**Obese BMI ≥ 30.0 kg/m²**		
	Median (min-max)	**Median (min-max)**	**Median (min-max)**	**χ^2**	***p****
Age (year)	28.5 (19-59)	38 (19-79)	47 (21-78)	23.22	**0.001**
Neck (cm)	36.5 (33-39.3)	37.3 (34-44)	38.5 (36.5-46)	57.39	**0.001**
SBP (mmHg)	130 (120-140)	130 (90-140)	130 (110-170)	20.55	0.151
DBP (mmHg)	80 (60-90)	80 (50-90)	80 (50-100)	3.78	**0.001**
FBG (mg/dl)	90.5 (70-125)	93 (74-194)	99.5 (79-158)	18.70	**0.001**
Insulin (µ/ml)	6.3 (1.6-30)	7.6 (0.9-80.5)	10.8 (3.6-95.4)	10.05	**0.007**
Omentin (ng/ml)	99 (5-1464)	89 (3-1375)	75.5(2-1275)	2.93	0.231
Ghrelin(pg/ml)	195.5 (62-4105)	213.5 (31-5385)	216 (102-4990)	1.005	0.369
HOMA-IR	1.5 (0.4-7.1)	1.7 (0.2-26.0)	2.6 (1-32.1)	12.31	**0.002**

* Kruskal–Wallis test

Table 4. Correlation between Omentin-1, Ghrelin and some parameters in OSAS patients.

		1	2	3	4	5	6	7	8
1.Omentin-1 (ng /ml)	r	1							
	p								
2.Ghrelin (pg/ml)	r	-.089	1						
	p	.450							
3.AHI	r	.142	-.010	1					
	p	.224	.932						
4.HOMA-IR	r	-.053	-.049	.196	1				
	p	.650	.678	.092					
5.BMI (kg/m^2)	r	.089	.012	.238**	.100	1			
	p	.450	.920	.040	.394				
6.ESS	r	-.026	.118	.259	.074	.138	1		
	p	.827	.312	.025	.528	.239			
7.Min. O_2 sat.	r	-.044	.149	-.523**	-.047	-.299	-.327	1	
	p	.709	.201	.000	.691	.009	.004		
8. Aver. O_2 sat.	r	-.065	.173	-.440**	.105	-.166	-.315	.695**	1
	p	.582	.138	.000	.371	.155	.006	.000	
9. ≤ 90 O_2 sat.	r	.184	-.245**	.298	-.151	.251	.214	-.537**	-.732**
	p	.114	.034	.010	.197	.030	.065	.000	.000

** Correlation significant at 0.01

Univariate and multivariate linear regression analysis was performed to determine the independent risk factors of dependent variable AHI in OSAS patients. According to the univariate model results, the effect of BMI on AHI was positive and statistically significant (β=0.238, p=0.040). One unit increase in BMI results in 23.8% increase in AHI. According to the results of multiple linear regression analysis, the effect of only Min. O2 sat. on AHI was found to be negative and statistically significant (β= -0.515, p<0.001). Linear regression analysis of variables for dependent variable AHI in OSAS patients was shown in Table 5.

Table 5. Linear regression analysis of variables for dependent variable AHI in OSAS patients

	One variable model				**Multivariate model (stepwise)**			
	R^2	**β**	**t**	**p**	**R^2**	**β**	**t**	**p**
BMI (kg/m^2)	0.057	0.238	2.092	**0.040**				
ESS	0.067	0.259	2.292	**0.025**				
Min. O_2 sat.	0.274	-0.523	-5.244	**<0.001**	0.303	-0.515	-5.230	**<0.001**
Aver. O_2 sat.	0.194	-0.440	-4.188	**<0.001**				
≤ 90 O_2 sat.	0.089	0.298	2.662	**0.010**				
Omentin-1 (ng /ml)	0.020	0.142	1.226	0.224				
Ghrelin (pg/ml)	-0.014	-0.010	-0.085	0.932				
HOMA-IR	0.038	0.196	1.708	0.092				

DISCUSSION

There is an increasing evidence of association between OSAS and cardiovascular diseases. A strong relationship has been reported between OSAS and arterial hypertension, especially in patients with resistant hypertension. OSAS is a disease which progressively comes into prominence as its clinical outcomes and relationship with other systemic disorders become clarified and tends to increase in prevalence by age. Recent research has suggested that the effects of sleep apnea on insulin dynamics can be completely explained by obesity. Omentin-1 plays an important role regulating insulin and has beneficial effects on IR, and ghrelin, which affects appetite. The literature suggests that omentin-1 insufficiency and high ghrelin levels may be associated with glucose intolerance, MetS, obesity and cardiovascular abnormalities (15,16). In this study, the results were similar to the literature. Omentin-1 levels were significantly lower in patients with OSAS than those without OSAS. Decreased levels of omentin-1 are also associated with insulin resistance type 2 diabetes mellitus, coronary artery disease, arterial stiffness and carotid plaque or in other words correlated inversely with the metabolic syndrome (17,18). However, in our study, omentin-1 levels were not associated with BMI, AHI, ESS, HOMA-IR levels.

It has been suggested that ghrelin may be of pathophysiological importance in the development of IR (19). Serum ghrelin levels are lower in patients with type 2 diabetes mellitus or IR and obese person (19,20). However, relationship

between serum ghrelin level and OSAS is controversial. Harsh et al.(21) and Ursavas et al.(22) reported that ghrelin levels were significantly increased in the OSAS group. Similar to that study, ghrelin levels were also higher in our OSAS group than without OSAS group. In Harsh et al study, BMI and total body fat were shown as predictors of ghrelin levels in both OSAS patients and controls, the minimal O2 saturation was a significant predictor in OSAS patients but not in without OSAS group (21). Ursavas et al. reported that there was a significant relationship between serum ghrelin levels and AHI, ESS (22). In our study, there was no correlation between ghrelin levels and ESS, IR and BMI. Apneic episodes are generally terminated by an arousal (brief awakening) which results in fragmented sleep. These arousals are believed to be an important contributor to the symptoms of excessive daytime sleepiness (EDS) and the neurocognitive impairment seen in sleep apnea (22,23). Some, but not all, studies have indicated that sleep loss is associated with increased sympathetic nervous system outflow. Increased cardiac sympathovagal balance could also reflect decreased vagal activity, which could explain increased ghrelin levels. Several studies have shown that the vagal activity has a negative influence on ghrelin (24,25).

In this study, the insulin resistance in patients with OSAS was not significantly different from the non-OSAS subjects. Although FBG levels were higher in OSAS than in controls, no difference was found between insulin and HOMA-IR scores. A positive correlation was found between HOMA-IR scores and BMI, AHI, ESS. Similar to our study, Sharma et al. reported no differences in IR between an obese OSAS group and obese control group. They reported that metabolic abnormalities were due to obesity rather than OSAS (23). In addition, two other controlled studies suggested that the relation between sleep apnea and plasma insulin levels or insulin resistance reflected the known effects of obesity (24,25). Contrarily, Makino et al. analyzed 213 patients with mild, moderate, and severe OSAS based on the AHI to investigate the relationship between AHI level and IR. They reported that sleep-disordered breathing was associated with insulin resistance independent of obesity (26). Bulcun et al. (27) reported that there was no difference between OSAS and control groups on IR, but IR was associated with AHI, BMI, arousal index and ESS score. In a study in which the IR scores were higher in OSAS, it was related to the values of IR AHI and minimum oxygen saturation (4). These contradictory results reported for IR in OSAS are probably due to the heterologous disease of sleep apnea in terms of properties reported to be associated with insulin resistance.

In conclusion, elderly age, increased BMI and increased AHI are risk factors for high ESS scores; this is associated with a decrease in omentin-1 and an increase in ghrelin and IR. It has been suggested that ghrelin and omentin-1 may have a pathophysiological prescription in the development of IR. Presented study showed that omentin-1 levels were lower and ghrelin levels were higher in the patients with OSAS. There was no relationship between omentin-1 and ghreline levels and IR. The essential approach in preventing obesity is use of anti-obesity medications. In the recent years, use of anti-ghrelin vaccine is a topical issue. Because, obesity is defined by low growth hormone and ghrelin levels. This vaccine prevents weight gain by inhibiting transmission of “ghrelin” hormone which sends hunger signal to brain via circulation (28). All studies to be conducted regarding detailed examination of the relationship between obesity, insulin resistance and biochemical molecules and evaluation of the results; It will contribute positively to the fight against other obesity-related comorbid conditions such as OSAS. However, more studies are needed to better assess the impact of OSAS, and possible benefit of treatment with continuous positive airway pressure (CPAP) on dyslipidemia, type 2 diabetes, insulin resistance and cardiovascular mortality.

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RESEARCH ARTICLE

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Evaluation of the Use of the Mistik Score and Modified Centor Score in Sore Throat

ABSTRACT

Objective: There are various clinical scoring systems in acute tonsillopharyngitis. The Modified Centor Score was developed to predict streptococcal pharyngitis and the Mistik score was developed to diagnose viral agents. This study aimed to determine cut-off values of the Modified Centor Score and Mistik Score according to rapid antigen detection test (RADT) results of patients admitted with sore throat in the primary care and evaluate the use of these scoring systems by family physicians.

Methods: A total of 125 patients between the ages of 3 and 80 who were admitted with sore throat to the family health centers were included in the study. Physical examinations of the patients were performed and their Mistik and Modified Centor Scores were detected. Patients underwent RADT. Treatments of the patients were recorded. In the second stage of the study, patients underwent clinical scoring and the physicians completed a questionnaire to evaluate the use of the Mistik Score, Modified Centor Score and RADT.

Results: The optimal cut-off value for the Modified Centor Score was 3, sensitivity was 87.5%, specificity was 86.2%, positive predictive value was 48.3% and negative predictive value was 97.9%. The optimal cut-off value for Mistik score was 3, sensitivity was 61%, specificity was 87.5%, positive predictive value was 97% and negative predictive value was 25%. Of the physicians, 75% used clinical scoring during routine examination and the most common scoring system used was the Modified Centor Score (88.9%). Of the physicians, 75% used clinical scoring before the decision of RADT.

Conclusions: The use of clinical scoring systems such as the Mistik Score and Modified Centor Score will primarily allow RADT be used in the appropriate patient for sore throat that is very common in daily family practice. In addition, it will help family physicians decide on the best treatment options for tonsillopharyngitis. Therefore, we recommend the use of clinical scoring systems especially the Mistik Score, which is a viral scoring system.

Keywords: Sore Throat, Diagnose, Viral, Score.

Boğaz Ağrısında Mıstık Skoru ve Modifiye Centor Skoru'nun Kullanımının Değerlendirilmesi

ÖZET

Amaç: Akut tonsillofarenjitte, kullanılan çeşitli klinik skorlamalar mevcuttur. Streptokoksik farenjitini öngörmek için Modifiye Centor Skoru ve viral ajanlara tanı koymak için Mıstık Skoru geliştirilmiştir. Bu çalışma da birinci basamakta boğaz ağrısı şikayeti ile gelen hastaların hızlı antijen testi (HAT) sonuçlarına göre Modifiye Centor Skoru ve Mıstık Skoru'nun cutoff noktalarının belirlenmesi ve aile hekimleri tarafından kullanımının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Aile sağlığı merkezlerine boğaz ağrısı şikayeti ile başvuran 3-80 yaş arasındaki 125 hasta dahil edildi. Hastaların fizik muayeneleri yapılıp Mıstık Skoru ve Modifiye Centor Skor'ları tespit edildi. Hastalara HAT uygulandı, verilen tedavi kaydedildi. Çalışmanın 2. aşamasında hastalara klinik skorlama uygulanıp Mıstık Skoru, Modifiye Centor Skoru ve HAT kullanımının değerlendirilmesi için doktorlara anket uygulandı.

Bulgular: Modifiye Centor Skoru için en uygun kesim noktası 3 olup duyarlılığı %87.5 ve seçiciliği %86.2, pozitif kestirim değeri %48.3, negatif kestirim değeri %97.9 bulunmuştur. Mıstık Skoru için en uygun cutoff değeri 3 olup duyarlılığı %61 ve seçiciliği %87.5, pozitif kestirim değeri %97, negatif kestirim değeri %25 bulunmuştur. Aile hekimlerinin %75'i klinik skorlamayı rutin muayenede kullanıyordu ve en çok kullanılan skorlama yöntemi de ModifiyeCentor Skoru idi (%88.9). Aile hekimlerinin %75'i HAT' a karar vermeden önce klinik skorlama kullanmaktadır.

Sonuç: Günlük aile hekimliği pratiğinde çok sık görülen boğaz ağrısında Mıstık Skoru, Modifiye Centor Skoru gibi klinik skor kullanımı öncelikle HAT testinin uygun hastada yapılmasını sağlayacaktır. İlaveten aile hekimlerinin tonsillofarenjit konusunda daha uygun tedavi kararları vermesini sağlayacaktır. Bu yüzden klinik skorlamaların kullanımını ve özellikle viral bir skorlama olan Mıstık Skoru'nun kullanımını önermekteyiz.

Anahtar Kelimeler: Boğaz Ağrısı, Teşhis, Viral, Skor.

INTRODUCTION

Sore throat is one of the most common patient complaints in primary care. Viral agents are the most common causes of sore throat. Group A beta-hemolytic streptococcus (GABHS) is the most common pathogen causing acute tonsillopharyngitis and accounts for 15-30% of all cases in children and 5-10% in adults (1). It is diagnosed by microbiological tests performed with clinical symptoms and findings. Rapid antigen detection test (RADT) and throat culture are diagnostic tests of the disease (2). RADT can give results within about 15-20 minutes and its usage is limited due to high costs (3). The criteria revealing in which clinical conditions these tests will be used in acute tonsillopharyngitis have also been determined. Whether the factors causing this infection are viral or bacterial is one of the most important points (4). A clinical scoring system has been developed to predict streptococcal pharyngitis. Centor was first to investigate how symptoms and findings were determinant in GABHS infection (5). The physicians can use RADT in patients with high possibility of streptococcus infection (e.g. Modified Centor Score of 3-4). Throat culture is not necessary after clinical scoring and negative test result in RADT negative patients (6). The Mistik Score has been developed to diagnose viral agents that are the most common reason of acute tonsillopharyngitis. A negative correlation between the Modified Centor Score and Mistik Score has been found. Physicians can use one of these scoring systems for the etiology of sore throat. However, using both of them will provide a better assessment in diagnosing bacterial and viral sore throat (7).

This study aimed to assess the use of the Modified Centor Score and Mistik score by family physicians in patients admitted with sore throat in the primary care and create a prediction for appropriate antibiotic prescribing in patients by increasing the use of these scoring systems.

MATERIAL AND METHODS

Study Design and Setting: Our study was performed as a cross-sectional study. It consisted of 2 stages. In the 1st stage, patients admitted with sore throat to the family medicine outpatient clinic were clinically assessed with the Modified Centor Score and Mistik Score. Scoring results of the patients were recorded into the patient assessment forms. Then, each patient underwent QuickVue-Strep A test, a rapid antigen detection test (RADT). Family physicians were immediately informed about the test results. Their treatment plans were recorded. In the 2nd stage of our study, family physicians completed a questionnaire. The questionnaire included questions about the use of clinical scoring systems and RADT. Statistical analysis of the data was performed with IBM SPSS 22.0 software program.

Our study was designed in compliance with the principles of Helsinki Declaration and regulation of patients' rights and approved by the clinical research ethics committee of Erciyes University, the Faculty of Medicine (15.05.2018/96681246).

Study Participants: A total of 125 patients between the ages of 3 and 80 were included in the 1st stage of the study. Those were patients who were admitted with sore throat to the Bunyamin Somyurek Family Health Center. The study was performed between the 1st of October and the 1st of November in 2018. Power analysis of the study was performed with G*power3.1 analysis program to determine the sample size. The minimum number of patients to participate in the study was determined as 125 (α- value:0.05, ß-value:0.80). The patients and their relatives were informed about the study and their informed consents were obtained.

Patients with the following characteristics were excluded from the study:

1.Patients below the age of 3 and above the age of 80,

2.Patients with noninfectious sore throat,

3.Patients who had used antibiotics in the last 3 days.

A total of 12 family physicians were included in the 2nd stage of the study. Those who used RADT and clinical scoring for patients in the 1st stage of the study were family physicians. Consents of all the physicians in the family health center where these 12 family physicians worked were obtained and the 12 family physicians completed a questionnaire between the 1st of November and 15th of November 2018.

Data Screening Process: It was reported that patients with the Modified Centor Score of 1 and below did not need antibiotics or further examination while throat culture was recommended for patients with the score of 2 and 3 or patients with the score of 4 and above needed empirical antibiotic therapy (Table 1).

Table 1. Modified Centor Score (5,9)

Criterion	Score
Fever (> 38ºC)	1
No cough	1
Painful and enlarged anterior cervical lymph node	1
Tonsillar hypertrophy and exudate	1
Age	
3-14 years	1
15-44 years	0
45 years and older	-1

The Mistik Score: The probabilities of positive viral analysis in the scores between 0 and 5

were 8.3%, 14.7-20.4%, 25.2-36.3%, 42.2-55.3%, 61.9-70.7%, and 82.1% respectively.

Table 2. Mistik Score (7)

Variables	Score
No headache	1
Nasal obstruction	1
Sneeze	1
Fever on physical examination (≥37.5°C)	1
No exudate and/or hypertrophy in tonsils	1

Antigen Detection Test: The swabs were analyzed within 10-20 minutes to detect antigen with immunoassay method. In brief, the swab is centrifuged and kept in the tube including extraction solution for five minutes. The whole solution including the extracted material was transferred to the sample well. After a color developed in the control well (approximately five minutes), the test was assessed. In case a pink color developed in the control well and a positive sign appeared on the sample well the antigen detection test was regarded as positive.

Questionnaire: The questionnaire included questions about the attitudes and behaviors of family physicians on the use of clinical scoring systems and RADT. Demographic data and employment durations of the family physicians were also questioned. The number of RADTs they used in daily routine, whether they used clinical scoring in routine practice for patients admitted with sore throat or not, whether they decided to use RADT after clinical scoring or not, which clinical scoring system they mostly used and which clinical scoring system they found more useful during the 1st stage of the study were questioned. The questionnaire was completed by family physicians after the clinical scoring and RADT were used in the 1st stage of the study.

Statistical Analysis: Descriptive statistics for continuous variables were expressed as median, minimum and maximum values while categorical variables were expressed as number and percentile. Whether numerical data of the variables were normally distributed or not was determined with one-sample Kolmogorov Smirnov test. Chi-square test was used to determine the relationship between the groups and categorical variables. The linear correlation between the variables was assessed with Pearson's correlation analysis. Receiver operating characteristic (ROC) curves were used to identify the differentiating effect of the Mistik Score on Modified Centor Score. Area under the ROC curves were calculated with 95% confidence intervals. Youden index was calculated for optimal cut-off value. Sensitivity, specificity, and positive and negative predictive values were calculated with 95% confidence intervals. Internal consistency was obtained by the correlation of each item in the Mistik Score with total the total score. The correlation of the Mistik Score with the Modified Centor Score was assessed by Spearman's correlation for convergent validity. All analyses were performed using TURCOSA (Turcosa Analytics Ltd. Co., www.turcosa.com.tr) statistical software, MVN (19) and easyROC (20) software. p values less than 5% were considered as statistically significant**.**

RESULTS

Results of the 1st Stage of the Study: This study was performed with patients admitted to Bunyamin Somyurek Family Health Center. Median age of the patients was 38 (min-max: 3-80). A total of 125 patients were included in the study and while 87 (70%) of them were female 38 (30%) were male. Clinical and demographic characteristics of the patients were given in Table 3.

Table 3. Demographic and clinical characteristics of the patients

	n	%
Gender		
Female	87	70
Male	38	30
Age Group		
Child	57	45.6
Adult	68	54.4
RADT		
Positive	16	12.8
Negative	109	87.2
Antibiotics		
Prescribed	26	20.8
Not Prescribed	99	79.2
Modified Centor scores		
-1	4	3.2
0	33	26.4
1	38	30.4
2	21	16.8
3	16	12.8
4	9	7.2
5	4	3.2
Mistik Scores		
1	19	15.2
2	37	29.6
3	43	34.4
4	23	18.2
5	3	2.4

The Modified Centor Score of 87.5% of RADT positive patients was 3 and above. The Modified Centor Score of 86% of RADT negative patients was 2 and below. Considering RADT positivity, the optimal cut-off value for the Modified Centor Score was found as 3, sensitivity as 87.5%, specificity as 86.2%, positive predictive value as 48.3% and negative predictive value as

97.9%. The ROC curve showed a significant sensitivity and specificity (Area under ROC curve= 0.918) (CI: 0.856-0.960) (p<0.001) (Figure 1) (Table 4).

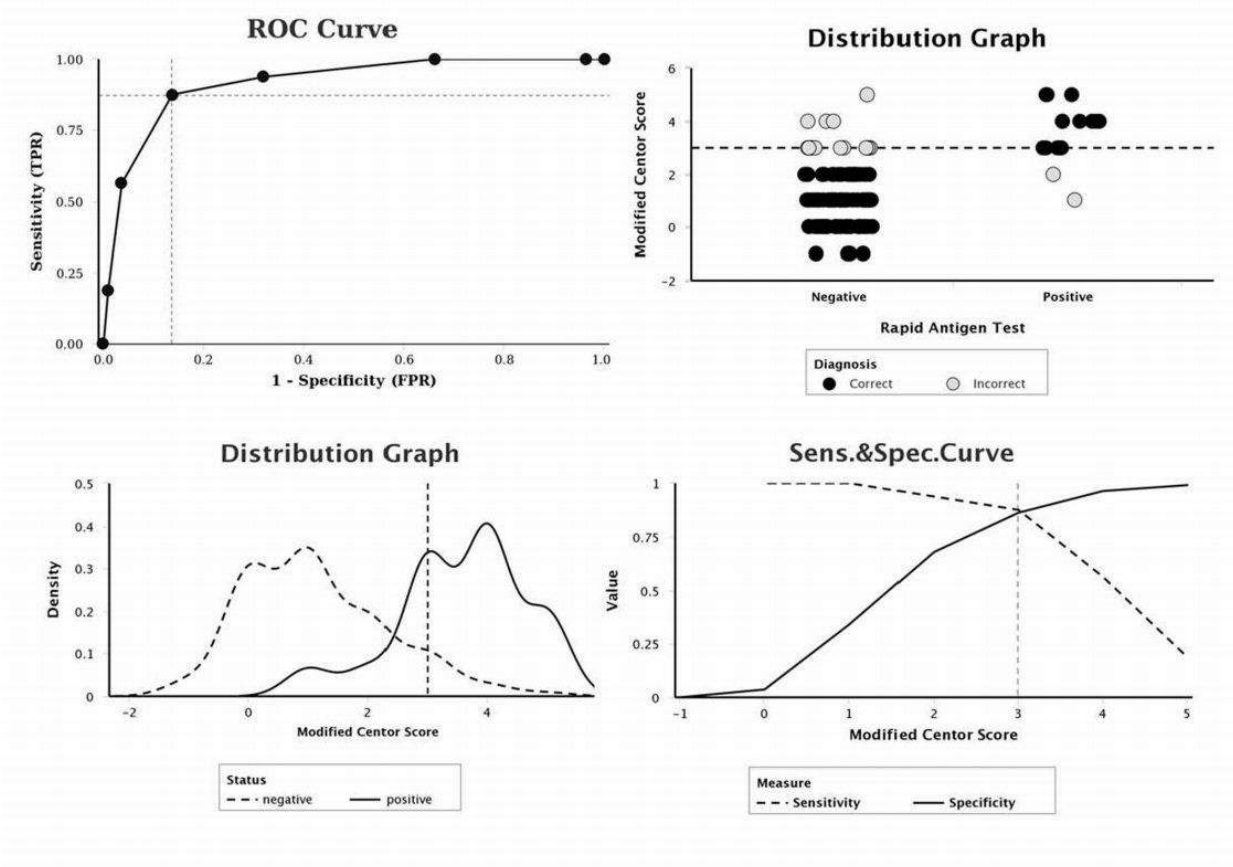

Figure 1. Specificity, sensitivity and ROC curve for the Modified Centor Score

Table 4. ROC curve results and statistical diagnostic measurements for (RADT positive) determining elderly with or without a normal Modified Centor Score.

Statistics	Estimate	Confidence Interval (95%)
ROC curve statistics		
Area under curve	0.918	0.856-0.960
p value	<0.001	
Diagnostic measurements		
Sensitivity	0.875	0.640-0.965
Specificity	0.862	0.785-0.914
Positive predictive value	0.482	0.314-0.656
Negative predictive value	0.979	0.927-0.994

The Mistik Score of 25 (23%) of RADT negative patients was 3 and above. The Mistik Score of 14 (87.5%) of RADT positive patients was 2 and below. There was a statistically significant difference between the groups (p<0.05). Considering RADT negativity, the optimal cut-off value for the Mistik score was found as 3, sensitivity as 87.5%, specificity as 61%, positive predictive value as 25%, and negative predictive value as 97%.

The ROC curve for the Mistik Score showed a significant sensitivity and specificity (Area under ROC curve= 0.760) (CI: 0.676-0.832) (p=0.001) (Table 5) (Figure 2).

Table 5. ROC curve results and statistical diagnostic measures for (RADT negative) determining elderly with or without a normal Mistik Score

Statistics	Estimate	Confidence Interval 95%)
ROC curve statistics		
Area under curve	0.760	0.676-0.833
p value	<0.001	
Diagnostic measurements		
Sensitivity	0.615	0.521-0.700
Specificity	0.875	0.640-0.965
Positive predictive value	0.971	0.900-0.992
Negative predictive value	0.250	0.155-0.377

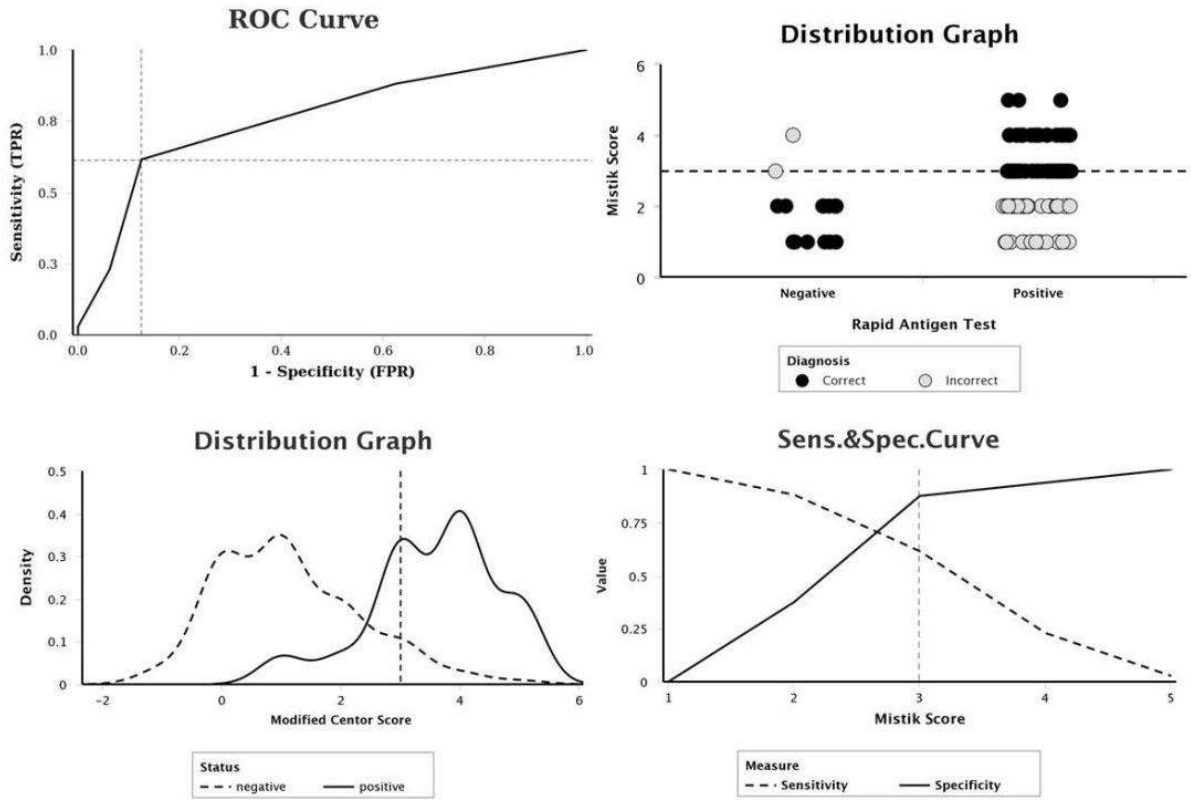

Figure 2. Specificity, sensitivity and ROC curve for the Mistik Score

There was a weak, negative and statistically significant correlation between the variables of the Modified Centor Score and Mistik Score (r value: -0.272) (p= 0.002).

A significant difference was found between the groups in terms of antibiotherapy according to RADT results. While 16 (100%) patients in the RADT positive group received a therapy 10 (9.2%) patients in the RADT negative group received a therapy (p<0.001). The number of patients with cervical node, hypertrophic and exudate tonsils, and fever (>38) was higher in the RADT positive group compared to the RADT negative group (p<0.001, p<0.001, and p<0.001 respectively). The number of patients with sneeze was higher in the RADT negative group (p<0.001). The Modified Centor Score was higher in the RADT positive group (p<0.001) and the Mistik Score was higher in the RADT negative group (p=0.001). Patients' demographic data, symptoms and data including clinical scoring points according to RADT results were given in Table 6.

Table 6. Comparison of RADT positive and RADT negative groups

Rapid Antigen Diagnostic Test	Positive (16)	Negative (109)	p value
Age, median (25-75 percentile)	12 (5-30.75)	23 (12.5-44.5)	0.045
Gender (Female)	11 (68.8)	76 (69.7)	0.930
Child	11 (68.80%)	47 (43.1)	0.490
No coughing	12 (75)	48 (44)	0.190
Cervical node	14 (87.5)	32 (29.4)	<0.001
38^0C fever	10 (62.5)	9 (8.3)	<0.001
Hypertrophy/Exudate in Tonsils	14 (87.5)	23 (21.1)	<0.001
No headache	6 (37.5)	46 (42.2)	0.471
Nasal obstruction	9 (56.3)	88 (80.7)	0.360
Sneeze	3 (18.8)	68 (62.4)	0.001
Fever (>37.5 º C)	10 (62.5)	32 (29.4)	0.011
The Modified Centor Score	4 (1-5)	1 (-1-5)	<0.001
The Mistik Score	1.81±0.8	2.75±1	0.001
Antibiotherapy	16 (%100)	10 (%9.2)	<0.001

Results of the 2nd Stage of the Study: Mean age of the family physicians who participated in the study was 51.5±7.9. Mean employment duration of the family physicians in this occupation was 25.5+7.4. Mean number of RADTs used by the family physicians while working was 10.4±4.1. Mean rate of the use of RADT in daily routine practice was 1.25±0.45. Responses of the family physicians about their attitudes and behaviors on clinical scoring and RADT were given in Table 7. The rate of those who found the Mistik score useful was higher.

Table 7. Attitudes and behaviors of the family physicians on clinical scoring and RADT at the end of the study

Do you use clinical scoring? (Yes)	9 (75%)
If yes, which one do you use?	
The Modified Centor Score	8 (88.9%)
Other scoring system	1 (11.1%)
Do you use RADT? (Yes)	9 (75%)
In which age range you prefer RADT?	
Age range of 3-17	6 (50%)
Age range of 18-65	3 (25%)
Ages above 65	1 (8.3%)
All ages	2 (16.7%)
Do you use clinical scoring before deciding on RADT? (Yes)	9 (75%)
Do you think clinical scoring systems are helpful in diagnosing? (Yes)	11 (91.7%)
Is the Modified Centor Score useful?	10 (83.3%)
Is the Mistik Score useful?	11 (91.6%)

DISCUSSION

Considering RADT positivity, the optimal cut-off value for the Modified Centor Score was found as 3 and considering RADT negativity, the optimal cut-off value for the Mistik Score was found as 3 in this study. It was found that 75% of the family physicians used clinical scoring in routine examination before deciding on RADT.

In the treatment of acute tonsillopharyngitis, which is one of the most common causes of sore throat, detecting whether it is bacterial or viral in an early period is important. The therapy should immediately be decided and initiated to prevent especially GABHS from leading to complications such as acute rheumatic fever. The Infectious Diseases Society of America recommends throat culture or RADT in detection of GABHS (8).

In detection of GABHS, rapid tests based on detecting the antigen are resulted in a short time. Positive result provides early diagnosis and treatment (9). Sensitivity rates of antigen detection tests are known to range from 75% to 90% while their specificity rates range from 86% to 100%. Specificity rates of these tests are higher than their sensitivity rates compared to throat culture. This means they have low rate of false positivity (10,11,12).

RADT used for detecting GABHS was positive in 14% in the study by Cannarpur et al. and in 18.2% in the study on children by Pontin et al. (13,14).

In our study, 12.8% of the patients were RADT positive and 19.3% of pediatric patients were RADT positive. These rates are consistent with those in literature.

As well as RADT, clinical scoring systems are also used in approximate diagnosing to predict streptococcus infection in patients with sore throat. It is commonly asserted that clinical scoring systems can be used alone or in combination with antigen detection tests (2). The use of clinical scoring for acute sore throat forms the treatment and decreases the use of antibiotics. In studies, the use of clinical scoring for adult patients with tonsillopharyngitis by family physicians is strongly recommended (15).

Palla et al. used the Modified Centor Score and collected throat culture in a group including 137 patients of a low-socioeconomic status community in Pakistan among patients between the ages of 14-65. While 88% of the patients had scores of 0, 1, 2 and 3 17 (12%) patients had the score of 4. Sensitivity rate of the Modified Centor Score was found as 80%, specificity as 68.7%, positive predictive value as 12.7% and negative predictive value as 100%. They also found a direct correlation between the results of the Modified Centor Score and throat culture (16). In our study, patients had the Modified Centor Scores between -1 and 5. Especially the following results were similar to the findings in literature: 96.3% of RADT negative patients had the score of 3 and below, the sensitivity of the Modified Centor Score was 87.5%, specificity was 86.2%, positive predictive value was 48.3%, and negative predictive value was 97.9%. Like in other studies, sensitivity of the Modified Centor Score was higher than its specificity in our study. RADT negative patients in our study had the Modified Centor Score of -1 and 0, which reveals that the Modified Centor Score is compliant with RADT. Negative RADT result in low Modified Centor Score increases the possibility of viral infection.

The optimal cut-off value for the Modified Centor Score in RADT positive patients is 3. McIsaac et al. recommend RADT for patients with the Modified Centor Score of 2-3. In our study, the optimal cut-off value for the Modified Centor Score in RADT positive patients was found as 3, which is consistent with the findings in literature. We think preferring RADT in case the Modified Centor Score is 2 and below is unnecessary while RADT must be preferred in case the Modified Centor Score is 3 and above.

Viral agents are the most common causes of sore throat. Mistik et al. developed a new scoring system to find out the rate of bacterial and viral causes, reveal seasonal changes and diagnose viral sore throat. They collected throat culture from each patient for GABHS and nasopharyngeal swab to detect 16 respiratory tract viruses and analyzed a total of 624 throat cultures and polymerase chain

reaction throughout 52 weeks. They detected viral infection in 277 (44.3%) patients and GABHS infection in 116 (18.5%) patient. These viruses had all the Modified Centor Scores between -1 and 5 and similarly all the Mistik Scores between 0 and 5. In the study by Mistik et al., sensitivity of the Mistik Score was 60.2%, specificity was 72.5%, positive predictive value was 62.5% and negative predictive value was 70.5%. Diagnostic accuracy of the Mistik Score was 68% while that of the Modified Centor Score was 75%. A negative correlation was found between the Modified Centor Score and Mistik Score (7). While the Mistik Score of all the patients in our study was between 1 and 5 that of RADT positive patients was observed to be between 1 and 4. RADT positivity never had the Mistik Score of 5. This is because the possibility of viral infection is high when the Mistik Score is 5. In the Mistik Score of 5, the possibility of viral infection is 82%, which is also consistent with these results. As in literature, specificity of the Mistik Score was higher than its sensitivity in our study. There are no similar studies finding that the optimal cut-off value for the Mistik Score in RADT negative patients is 3 and above. We think RADT must not be used in case the Mistik Score is 3 and above while we recommend the use of RADT in case the score is 2 and below. The significant and negative correlation between the Mistik Score and Modified Centor Score is similar to findings in literature.

In the study including 223 pediatric patients admitted with the diagnosis of pharyngitis by Kose et al., the number of patients without cough and with cervical lymph node were higher in GABHS positive patients compared to the GABHS negative group ($p<0.05$ and $p<0.05$ respectively). The symptoms of fever (above 38 degrees), nasal obstruction, and tonsillar crypt and hypertrophy were similar ($p>0.05$, $p>0.05$, $p>0.05$, and $p>0.05$ respectively). Median (25p-75p) Modified Centor Score in GABHS positive group was 3 (3-4) and lower than that of GABHS negative group ($p<0.05$). In our study, the number of patients with cervical lymphadenopathy was higher in RADT positive group, which is similar to findings in literature. The number of patients with fever (above 38 degrees) and tonsillar exudate/hypertrophy was higher in RADT positive group, which is different from findings in literature. The number of patients with nasal obstruction was lower in RADT positive group, which is different from findings in literature. These may be because RADT was used instead of throat culture and a mixed population including adults and children was included in our study.

Clinical scoring systems in patients admitted with sore throat are easy-to-apply and useful and decreases the cost of RADT (17). The number of studies assessing the use of clinical scoring is limited in literature. In a study in which the Modified Centor Score was followed by RADT, utility of clinical scoring systems and RADT was assessed by healthcare workers and patients. All the patients (100%) who underwent RADT found it useful. They stated that the test was satisfactory (99.3%) and that they would undergo the test again in the future if necessary. All the pharmacists who participated in that study also found the intervention useful. The pharmacists spent 6-15 minutes to apply clinical scoring systems and 98.6% of the pharmacists who gave feedback explained that they were ready to use clinical scoring systems in daily practice. All the pharmacists stated that RADT was easy-to-use, 75.7% stated that it was not hard to collect pharyngeal swab and 97.3% stated that scoring systems were sufficient to guide clinical treatments. Patients' level of accepting RADT and compliance with the recommendations were observed to be very high. Putting a combination of clinical scoring and RADT into use in the management of sore throat has been positively responded by both patients and pharmacists (18). In our study, family physicians stated at the end of the study that clinical scoring systems were satisfactory in 91.7% of patients admitted with sore throat, which is consistent with findings in literature. Family physicians stated that they mostly used the Modified Centor Score, which may be because the Modified Centor Score was the most commonly used and known clinical scoring system in the United States of America and European countries. Family physicians stated at the end of the study that the Mistik Score was more useful, which may be because the rate of viral infections was higher and there was no other scoring system for viral infections in literature.

Another study in France revealed that RADTs were not commonly used by family physicians although they were provided free of charge by health insurance. The time needed for the test was one of the most common obstacles reported for the use of RADT (18). Another study revealed that French family physicians used RADT in only 60.1% of the pediatric patients with tonsillopharyngitis (19). The number of RADTs used by family physicians in that study was higher than the rates of its daily use. Family physicians stated that they preferred RADT mostly in pediatric age group, which is consistent with findings in literature. In our study, the reason why RADT was not commonly used was similar to that in literature and may be the time insufficiency for examination. Moreover, family physicians may also have used RADT more in the pediatric age group due to the sequela of acute rheumatic fever.

Limitations of the Study: The most important limitation of the study is using RADT, which helps diagnosis, for diagnosis in the 1st stage of the study instead of throat culture which is the gold standard test. Other limitations are that the number of patients participating in the study was

low and the study group was a mix of children and adults. Another limitation is that the number of family physicians participating in the 2nd stage of the study was low.

Strengths of the Study: The number of studies assessing the use of clinical scoring and RADT used in the primary care in patients with sore throat is low and there are ongoing studies. One of the strengths of this study is that it was performed with RADT giving rapid results and clinical scoring was used for patients before RADT.

CONCLUSION

In conclusion, the use of clinical scoring systems and RADT makes diagnosis more approximate in GABHS tonsillopharyngitis. The use of clinical scoring systems such as the Mistik Score and Modified Centor Score in sore throat commonly seen in daily family medicine practice will help the decision on whether additional RADT is necessary or not and most importantly help family physicians decide on the better therapy options.

We believe allowing the use of RADT and clinical scoring in Family Healthcare Centers will be effective in reducing antibiotic use and help patients who only need symptomatic treatment receive therapy without waiting. Therefore, we recommend the use of clinical scoring systems. The use of the Mistik Score may be better in diagnosing viral sore throat especially due the high rate of viral tonsillopharyngitis.

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RESEARCH ARTICLE

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The Effects of Heart-to-Mobile Phone Distance on the Circulatory System

ABSTRACT

Objective: The effect of the change in the distance between the mobile phone(MP) and the heart on heart rate variability (HRV) was examined, and the influence of the MP's distance to heart on the circulatory system was investigated.

Methods: Healthy volunteers using MPs were included in this study. The distance from the heart to the right ear is about four centimeters greater than its distance to the left ear. Taking advantage of this distance difference, the volunteers were divided into two groups: right-hand dominant and left-hand dominant individuals. A total of 31 right-hand dominant (Group 1) and 32 left-hand dominant (Group 2) volunteers were enrolled. HRV was automatically calculated by a commercially available FDA-approved three-channel Holter monitoring device (Holter ECG Recorder DMS300-4A.

Results: The mean tragus to apex distance of the first group was 37.5 cm and the mean of the second group was 33.6 cm. There was no statistically significant difference between standard deviation of N–Ns (SDNN), the standard deviation of the 5-min mean values of N–Ns (SDANN), root mean square successive difference of N–Ns (RMSSD), low-frequency (LF), and high frequency (HF) values and LF/HF ratio between the two groups. The percentage of successive N–N differences that were greater than 50 ms for each 5-min interval (pNN50%) was significantly lower in Group 2 compared to Group 1 ($p = 0.014$).

Conclusions: Our findings show that even a small increase in MPs to heart distance can reduce its negative effects on the cardiovascular system.

Keywords: Cardiac Autonomic Modulation, Electromagnetic Field, Heart Rate Variability, Mobile Phone

Kalp-Cep Telefonu Mesafesinin Dolaşım Sistemine Etkileri

ÖZET

Amaç: Cep telefonu ile kalp arasındaki mesafenin kalp hızı değişkenliğine (KHD) etkisi incelenerek cep telefonunun kalbe olan uzaklığının dolaşım sistemine etkisi araştırıldı.

Gereç ve Yöntem: Cep telefonu kullanan sağlıklı gönüllüler çalışmaya dahil edildi. Kalbin sağ kulağa uzaklığı, sol kulağa olan uzaklığından yaklaşık dört santimetre daha fazladır. Bu mesafe farkından yararlanılarak gönüllüler sağ el baskın ve sol el baskın bireyler olarak iki gruba ayrıldı. Toplam 31 sağ el baskın (Grup 1) ve 32 sol el baskın (Grup 2) gönüllü kaydedildi. KHD, piyasada bulunan FDA onaylı üç kanallı Holter izleme cihazı (Holter ECG Kaydedici DMS300-4A) tarafından otomatik olarak hesaplandı.

Bulgular: Birinci grubun ortalama tragustan apekse mesafesi 37,5 cm ve ikinci grubun ortalaması 33,6 cm idi. N – Ns (SDNN) standart sapması, N – Ns (SDANN) 5 dakikalık ortalama değerlerinin standart sapması, N – Ns (RMSSD) karekök ortalama ardışık farkı (RMSSD), düşük frekans (LF) ve yüksek frekans (HF) değerleri ve iki grup arasındaki LF / HF oranı arasında istatistiksel olarak anlamlı bir fark yoktu. Her 5 dakikalık aralık için 50 ms'den büyük olan ardışık N-N farklarının yüzdesi (pNN% 50) Grup 2'de Grup 1'e göre anlamlı olarak daha düşüktü ($p = 0,014$).

Sonuç: Bulgularımız, cep telefonu kalp mesafesindeki küçük bir artışın bile kardiyovasküler sistem üzerindeki olumsuz etkilerini azaltabileceğini göstermektedir.

Anahtar Kelimeler: Kardiyak Otonomik Modülasyon, Elektromanyetik Alan, Kalp Hızı Değişkenliği, Cep Telefonu

INTRODUCTION

Mobile phones (MPs) emit electromagnetic waves, which may have an influence on human tissues (1). Electromagnetic field (EMF) caused by MPs can affect the autonomic nervous system (ANS) which regulates the function of the circulatory system (2). There are many studies showing the effects of EMF from MP on the cardiovascular system (3-5). These studies investigated the duration of cardiovascular exposure to EMF. However, the distance between the EMF-source and the tissue is also an important factor (6). For example, in a study by Forouharmajd et al, it was shown that a four-centimeter (cm) MP to head distance could decrease heat exposure in brain tissues when compared to four-millimeter MP to head distance (7).

Heart rate variability (HRV) is a rough measure of the variation in time between each heartbeat, and is regulated by the ANS. Previous data indicate that MP use and related EMF exposure may change the autonomic balance in healthy subjects and thus influence HRV (8). The distance between the right ear and the heart is about 4 cm greater than the distance between the left ear and the heart. Therefore, the impact of the EMF originating from a MP may be different in subjects using their MPs on the right or left ears. Although there is sufficient evidence that MP use influences HRV, data concerning the impact of MP distance on HRV during phone calls has not been studied yet. Given the significant effects of EMF on HRV, we hypothesized that a shorter MP-to-heart distance would result in greater HRV change compared to longer MP-to-heart distance.

Thus, this study was conducted to examine the effect of the distance between MP and the heart (with regard to which ear was used during phone calls) on HRV values using Holter monitoring.

MATERIAL AND METHODS

The sample was obtained from a population of 485 sequential volunteers who were scheduled for 24-h ambulatory ECG monitoring. All volunteers were gathered from medical school students and hospital staff. The inclusion criteria were, having completed interference-free 24-h Holter ECG monitoring and being aged older than 18 years. All of the participants were healthy and none of them were on any medications. There were no individuals with sedentary lifestyle and none were professional athletes. To ensure that the phones' specific absorption rate (SAR) values remained the same, only those who had been using cell phones of the same brand and model for the preceding year were included in the study. Subjects were included in this study if right-hand dominant subjects used MPs to their right ear and left-hand dominant subjects to their left ear. All participants underwent electrocardiography and echocardiography before the study and laboratory analysis values were recorded from the hospital's electronic database. Patients with arrhythmia, structural heart disease, anemia or endocrine disease were excluded from the study. After these qualifications, 63 individuals were enrolled in the study.

The volunteers were divided into two groups: right-hand dominant and left-hand dominant individuals. There were 31 right-hand dominant (Group 1) and 32 left-hand dominant (Group 2) volunteers. All individuals reported that they were using their MPs on their dominant side for calls. The phone-heart distance was defined as the distance from the left nipple to the tragus of the right ear for the right-hand dominant group, and the distance from the left nipple to the tragus of the left ear for the left-hand dominant group. The duration of MP use (only including call duration) was recorded from the individuals' phone histories after completing holter monitoring.

We carried out 24-h ECG monitoring using a three-channel amplitude-modulation tape recorder (Holter ECG Recorder DMS300-4A). The 24-h Holter recordings were used to assess HRV parameters. The program specified and labeled each QRS complex that was automatically detected. Each holter recording was assessed by experienced cardiologists for QRS results and consistency. R-R intervals from the 24-hour ECG Holter monitoring were observed visually and the artifacts were detected and removed. As described in the literature, estimation of the time and frequency domain characteristics of HRV was taken (9). The time domain analysis of HRV contained the standard deviation of N–Ns (SDNN), the standard deviation of the 5-min mean values of N–Ns (SDANN), the percentage of successive N–N differences >50 ms for each 5-min interval (pNN50%) and the root mean square successive difference of N–Ns (RMSSD). The frequency domain analysis of HRV involved total power components (0.01–1.00 Hz), high-frequency power (HF: 0.16–0.40 Hz), low-frequency power (LF: 0.04–0.15 Hz) and the LF/HF ratio (10-12).

Comparison of HRV results in subjects using their right or left ears for MP calls during the Holter recording was the primary outcome measure of this study.

This study protocol was approved by the local ethics committee (Decision no: 2011-KAEK-27/2019-E.1900088400). Written and verbal information was given to all individuals before testing.

Statistical Analysis: The data obtained during the study were transferred to a computer spreadsheet file and were evaluated in the SPSS Version 15.0 package program. Distribution of data was tested using the Shapiro-Wilk test. In the analysis of the data, chi-square test was used to compare qualitative variables, and the independent sample t-test was used to compare quantitative variables. P value <0.05 was considered statistically significant**.**

RESULTS

A total of 63 subjects were enrolled in the study. 31 of them used MP with their right hand on their right ear (Group 1) and 32 used it with their left hand on their left ear (Group 2). Demographic information of the participants are presented in Table 1. All volunteers included in the study had normal sinus rhythm with a mean heart rate of 77 beats/min. There were no significant differences between the two groups in terms of echocardiography parameters, alcohol use and smoking. The mean MP call time of the two groups during the Holter monitoring was 128.2 ± 16.6 and 122.2 ± 16.9 minutes, respectively (p=0.161). The mean tragus to apex distance of Group 1 was 37.5 ± 0.8 cm and the mean of the Group 2 was 33.6 ± 0.9 cm (p<0.001).

Table 1. Sociodemographic Distributions and Clinical Features of the Study Groups

	Group 1 (n=31)	**Group 2 (n=32)**	**p values**
Age (year)	22.5±1.8	22.5±1.8	0.862
Sex (n, %)			
Male	15(48.4)	15(46.9)	0.904
Female	16(51.6)	17(53.1)	
BMI (kg/m^2)			
Normal	27(87.2)	30(93.8)	0.368
Overweight-obese	4(12.9)	2(6.2)	
Smoking	12(38.7)	10 (31.2)	0.535
Alcohol	12(38.7)	7(21.9)	0.146
SBP (mmHg)	115.3±9.8	114.1±10.9	0.632
DBP (mmHg)	72.4±8.4	71.6±8.8	0.694
LVEDD (mm)	43.3±2.6	42.6±2.8	0.352
LVESD (mm)	28.2±2.3	27.9±2.6	0.607
EF (%)	62.9±3.1	63.7±2.9	0.306
RA (mm)	40.4±2.5	40.2±1.9	0.766
RV (mm)	37.8±3.2	38.6±2.0	0.256
PAP (mmHg)	23.6±6.6	24.2±5.4	0.675
LA (mm)	32.1±3.5	32.0±3.1	0.938
IVS(mm)	8.4±1.1	8.3±1.1	0.968
Distance of Tragus to apex (cm)	37.5±0.8	33.6±0.9	**<0.001**
Call history (hour)	128.2±16.6	122.2±16.9	0.161

Data are presented as number (percentage), mean ± standard deviation
SBP, Sistolik Blood Presure; DBP, Diastolic blood pressure; LVEDD, Left Ventricule End Diastolik Daimeter; LVSDD, Left Ventricular End Systolic Diameter; EF, Ejection Fraction; RA, Right Atrium; RV, Right Ventricular; PAP, Pulmonary Artery Pressure; LA, Left Atrium; IVS, Interventricular Septum.

There was no statistically significant difference between SDNN, SDANN, RMSSD, LF, HF and LF/HF between the two groups. However, PNN50 was significantly lower in Group 2 compared to that of the Group 1 (15.5 ± 9.8 vs. 22.7 ± 11.4, p = 0.014) (Table 2).

Table 2. Heart Rate Variability Values of the Study Groups

	Group 1 n (%)	**Group 2 n(%)**	**P values**
Min HR (beats/minute)	45.1±8.9	46.4±7.3	0.525
Max HR (beats/minute)	149.1±18.2	152.5±27.3	0.526
Mean HR (beats/minute)	77.2±10.1	77.5±10.9	0.918
SDNN	152.8±33.2	152.9±39.0	0.991
SDANN	136.3±33.6	139.7±37.1	0.705
RMSSD	42.8±14.9	38.6±13.3	0.245
PNN50	22.7±11.4	15.5±9.8	**0.014**
LF	1161.5±442.5	1014.7±471.7	0.208
HF	527.8±293.3	479.8±305.3	0.527
LF/HF ratio	2.65±1.16	2.46±0.88	0.483

Data are presented as mean ± standard deviation
Min HR, Minimal Heart Rate; Max HR: Maximum Heart Rate; SDNN, standard deviation of N–Ns; SDANN, standard deviation of the 5-min mean values of N–Ns; RMSSD, the root mean square successive difference of N–Ns, PNN50, the percentage of successive N– N

DISCUSSION

This study aimed to investigate the effects of MP to heart distance on HRV and showed that a mean 4-cm reduction in MP to heart distance during phone call resulted in a significant reduction of the PNN50 value; however, SDNN, SDANN, RMSSD, LF, HF and LF/HF values remained similar.

The signals sent and received by an MP is in the form of microwave radiation which may lead to changes in tissues and water molecules. These changes have been associated with thermal and non-thermal effects in human tissues (13-15). Cardiovascular mortality, cognitive disability and birth defects are among the negative outcomes that have been associated with these thermal and non-thermal effects (16). Additionally, emission of these microwaves has been shown to be related with the development of some symptoms, such extreme irritation, decreased reflexes and an increase in arterial blood pressure, albeit rarely (13). However, while there are many studies investigating the relationship between cell phone call time and HRV, the changes in these effects with regard to distance have not been adequately studied yet (2, 3, 10).

The literature on this topic has mainly assessed the effect of MP on the heart by examining HRV. It is considered that HF is associated with parasympathetic activity and LF with sympathetic activity. The LF/HF ratio reflects sympathovagal balance and sympathetic regulation (17). RMSDD and PNN50 are known to reflect parasympathetic tone activity and these are considered as the 'short-term' HRV parameters. SDNN and SDANN represent both sympathetic and parasympathetic activities and are indicative of 'long-term' HRV. A reduced HRV has been shown to be a risk factor for the onset of malignant arrhythmias associated with sympathetic over activity in patients with cardiovascular disease (18). It has been shown that radiofrequency waves emitted by MP may affect HRV through their influence on the ANS (19).

The present study examined HRV values in two groups included. In the second group with shorter MP-to-heart distance, a decrease in PNN50 was observed. This decrease indicates a reduction in parasympathetic nervous system activity and an increased risk for cardiovascular disease.

The distance from the target organ to MP is relevant to EMF exposure, and therefore, its effects. A recent study by Kivekas et al. revealed that the easiest way to decrease SAR exposure was to increase the distance between the body and MP (20). Another study conducted by Hirata et al. identified significant factors affecting the absorption rate of electromagnetic waves. The authors noted that the electrical properties of the tissue, the size of the withstanding tissue and the distance between the source of the electromagnetic wave and user's body were effective on absorption (18). Furthermore, even changes of a few centimeters have been shown to result in significant changes in the impact of EMF on the tissues (7, 21). In our study, we used an objective and continuous measure of the distance from the heart to the MP using the ears as the reference points. Our findings show that subjects using the MP with their right hands had lower PNN50 values compared to those using MP with their left hands. This significant difference between the two groups of PPN50 alone may not be clinically significant because there was no difference in other HRV values. However, the difference in this parameter may be predictive of the decrease in other HRV parameters if this study was to be repeated in a larger population.

From this point of view, our results support the evidence put forth by previous studies which showed that electromagnetic interference on pacemakers was most significant when the phone antenna was placed in close proximity (22). Our findings also support the international guidelines which recommend using MPs via the ipsilateral ear in patients with implanted devices (23).

The present study has some limitations to be mentioned. Firstly, this study used the same MP brand and model and selected individuals with similar BMI to avoid differences between SAR values. This led to a limited sample size. Secondly, in this study, the duration of telephone calls was taken into consideration, but nowadays telephones are used for many purposes such as internet, watching videos and taking photos and also used for other usages like a health applications(24). Thirdly, the lack of iterated long-term HRV analysis (25) may have caused some misinterpretations in HRV analysis. Lastly, although the participants stated that their MP use was similar, it may have affected the results since there was no objective evidence to confirm this.

With this study, we call attention to the impact of MP-to-heart distance on HRV parameters. Our findings show that even a small increase in distance can reduce possible negative effects on the cardiovascular system.

CONCLUSION

It is well-established that SAR value decreases with increased device-to-tissue distance. Therefore, increasing the distance between the heart and MP, at least during use, can be effective in reducing heart-related possible adverse effects caused by MPs. The significance of MP-to-heart distance has not been directly investigated. In order to determine the optimal distance, there is a need for a wide range of studies.

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RESEARCH ARTICLE

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Clinical Approach to Patients with Klippel-Feil Syndrome

ABSTRACT

Objective: Klippel-Feil Syndrome (KFS) is a congenital syndrome that have short neck, fusion in the at least 2 cervical vertebrae and decrease in neck movements in the classic triad. KFS patients may have higher risk for mechanical spinal cord injury because of unstable cervical segments. Patients should be examined extensively, since many anomalies may accompany KFS. In the current article, we aimed to present our cases with KFS with clinical, radiological and genetic results.

Methods: In this study, a total of 317 cervical MRIs required by Pediatric Surgery between 2012-2019 years with various indications and 90 cervical CT reports in 2019 were evaluated. Information was collected retrospectively with a file scan. Clinical, radiological, biochemical and genetic evaluation of KFS cases were performed.

Results: All of our patients with KFS had Sprengel deformity, cervical fusion anomaly, restricted neck movements, short neck, low hairline and growth retardation. Growth Differentiating Factor 6 (GDF6) gene was negative in all our patients.

Conclusions: The necessity of treatment depends more on the pathologies that may be caused by deformity and other systemic findings. The patient should be cautious and avoid heavy exercise because of neurological deficits seen after minor trauma in patients with KFS.

Keywords: Klippel-Feil Syndrome, Sprengel Deformity, Cervical Fusion Anomaly, Short Neck

Klippel-Feil Sendromlu Hastalara Klinik Yaklaşım

ÖZET

Amaç: Klippel-Feil Sendromu (KFS), klasik triadında kısa boyun, en az 2 servikal omurda füzyon anomalisi ve boyun hareketlerinde azalmanın olduğu konjenital bir malformasyondur. KFS'li hastalar stabil olmayan boyun segmentleri nedeniyle, mekanik olarak yüksek spinal kord hasarı riskine sahiptirler. Bu hastalar, KFS'ye birçok anomalinin eşlik etmesi muhtemel olduğundan kapsamlı bir şekilde muayene edilmelidir. Biz bu çalışmada KFS olgularımızı klinik, radyolojik, biyokimyasal ve genetik sonuçlarıyla sunmayı amaçladık.

Gereç ve Yöntem: 2012-2019 yılları arasında çeşitli endikasyonlarla; Çocuk Cerrahisi kliniği tarafından istenen toplam 317 servikal MR ve 2019 yılı içerisinde çekilen toplam 90 servikal BT raporu değerlendirildi. Saptanan KFS olgularının bilgileri dosya taraması yöntemiyle toplandı. KFS olgularının klinik, radyolojik, biyokimyasal ve genetik değerlendirmeleri yapıldı.

Bulgular: Hastalarımızın hepsinde Sprengel deformitesi (yüksek skapula), servikal füzyon anomalisi, boyun hareketlerinde kısıtlılık, kısa boyun, düşük saç çizgisi ve büyüme geriliği bulguları mevcuttu. Tüm hastalarımızda Büyüme Farklılaştırıcı Faktör 6 (GDF6) geni negatifti.

Sonuç: Tedavinin gerekliliği deformite ve diğer sistemik bulguların neden olabileceği patolojilere bağlıdır. KFS hastalarında küçük travma sonrası görülen nörolojik defisitler nedeniyle hasta temkinli olmalı ve ağır egzersizden kaçınmalıdır.

Anahtar Kelimeler: Klippel-Feil Sendromu, Sprengel Deformitesi, Servikal Füzyon Anomalisi, Kısa Boyun

INTRODUCTION

Klippel-Feil Syndrome (KFS) is a congenital malformation caused by insufficiency in the segmentation of cervical somites on the 3-8th week of gestation. There is a short neck, fusion in the at least 2 cervical vertebrae and a decrease in neck movements in the classic triad. KFS patients may have higher risk for mechanical spinal cord injury because of unstable cervical segments. Although KFS generally occur as sporadic, the autosomal dominant, recessive and X-linked forms also can be occurred (1,2). Autosomal recessive (*MEOX1* and *RIPPLY2*) and dominant (*GDF6* and *GDF3*) inheritance patterns were announced in KFS families (3). In the current article, we aimed to present our cases with KFS syndrome with clinical, radiological and genetic results.

MATERIAL AND METHODS

A total of 317 cervical MRIs between 2012-2019 years with various indications and 90 cervical CT reports in Duzce University, Faculty of Medicine, Research and Practice Hospital, Pediatric Surgery Department were included in the study. Ethical approval was obtained from the local ethics committee before starting work. Information was collected retrospectively with a file scan. Informed consent was obtained from all patients. The demographic, physical, radiological, genetic, biochemical, audiological, ocular, cardiologic examination of four cases with KFS (Sprengel deformity and cervical fusion anomalies) were carried out. For chromosomal analysis, 5 cc peripheral blood samples of cases were cultured and metaphase plaques were obtained and G banding was carried out. Also DNA was isolated from the peripheral blood samples of cases with KFS and *GDF6* gene was sequenced.

Statistical Analysis: All statistical analyses were performed using IBM SPSS® Statistics for Windows®, version 23.0 (IBM Corp., Armonk, NY, USA). The descriptive statistic was carried out. All data are expressed as mean±SD, min, max and percentage unless otherwise stated.

RESULTS

A total of 317 cervical MRIs required by Pediatric Surgery between 2012-2019 years with various indications and 90 cervical CT reports in 2019 were evaluated. 208 patients were female and 199 were male. In terms of gender, there was no significant difference (p=0.173) between the mean age of girls (11.1±5.9) and the mean age of boys (10.3±5.5). KFS findings were detected in 4 of these patients.

First case of KFS is 6-year-old female patient, first child of a 25-year-old mother (Gravidity3, Parturition2 and Alive2). Parents have not relationship and chronic diseases. Her parents age were 25-year-old. Her mother did not take any drugs during her pregnancy. She was born with normal spontaneous vaginal delivery (NSVD) at 40 weeks gestation. Patient's weight was 18 kg (lower than -0.67 SD) and her length was 112 cm (lower than -0.35 SD). She had normal mental level. Recurrent urinary tract infections and complaints in patients admitted to the pediatric surgery clinic with fecal incontinence were assessed as secondary to both constipation and there was no surgical pathology in patient. The patient's complaints improved with constipation and anal fissure treatment. Chest X - ray was showed Sprengel deformity (also known as High scapula) and cervical fusion anomalies. MRI, which was taken due to left shoulder impairment and scoliosis was present at the cervicothoracic level, and was consistent with the intramedullary syringohydromyelitis at the Th7-8 level, and was posterior fusion and anterior hemi vertebra at the C7-Th1 level. Also there was increase in the diameter of the vertebrae and spinal canal at the C3-4 level, and was a hemi vertebra view at the posterior of Th4-5 and a spinal canal at the midline of the bone septa (Diastometamyelia) (Figure 1).

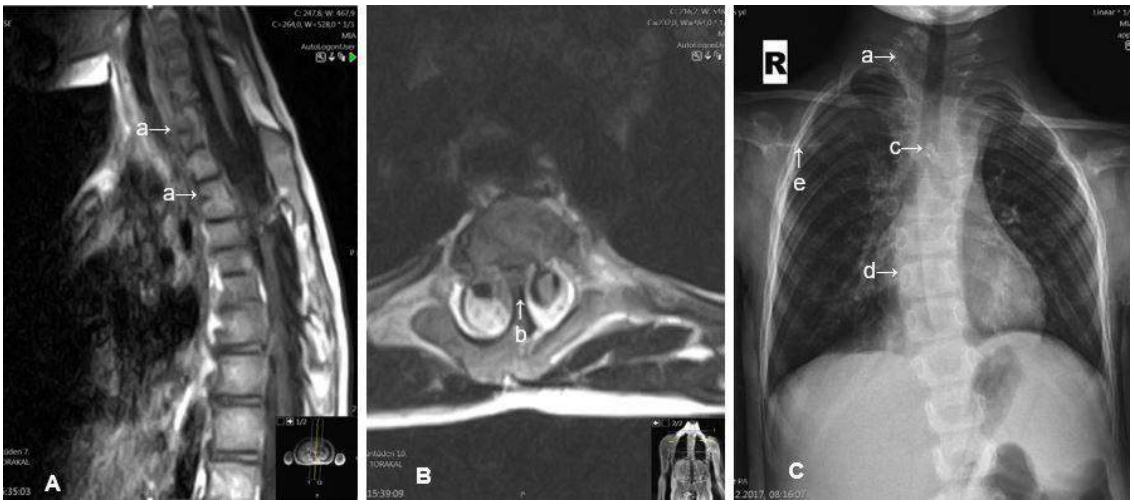

Figure 1. A; cervical fusion anomaly (a), B; hemi vertebra (b), C; bone septa image of diastematomyelia (c), scoliosis (d), high scapula (e)

She has been treated with thyroid hormone replacement therapy for congenital hypothyroidism until the age of 3, but currently not using drugs. The patient's audiological and ocular examination was normal. Electrocardiography and echocardiography findings were normal. Abdominal USG of the patient had a liver longitudinal dimension of 126 mm. (N:70-120). Routine biochemical analyses were normal. Her karyotype was 46, XX and sequencing analysis of *GDF6* gene was normal. The clinical features of the case were given in Table 1.

Table 1. Clinical features of cases with KFS

	HS	CFA	LNM	SN	LH	GR	S	OA	K	FR	GDF6	AF
Case1	+	+	+	+	+	+	+	HM	46,XX	-	-	Diastometamyelia and hypothyroidism
Case2	+	+	+	+	+	+	-	-	46,XX	-	-	Numerical deficiency in the right upper ribs
Case3	+	+	+	+	+	+	-	-	46,XX	-	-	Hydrocephalus, right choanal atresia, high palate, low ear and MMR
Case4	bilateral	+	+	+	+	+	+	RA	46,XX	-	-	Strabismus and amblyopia

HS: High scapula (Sprengel deformity); **CFA**: Cervical fusion anomaly; **LNM**: Limited neck motion; **SN**: Short neck; **LH**: Low hairline; **GR**: Growth retardation; **S**: Scoliosis; **OA**: Organ anomaly; **K**: Karyotype; **FR**: Family relationship; **AF**: Additional features; **GDF6**: Growth differentiation factor 6 gene; **MMR**: Mental Motor Retardation; **HM:** Hepatomegaly; **RA:** Renal agenesis.

Second case of KFS is 7 months old female patient, last child of a 27-year-old mother (Gravidity3, Parturition3 and Alive3). She was born with NSVD at 41 weeks gestation. Parents have not relationship and chronic diseases. Her parents age were 27-year-old. Her mother did not take any drugs during her pregnancy. Patient's weight was 7 kg (lower than -0.44 SD) and her length was 67 cm (higher than 0.76 SD). Her mental level was normal. Difficult defecation and anal bleedings were evaluated as secondary to both constipation and anal fissure in patients admitted to pediatric surgery and there was no surgical pathology in patient. The patient's complaints improved with constipation and anal fissure treatment. Chest X-ray was showed Sprengel deformity, cervical fusion and numerical deficiency in the right upper ribs anomalies (Figure2).

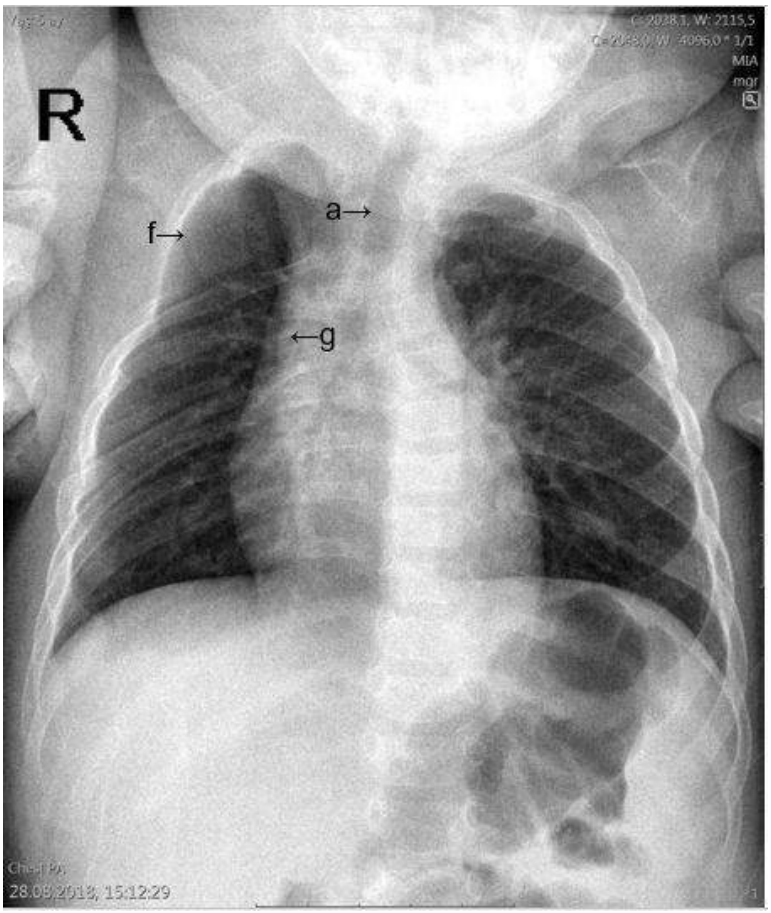

Figure 2. Cervical fusion anomaly (a), numerical deficiency in the right upper ribs (11 ribs) (f), fusion anomaly in ribs 2 and 3 (g)

The patient's audiological and ocular examination was normal. Abdominal ultrasonography, electrocardiography, echocardiography and routine biochemical analyses were normal. Her karyotype was 46, XX and sequencing analysis of *GDF6* gene was normal. The clinical features of the case were given in Table 1.

Third case of KFS is last child of a 30-year-old mother (Gravidity5, Parturition2, Abortus2 and Alive1). There was no family relationship between her mother and father. The parents have not chronic diseases. Her mother's was 30-year-old and father's was 35-year-old. Her mother did not take any drugs during her pregnancy. First gravidity was 9-year-old male, alive and healthy. Second gravidity was aborts. Third gravidity was aborts. Fourth gravidity was curettage. Fifth gravidity was our patient. She was born at 39 weeks gestation with a birth weight of 4200 gr by cesarean section (C/S) due to diagnosis of intrauterine hydrocephalus. Her first Apgar score was 8 at the time of birth. Macrocephaly, short neck, right choanal atresia, high palate and low ears findings were detected in the patient's physical examination. The patient was admitted to the neonatal intensive care unit for respiratory distress and remained about 1 month. Patient's weight was 5300 gr (lower than -0.11 SD) and her length was 50 cm (lower than -5.21 SD). The patient had severe mental motor retardation (MMR). Chest X - ray was showed Sprengel deformity and cervical fusion anomalies. The patient's audiological and ocular examination was normal. Her cranial computerized tomography revealed a significant enlargement in the third and bilateral lateral ventricles consistent with massive hydrocephalus and there was severe thinning of the brain parenchymal thickness, especially in the lateral and posterior segments, and this view was evaluated as significant in terms of hydrocephaly (Figure3).

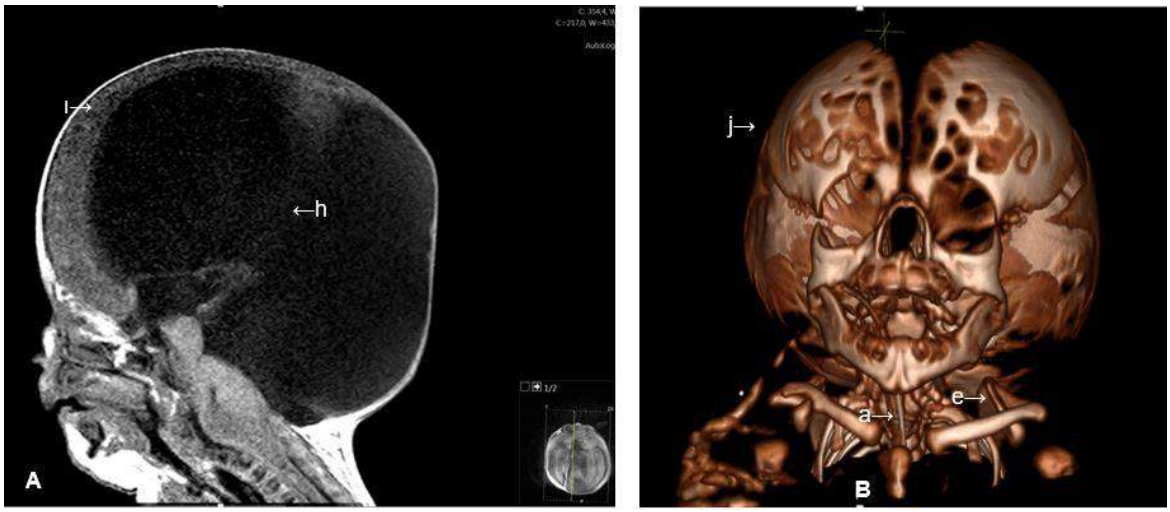

Figure 3. A; severe hydrocephalus (h) and severe thinning of the brain parenchyma (ı), B; cervical fusion anomaly (a), high scapula (e), massive macrocephaly (j)

Abdominal ultrasonography, electrocardiography, echocardiography findings and routine biochemical analyses were normal. Her karyotype was 46, XX and sequencing analysis of *GDF6* gene was normal. Because of the possibility of difficult intubation due to short neck and cervical fusion anomaly, anesthesia approval could not be obtained. Thus ventriculoperitoneal shunt planned by brain surgery and the gastrostomy opening planned by the children's surgery was not done. The patient underwent ventriculostomy by brain surgery at the bedside and she referred to the external center for shunt surgery. The patient was admitted to the pediatric intensive care unit with respiratory distress again 2 months later. In her anamnesis, since the brain parenchymal thickness was severely thinner, she was considered inoperable, and shunt operation was not performed. On day 3 of hospitalization, patient was died because of the cardiopulmonary arrest. The clinical features of the case were given in Table 1. Fourth case of KFS is last child of a 42-year-old mother (Gravidity2, Parturition2 and Alive2). She had a healthy sister at 16-year-old. Her parents were 42-years old and have not relationship and chronic diseases. The cases' mother had used antibiotics due to the bronchitis during pregnancy. She was born at 38 weeks gestation by C/S because of fetal distress. She was 9-year-old girl and had growth retardation and congenital unilateral renal agenesis. Patient's weight was 19.6 kg (lower than -2.33 SD) and her length was 118 cm (lower than -2.44 SD). She had normal mental level. She had prominent forehead, short neck, and low posterior hairline, limitation of the movement of head and neck, scoliosis, bilateral Sprengel deformity and congenital unilateral renal agenesis. She had strabismus, amblyopia, normal hearing and pediatric echocardiography. Vertebral fusion related with C7-T1 and also spinous process fusion at C5-T1 and T3-T5 levels were detected in

cervical MR. Cervical kyphosis, the fusiform enlargement of the spinal canal between C6-T2, increased thickness of the cervical spinal cord at C5-T1 level were detected (Figure 4). According to renal scan, both kidneys were not seen normal position. Also while one of them showed renal agenesis, the other had ectopic location and normal function. No surgical pathology was found in the pediatric patient due to abdominal pain. Routine biochemical analyses were normal. Her karyotype was 46, XX and sequencing analysis of *GDF6* gene was normal. The clinical features of the case were given in table 1.Writing informed consent was taken from all cases.

Figure 4. Cervical fusion anomaly (a), scoliosis (d), high scapula (e), deformity in the left upper ribs (k)

DISCUSSION

KFS was first described in 1912 by Klippel and Feil. It is occurred in common heterogeneous group of cases with cervical spine fusion. The frequency of disease is one in 40000-42000 births (4). Radiographic examination is difficult in infants and children and so there are differences between patients compared to adults. Patients should be examined extensively, since it is likely that many anomalies may accompany to KFS. In addition to cervical spine radiographs, thoracic and lumbar spine radiographs should be performed in order to investigate possible other anomalies and scoliosis development in all patients with KFS. Scoliosis and/or kyphosis (60%), Sprengel deformity (30%) and skeletal anomalies such as torticollis, urinary system anomalies (35%), hearing loss (30%), asymmetry on face and ascension (20%), synkinesis or mirror movements (20%), congenital heart diseases (4%) can be detected in cases with KFS (5). All of our patients had Sprengel deformity, cervical fusion anomaly, restricted neck movements, short neck, low hairline and growth retardation. Scoliosis was present in our first and fourth patients. Hepatomegaly in our first patient and unilateral renal agenesis in our fourth patients were detected. The karyotypes of all our patients were 46, XX. There was no relationship between their mothers and their fathers. *GDF6* gene was negative in all our patients. In our first patient, there were hypothyroidism and diastometamyelia in addition to routine findings. In our second patient, there was numerical deficiency in the right upper ribs in addition to routine findings. In our third patient, there were hydrocephalus, right choanal atresia, high palate, low ear and MMR in addition to routine findings. In our fourth patient, there were strabismus and amblyopia in addition to routine findings (Table 1).

KFS generally appear as sporadic but also autosomal dominant, recessive, and X-linked forms are seen (6). Mutations in KFS and *GDF6* (Growth Differentiating Factor 6) and *GDF3* (Growth Differentiating Factor 3) genes were found to be closely related (7). These growth factors are involved in the transforming growth factor beta group and play a role in the formation and elongation of cartilage and bone. *GDF6* is primarily involved in the formation of the spine. However, it is not possible to find these gene mutations in all patients with KFS. This explains a wide variety of

anomalies in KFS. The resulting *GDF6* and *GDF3* mutations are inherited in two ways; C2-C3 is autosomal dominant in segmentation defect, or is autosomal recessive in C5-C6 segmentation defect (8). An autosomal dominant transient gene copy is sufficient for the development of the disease, but in autosomal recessive transition, there is a need for double corrupted gene copy. In our cases, the *GDF6* gene was evaluated but the mutations were not detected in this gen. Also family history was not detected in each case.

Because of airway management involved difficult tracheal intubation, it was obligated to secure the field of operation. Additionally careful postoperative care and respiratory management are necessary for the patient with KFS (9). In our case 3, ventriculoperitoneal shunt planned by brain surgery and the gastrostomy opening planned by the children's surgery was not performed because of the short neck and cervical fusion anomaly; anesthesia approval could not be obtained.

CONCLUSIONS

The necessity of treatment depends more on the pathologies that may be caused by deformity and other systemic findings. The patient should be cautious and avoid heavy exercise because of neurological deficits seen after minor trauma in patients with KFS.

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RESEARCH ARTICLE

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Antibiotic Susceptibilities of *Escherichia coli* Strains Isolated From Urine Samples in a Tertiary Hospital in Eastern Turkey

ABSTRACT

Objective: *Escherichia coli* is the most common cause of urinary tract infections. The increase and spread of resistance to antibiotics is a serious problem in our country as well as in the world. The aim of this study was to determine the antimicrobial susceptibility profiles of *E. coli* strains that cause urinary tract infection.

Methods: The antibiotic susceptibilities of 1850 *E. coli* strains isolated from urine samples of patients who applied to Urology service and outpatient clinics of Malatya Education and Research Hospital, which is a tertiary care hospital in eastern part of Turkey, over the 3 years between July 2016 and July 2019, were evaluated retrospectively from computer records using the Vitek 2.0 Compact automated system (BioMérieux, France). Antibiogram results were classified as susceptible, moderately susceptible and resistant.

Result: Of the total 1850 samples, 1300 (70.3%) were female and 550 (29.7%) were male, with a mean age of 52 (18-104) years. 73 (3.9%) of the patients were inpatients and 1777 were outpatients. All the isolated *E. coli* strains were found to be susceptible to ampicillin 39.2%, amoxicillin-clavulanic acid 67.9%, piperacillin-tazobactam 80.8%, cefuroxime 67.1%, cefotaxime 68.8%, cefixime 67.7%, ceftazidime 71.2%, cefepime 73.9%, ertapeneme 97.9%, imipeneme 99.5%, meropeneme 99.7%, amikacin 89.7%, gentamicin 78.4%, norfloxacine 65.8%, ciprofloxacine 65.9%, nitrofurantoin 94.2% and fosfomycin 96.5%. Resistance rates were higher in inpatients as compared to outpatients. Antibiotic susceptibilities were found to be decreased in different age ranges with increasing age.

Conclusion: When selecting the antimicrobial treatment in community-acquired urinary tract infections, considering the antimicrobial resistance data in the region, the choice of cost-effective treatment with high patient compliance and low resistance rates will increase treatment success. It is thought that antibiotic treatment should be determined according to the results of culture antibiograms, especially in nosocomial infections due to high resistance rates.

Keywords: Antibiotic Resistance, *Escherichia coli*, Urinary Tract Infections

Türkiye'nin Doğusunda Üçüncü Basamak Bir Hastanede İdrar Örneklerinden İzole Edilen *Escherichia coli* Suşlarının Antibiyotiklere Duyarlılıkları

ÖZET

Amaç: Üriner sistem enfeksiyonlarının etkenleri arasında en sık *Escherichia coli* izole edilmektedir. Antibiyotiklere karşı gelişen direncin artması ve yayılması bütün dünyada olduğu gibi ülkemizde de ciddi bir sorundur. Çalışmamızda, üriner sistem enfeksiyonu etkeni olan *E. coli* suşlarının antimikrobiyal duyarlılık profillerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Temmuz 2016–Temmuz 2019 tarihleri arasında 3 yıllık süreçte, Malatya Eğitim ve Araştırma Hastanesi Üroloji servis ve polikliniklerine başvuran hastaların, idrar örneklerinden izole edilen 1850 *Escherichia coli* suşunun, Vitek 2.0 Compact otomatize sistemi (BioMérieux, Fransa) kullanılarak belirlenen antibiyotik duyarlılıkları bilgisayar kayıtları üzerinden retrospektif olarak incelenmiştir. Antibiyogram sonuçları duyarlı, orta duyarlı ve dirençli olarak sınıflandırılmıştır. Verilerin değerlendirilmesi aşamasında orta duyarlı suşlar dirençli olarak kabul edilmiştir.

Bulgular: Toplam 1850 örneğin 1300'ü (%70,3) kadın, 550'si (%29,7) erkek hastaya ait olup tüm hastaların yaş ortalaması 52 (18-104) idi. Hastaların 73'ü (%3,9) yatan hasta, 1777'si poliklinik hastasıydı. İzole edilen tüm *Escherichia coli* suşlarının ampisiline %39,2, amoksisilin-klavulonik asite %67,9, piperasilin-tazobaktama %80,8, sefuroksime %67,1, sefotaksime %68,8, sefiksime %67,7, seftazidime %71,2, sefepime %73,9, ertapeneme %97,9, imipeneme %99,5, meropeneme %99,7, amikasine %89,7, gentamisine %78,4, norfloksasine %65,8, siprofloksasine %65,9, nitrofurantoine %94,2 ve fosfomisine %96,5 duyarlı olduğu saptanmıştır. Yatan hastalarda direnç oranları ayaktan tedavi olanlara kıyasla yüksek bulunmuştur. Değişen yaş aralıklarında yaş ilerledikçe antibiyotik duyarlılıklarının düştüğü görülmüştür.

Sonuç: Toplum kökenli üriner sistem enfeksiyonlarında antimikrobiyal tedavi seçiminde, bölgesel antimikrobiyal direnç verileri göz önüne alınarak; direnç oranlarının düşük, hasta uyumunun yüksek olduğu, maliyet etkin tedavilerin tercihi tedavi başarısını artıracaktır. Özellikle nozokomiyal enfeksiyonlarda yüksek direnç oranları nedeni ile kültür antibiyogram sonucuna göre antibiyotik tedavisinin belirlenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Antibiyotik Direnci, *Escherichia coli*, Üriner Sistem Enfeksiyonları

INTRODUCTION

Urinary tract infections (UTIs), which are reported to affect 150 million people annually, are the second most common cause of infectious diseases. The incidence of UTI in all age groups from neonates to elderly increases with age. It is more commonly seen in neonates, pregnant women or elderly patients. Approximately 15% of all prescribed antibiotics are for UTI treatment. The clinical spectrum of infections includes urethritis, cystitis, prostatitis and pyelonephritis. If left untreated, it may cause serious complications such as kidney damage and kidney failure (1, 2).

UTI is usually caused by Gram negative bacteria such as *E. coli*, *Proteus* species, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Klebsiella* species, *Enterobacter* and *Citrobacter* species. Gram-positive bacteria include *Staphylococcus saprophyticus*, *Enterococcus* species and coagulase-negative *Staphylococcus* species. Bacteria responsible for causing UTI, have more aggressive virulence factors. *Escherichia coli* is the most common cause of community-acquired and healthcare related urinary tract infections. Uropathogenic *E. coli* (UPEC) strains show their pathogenicity in the urinary tract by different virulence factors such as fimbria, capsule, flagella, toxins and lipopolysaccharide (1, 3).

Antibiotic susceptibility of uropathogenic bacteria varies over time and in different regions. In almost all uncomplicated UTI cases, antimicrobial treatment is initiated empirically without urine culture results. Inappropriate use of antibiotics has resulted in the development of antibiotic resistance in bacteria worldwide. Antimicrobial resistance is an important public health concern that increases patient care costs, prolongs hospital stay and causes treatment failures. Antimicrobial resistance surveillance studies at national level are of great importance in the fight against resistance problem. (4-6).

Fosfomycin, which is commonly used in the treatment of UTI, is an agent with a broad spectrum of antimicrobial activity that inhibits enolpyruvile transferase irreversibly in the first stage of bacterial cell wall synthesis and is often used in the treatment of uncomplicated urinary tract infections. Since its discovery in 1969, there has been an increasing interest in the use of fosfomycin in the treatment of urinary and systemic infections caused by multi-drug resistant Gram negative bacteria, especially *Enterobacteriaceae*, which are resistant to agents used conventionally (7, 8).

The most effective way to maintain the effectiveness of antibiotics in future generations, to prevent increasing resistance rates and to prevent the spread of resistant bacteria is the rational use of antibiotics. In this study, we aimed to determine the susceptibility of E. coli strains, which are the causative agents of urinary system infection, to the most commonly used antibiotics in the clinic, to raise awareness for the effective use of antibiotics and to guide empirical treatment.

In our study, in vitro antibiotic susceptibility of *E. coli* strains isolated from urology outpatient clinics and wards of our hospital over the 3 years, to drugs such as ampicillin (AM), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefuroxime (CXM), cefotaxime (CTX), cefixime (CFM), ceftazidime (CAZ), cefepime (FEP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), amikacin (AN), gentamicin (GN), norfloxacin (NOR), ciprofloxacin (CIP), nitrofurantoin (FT) and fosfomycin (FOS), were investigated retrospectively. The aim of this study was to determine the antimicrobial susceptibility profiles of *E. coli* strains that cause urinary tract infection.

MATERIAL AND METHODS

The patients admitted to the Urology service and outpatient clinics of Malatya Education and Research Hospital, with a capacity of 1040 beds, with the suspicion of UTI and diagnosed as urinary infection between July 2016 and July 2019, were retrospectively evaluated. Patients with urine culture growth were included in the study.

In vitro antimicrobial susceptibility of urine cultures, sent to the Microbiology Laboratory of our hospital and found to have growth, to drugs such as ampicillin (AM), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefuroxime (CXM), cefotaxime (CTX), cefixime (CFM), ceftazidime (CAZ), cefepime (FEP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), amikacin (AN), gentamicin (GN), norfloxacin (NOR), ciprofloxacin (CIP), nitrofurantoin (FT) and fosfomycin (FOS), was determined using the Vitek 2.0 Compact automated system (BioMérieux, France). Antibiogram results were classified as susceptible, moderately susceptible and resistant. Moderately susceptible strains were considered to be resistant during the evaluation of the data.

The approval was obtained from Malatya *Clinical Research Ethics Committee* on June 3th, 2020 with the protocol number 2020/55.

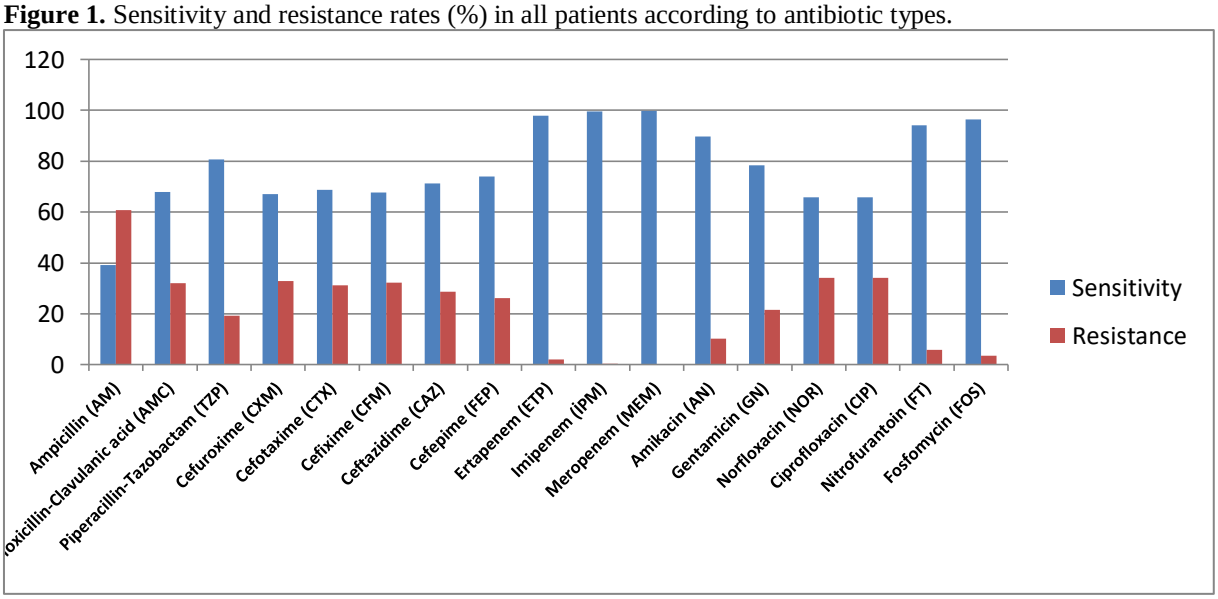
All necessary data for the study were obtained from hospital information system. SPSS 22 program was used for data analysis. Data were analyzed by Pearson Chi-square, Yates chi-square and Fisher chi-square tests. $p<0.05$ was considered to be significant. Percentage (%) of descriptive statistics was used in order to show the distribution of antibiotic susceptibility and resistance.

RESULTS

1850 patients (1300 females and 550 males) with *E. coli* growth in their urine culture were included in the study, and the mean ages were 52 ± 19.4 (18-104). The susceptibility of isolated *E. coli* to antibiotics was very high for carbapenems such

as meropenem, imipenem and ertapenem (99.7%, 99.5% and 97.9%, respectively). In terms of susceptibility, these antibiotics were followed by fosfomycin (96.5%), nitrofurantoin (94.2%), amikacin (89.7%), piperacillin-tazobactam (80.8%), gentamicin (78.4%), cefepime (73.9%), ceftazidime (71.2%), cefotaxime (68.8%) and amoxicillin-clavulanic acid (67.9%). Ampicillin 60.8%, norfloxacin 34.2%, ciprofloxacin 34.1%, cefuroxime 32.9% and cefixime 32.3% were the most resistant antibiotics with high resistance rates (Figure 1).

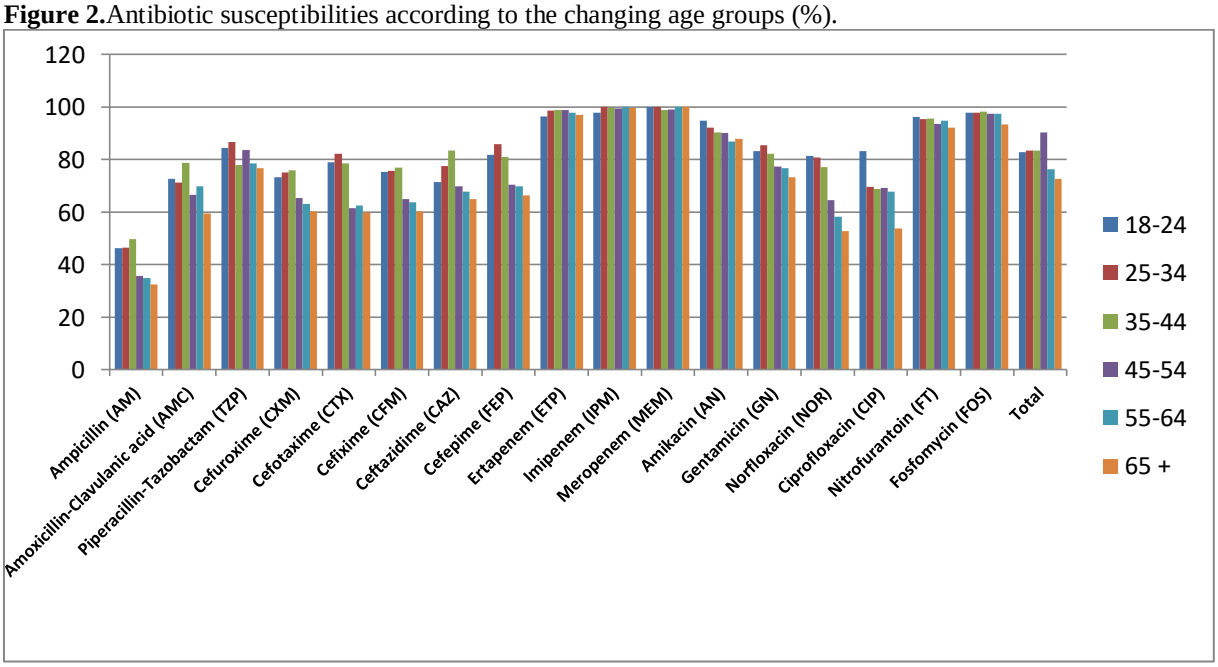
Figure 1. Sensitivity and resistance rates (%) in all patients according to antibiotic types.



Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CXM, cefuroxime; CTX, cefotaxime; CFM, cefixime; CAZ, ceftazidime; FEP, cefepime; ETP ertapenem; IPM, imipenem; MEM, meropenem; AN, amikacin; GN, gentamicin; NOR, norfloxacin; CIP, ciprofloxacin; FT, nitrofurantoin; FOS, fosfomycin.

We divided our patients into 6 age groups as 18-24, 25-34, 35-44, 45-54, 55-64 and over 65 years of age and showed the effects of age changes on antibiotic susceptibility. In general, we found that the antibiotic susceptibility decreased with increasing age in all antibiotics (Figure 2).

Figure 2. Antibiotic susceptibilities according to the changing age groups (%).



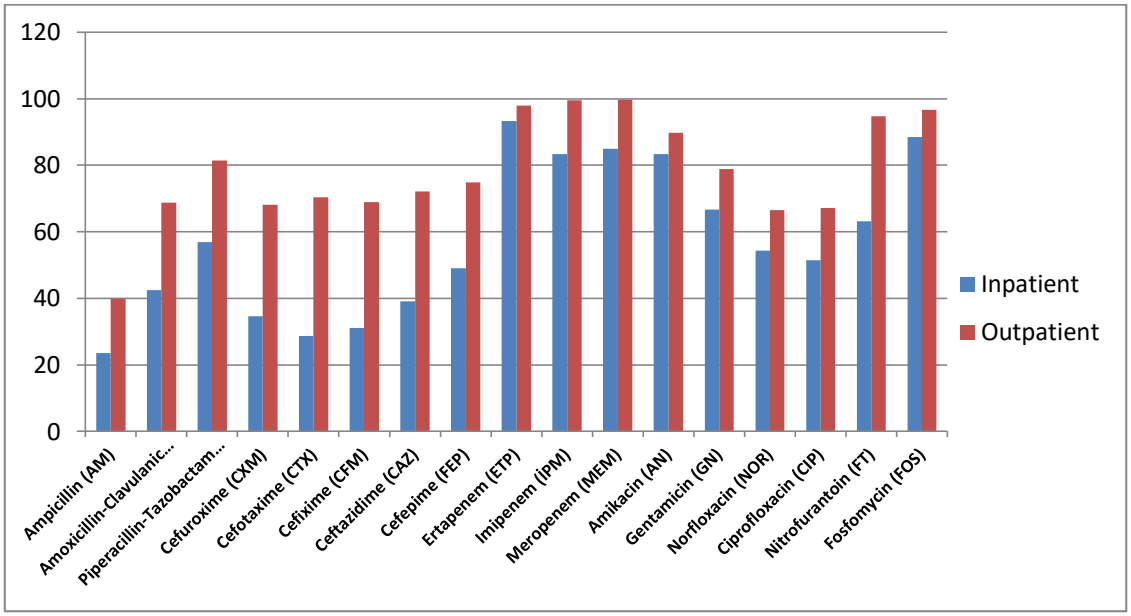
When the antibiotic susceptibilities were examined according to the changing age groups, the relationship between ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefotaxime, cefixime, ceftazidime, cefepime, gentamicin, norfloxacin, ciprofloxacin and fosfomycin antibiotics and age groups, was found to be statistically significant (Table 1).

Table 1. Antibiotic susceptibilities according to the changing age groups.

	N / n (%)	N / n (%)	N / n (%)	N / n (%)	N / n (%)	N / n (%)	
Name of Antibiotic	**18-24**	**25-34**	**35-44**	**45-54**	**55-64**	**65<**	**p**
Ampicillin (AM)	177/82(46,3)	235/109(46,4)	273/136(49,8)	266/95(35,7)	278/97(34,9)	556/18132,5	**<0,001**
Amoxicillin-Clavulanic Acid (AMC)	164/119(72,6)	219/156(71,2)	255/201(78,8)	254/169(66,5)	259/181(69,9)	508/302(59,5)	**<0,001**
Piperacillin-Tazobactam (TZP)	90/76(84,4)	113/98(86,7)	100/78(78)	129/108(83,7)	112/88(78,6)	219/168(76,7)	0,203
Cefuroxime (CXM)	187/137(73,3)	238/179(75,2)	279/212(76)	277/181(65,3)	290/183(63,1)	571/344(60,2)	**<0,001**
Cefotaxime (CTX)	95/75(78,9)	113/93(82,3)	126/99(78,6)	138/85(61,6)	99/82(62,6)	229/137(59,8)	**<0,001**
Cefixime (CFM)	178/134(75,3)	235/178(75,7)	274/211(77)	263/171(65)	279/178(63,8)	554/334(60,3)	**<0,001**
Ceftazidime (CAZ)	119/85(71,4)	152/118(77,6)	211/176(83,4)	183/128(69,9)	218/148(67,9)	428/278(64,9)	**<0,001**
Cefepime (FEP)	148/121(81,8)	198/170(85,9)	233/189(81,1)	220/155(70,4)	223/156(69,9)	467/310(66,4)	**<0,001**
Ertapenem (ETP)	170/164(96,5)	229/226(98,7)	264/261(98,9)	257/254(98,8)	269/263(97,8)	541/525(97)	0,261
Imipenem (IPM)	136/133(97,8)	175/175(100)	226/225(99,6)	201/200(99,5)	228/228(100)	4627460(99,6)	0,069
Meropenem (MEM)	74/74(100)	90/90(100)	87/86(98,8)	115/114(99,1)	102/102(100)	185/185(100)	0,466
Amikacin (AN)	173/164(94,8)	228/210(92,1)	269/243(90,3)	253/228(90,1)	265/230(86,8)	543/478(88)	0,061
Gentamicin (GN)	180/150(83,3)	240/205(85,4)	275/226(82,2)	277/214(77,3)	289/222(76,8)	564/413(73,2)	**0,001**
Norfloxacin (NOR)	145/118(81,4)	203/164(80,8)	233/180(77,2)	220/142(64,5)	228/133(58,3)	463/244(52,7)	**<0,001**
Ciprofloxacin (CIP)	42/35(83,3)	33/23(69,7)	45/31(68,9)	52/36(69,2)	59/40(67,8)	106/57(53,8)	**0,020**
Nitrofurantoin (FT)	188/181(96,3)	241/230(95,4)	278/266(95,7)	280/262(93,6)	291/276(94,8)	568/524(92,2)	0,177
Fosfomycin (FOS)	177/175(97,9)	237/232(97,9)	271/266(98,2)	270/263(97,4)	281/274(97,5)	552/515(93,3)	**<0,001**

Inpatients and outpatients were also evaluated in our study. 73 (3.9%) of the patients were inpatients and 1777 were outpatients. In all comparisons for all antibiotics, antibiotic susceptibility was observed to be higher in outpatients than in inpatients (Figure 3).

Figure 3. Antibiotic susceptibilities in inpatients and outpatients (%).

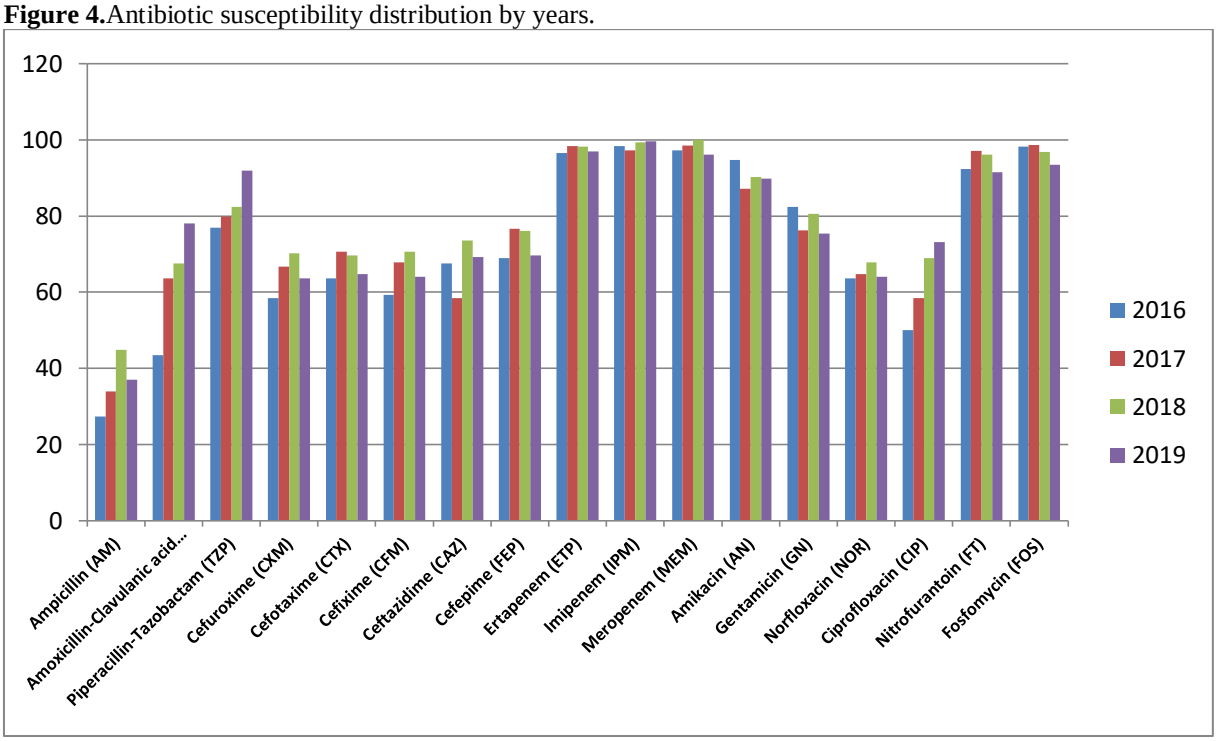


When the antibiotic susceptibilities in inpatients and outpatients were observed, the relationship between them in terms of ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefotaxime, cefixime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, nitrofurantoin and fosfomycin antibiotics, was found to be statistically significant (Table 2). In our study, six months data for 2016 and 2019 and all data for 2017 and 2018, were evaluated separately. We have shown antibiotic susceptibility distribution by years atFigure 4.

Table 2. Antibiotic susceptibilities in inpatients and outpatients**.**

	N / n (%)	N / n (%)	
Antibiotic Name	**Inpatient**	**Outpatient**	**p**
Ampicillin (AM)	55/13 (23,6)	1722/687 (39,9)	**0,022**
Amoxicillin-Clavulanic acid (AMC)	47/20 (42,5)	1599/1099 (68,7)	**<0,001**
Piperacillin-Tazobactam (TZP)	51/29 (56,9)	730/595 (81,5)	**<0,001**
Cefuroxime (CXM)	52/18 (34,6)	1776/1211 (68,2)	**<0,001**
Cefotaxime (CTX)	28/8 (28,6)	767/539 (70,3)	**<0,001**
Cefixime (CFM)	45/14 (31,1)	1722/1187 (68,9)	**<0,001**
Ceftazidime (CAZ)	46/18 (39,1)	1265/913 (72,2)	**<0,001**
Cefepime (FEP)	53/26 (49,1)	1437/1077 (74,9)	**<0,001**
Ertapenem (ETP)	45/42 (93,3)	1669/1635 (98,0)	0,071
Imipenem (IPM)	60/50 (83,3)	1370/1363 (99,5)	**<0,001**
Meropenem (MEM)	40/34 (85,0)	633/631 (99,7)	**<0,001**
Amikacin (AN)	60/50 (83,3)	1670/1500 (89,8)	0,161
Gentamicin (GN)	72/48 (66,7)	1756/1385 (78,9)	**0,020**
Norfloxacin (NOR)	46/25 (54,3)	1443/960 (66,5)	0,119
Ciprofloxacin (CIP)	33/17 (51,5)	316/212 (67,1)	0,971
Nitrofurantoin (FT)	57/36 (63,2)	1779/1684 (94,7)	**<0,001**
Fosfomycin (FOS)	52/46 (88,5)	1723/1664 (96,6)	**0,010**

Figure 4. Antibiotic susceptibility distribution by years.



When the antibiotic susceptibility and resistance rates were examined by years, it was found that the change in the resistance of isolates against ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefixime, ceftazidime, cefepime, imipenem, gentamicin, norfloxacin, nitrofurantoin, and fosfomycin was statistically significant (Table 3).

DISCUSSION

Antibiotic is the most commonly used as well as misused drug in our country as in the world. Overuse and misuse of antimicrobials bring about resistance problems. Antimicrobial resistance reduces the efficacy of antimicrobial drugs and makes the treatment of the patients difficult, costly, and even impossible. Turkey is the country with the highest consumption level of antibiotic in the world. In addition, it is the second country with the highest antimicrobial resistance rate among the countries with data in the world. For these reasons, antimicrobial resistance is a serious threat for our country (9).

Table 3. Antibiotic susceptibility distribution by years.

	N / n (%)				
Antibiotic Name	**2016**	**2017**	**2018**	**2019**	**p**
Ampicillin (AM)	113/31 (27,4)	438/149 (34,0)	780/350 (44,9)	462/ 171 (37,0)	**<0,001**
Amoxicillin-Clavulanic acid (AMC)	113/49 (43,4)	386/246 (63,7)	715/483 (67,5)	453/354 (78,1)	**<0,001**
Piperacillin-Tazobactam (TZP)	96/74 (77,0)	388/310 (79,9)	256/211 (82,4)	26/24 (92,0)	0,294
Cefuroxime (CXM)	113/66 (58,4)	435/290 (66,7)	839/589 (70,2)	463/295 (63,7)	**0,019**
Cefotaxime (CTX)	102/65 (63,7)	358/253 (70,7)	155/108 (69,7)	649/420 (64,7)	0,195
Cefixime (CFM)	113/67 (59,3)	438/297 (67,8)	778/550 (70,7)	460/295 (64,1)	**0,023**
Ceftazidime (CAZ)	40/27 (67,5)	82/48 (58,5)	732/539 (73,6)	464/321 (69,2)	**0,022**
Cefepime (FEP)	103/71 (68,9)	381/292 (76,6)	570/434 (76,1)	442/308 (69,7)	**0,039**
Ertapenem (ETP)	113/109 (96,5)	383/377 (98,4)	779/765 (98,2)	463/449 (97,0)	0,293
Imipenem (İPM)	125/123 (98,4)	182/177 (97,2)	728/724 (99,4)	461/459 (99,6)	**0,019**
Meropenem (MEM)	36/35 (97,2)	390/384 (98,5)	267/267 (100,0)	26/25 (96,1)	0,114
Amikacin (AN)	113/107 (94,7)	415/362 (87,2)	746/673 (90,2)	464/417 (89,9)	0,111
Gentamicin (GN)	125/103 (82,4)	414/316 (76,3)	834/672 (80,6)	460/347 (75,4)	**0,049**
Norfloxacin (NOR)	102/65 (63,7)	358/232 (64,8)	602/408 (67,8)	436/279 (64,0)	**<0,001**
Ciprofloxacin (CIP)	12/6 (50,0)	82/48 (58,5)	219/151 (69,0)	26/19 (73,1)	0,184
Nitrofurantoin (FT)	119/110 (92,4)	438/412 (97,1)	838/805 (96,1)	457/418 (91,5)	**0,007**
Fosfomycin (FOS)	113/11 (98,2)	384/379 (98,7)	837/810 (96,8)	460/430 (93,5)	**0,001**

E. coli is the most common bacterial pathogen among community-acquired and hospital-acquired UTI agents. Today, as in the world, there is a dramatic increase in resistance to some antibiotics used in the treatment of urinary tract infections caused by uropathogen *E. coli* strains in our country. Due to the rapid spread of antimicrobial resistance problem, treatment failures are encountered while treating UTI caused by *E. coli*. Treatment failures lead to prolongation, recurrence or chronicity of the infection, thus leading to increased treatment costs and reduced life quality of the patient.

The differences are observed in antibiotic susceptibility or resistance of *E.coli* strains, which are primary pathogens, between regions and even between centers of the same region as a result of the differences in the frequency and policies of antibiotic use. It is noteworthy that resistance rates are higher in inpatients than in outpatients. Periodic detection of antibiotic resistance is important in terms of determining effective treatment and preventing loss of time and money in treatment. The Infectious Diseases Society of America recommends regional surveillance to monitor changes in the susceptibilities of uropathogens in certain regions (1). According to the EARSS-Net 2016 report, the mean resistance of invasive *E. coli* isolates of the European Union (EU) countries was determined to be 12.4% for third generation cephalosporins, 10.4% for aminoglycosides and 0.1% for carbapenem. Our data are well above EU averages (10).

In a study conducted in US showing antibiotic resistance in UTIs in 2018, it was reported that the susceptibility of nitrofurantoin had not changed for 10 years and it was around 92.2%, the susceptibility of fosfomycin was 98.9%, the resistance to fluoroquinolones increased to 11.8%, the amoxicillin-clavulanic acid resistance was 4% and the susceptibility levels in cephalosporins were approximately 90%. These sensitivity rates in America are quite good compared to the data of our country (2).

In a multicenter study conducted in Russia in 2019, *E. coli* (49.2%) was the most frequently isolated species among uropathogens. Resistance to ampicillin was 50%, resistance to levofloxacin was 28.8%, and resistance to cefuroxime was found to be 21%, whereas resistance to imipenem (0.7%), amikacin (0.9%), nitrofurantoin (4.5%) and fosfomycin (1.2%) were found to be very low (11). Resistance data of our country are quite above these rates.

According to the 2016 surveillance data of the National Antimicrobial Resistance Surveillance System (NAMRSS) including 4342 *E. coli* isolates, ceftriaxone/cefotaxime resistance from 3rd generation cephalosporins was found to be 51.1%, ceftazidime resistance was 54.2%, gentamicin resistance from aminoglycosides was 29.3% and amikacin resistance was 8.7% in the invasive *E. coli* isolates. Ciprofloxacin resistance from

fluoroquinolone group was found to be 54.2%, levofloxacin resistance was 53.5%, and imipenem, meropenem and ertapenem resistance from the carbapenem group were 5.2%, 3.1% and 8.2%, respectively. Colistin resistance was found to be 3% in 2742 isolates. Multidrug resistance (resistance to fluoroquinolones, 3rd generation cephalosporins and aminoglycosides) was found in 25% of isolates (10). In our study, amikacin resistance was found to be 10.3% higher than this study, whereas, the resistance rates to all other antibiotics were found to be lower.

In a study conducted by Yousefi Rad et al. in Ankara, 29.2% of 677 *E. coli* strains were found to be resistant to ciprofloxacin, 64.8% to ampicillin, 38.6% to trimethoprim / sulfamethoxazole, 13.9% to ceftriaxone, 4.6% to amikacin and 6.1% to nitrofurantoin (12).

In a study conducted by Deveci et al. in Mardin Kiziltepe, all *E. coli* strains isolated from urine samples were susceptible to fosfomycin, whereas their resistance to ciprofloxacin, imipenem, piperacillin-tazobactam and ceftazidime were found to be 33.3%, 49.1%, 36.8% and 45.6%, respectively (13).

In a study conducted by Gozukucuk et al. in Istanbul, 191 *E. coli* strains were isolated and the resistance rates were found to be 40% in trimethoprim-sulfamethoxazole, 19.8% in ciprofloxacin, 19.2% in ceftriaxonde and 12.4% in nitrofurantoin (14). In our study, nitrofurantoin resistance with 5.8% was lower than this study, whereas, Ciprofloxacin resistance was higher with 34.1%.

In a study of 285 *E. coli* isolates in the province of Van, Zengin et al. found the resistance rates of ampicillin, amoxicillin-clavulanic acid, cefuroxime axetil, ceftazidime, ceftriaxone, cefaperozone/sulbactam, gentamicin, amikacin, ciprofloxacin, norfloxacin to be 55%, 42%, 30%, 15%, 18%, 6%, 10%, 5%, 33%, 34% and 45%, respectively. All strains were found to be susceptible to meropenem and imipenem (15). In our study, common antibiotics were generally found to have higher resistance.

In a study conducted by Tekin et al. in Diyarbakir, 3279 *E. coli* strains were isolated from urine samples and 97.8% of the isolated strains were susceptible to fosfomycin, 41.1% to ciprofloxacin, 39.8% to trimetoprim-sulfamethoxazole and 22.2% were found to be susceptible to amoxicillin-clavulanic acid (16). Higher susceptibilities were determined in our study.

In a study conducted by Yilmaz et al. in Izmir, 8975 isolated *E. coli* strains were resistant to antimicrobial drugs; ampicillin 66.9%, cefazolin 42.9%, cefuroxime 30.9%, ceftazidime 14.9%, ceftriaxone 28%, cefepime 12%, amoxicillin-clavulanic acid 36.9%, trimethoprim sulfamethoxazole (TMP-SXT) 20% , ciprofloxacin 49.9%, amikacin 0.3%, gentamicin 24%, nitrofurantoin 0.9% and fosfomycin 4.3%, whereas, there was no resistance detected to imipenem and meropenem (17). All these studies show that the susceptibility results of different time and centers are quite different from each other. In our study, resistance rates were found to be the highest in ampicillin which is an aminopenicillin, ciprofloxacin and norfloxacin from fluoroquinolone group, cefuroxime, cefixime, cefotaxime, ceftazidime and cefepime which are the 2nd, 3rd and 4th generation cephalosporins, and amoxicillin-clavulanic acid which is a penicillin group. Amikacin susceptibilities were found to be lower in our study as compared to other studies conducted in our country. Considering the socioeconomic structure of our region, the use of unconscious antibiotics and the selection of antibiotics for the treatment without adequate evaluation of the susceptibility data by the physician, may be considered among the causes of resistance development. Therefore, we believe that regular surveillance of available antibiotic data at different time intervals will help to select the appropriate antibiotic and prevent the increase of antibiotic resistance rates in our region.

In particular, the resistance developed to amoxicillin-clavulanic acid and fluoroquinolones prescribed empirically in routine treatment, has led to the frequent use of both more expensive and parenterally administered cephalosporins. This situation has both increased treatment and care costs and reduced patient compliance, and inappropriate and excessive use of these antibiotics has led to new antimicrobial resistance problems.

When the results obtained from our hospital data are evaluated, fosfomycin may be the first choice in the empirical treatment of community-acquired urinary tract infections. It can be used as the first choice in the empirical treatment of UTIs developed with *E. coli* strains, due to its high patient compliance with its single-dose ease of use, extremely low resistance rates, high clinical eradication rates, and low side effects. We also believe that nitrofurantoin should be among the drugs that can be preferred in uncomplicated UTI due to its low resistance rates and ease of use. It is thought that antibiotic treatment should be determined according to the results of culture antibiogram, especially in nosocomial infections due to their high resistance rates.

The principal limitation of this study are that we conducted this research in a single center and that we included only adult patients in the study. Another limitation related to our research work can not be added to the post-treatment patient data.

CONCLUSION

As a result, high resistance rates have been observed to various antibiotics commonly used in the empirical treatment of urinary tract infections in the past years. Regular surveillance of regional and

temporal antibiotic susceptibility data will guide the empirical treatment of urinary tract infections caused by *E. coli* . Our study is the most comprehensive and recent study in our province. Considering the data of our hospital, due to low resistance rates, fosfomycin and nitrofurantoin may be preferred primarily in primary care and the empirical treatment of uropathogen *E. coli* bacteria. According to the obtained results, new antibiotic usage policies should be developed urgently and regional antibiotic resistance rates should be prevented.

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RESEARCH ARTICLE

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The Prognostic Evaluation of West Syndrome Patients: A Retrospective Observational Study

ABSTRACT
Objective: To determine neurodevelopmental and seizure prognoses in our patients with West syndrome receiving adrenocorticotropic hormone (ACTH) therapy, and to identify the factors affecting these.
Methods: We determined the demographic factors, previous seizure histories, ACTH use and drug response times, and etiological reasons of 34 patients diagnosed with West syndrome in our clinic at 3-24 months old and receiving ACTH therapy. We also investigated their neurological development and its effect on seizure prognosis.
Results: We found a significant relationship between patients experiencing seizures before diagnosis and subsequent seizure prognosis. We also defined a later response to ACTH and poorer neurodevelopment and seizure prognoses in patients with symptomatic etiologies. Global developmental delay was identified in 76% of all cases, and seizures persisted despite antiepileptic drugs in 62%.
Conclusions: Symptomatic etiological factors in West syndrome adversely affect the neurodevelopmental process and subsequent seizure prognosis.

Keywords: West Syndrome, ACTH Therapy, Spasm, Neurodevelopmental Delay, Prognosis

West Sendromlu Hastaların Prognostik Değerlendirilmesi: Retrospektif Gözlemsel Çalışma

ÖZET
Amaç: ACTH tedavisi alan West sendromlu hastalarımızın ileri dönemdeki nörogelişimsel prognozu ve nöbet prognozunun saptanması ve bunlara katkı sunan faktörlerin belirlenmesidir.
Gereç ve Yöntem: Kliniğimizde 3-24.aylarında West sendromu tanısı alarak ACTH tedavisi uygulanan 34 hastanın demografik faktörleri, nöbet şekilleri, öncesindeki nöbet öyküleri, ACTH kullanımı ve yanıt süreleri, etyolojik faktörlerini belirledik. Bunların nörolojik gelişim ve nöbet prognozuna etkisini araştırdık.
Bulgular: Hastaların tanı öncesinde nöbeti olmasının ilerideki nöbet prognozu ile ilişkisini anlamlı bulduk. Ayrıca semptomatik etyolojili hastalarda ACTH yanıtını daha geç; nörogelişim ve nöbet prognozunu ise daha kötü saptadık. Tüm hastaların %76'sında global gelişme geriliği, % 62'sinde antiepileptik ilaclara rağmen devam eden nöbetler belirledik.
Sonuç: West sendromunda semptomatik etyolojik faktörler nörogelişimsel süreci ve ileriki dönemdeki nöbet prognozunu olumsuz etkilemektedir.

Anahtar Kelimeler: West Sendromu, ACTH Tedavisi, Spazm, Nörogelişimsel Gerilik, Prognoz

INTRODUCTION

West syndrome (WS) is the most common infantile devastating epileptic encephalopathy, with an incidence of 3-4.5/100,000. It is characterized by hypsarrhythmia on electroencephalograms (EEG), spasm seizures, and developmental arrest or delay (1,2). Epilepsy is difficult to treat due to various factors, including late recognition of spasms by both parents and physicians, and weak response to antiepileptic drugs. Although neurodevelopmental prognosis in children with WS can range from normal to severe neuromotor retardation, prognosis is generally poor (3-5). Treatment of spasms in the early period has been linked to good prognosis (6). The purpose of this study was to determine prognoses concerning subsequent neurodevelopment and persistence of seizures in our patients with West syndrome and the factors affecting these.

MATERIAL AND METHODS

Thirty-four patients with WS diagnosed at 3-24 months in 2015-2018 in our university hospital's pediatric neurology clinic and receiving adrenocorticotropic hormone (ACTH) therapy were investigated retrospectively. WS was diagnosed in patients with clinical developmental arrest, spasm seizures, and hypsarrhythmia at EEG examination (1,2). Patients with inadequate attendance for clinical controls or exhibiting spasm but with no hypsarrhythmia determined at EEG were excluded from the study. Our patients were followed-up for a mean two years. Approval for the study was granted by our medical faculty ethical committee (No. 2020/138).

Patient age, sex, and age at diagnosis were determined. Patients were divided based on type of seizure, those with spasm and those with other seizure types. Patients with histories of seizure before WS were identified.

Complete blood count, blood biochemistry, lactate, pyruvate, ammonia, serum amino acids, urine organic acids, free and total carnitine analysis, plasma acylcarnitine analysis, uric acid, and biotinidase activity were investigated to determine etiology in all cases. Various genetic examinations were performed as required in case of dysmorphic findings and other data (chromosome analysis, CGH array, single-gene sequencing, etc.). Whole exome sequencing/whole genome analysis could not be performed due to unavailability. EEG examinations were carried out before and after ACTH therapy. Patients were classified at cryptogenic or symptomatic, depending on their etiology. Cryptogenic cases were defined as those with no previous history of convulsion, exhibiting normal development, with normal neuroimaging, and with no underlying disease. Patients with previous seizures, abnormal neurological development, with anomalies detected at neuroimaging, or with abnormality detected at other examinations were defined as symptomatic. Patients diagnosed with WS due to diagnosis of tuberous sclerosis were also excluded.

Our unit's standard ACTH (tetracosactide) therapy protocol was applied intramuscularly in the form of 1 mg for patients weighing >10 kg and 0.5 mg for those weighing <10 kg administered three times weekly in the first two weeks, twice weekly in the subsequent two weeks, and once weekly in the following four weeks. Patients were assessed in terms of ACTH response at the end of the first and second weeks and the first and second months. Times elapsing to reactions to ACTH were also determined. At the end of ACTH therapy, types of response were divided into four categories – patients exhibiting complete response (patients whose spasms disappeared and whose hypsarrhythmia patterns at EEG resolved with ACTH therapy alone), those exhibiting partial response (spasms decreasing by more than 50% with additional drug requirements), those with no response to ACTH, and patients with recurrence of spasms one month after ACTH therapy. All patients started on ACTH therapy were also given pyridoxine from the outset.

Seizure prognosis was assessed at one year. Patents were divided into four groups – seizure-free without medication (not using any antiepileptic drug (AED)), seizure-free with medication (using AEDs and without seizures), partial control (fewer than two seizures a month with AEDs), and uncontrolled (patients with refractory seizures).

Neurological prognosis was assessed after one year using Denver II developmental tests and clinical examinations (7). Patients were divided into three groups - normal neurological development, isolated speech delay (delay in a single area), and global developmental delay (GDD).

Age, sex, age at diagnosis, time elapsed between diagnosis and spasms, presence of seizure prior to spasms, type of seizure, effects of etiology of seizure prognosis and neurological prognosis, and type of response to ACTH were determined.

Statistical Analysis: The general characteristics of the participants were summarized using descriptive statistics. Continuous variables were calculated as mean ± standard deviation and median (range). Categorical variables were expressed as frequency (n) and percentages (%). Categorical variables were analyzed using Fisher's exact test. The Wallis and Mann Whitney U tests were applied to compare continuous variables between groups due to non-normality of distribution. Spearman's correlation coefficient was calculated to assess correlations between continuous variables. Significance was set at $p<0.05$, and all analyses were performed on Statistical Package for Social Sciences 25.0 for Windows (SPSS Inc., Chicago, Illinois, USA) software.

RESULTS

The patients consisted of 15 girls and 19 boys. Median age at start of treatment was nine months (2-36). The mean time elapsing to response to ACTH therapy was 12 days. Treatment lasted between two and eight weeks, depending on the response (Table 1).

Table 1. Descriptive Statistics

Age at onset (months)	
Mean ± SD	14.03 ± 11.26
Median (range)	9.0 (2-36)
Length of follow-up (years)	
Mean ± SD	1.92 ± 1.58
Median (range)	2 (0.2-9)
Presence of seizure before infantile spasm n(%)	15 (44.1)
Seizure types n(%)	
Spasm	21 (61.8)
Generalized tonic seizure	3 (8.8)
Multiple seizure types	10 (29.4)
Cryptogenic/symptomatic n(%)	
Cryptogenic	10 (29.4)
Symptomatic	24 (70.6)
Response to treatment(days)	
Mean ± SD	11.70 ± 6.51
Median (range)	12 (3-28)
Length of treatment (weeks)	
Mean ± SD	5.5 ± 2.11
Median (range)	4 (2-8)
Etiology n(%)	
Unknown	10 (29.4)
Prenatal	11 (32.3)
Perinatal	8(23.5)
Postnatal	5(14.7)

Treatment was discontinued at the end of two weeks in two patients with no response. Twenty patients exhibited complete response to ACTH therapy, eight exhibited partial response, and two exhibited no response, while recurrence was observed in four. Time between diagnosis of WS and onset of spasms ranged between two and 90 days. No relationships were determined between type of response to ACTH therapy and age, sex, age at diagnosis, time between diagnosis and spasms, time to response to ACTH therapy, or type of response to ACTH. These parameters exhibited no correlation with prognosis (Table 2).

Table 2. Parameters compared in terms of responses

Time(days)	**Recurrence(n=4).**	**Partial (n=8)**	**Full(n=20)**	**Absent(n=2)**	**p**
Diagnosis time	8 (3-12)	12 (5-36)	8 (4-36)	19 (2-36)	0.596
Lag time	44 (3-60)	30 (7-90)	14 (2-90)	52 (14-90)	0.354
Day of response	7 (6-10)	14 (7-28)	14(3-28)		0.202

Data are expressed as median (range). The Kruskal-Wallis test was performed.

Twenty-one patients presented with spasm only, three with generalized tonic seizure, and 10 with multiple seizures. Fifteen (44%) patients had seizures prior to spasms. No relationship was observed between presence of preceding seizure and type of response to ACTH, duration of response, and neurological prognosis. However, it was correlated with seizure prognosis (p=0.04). No correlation was also observed between type of seizure and type of response to ACTH, duration of response, neurological prognosis, and seizure prognosis.

Ten patients were cryptogenic, and 24 were symptomatic. Later response to ACTH was determined in the symptomatic group (p=0.04). Seizure prognosis (p=0.01) and neurological prognosis (p<0.01) were also markedly worse in this group. Neurodevelopmental delay was also more severe in the symptomatic group. Twenty-three of the 24 symptomatic patients exhibited moderate or severe developmental delay. However, neurodevelopmental delay was not observed in the cryptogenic group. No relationship was determined between type of response and etiology (Table 3).

Underlying etiological diagnoses in the 24 patients (70.5%) were Dandy-Walker in one, genetic syndromes (Kleefstra, Down, and Aicardi) in three, cortical dysplasia in four, congenital metabolic disease in two, meningomyelocele in one, perinatal hypoxia in eight, and postnatal events in five. The remaining 10 patients (29.5%) were in the cryptogenic group.

Table 3. Response and prognosis by etiology

Category	Cryptogenic (n=10)	Symptomatic (n=24)	p
Response type			0.063
Recurrence	1 (10.0)	3 (12.5)	
Partial	0 (0)	8 (33.3)	
Full	9 (90.0)	11 (45.8)	
Absent	0 (0)	2 (8.3)	
Day of response	7.0 (3-14	14(5-28)	**0.040**
Neurodev prognosis			**<0.001**
Normal	4 (40.0)	1 (4.2)	
Speech delay	3 (30.0)	0 (0)	
Mild neuromotor retardation	3 (30.0)	3 (12.5)	
Moderate neuromotor retardation	0 (0)	7 (29.2)	
Severe neuromotor retardation	0 (0)	13 (54.2)	
Seizure prognosis			**0.001**
Seizure-free without medication	3 (30.0)	0 (0)	
Seizure-free with medication	6 (60.0)	4 (16.7)	
Controlled seizure with medication	0 (0)	6 (25.0)	
Partially controlled seizure	0 (0)	1 (4.2)	
No control	1 (10.0)	13 (54.1)	

Data are expressed as n(%) and median (range). The Mann–Whitney U test and Fisher's exact test were applied, and p values < 0.05 were significant

In terms of seizure prognosis, three patients were seizure-free without medication, 10 were seizure-free with medication, six were under control with medication, one was under partial control, and 14 were uncontrolled. Generally, prospective prognosis was poorer in WS. The number of seizure-free patients with or without medication was only 13 (38%) (Table 4). A single AED had been used in addition to ACTH by 12 patients, two AEDs by two, and multiple AEDs by 17. The most frequently used single AED was phenobarbital, employed in five patients, followed by vigabatrin in four, and valproic acid in three. Other additionally employed multiple AEDS included levetiracetam, clobazam, and sultiame.

No association was observed between these and seizure prognosis or neurological development. When patients were divided in terms of neurological prognosis, normal neurological development was present in five (15%), isolated speech delay in three (9%), and GDD in 26 (76%). Delay in more than three developmental areas was present in 13 patients with global developmental delay.

Our patients generally exhibited more than one side-effect due to ACTH use, the most common being irritability, weight gain, and infection. No statistical comparison was possible due to the retrospective nature of the data.

Table 4. Response and prognosis by presence of seizure before infantile spasm

Seizure history	No (n=19)	Yes (n=15)	p
Response type			0.154
Recurrence	2 (10.5)	2 (13.3)	
Partial	3 (15.8)	5 (33.3)	
Full	14 (73.7)	6 (40)	
Absent	0 (0)	2 (13.3)	
Day of response	10.0 (3-28)	14(6-28)	**0.363**
Neurological Prognosis			**0.063**
Normal	4 (21.1)	1 (6.7)	
Speech delay	3 (15.8)	0 (0)	
Mild neuromotor retardation	5 (26.3)	1 (6.7)	
Moderate neuromotor retardation	3 (15.8)	4 (26.7)	
Severe neuromotor retardation	4 (21.1)	9 (60.0)	
Seizure Prognosis			**0.042**
Seizure-free without medication	3 (15.8)	0 (0)	
Seizure-free with medication	6 (31.6)	4 (26.7)	
Controlled seizure with medication	5 (26.3)	1 (6.7)	
Partially controlled seizure	1 (5.3)	0 (0)	
No control	4 (21.1)	10 (66.7)	

Data are expressed as n(%) and median (range). The Mann–Whitney U test and Fisher's exact test were applied, and p values < 0.05 were considered significant.

DISCUSSION

The most important aim in the treatment of infantile spasm is to achieve good cognitive development. Unfortunately, however, neurological development is significantly affected in a large proportion of patients, depending on the etiology (8). The present study identified factors associated with neurodevelopmental and subsequent seizure prognosis in patients diagnosed with infantile spasm receiving ACTH. The male:female ratio in this study was 56/44, a figure compatible with the previous literature (9). Consistent with the present research, many previous studies have found no effect of gender on prognosis (6,10,11). There are also studies suggesting that male gender leads to poorer prognosis (12). Mean age at diagnosis was nine months. WS emerges in infancy, peaking between four and 10 months (8). We attribute the higher value in the present study compared to previous research to late recognition of the disease by both physicians and families due to low awareness concerning it, particularly in developing countries (5).

Analysis of responses to ACTH therapy revealed that the time elapsing between onset and diagnosis of spasms ranged between two and 90 days. Times ranging between 25 and 45 days have been reported in the previous literature (13). The pronounced difference between these figures and our findings may be due to our patient population being from various different provinces since ours is the only pediatric neurology center in the Western Black Sea region of Turkey. The mean response time to ACTH therapy was 12 days, with differing figures being reported depending on patient evaluation times in previous studies (14). Fifty-seven percent (20 patients) of our patients exhibited complete response to ACTH therapy. While figures consistent with those of the present study have been reported, rates as high as 76% have also been observed (15,16). No correlation was observed in the present study between time elapsing between onset and diagnosis of spasms and type of response to ACTH. Times between onset and treatment in the literature have generally been compared with neurodevelopmental prognosis, and a time less than four weeks has been linked to better neurological prognosis (17). A longer period has been associated with poor neurological development (14,18). As in the present research, this period has been associated with prognosis in some studies (10).

Seizure was present before spasms in 44% (15 patients) of patients. Previous seizure was also associated with subsequent seizure prognosis ($p=0.04$). In contrast to the previous literature, we observed no relationship between neurological prognosis and seizures prior to WS. However, several studies have reported that the presence of seizures before WS is associated with poor neurodevelopmental prognosis and also seizure prognosis (6,10,18).

Etiology has been identified as the most important factor affecting prognosis (14). Under the current classification, known etiology is described as symptomatic, and unknown etiology as cryptogenic (19). In the present study, the etiology was cryptogenic in 29% of cases and symptomatic in 71%. The proportion of WS cases classified as symptomatic has gradually risen with the use of improved metabolic and genetic neuroimaging techniques. Underlying disorder has been reported in approximately 60% of surviving patients and 90% of autopsy cases (20,21). Prenatal causes such as central nervous system malformations, intrauterine events, tuberosclerous complex (TSC), metabolic disorders, or genetic syndromes are present in approximately 50% of cases. Hypoxic-ischemic encephalopathy is one of the neonatal causes, while postnatal causes include trauma, infection, and rarely tumors (4). TSC is an important cause of WS (22). Patients with TSC were not included in the present study. Our patients' diagnoses were prenatal in 11 cases, perinatal in eight, postnatal in five, and unknown in 10. Consistent with previous research, the rate of hypoxia-related etiology in the present study was 24%. Rates between 6% and 69% have been reported in the literature (11-12,23-25).

Later responses to ACTH were observed in our symptomatic patient group ($p=0.04$), and subsequent seizure prognosis and neurodevelopmental prognosis were both poorer ($p<0.01$). We encountered no data in the literature concerning later responses to ACTH in symptomatic groups. Symptomatic etiology is known to worsen subsequent seizure prognosis and neurodevelopmental prognosis (10,26,27).

Eighty percent of the cases in the cryptogenic group with normal development prior to onset of spasm seizures also exhibited almost normal neurological development in the subsequent period (14). All three of our seizure-free patients, and four of the five with normal neurological development, were in the cryptogenic group. Close to normal development may be observed in 20% of patients in the symptomatic group (9,14,28). Hypoglycemia was determined in the etiology of our sole patient with normal neurological development and controlled seizures in the symptomatic group. Etiological factors exhibiting good prognosis with symptomatic etiology in previous studies include neonatal hypoglycemia, Down syndrome, stroke, periventricular leukomalacia, and neurofibromatosis (14).

Generally, and irrespective of etiology, 76% of all our patients had poor neurological development, and seizures other than spams were present in the subsequent period in 21 patients. Riikonen showed similar rates in several studies in a review article from 2020 (14). Despite advances

in treatment, WS still has a poor reputation from that perspective.

The limitations of this study include its retrospective nature and the low patient numbers. ACTH was used as the first-choice treatment in all our patients. Medications such as vigabatrin, phenobarbital, valproic acid, levetiracetam, clobazam, and sultiame were employed in cases with insufficient responses to ACTH. However, these data could not be subjected to statistical comparison due to our low patient numbers. Vigabatrin is the most commonly used agent in the treatment of tuberous sclerosis-related WS, and the second most common in WS associated with other etiological factors (2,8). However, despite being an effective option, vigabatrin is not frequently used in our clinic since it is not produced in Turkey, and is difficult to obtain. The most common side-effects of ACTH in our patients were irritability, weight gain, and infection, and the majority exhibited more than one symptom. Since data on this subject were insufficient in some patients, it was not subjected to statistical analysis.

CONCLUSIONS

The presence of seizure before diagnosis of WS and symptomatic etiological factors were found to be related to unfavorable prognosis. Consistent with previous studies, response to ACTH therapy was achieved later in WS patients with symptomatic etiologies. The prevention of diseases such and infection, which occupy an important place in the etiology, the early diagnosis of treatable metabolic diseases, and prenatal counseling management in patients at genetic are at least as important as treatment. Forms of treatment supported by new studies are needed for a promising diagnosis in WS.

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CASE REPORT

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The Use of Hypnosis to Prevent the Gag Reflex in Dentistry: Two Case Reports

ABSTRACT
In dental practice, gagging can become a severe problem, and various methods are used for treatment of this problem. In this study, hypnotherapy was applied to two patients to whom dental procedures could not be carried out due to gagging. The first case was one in which it was thought that the patient would be unable to use dentures due to gagging, and, after hypnotherapy, it was ensured that she was able to use her dentures without any problem. The second case was a hypnotic-resistant case, and the impression procedure was carried out only after hypnotherapeutic suggestion. Our study revealed that hypnosis can be used successfully in the treatment of a gagging problem that is observed during dental practices and cannot be overcome.

Keywords: Gag Reflex, Dental Procedures, Hypnosis.

Diş Hekimliğinde Öğürme Refleksinin Önlenmesinde Hipnozun Kullanımı: İki Olgu Sunumu

ÖZET
Diş hekimliği uygulamalarında öğürme ciddi bir sorun haline gelebilir. Tedavide değişik yöntemler kullanılmaktadır. Bu çalışmada öğürme nedeniyle uygulamaları yapılamayan iki hastada hipnoterapi uygulanmıştır. Birinci olgu protezini öğürme nedeniyle kullanamayacağı düşünülen bir olgudur ve hipnoterapi sonrasında protezlerini sorunsuz bir şekilde kullanması sağlanmıştır. İkinci olgu hipnoza dirençli bir olgu olup ölçü alma işlemi ancak hipnoterapötik telkin sonucunda sağlanmıştır. Çalışmamız diş hekimliği uygulamaları sırasında görülen ve baş edilemeyen öğürme probleminin tedavisinde hipnoz uygulamasının başarı ile kullanılabileceğini göstermesi açısından önemlidir.

Anahtar Kelimeler: Öğürme Refleksi, Dental İşlemler, Hipnoz.

INTRODUCTION

Gagging can become a significant problem during taking impression for dental dentures, periapical radiography, the restoration of posterior teeth, and the use of full removable dentures. In dental applications, some precautions can be taken when gagging is at a level that prevents the procedure. Breathing exercises, sedation, acupuncture, hypnosis and general anesthesia practices in severe cases are recommended (1-2). Hypnosis is a traditional and complementary medicine method that can make medical procedures more comfortable by relieving patients in many areas (3). In this study, the results of hypnosis practice in two patients who experienced severe gagging problems during taking impression for the denture were presented.

CASES

CASE 1

A 65-year-old female patient who required that prosthetic procedures be performed on the maxillar jaw had tooth supported fixed dental prosthesis on her jaw, and the abutment teeth were mobile. It was observed that the patient experienced severe gagging during the examination. No psychodynamic cause was detected in the patient's anamnesis. The extraction of the teeth numbered 11,12,15,16,22,23,25, and 26, which were abutment teeth after the removal of our patient's bridge, was planned, and then an immediate denture was planned. Topical anesthetic was applied to the upper palate of our patient during taking an impression. The impression was taken after this short-term procedure. The complete palatal denture was planned for the patient with an insufficient tuber area; however, the patient seemed to have difficulty in wearing this denture in the long term. Hypnotherapy was planned to allow the patient to use the denture that would be made.

First Session: The patient was informed about hypnosis in order to relieve the patient's tension and anxiety, her questions about hypnosis were answered and she signed the informed consent form.

In the first session, the patient was put into a state of hypnosis. Suggestions of relaxation were given. The patient's anxiety was decreased by means of positive imagination suggestions, and the session was terminated by giving a post-hypnotic suggestion so that the patient would to enter into a deeper state of hypnosis in a shorter time in the next hypnosis session.

Second Session: The patient was put under deep hypnosis by using a fast hypnosis technique. The patient was relieved, and her anxiety was decreased with the suggestions of relaxation by using the imagination method. The impression tray was placed in the mouth without any impression material and kept in the mouth for thirty seconds. The procedure was repeated five times, and the patient was observed not to gag. In the meantime, therapeutically positive suggestions continued to be given to the patient.

A denture was prepared and delivered.In the follow-up, it was revealed that the patient had no denture-related gagging problems.

CASE 2

A 40-year-old female patient who was referred to our clinic planned to undergo implant treatment due to the lack of single tooth numbered 26. It was observed that even the contact of the impression tray on the palatal area of the patient during the implant-supported denture process triggered the gag reflex. This situation taking impression; therefore, hypnotherapy was recommended to the patient.

The patient was informed about hypnosis in order to relieve the patient's tension and anxiety, her questions about hypnosis were answered, and she signed the informed consent form.

In the first session, an attempt was made to put the patient into a state of hypnosis. However, it was determined that the patient was resistant. At this point, the authoritarian awakening method, which is applied in resistant cases, was employed (4). For this purpose, the patient was given the suggestion that she would overcome the gag reflex if she breathed loudly and held her breath for a certain period of time while gagging. Hypnotherapeutic suggestion was initiated due to the patient's resistance to the suggestion. Glove anesthesia was obtained in the patient with this practice. After hypoanesthesia was performed on the patient's right hand, she was asked to transfer this anesthesia to her throat, and the suggestion that she could not gag even though she wanted to gag was repeated many times. No gagging was observed when the impression procedure was initiated, and the fixed implant supported denture was completed successfully.

DISCUSSION

There may be many different reasons for the gagging problem. It is possible to examine them by listing them as follows: a central state caused by signals coming from the brain; a migraine or other types of headaches that can cause problems such as vomiting, nausea, and gagging; inner ear disorders that can cause balance problems (changes in the amount of fluid in the inner ear, an increase or decrease, and other conditions leading to balance problems); meningitis and other head traumas that cause the intracranial pressure to change and possibly lead to gagging or vomiting; tumors, concussions of the brain, and other diseases/conditions that affect and destabilize blood circulation or blood pressure; seasickness or motion sickness; diseases such as stomach ulcers and reflux disease that irritate the stomach; and habits or medications such as smoking, ibuprofen, aspirin, and anti-inflammatory drugs (2).

Although gagging is a common temporary problem during dental practices (taking a impression for dentures, periapical radiography images, restorations of the posterior teeth, the use of removable complete dentures, etc.), in some patients, it can occur at a level that prevents performing procedures in some patients.

The gag reflex can be frustrating for some individuals during dental treatments. This may be due to the physiological or psychological reasons of the reflex as well as fear of the dentist. Individuals with this condition are panicked, fearing that they will have difficulty in breathing or swallowing. Individuals with an overactive gag reflex can also have difficulty in brushing teeth (5).

If the denture bases cannot be tolerated in patients using dentures, liquids with topical anesthetic content (benzocaine (14%), butyl aminobenzoate, (2%), tetracaine hydrochloride 2%)) can be dropped on gauze pad and applied to the back of the arch. Making the post-dam area of the denture inclined can also reduce gagging problems and increase comfort (5).

Several treatment methods have been recommended in the case of the stimulation of severe gag reflex while working in the mouth. In the marble technique, the patient puts marbles in her mouth and keeps the marbles in her mouth except for sleeping and eating, and, after a while, she becomes unresponsive to the stimulus. İn the button technique the patient keeps buttons or plastic discs in her mouth for two hours a day and keep a diary about it. If the patient is involved in the gagging process during the taking impression, she/he should be guided to take a slow and deep breath (6).

Considering that severe gagging is due to a psychogenic cause, therapy methods can be used.

The psychotherapy knowledge and training of dentists are generally inadequate (7).

One of the most effective therapy methods is hypnotherapy (2). Hypnotherapy is a traditional and complementary medicine method that can be applied by trained dentists.

In our study, the dentist who applied hypnotherapy in both cases had a certified hypnotherapy certificate from the Ministry of Health. Before hypnosis, suggestion-based techniques can be another approach for overcoming the gag reflex. Relaxation exercises can be offered to the patient several times a week, so it may be advisable for the patient to listen to relaxation procedures and a voice recording in which the muscles relax. The stimulation of the maxillary region, alveolar region, and palatal dome with a toothbrush, a spoon, mouthwash, or by other means can be recommended.

In the stick technique, a broom handle is placed in front of the patient so that the hole is in the middle, and the patient is encouraged to look at that point until the impression process is over. As long as the patient is focused on that point, reportedly the gagging will disappear (this technique is a combination of distraction and suggestion) (8). If the denture base cannot be tolerated by the patient, the patient should practice at least five days a week, three times a day. The patient should be encouraged to keep the dentures in their mouths at intervals of thirty seconds for increasingly longer time periods until the patient's gag reflex is eliminated (5). At least four or five sessions may be required for the patient to overcome the gag reflex (7). The number of sessions was reduced in the patients with whom we performed hypnotherapy. This may be due to therapeutic suggestions given to the patient during hypnosis. Clarke, Stephen, & Persichetti (9) agree that the suggestions given in the gag reflex reduces the condition from day to day.

The first of our cases was an individual inclined to hypnosis. The score of Tastan Suggestibility Scale was four point(10). For this reason, relaxation was provided only by relaxation patterns, the gagging problem was successfully treated with the positive imagination technique, and the denture was used without any problems.

Individuals have varying tendencies to hypnotic suggestions related to factors such as personality type, sociocultural background, age, gender, previous experiences of personal discoveries such as hypnosis, desire to be hypnotized, and personal attitude to hypnosis (11). Since the second case was resistant to hypnosis, the authoritarian awakening method was applied, but the practice of hypnotherapeutic suggestion was initiated due to the resistance of the patient, and success was achieved. . The score of Tastan Suggestibility Scale was three point.

If the appropriate information is obtained from the patient before hypnosis, past events can be recalled, and the cause of the problem can be revealed by applying regression hypnosis. In our cases, regression hypnosis was not needed.

In conclusion, our study indicates that hypnosis can be used successfully in the treatment of the gagging problem that is observed during dental practices and cannot be overcome.

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REVIEW

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Are Blood Groups Protective Against COVID-19?

ABSTRACT
The SARS-CoV-2 or COVID-19 disease, which has spread rapidly since its first appearance and caused pandemic, has become more dangerous day by day, and by infecting large masses caused the death of many people. The numbers of cases and deaths reaching dangerous levels globally have pushed scientists to get to know this infection more closely and to investigate preventive and therapeutic methods. At this point, scientists have discovered, based on past infectious disease researches, that some individuals are more susceptible to certain infections. Importantly, in the light of this information, it has been determined that there is a relationship between infectious diseases and blood groups, and individuals with certain blood groups are more susceptible to these infectious diseases. The obtained data suggested that there may be a relationship between blood groups and SARS-CoV-2, and research has been shifted in this direction in order to quickly determine susceptibility to the disease. Indeed, relationships between SARS-CoV-2 patients' blood groups, from hospitals in China, US, Italy, Spain and Turkey, and caught this infectious were investigated. It has been demonstrated that blood groups have an effect on getting this disease. With the discovery of this relationship, it has been revealed in studies that A, B, AB and O blood groups can be a potential biomarker in determining the sensitivity to COVID-19 infection. Studies have determined that individuals with blood type A have an increased sensitivity to COVID-19, and individuals with blood type O have a decreased sensitivity to it. It is thought that the reason for the decreased sensitivity to COVID-19 in individuals with that blood group, and the increased sensitivity seen in individuals with blood group A is due to the antibody A in the blood. This antibody can inhibit virus-cell adhesion in individuals with antibody A. Therefore, it is very important for individuals with blood group A, who do not carry this antibody in their blood, to use personal protective equipment to protect themselves from COVID-19. The purpose of this review is to bring together studies that reveal the relationship between COVID-19 and blood types.
Keywords: COVID-19, ABO Blood Groups, ACE2, S Protein.

Kan Grupları COVID-19'a Karşı Koruyucu Mu?

ÖZET
İlk ortaya çıktığı andan itibaren hızla yayılan ve pandemiye neden olan SARS-CoV-2 veya COVID-19 hastalığı, gün geçtikçe daha tehlikeli bir hale gelmiş ve geniş kitlelere bulaşarak birçok insanın ölümüne neden olmuştur. Küresel olarak tehlikeli seviyelere ulaşan vaka ve ölümlerin sayısı, bilim insanlarını bu enfeksiyonu daha yakından tanımaya ve önleyici ve tedavi edici yöntemleri araştırmaya itmiştir. Bu noktada bilim insanları, geçmiş enfeksiyöz hastalıkları araştırmalarına dayanarak bazı bireylerin bazı enfeksiyonlara daha duyarlı olduğunu keşfetmişlerdir. Önemli olarak, bu edinilen bilgiler ışığında geçtiğimiz yıllarda yapılan çalışmalarla enfeksiyöz hastalıklar ile kan grupları arasında bir ilişki olduğu saptanmış ve belirli kan gruplarına sahip bireylerin bu bulaşıcı hastalıklara daha duyarlı oldukları saptanmıştır. Elde edilen veriler kan grupları ile SARS-CoV-2 arasında bir ilişki olabileceğini düşündürmüş ve araştırmalar hastalığa karşı duyarlılığın hızlı bir şekilde belirlenmesi amacıyla bu yöne kaydırılmıştır. Nitekim Çin'in farklı bölgelerinde, Amerika Birleşik Devletleri'nde, İtalya, İspanya ve Türkiye'deki hastanelerde SARS-CoV-2 hastalarının kan grupları ile bu hastalığa yakalanma ilişkileri araştırılmış ve kan gruplarının bu hastalığa yakalanmada etkisinin olduğu ortaya konmuştur. Bu ilişkinin keşfedilmesiyle birlikte insanlarda bulunan A, B, AB ve O kan gruplarının COVID-19 enfeksiyonuna duyarlılığın belirlenmesinde potansiyel bir biyobelirteç olabileceği araştırmalarda ortaya çıkmıştır. Yapılan çalışmalarda kan grubu A olan bireylerin COVID-19'a karşı artmış bir duyarlılığının olduğu ve kan grubu O olan bireylerin ise azalmış bir duyarlılığa sahip olduğu belirlendi. O kan grubuna sahip bireylerde COVID-19'a karşı görülen azalmış duyarlılığın ve A kan grubuna sahip bireylerde görülen artmış duyarlılığın nedeninin kanda bulunan A antikorundan kaynaklandığı düşünülmektedir. A antikoruna sahip bireylerde bu antikor virus-hücre adezyonunu inhibe edebilmektedir. Bu nedenledir ki özellikle bu antikoru kanında taşımayan A kan grubuna sahip bireylerin COVID-19'dan korunması için kişisel koruyucu ekipman kullanmaları çok önemlidir. Bu derlemenin amacı COVID-19 ile kan grubu arasındaki ilişkiyi ortaya koyan çalışmaları bir araya getirmektir.
Anahtar Kelimeler: COVID-19, ABO Kan Grupları, ACE2, S Protein.

INTRODUCTION

SARS-CoV-2, which has been spreading around the world since December 2019 and declared as a pandemic by the World Health Organization (WHO), causes new type of coronavirus infection disease-2019 (COVID-19). Today, more than 200 countries worldwide have been affected by COVID-19, over 64.5 million people have been infected and approximately 1.500.000 patients have died from this virus (the number of cases and deaths were reported from https://coronavirus.jhu.edu/map.html, as of 03 December 2020). As of December 2020, for the number of COVID-19 cases Turkey is in the 18th place, and for the number of the death is in the 20th place between the world countries.

SARS-CoV-2 first passed from bat to people and continued to spread from person to person in the following period. As a matter of fact, the virus spread by the sneezing or coughing of the infected individual may hang in the air in droplets for a while, which means that the virus; by entering the system of a healthy individual through the eyes, mouth, respiratory tract or food, it leads to infection. The virus can then infect the induvidiuals very quickly.

Previous studies have revealed that blood group antigens are effective receptors for various infectious microorganisms (1). Specific ABO glycan antigen receptors that bind to the spike (S) proteins of the virus during infection can support the entry of the virus into the cell (2).

Generally SARS-CoV-2 can enter the human cells in two ways. In first and main way, viral spike glycoproteins attach to the receptors of the target cell (3). Target cell for SARS-CoV-2; while pulmonary cells, the target receptor is angiotensin converting enzyme-2 (ACE2), which is expressed in mucosal cells. Angiotensin converting enzyme-2 is an enzyme that converts angiotensin to 1-7 by hydrolyzing angiotensin II, causing blood pressure to drop. The COVID-19 virus binds to this receptor and enters the cell (3). In addition to ACE2, CD147 and TMPRSS2 are two of the membrane proteins used by the virus to enter the cell. CD147 is a heavily glycosylated type I transmembrane protein involved in spermatogenesis and fertilization, neural network formation and development, tumor metastasis and angiogenesis, and cardiovascular disease. On the other hand TMPRSS2, is a slightly glycolyzed membrane protein that plays important roles in human and mammal development and homeostasis, as well as in various diseases such as cancer and influenza infection(4). In the second way, viral entry is dependent on the antibody. These antibodies bind to the virus in one hand, and on the other hand binds to the Fcγ receptor. By this way virus can enter to the immunoglobulin cells which express the Fcγ receptor (3).

In general, virus-specific antibodies are considered antiviral and play an important role in various ways of controlling virus infections. In some cases, however, the presence of specific antibodies may be beneficial for the virus. This activity is known as antibody-dependent enhancement (ADE) of virus infection. The antibody-dependent enhancement of virus infection is a phenomenon in which virus-specific antibodies improve the entry of the virus into the cell, and in some cases the virus' proliferation in monocytes/macrophages and granulocytic cells by interacting with Fcγ and/or complement receptors (5).

Pulmonary macrophages are at the center of the inflammatory response in COVID-19 infection. As a result, beside the binding to ACE2, CD147 and TMPRSS2 membran proteins to enter mucosal cells, enter to the macrophages with the ADE is the second way.

With more than 68.3 millions people infected today and about 1.5 millions deaths, scientists have begun to investigate the pathology, diagnosis, treatment and individuals' susceptibility to COVID-19. Fever, cough and weakness are among the main symptoms of COVID-19 (2).

Clinical observations on cases have been shown that the age, male gender, chronic diseases such as cardiovascular disease, diabetes, chronic obstructive pulmonary disease (COPD) and hypertension, constitute increased disease severity beside with an increased risk of disease for COVID-19 infection (6). Today, there is not biomarker has been found yet to fully reveal the susceptibility to COVID-19 (7). However, studies have shown that A, B, AB and O blood groups can be a potential biomarker in determining the susceptibility to COVID-19 infection.

Blood groups were first described as A, B, AB and O by Karl Landsteiner, an Austrian immunologist in 1901. Landsteiner also identified as carbohydrate epitopes on erythrocytes. These carbohydrate epitopes are also called antigens, and blood groups differ from person to person depending on the type of these antigens and whether they are present on the surface of the erythrocyte. The trisaccharide parts, which are the antigenic determinants of blood group A and B, are GalNAcα1-3- (Fucα1,2) -Galβ- and Galα1-3- (Fucα1,2) -Galβ-, respectively, while the determinative disaccharide portion of O blood group is Fucα1,2-Galβ- (7). The genetic factor plays an important role in determining blood groups.

The ABO blood group system is a very stable genetic material found in the third region fourth band (9q34) on the long arm of the 9th chromosome, and is closely related to many diseases (8). Davidson et al. stated that the

expression of blood group antigens varies between human populations and geographic regions (9). The surface of the red blood cell has many surface antigens that can affect susceptibility to many diseases.

Researches attempting to reveal the relationship between blood groups and human health began in the early 1900s. As a result, blood groups are; it has been associated with the emergence of various diseases such as cardiovascular disorders, neurological disorders, cancer and infections (10). As a matter of fact, it has been reported in previous studies that individuals without O blood group are more susceptible to coronary diseases (11), and individuals with B blood group are more likely to have type 2 diabetes compared to other blood groups, and also the Rh factor has no effect on susceptibility (12).

Blood groups can determine the risk of developing the disease, affect the progression of the disease, as well as the severity and consequences of the symptoms shown (2). To determine this, a retrospective study was conducted by a group of Japanese scientists to analyze the effect of the ABO blood group on the disease in patients with severe trauma. As a result, higher mortality and less ventilator time have been reported in patients with O blood group. In addition (13), blood groups have been shown to be associated with chronic heart failure (14) and esophageal squamous cell carcinoma development and outcomes (15). However, other studies have provided contradictory evidence that blood groups are not a risk factor for various diseases (16).

In addition, previous researches have revealed that there is a relationship between ABO blood groups and many infectious diseases and the severity of these diseases. As an example of these diseases; SARS-CoV-1(17), *P. falciparum* (18), *H. pylori*(19), *Norwalk virus* (20), *hepatitis B virus* (21) can be given.

Studies examining the relationship between blood group and SARS-CoV-2 have gained great importance recently, and revealing this relationship has become the focus of many researchers. When the SARS virus first appeared in 2003, as the first of the studies on the association of blood groups with SARS virus can be given as Cheng et al.'s study that revealed a relationship between this virus and blood groups (17). According to the results obtained, individuals belonging to the blood group O showed less susceptibility to SARS virus compared to other blood groups (17). Moreover, natural mechanisms have been investigated, and it has been demonstrated that antibodies of the human anti-histo-blood groups block the interaction of the virus and the cell by binding to the S protein of the virus (22).

In order to reveal the association between SARS-CoV-2 and blood groups, the study done by Zhao et al.'s by is of great importance. In this study, blood groups data obtained from 2173 patients diagnosed with COVID-19 and blood groups data obtained from 27.080 healthy individuals for control purposes were compared in Wuhan and Shenzhen cities of China (7). As a result, the distribution of blood groups of healthy individuals collected from these two cities; It was found as 32.16% blood group A, 24.90% blood group B, 9.10% blood group AB, 33.84% blood group O (7). On the other hand, the blood group distribution of patients diagnosed with COVID-19 has determined as 37.75% blood group A, 26.42% blood group B, 10.03% blood group AB, 25.80% blood group O (7) As a result of this study, it was found that there is an association between COVID-19 and blood groups. Specifically, in this relationship, blood group A was associated with an increased risk of developing COVID-19, while blood group O was associated with a reduced risk. It has been demonstrated with this study that different blood groups can be a biomarker for differential susceptible for COVID-19 (7). In another study, which is similar to this study, the rates of infection of the hospital staff of blood group O and non-blood group O were compared, and it was reported that those with blood group O were infected with a lower risk (17). In this study also looked at the distribution of the blood group between sex and different age groups. Patients were divided into three different groups as under the age 40, between the age 41-59 and over the age 60. The distribution of blood groups showed similar rates in different age groups and in male and female patients. This situation revealed that the distribution of blood group does not depend on a certain gender or a certain age range (7).

Another study, similar to Zhao et al.'s study and supported it, was conducted by Zietz and Tatonetti, and the research was conducted in the New York Presbiterian (NYP) hospital (23). In this study, the relationship between the SARS-CoV-2 and infection status of individuals with ABO Rh+ blood group was investigated on 1559 individuals, 682 of whom were diagnosed with COVID-19 (23). In both cases, the result was significant only in Rh+, but blood group A was found higher and blood group O was found lower in patients diagnosed with COVID-19 which compare to the healthy individuals (23). As a result of the study, a negative relationship was found between O blood group and COVID-19. Indeed, in patients with SARS, O blood group has been identified as a rare blood group (17). In addition, no significant relationship was found between blood groups and mortality of cases (17). Similar studies support these findings with their studies(24-26).

Indeed, one another study performed by Zietz et al has conducted with 14.112 individuals tested for SARS-CoV-2 with known blood type in November 2020 found in New York Presbyterian

(NYP) hospital system. Results have showed that A,B and AB blood types have slightly increased infection prevalence than the O blood type . Especially blood type A has higher prevalence to infection than the other blood groups. When compare the blood groups for intubation, it was found that risk factor of the intubation prevalence is more higher in blood group B than the other groups. It also was found that blood group AB has most higher prevalence for risk of death than the other groups. Rh negative factor was found protective for risk of infection, intubation and death(27).

To reveal the association between blood groups and SARS-CoV-2 a study was carried out by Li et al in China. In a retrospective cohort study, which include 265 patients diagnosed with COVID-19 pneumonia in Wuhan Central Hospital, 39% are in blood group A, 25% are in the blood group B, 10% are in the blood group AB, and 26% are in the blood group O of the patients were reported. Also, blood group distributions are found as 32% blood type A, 25% blood type B, 9% blood type AB and 34% blood type O on 3694 healthy individuals. This results supports Zhao et al's data (28). It has been demonstrated that the rate of patients with blood group A diagnosed with COVID-19 is significantly higher than that of the control group, with a ratio of 39% to 32% (the rate of healthy individuals versus the patient). On the other hand, the rate of patients with blood group O was found significantly lower, compared to the control group, with 26% and 34% (the rate of healthy individuals versus the patient). These distributions were consistent in terms of age and gender according to blood groups. However, there was no significant difference in mortality rates compared to blood groups (28).

Peng et al. also conducted a retrospective study to understand the importance of the blood group in COVID-19 at the Public Hygiene Center at Taizhou Hospital in China between January 21, 2020 and February 20, 2020 (29). This study was conducted with 138 patients who diagnosed with COVID-19, and 82 patients with who undiagnosed with COVID-19. All the patients are compared in terms of blood groups distribution, gender distribution and severity of the disease (29). Diagnoses of the COVID-19 patients are based on Real Time PCR, CT radiography and clinical symptoms. While the average age of the patients was 50, 74 of the patients (53.6%) were male in terms of gender. Clinical symptoms were observed as 70.3% fever, 57.2% cough, 44% sputum removal, 22.5% weakness, 10.9% headache, diarrhea and chest pain (29). The blood group distributions of patients with COVID-19 are those who have severe disease; while 34.4% were in the blood group A, 34.4% were in the blood group B, 12.5% were in the blood group AB, 18.8% were in the blood group O, and are those who have mild disease; 28.3% were in blood group A, 27.4% were in blood group B, 11.3% were in blood group AB, 33.0% were in blood group O (29). The blood group distribution of those non-COVID-19 patients; it was reported that 26.8% were in the blood group A, 28.0% were in the blood group B, 7.3% were in the blood group AB, 37.8% were in the blood group O. As a result of the blood group distributions of the patients with COVID-19 and the statistical analysis, the risk of infection of the patients belonging to blood group O was found to be lower compared to other blood groups ($p = 0.044$) (29). Consequently, researchers have revealed that individuals with blood group O have a low risk of COVID-19 infection, especially in women with blood group O (29).

In addition to other studies, Zeng et al. conducted a research to determined the association between SARS-CoV-2 and blood groups on patients with COVID-19 from a total of 5 hospitals in the cities of Tianjin, Wuhan and Beijing in China (2). The diagnosis of COVID-19 of the hospitalized cases was made by positive real-time reverse transcriptase polymerase-chain-reaction (PCR) test of the nasal and pharyngeal swab samples taken from individuals. The blood groups of the patients were determined by the blood group test performed clinically. In the study, besides the blood group, the patients' age, gender, medical history, hospital reports and chest radiography were also taken. 137 patients with mild pneumonia and 97 patients with severe pneumonia were included in the study. The blood group AB was not included in the study, as it covered only a small proportion of the Chinese population, such as only 9% of the population, due to insufficient number of patients. When the patients were compared in terms of gender, 133 (56.8%) of 234 patients in total were male. When the patients are compared in terms of age, the age of 57-75 years, especially 67, is a critical age for the risk of infection. In this age and older age groups have a severe course of COVID-19, and most of the patients identified in the study were over 60 (73.2%). In addition, it was determined that patients between the ages of 40-64 (36.5%) had a mild infection compared to older age groups. Severe pneumonia was accompanied by acute distressed respiratory syndrome in approximately 67% of patients diagnosed with COVID-19 (2). The cause of death of patients in intensive care unit was recorded as multiple organ failure. When the association between COVID-19 and blood groups is examined, it was reported that 35.76% of patients with coronavirus with blood group A had mild infection and 39.22% of them had severe infection. Considering that a large part of the Chinese population consists of individuals with blood group A, it was found as a result of the study that individuals with this blood group were more susceptible to COVID-19 than other blood groups (2). Indeed, previous studies have revealed that the blood group O is the least susceptible to the SARS

virus (17). However in the new coronavirus study which is conducted by Zeng et al., the finding that blood group O had the least sensitivity could not be supported. Instead, they reported in their study that individuals with blood type A were more susceptible to SARS-CoV-2 infection. As the main reason for this may be the specific viral protein structure is thought to cause different susceptibility in different blood groups. Moreover, it was not found any significant effect on the mortality rates of different blood groups in their study. In addition to this, most of the patients with COVID-19, who have mild or severe symptoms, patients' blood type was found as blood type A. As a result, individuals with A blood group are more susceptible to COVID-19 infection than other blood groups (2).

In another study, which is conducted by Arac et al. (30), it was researched the association between COVID-19 and ABO blood group and Rh factor in a hospital in the city of Diyarbakir, Turkey. A total of 392 patients were included to the study. PCR test of the 227 patients, which are included to the study, were found as positive for SARS-CoV-2, and CT findings of the 165 patients in favor of COVID-19 (30). As a control group, 127,091 people' blood group data in Diyarbakır in 2019 were used. In the study, the blood group A was found to be higher in patients diagnosed with COVID-19, while B and AB blood groups, especially O, were lower. However statistical analysis showed no significant difference between COVID-19 patients and healthy individuals in terms of ABO blood group system (30). When analyzed in terms of Rh blood group system, it was found that Rh positivity was significantly higher in patients with COVID-19 (30). A study, which supports to this study, is conducted by Goker et al. at Hacettepe University in Ankara, Turkey. 186 PCR confirmed for COVID-19 patients and 1881 healthy individuals' blood group data included to the study. Most of the COVID-19 patients' blood groups were A (57%) was found. Result of the study has showed that blood group A might have effect in increased susceptibility to SARS-CoV-2 (COVID-19) and blood group O may have protective effect on COVID-19(26).

Clinical And Research Consequences: A literature search was performed in English databases including PubMed/Medline, ISI Web of Science, SCOPUS, and Google Scholar etc…from 2000 to March 2020. The following key words were used: COVID-19, ABO Blood Groups, ACE2, S Protein, etc..

CONCLUSION

The SARS-CoV-2 virus enters the target cells through an enzyme receptor, specifically called the angiotensin converting enzyme 2 (ACE 2), located on the surface of the lung alveolar cell. In addition to ACE2, CD147 and TMPRSS2 are two of the membrane proteins used by the virus to enter the cell. At this point, the S protein of SARS-CoV-2 acts as an important key for the virus to enter the host and transfers its genetic material to the target cell via membran proteins. In a related study, it was revealed that antibody A inhibits the binding of cells expressing S protein of the SARS-CoV to cells expressing ACE2 (22). Given the nucleic acid sequence (31) between SARS-CoV and SARS-CoV-2 (32, 33)and the similarity of binding to receptor angiotensin converting enzyme 2 (ACE2), the decreased susceptibility of the blood group O and the increased susceptibility of the blood group A for COVID-19 can be related with the presence of the specific Anti-A antibody in the blood.

Previous studies have revealed that the ABO blood group distribution also differs significantly in other viral infections (17). Indeed, Chen et al. reported that individuals with blood group O are less likely to become infected by the SARS coronavirus, and Batool et al. suggested that individuals belonging to blood group O may have developed a defense against blood-borne infections and while individuals with blood group A are more likely to become infected with the hepatitis B and HIV virus (34). Jing et al. found that individuals with B blood group had a low risk of catching Hepatitis B (35).

Guillon et al. reported that binding S proteins, which produced by SARS-CoV-2, to ACE2 receptors inhibits specifically by anti-A antibodies (22). That is, anti-A antibodies in the circulatory system can interrupt or inhibit the virus-cell adhesion process (22). This situation revealed that why the O blood group is not susceptible to infection and the A blood group is susceptible. Moreover Silva-Filho et al. has been found that sialic acid containing receptors, which induced by ABO antigens in host cells, maximize the interaction of the cells with COVID-19(4).

In the studies conducted, it was also examined whether the blood groups are susceptible to SARS-CoV-2 depending on the A antigen. However, in other studies conducted since the discovery that anti-A antibodies can inhibit virus-cell adhesion, blood groups B and O carrying anti-A antibodies in their serums were compared with the A and AB blood group patients who did not carry the anti-A antibody in terms of the prevalence of the disease (36). The results showed that the incidence of COVID-19 was lower in individuals with blood groups B and O with anti-A antibodies in their serum compared to individuals with A and AB blood groups who did not have anti-A antibodies in their serum (36). Subsequently, the blood group O with anti-A antibody was compared in terms of its protective effect against COVID-19 compared to the blood group B which carry the same antibody (36). The data obtained showed that anti-A antibodies in the blood group O had a more protective effect than the anti-A antibody in the blood group B. This result is probably related to the

fact that the immunoglobulin dominant isotype of anti-B/anti-A in the serum of individuals with A and B blood groups is IgM, and the immunoglobulin dominant isotype of the individuals with O blood group is IgG. That is, the presence of anti-A antibodies in the serum, and specifically the presence of IgG with Anti-A, is thought to be of greater importance in the susceptible to COVID-19 than the blood groups themselves (36).

Beside the all studies about susceptibility to SARS-CoV-2, several other studies also have shown that the A allele in the blood groups is associated with an increased risk of cardiovascular disease (37). The antigen A can protect P-selectin and intercellular cell adhesion molecule 1 (ICAM1) from enzymatic cleavage by promoting stronger and longer binding of leukocytes on the vascular wall. Therefore, more adhesion molecules connected to endothelial cells will increase adhesion and inflammation, while reducing circulation (38). While this makes the individuals with blood group A more likely to develop cardiovascular disease, individuals may develop multiple disease states when they are exposed to a redox stress such as a virus infection. Therefore, individuals with blood group O are less likely to develop cardiovascular diseases and severe COVID-19. In contrast, patients with blood type A, who have hypertension and accompanying heart disease, tend to have the disease quite severely once they are infected with COVID-19. Therefore, these individuals should be placed under special medical care in order to be quarantined and protected from SARS-CoV-2 infection as soon as possible (39).

Zietz et al has found association between blood groups and SARS-CoV-2. It was reported that increased infection prevalence was found especially in blood group A than B, AB and Rh+ blood groups. Increased intubation risk was showed in blood group B and AB. Also risk of death increased in blood groups AB than the other blood groups (27).

Clinical significance of blood groups that can be used as a biomarker regarding the susceptibility of individuals to COVID-19 as a result of the study conducted by Zhao et al.are; individuals with blood type A should provide more personal protection to reduce the risk of transmission, patients with SARS-CoV-2, which are blood type A, should get more observation and get the aggressive treatment, and finally as the introduction of ABO blood group classification to the system for manage the COVID-19 can be listed.

Consequently, association between blood groups and SARS-CoV-2 has been found in case of susceptibility to the COVID-19. Having different degrees of infection susceptibility of different blood groups means more protection of the individual who has the most susceptible blood group. Studies have showed that blood group A was found most susceptible to the COVID-19, and who have this blood group should be more careful and should use personal protective equipment for not get infected by the virus. Due to it is important to understand susceptibility of blood groups to COVID-19 for decrease the number of death cases. However it needs further investigation to enlighten the association between blood groups and SARS-CoV-2.

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Konuralp Med Journal

Erratum to: Examination of Systemic Inflammation Related Hemogram Biomarkers in Children and Adolescents with Generalized Anxiety Disorder

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Konuralp Medical Journal 2020; The date of arrival of the article titled "Examination of Systemic Inflammation Related Hemogram Biomarkers in Children and Adolescents with Generalized Anxiety Disorder" published in 12 (3) issue was wrongly written by the journal. The incorrectly written is "Received: 22.09.2020" and the date of arrival of the article has been changed to "Recevied: 02. 09.2020". We apologize to the reader for this mistake.

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