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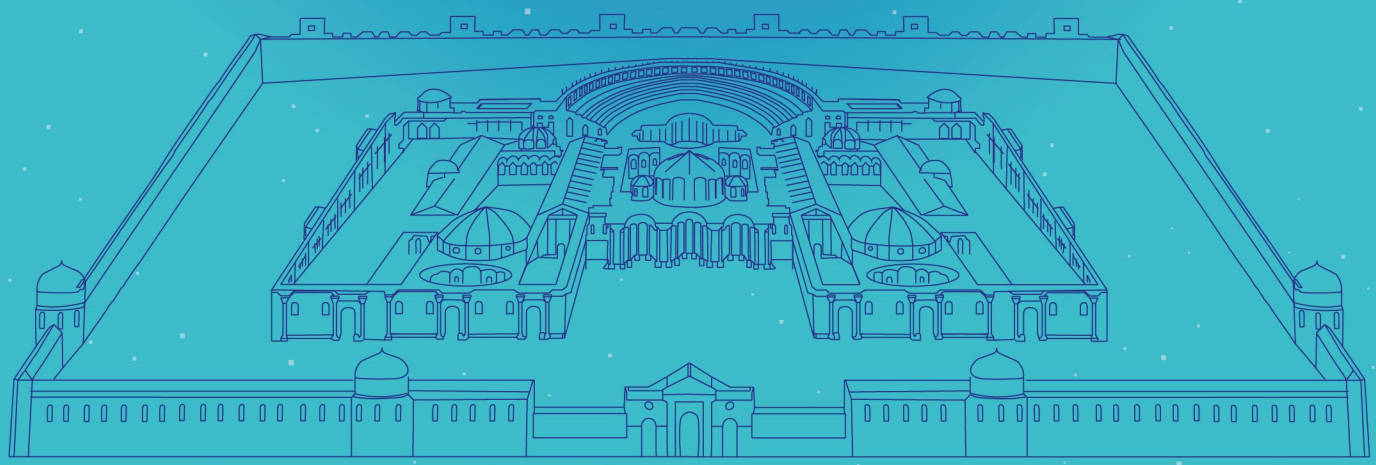

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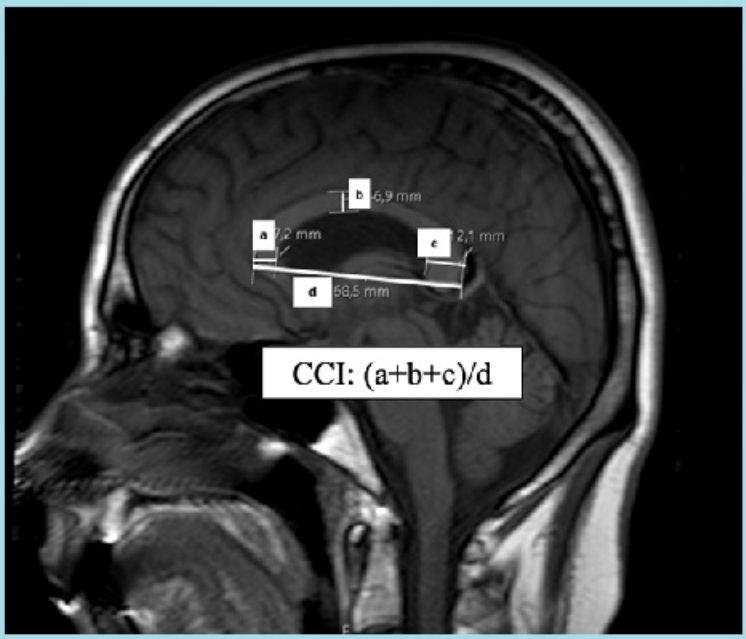

Corpus Callosum Index Measurement (CCI): Corpus Callosum Index, a. Genu thickness; b. Truncus thickness; c. Splenium thickness; d. Total anteroposterior length of the corpus callosum

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Research Article | Araştırma Makalesi

THE EFFECT OF ANTENATAL CORTICOSTEROID ADMINISTRATION ON UMBILICAL ARTERY DOPPLER VELOCIMETRY IN PREGNANCIES COMPLICATED WITH FETAL GROWTH RESTRICTION

FETAL BÜYÜME KISITLILIĞI İLE KOMPLİKE OLAN GEBELİKLERDE ANTENATAL KORTİKOSTEROİD UYGULAMASININ UMBİLİKAL ARTER DOPPLER VELOSİMETRİSİ ÜZERİNE ETKİSİ

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ABSTRACT

Objective: To examine the effect of antenatal corticosteroid administration on umbilical artery (UA) Doppler measurements in pregnancies complicated with fetal growth restriction (FGR).

Methods: This cross-sectional study was conducted with 149 pregnant women scheduled for antenatal corticosteroid therapy because of the possibility of preterm birth. UA Doppler measurements (PI, S/D ratio, RI) before antenatal corticosteroid administration and 24 and 48 hours after the last dose of corticosteroid administration were evaluated and compared with each other in pregnant groups complicated with FGR and uncomplicated with FGR.

Results: No statistically significant change was observed in UA Doppler parameters 24 and 48 hours after antenatal corticosteroid treatment in each group with and without FGR. While there was no significant difference between the precorticosteroid UA Doppler parameters (PI, S/D ratio, RI) of the two groups with and without FGR, the values of these parameters 24 hours after the last dose of treatment were statistically higher in the group complicated with FGR than in the uncomplicated group. However, no statistical difference was observed in UA Doppler parameters between the two groups 48 hours after the last dose of treatment.

Conclusion: Antenatal corticosteroid does not permanently affect UA Doppler parameters in the case of FGR. Close monitoring of the fetus for 72 hours after the first dose of antenatal corticosteroid may be helpful in pregnant women complicated by FGR.

Keywords: Antenatal corticosteroid, fetal growth restriction, fetal doppler, umbilical artery

ÖZ

Amaç: Fetal büyüme kısıtlılığı (FBK) ile komplike olan gebeliklerde antenatal kortikosteroid uygulamasının umbilikal arter (UA) Doppler ölçümleri üzerine etkisini incelemek.

Yöntem: Bu kesitsel çalışma, erken doğum olasılığı nedeniyle antenatal kortikosteroid tedavisi planlanan 149 gebe kadın ile gerçekleştirildi. FBK ile komplike olan ve FBK ile komplike olmayan gebe gruplarında, antenatal kortikosteroid uygulamasından önceki ve son doz kortikosteroid uygulamasından 24 ve 48 saat sonraki UA Doppler ölçümleri (PI, S/D oranı, RI) değerlendirildi ve birbirleriyle karşılaştırıldı.

Bulgular: FBK olan ve olmayan her grupta, antenatal kortikosteroid tedavisinden 24 ve 48 saat sonra UA Doppler parametrelerinde istatistiksel olarak anlamlı bir değişiklik gözlenmedi. FBK olan ve olmayan iki grubun prekortikosteroid UA Doppler parametreleri (PI, S/D oranı, RI) arasında anlamlı fark bulunmazken, son tedavi dozundan 24 saat sonra bu parametrelerin değerleri FBK ile komplike olan grupta komplike olmayan gruba göre istatistiksel olarak yüksekti. Ancak son tedavi dozundan 48 saat sonra iki grup arasında UA Doppler parametrelerinde istatistiksel fark gözlenmedi.

Sonuç: Antenatal kortikosteroid, FBK durumunda UA Doppler parametrelerini kalıcı olarak etkilememektedir. Doğum öncesi kortikosteroidin ilk dozundan sonra 72 saat boyunca fetüsün yakından izlenmesi, FBK ile komplike olan gebe kadınlarda faydalı olabilir.

Anahtar kelimeler: Antenatal kortikosteroid, fetal büyüme kısıtlılığı, fetal doppler, umbilikal arter

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Introduction

Fetal growth restriction (FGR), generally means that the estimated fetal weight measured ultrasonographically is below the 10th percentile for gestational age.[1-3] Maternal, fetal or placental causes may play a role in the occurrence of fetal growth restriction. Poor placental perfusion (ie placental insufficiency) is one of the most common pathologies associated with fetal growth restriction.[2,3] Adverse changes in uteroplacental and fetal-placental circulation cause fetal growth restriction. Uteroplacental and fetal placental circulation can be evaluated by ultrasonographic Doppler parameters. These evaluations can generally be made by examining the Doppler indexes of the uterine artery (UtA), umbilical artery (UA), middle cerebral artery (MCA) and ductus venosus (DV) vessels.[2,4]

Perfusion to the fetoplacental unit can be evaluated by Doppler velocimetry of the umbilical artery (UA).[5] In a normal pregnancy, as the pregnancy progresses, a decrease in the commonly used UA Doppler indices systolic:diastolic ratio (S/D ratio), pulsatility index (PI) and resistance index (RI) is observed in parallel with the increase in end-diastolic flow and decrease in vascular resistance in the UA.[6-8] However, in FGR, there is a decrease in diastolic flow in the UA initially due to increased vascular resistance. Depending on this situation, the S/D ratio, PI and RI indices of the UA may increase.[6,9] In the early stages of FGR, the PI of UA increases due to the decrease in the end-diastolic velocity. In later stages of FGR, absence or reversal of end-diastolic flow in UA occurs.[5] Absence or reversal of end-diastolic flow in the UA increases the risk of perinatal mortality.[3] Redistribution of blood flow occurs when fetal hypoxemia is present in fetuses with FGR. In this condition, known as the brain-sparing reflex, increased blood flow to the brain, heart, and adrenal glands and decreased flow to the peripheral circulations occur.[1,4] The increase in blood flow to the brain during end-diastole with this redistribution in fetuses with FGR is marked by a decreased pulsatility index [PI] in the middle cerebral artery (MCA).[4,5] Likewise, various changes can occur in the sonographic Doppler findings of different vessels in fetuses with FGR. However, the UA is the most commonly studied vessel in Doppler velocimetry due to its accessibility and strength of association with fetal outcomes.[5] In addition, it is thought that the use of UA Doppler velocimetry during antepartum evaluation in fetuses with FGR reduces perinatal mortality.[3,4] Given these circumstances, the UA is the preferred vessel in which Doppler flow velocity is evaluated to guide management in pregnancies complicated by suspected FGR.[4]

Antenatal corticosteroid administration is carried out in order to accelerate fetal lung maturation in pregnant women with a possibility of preterm birth for any reason. This practice reduces the risks of fetal and neonatal death and respiratory distress syndrome.[10] However, antenatal corticosteroid administration also places significant physiological and metabolic demands on the fetus.[11] Studies have shown that this practice may cause temporary reductions in fetal heart rate, fetal respiration and trunk movements.[12,13] In addition, animal experiments have shown that this application may cause fetal hypertension with increased vascular resistance[14], decreased cerebral blood flow with decreased oxygen delivery[15], and an increase in fetal lactate levels.[16] Although healthy fetuses can tolerate the physiological and metabolic demands of antenatal corticosteroids, fetuses with FGR may not cope easily with these problems.[11]

The purpose of this study is to evaluate the effect of antenatal corticosteroid administration on umbilical artery Doppler velocimetry measurements in pregnancies complicated by FGR. Thus, we aim to better understand the effects of antenatal corticosteroid administration on the fetus with FGR.

Methods

Study design and participants

This cross-sectional study was carried out with pregnant women who were planned for antenatal corticosteroid treatment between 2018-2019 due to the possibility of preterm delivery. 149 pregnant women who were treated with antenatal corticosteroid were included in the study. FGR was diagnosed in 49 of 149 pregnant women who received antenatal corticosteroid therapy, and the remaining 100 pregnant women who received antenatal corticosteroid therapy were diagnosed to be at risk of preterm delivery for reasons other than FGR (spontaneous preterm contraction, premature rupture of membranes, gestational cholestasis, preeclampsia, placenta previa etc.).

Pregnant women carrying a fetus whose estimated fetal weight was below the 10 percentile for gestational age, with or without a Doppler abnormality such as increased resistance in the umbilical artery, were included in the FGR group. Gestational age was calculated according to the last menstrual period and confirmed according to the crown-rump length, which was the first trimester ultrasonography measurement.

Pregnant women who had complicated pregnancy with fetal anomaly, who had multiple pregnancies, who left their pregnancy follow-up unfinished, and those under 18 years of age were excluded from the study.

The study was approved by the local ethics committee on 02.02.2018. Informed consent was obtained from participants.

Protocols

Demographic characteristics of the patients and ultrasonographic fetal biometric measurements were recorded just before antenatal corticosteroid administration. The data obtained were compared between pregnant women with FGR complicated and FGR uncomplicated.

Before antenatal corticosteroid administration, UA Doppler measurements (PI, S/D ratio, RI) were made and

recorded. As a corticosteroid, 12 mg betamethasone (Celestone chronodose ampul) was administered intramuscularly twice with 24-hour intervals. UA Doppler measurements were repeated 24 and 48 hours after the last dose of corticosteroid administration. The obtained Doppler measurements were evaluated and compared with each other in pregnant groups complicated with FGR and uncomplicated with FGR.

Ultrasound examinations were performed transabdominally with a 5 MHz probe (Toshiba, Aplio 500 device). Examination of the UA was performed when the mother was in the supine and slightly left-leaning position, while there was no significant fetal movement. The insonation angle was adjusted to be parallel to the blood flow. The color Doppler window was set to a minimum size, surrounding the vascular structure to be examined. At least three measurements were made. In each measurement, 10-15 waveforms were taken and the averages measured from three different consecutive cardiac cycles were recorded.

Statistical analysis

Analyzes were performed with IBM© SPSS program version 20. Measurement data were tested with Kolmogrov-Smirnov tests for the assumption of normal distribution. Variables are given as median (interquartile range [IQR]). Mann-Whitney U test and Friedman tests were used in appropriate places for the comparison of measurement data that did not show normal distribution. $p<0.05$ was accepted for statistical significance in all analyzes.

Results

Demographic characteristics and ultrasonographic fetal biometric measurements recorded just before antenatal corticosteroid administration were compared in Table 1 between pregnancies complicated with and uncomplicated with FGR.

In Table 2, UA Doppler parameters (S/D ratio, PI, RI) of pregnant women with and without FGR were evaluated just before the antenatal corticosteroid and 24 and 28 hours after the last dose of antenatal corticosteroid. No statistically significant change was observed in UA Doppler parameters at 24 and 48 hours after antenatal corticosteroid treatment in each group with and without FGR ($p>0.05$ for all). UA Doppler parameters S/D ratio and PI were found to be higher 24 hours after antenatal corticosteroid in the group complicated with FGR than before treatment, but this increase was not statistically significant. While there was no significant difference between the precorticosteroid UA Doppler parameters of the two groups complicated and uncomplicated with FGR ($p>0.05$ for all), the values of these parameters 24 hours after antenatal corticosteroid treatment were found to be statistically higher in the group complicated with FGR than in the group uncomplicated with FGR ($p=0.022$ for UA-S/D ratio, $p=0.001$ for UA PI, $p=0.014$ for UA-RI). No statistical difference was observed between the two groups in UA Doppler parameters 48 hours after antenatal corticosteroid treatment ($p>0.05$ for all).

Discussion

There are few studies in the literature examining the effect of antenatal corticosteroid administered on Doppler parameters in pregnant women with fetal growth restriction and preterm birth risk.[17-20] As in our study, there are almost no studies comparing the effects of antenatal corticosteroid therapy on the Doppler parameters of pregnant women complicated with and uncomplicated with FGR. We determined that antenatal corticosteroids did not statistically affect UA Doppler parameters at 24 and 48 hours for each group with and without growth restriction. In our study, although it was observed that the UA Doppler parameters S/D ratio and PI were higher in the FGR group 24 hours after the last dose than before treatment, this increase was not found to be statistically significant. While there was no significant difference between the precorticosteroid UA Doppler parameters (S/D ratio, PI and RI) of the two groups, complicated with and without FGR, the values of these parameters 24 hours after the last treatment dose were found to be statistically significantly higher (worse) in the group complicated with FGR than in the group uncomplicated with FGR. No such difference was observed between the two groups in parameters after 48 hours. For this reason, we think that although antenatal corticosteroid affects UA Doppler parameters negatively when first applied in fetuses with growth restriction, this situation improves 48 hours after the last dose. Therefore, if growth restriction is detected in the fetus of the mother to whom antenatal corticosteroids will be administered, it may be useful to follow it more closely in the first 72 hours from the beginning of the treatment.

Wijnberger et al.[17] compared the Doppler parameters of 55 patients with growth restriction, which were measured in the last 5 days before betamethasone administration and in the first 5 days after betamethasone administration. If more than one measurement was obtained, the time closest to betamethasone administration was evaluated. The compared Doppler parameters of the study were UA-PI, MCA-PI, DV-PI and the UA-PI/MCA-PI ratio. It was determined that these values did not show a significant difference between the first values before and after betamethasone. In addition, the course of these Doppler parameters over time was also evaluated in the studies of Wijnberger et al. Doppler values measured 5 days before and 9 days after betamethasone administration were compared with the values measured on the day of betamethasone administration. The UA-PI value did not change significantly over time. MCA-PI values showed a significant and gradual decrease over time. At days 5, 6, 8 and 9, MCA-PI values were significantly lower than at day 0. The UA-PI/MCA-PI ratio increased significantly over time. At day 8, the UA-PI/MCA-PI ratio was

Table 1. Comparison of demographic characteristics and ultrasonographic fetal biometric measurements recorded just before antenatal corticosteroid between pregnant women complicated with and uncomplicated with FGR

	With FGR (n=49)	Without FGR (n=100)	P[1]
Age	30 (24-35)	29 (25-35)	0.945
Weight (kg)	74.0 (67.0-82,0)	74.0 (64.0-80.0)	0.630
Height (mm)	159 (157-163)	161 (158-165)	0,060
Body mass index (kg/m²)	29.3 (26.6-31,2)	28.4 (25.0-30.8)	0.215
Gravida	2 (1-3)	2 (1-3)	0.938
Parity	1 (0-1)	1 (0-2)	0.718
Gestational age based on last menstrual period	34.6 (32.2-36)	32.5 (30-34.6)	***<0.001****
Gestational age based on ultrasound measurements	32 (30-33.4)	33 (30.1-34.5)	0.060
Gestational ages based on each ultrasonographic fetal biometric measurement			
Biparietal Diameter	32.1 (30.4-33.4)	33 (30.1-34.5)	0.111
Head Circumference	32.4 (30.4-34.1)	33.1 (29.6-34.3)	0.522
Abdominal circumference	30.4 (29.3-32.5)	32.5 (29.6-34.5)	***0.003****
Femur Length	32 (30.1-34)	32.4 (30.0-34.3)	0.505
Estimated Fetal Weight	1843 (1393-2124)	2076 (1488-2429)	***0.037****

Variables are given as median (interquartile range [IQR]). [1]Mann–Whitney U test.*Signifies statistical significance. FGR, Fetal growth restriction; With FGR, Pregnant women complicated with FGR; Without FGR, Pregnant women uncomplicated with FGR.

Table 2. Evaluation of umbilical artery Doppler parameters of pregnant women with and without FGR just before the antenatal corticosteroid and 24 and 28 hours after the last dose of antenatal corticosteroid

		With FGR (n=49)	Without FGR (n=100)	P[1]
UA-S/D ratio	**Prior**	2.6 (2.3-3.1)	2.4 (2.2-2.9)	0.319
	After 24 hour	2.6 (2,3-3,2)	2.4 (2.1-2.8)	***0.022****
	After 48 hour	2.5 (2,1-3,0)	2.4 (2,1-2.8)	0.364
	p[2]	0.290	0.143	
US-PI	**Prior**	1.0 (0.8-1.1)	0.9 (0.8-1.1)	0.083
	After 24 hour	1.0 (0.9-1.2)	0.9 (0.7-1.0)	***0.001****
	After 48 hour	0.9 (0.7-1.1)	0.9 (0.7-1.0)	0.104
	p[2]	0.092	0.102	
UA-RI	**Prior**	0.6 (0.6-0.7)	0.6 (0.5-0.7)	0.110
	After 24 hour	0.6 (0.6-0.7)	0.6 (0.5-0.7)	***0.014****
	After 48 hour	0.6 (0.5-0.7)	0.6 (0.5-0.6)	0.475
	p[2]	0.082	0.318	

Variables are given as median (interquartile range [IQR]). [1]Mann–Whitney U test. [2]Friedman test. *Signifies statistical significance. FGR, Fetal growth restriction; With FGR, Pregnant women complicated with FGR; Without FGR, Pregnant women uncomplicated with FGR. UA-S/D ratio, The umbilical artery systolic to diastolic ratio; UA-PI, The umbilical artery pulsatility index; UA-RI, The umbilical artery resistance index.

significantly higher than on the day of betamethasone administration. DV-PI values increased gradually over time. The DV-PI values on days 7 and 8 were significantly higher than the values on day 0.[17] In our study, only UA Doppler parameters were evaluated, and the latest evaluation was made 48 hours after corticosterone. In our study, while MCA-PI and DP-PI values were not analyzed as in the Wijnberger study, UA-SD and US-RI parameters were analyzed in addition to the UA-PI value. Senat et al.[18] examined how 40 FGR fetuses were affected by antenatal corticosteroid in terms of Doppler parameters. Betamethasone was used as corticosteroid in the group with 25 fetuses, and dexamethasone was used in the group with 15 fetuses. Doppler measurements were made before the treatment (day 0), 24-48 hours after the mother's first injection of corticosteroid, and 4-7 days later. UA-PI, descending aorta-PI and MCA-PI were evaluated as Doppler parameters. No statistically significant changes were observed over time in the Doppler parameters examined in both groups treated with corticosteroids. However, PI in MCA tended to decrease 24-48 hours and 4-7 days after maternal steroid administration compared to pretreatment values in both groups. In our study, groups were not separated according to the type of corticosteroid administered and only UA Doppler parameters were examined. In our study, UA-PI value after corticosterone in fetuses with FGR did not show statistically significant changes, consistent with the studies of both Senat et al. and Wijnberger et al. However, in our study, unlike the studies of Wijnber et al. and Senat et al., Doppler parameters of fetuses without growth restriction and corticosterone administered were also evaluated as a control group. Accordingly, 24 hours

after the last dose of antenatal corticosteroid, the UA-PI value in the group with FGR was significantly higher, that is, more negative, than the UA-PI value in the group without FGR.

In the study of Niroomanesh et al.[19], published in 2015, some Doppler parameters of the UA, uterine artery and MCA vessels were evaluated in 40 patients with FGR before betamethasone, 24 hours and 5 days after the completion of betamethasone doses. Although a statistically significant decrease was observed in the uterine artery PI value 24 hours after the treatment, it was found that the value returned to the pre-treatment value after 5 days and no significant difference was observed with the value before the treatment. This temporary decrease in the uterine artery may be important, as 16 (40%) of the 40 people included in the study had preeclampsia along with FGR. In the UA-PI value, a significant decrease, or improvement, was observed 24 hours after the completion of betamethasone and 5 days after the completion of betamethasone. The results of this study are not consistent with our study, in which UA-PI in fetuses with FGR did not show significant changes at 24 hours and 48 hours after the last dose of corticosterone.

In the case of FGR, adverse changes in UA dopplers are associated with stillbirth and neurological disorders. Therefore, UA Doppler changes in fetuses with FGR may play a role in determining the time of delivery.[4,21] Absence or reversal of end-diastolic flow in the UA has been associated with severe FGR.[4] In recent studies, the effect of corticosteroids has been started to be investigated in FGR fetuses with UA end-diastolic flow loss or abnormal UA Doppler findings. In the prospective study performed by Nozaki et al.[20], the values of the Doppler parameters (UA, DV and MCA) of 32 fetuses with FGR and end-diastolic flow loss in the UA before and after betamethasone were examined. In their studies, the values immediately before, 24 hours and 48 hours after the first dose of betamethasone were examined. In their study, flow loss in UA Doppler returned in 22 cases after 24 hours. A statistically significant decrease, that is, improvement, was observed after 24 hours in UA PI. Although an increase was observed in UA PI values compared to the values after 24 hours in the evaluation after 48 hours, it was still significantly lower than before betamethasone, that is, it was observed as better. DV value was also lower after 24 hours compared to baseline. There was no significant difference in DV-PI between the evaluations after 24 hours and after 48 hours. For MCA PI, however, no significant differences were observed between repeated measurements.[20] The results of this study are inconsistent with our study in terms of post-corticosteroid UA-PI values in case of FGR. In our study, unlike this study, the UA-PI value in FGR did not change significantly before the corticosteroid administration, 24 hours and 48 hours after the last dose of corticosteroid administration. In fact, in the case of FGR in our study, UA Doppler parameters were observed to be higher (worse) than before treatment 24 hours after the last dose of corticosteroid administration, but this could not be proven statistically. And these parameters are statistically significantly higher, that is, worse, in the FGR group than the UA Doppler parameters of the non-FGR group 24 hours after the last dose of betamethasone. In our study, unlike Nozakinin's study, UA Doppler parameters before the corticosteroid administration, 24 hours and 48 hours after the last dose of corticosteroid were examined in all fetuses with growth restriction with or without end-diastolic flow loss. Only those with end-diastolic flow loss in the UA were not taken into account in our analyses.

In 2004, Simshenet al.[22] evaluated the perinatal outcomes after antenatal corticosteroids in a small sample prospective study of 25 people. Fetuses of 19 patients had end diastolic Doppler flow loss or reverse flow in the UA with FGR. In this group with FGR, they observed that fetuses with return of end-diastolic flow after antenatal corticosteroid had a better perinatal outcome compared with those with permanent loss of end-diastolic flow or reversed end-diastolic flow. In 2009, Robertson et al.[23] published a new study with a larger number of patients similar to the study by Simshen et al. They performed a retrospective cohort study of betamethasone administration in FGR pregnancies without end-diastolic flow of the UA. Transient return of end-diastolic flow after betamethasone was observed in the majority of pregnant women included in the study, approximately two-thirds. Persistent flow loss was present in about one-third. Pre-pregnancy medical disorder was more common in pregnant women with persistent loss of flow. Some perinatal outcomes were better in the group with transient return of UA end-diastolic flow, neonates of this group required less assisted ventilation, assisted ventilation for a shorter duration, and supplemental oxygen for a shorter duration. In other words, they found that fetuses with FGR with permanent loss of diastolic flow in the UA after betamethasone administration were at higher perinatal risk compared to the group with transient return of flow loss. Although the exact cause has not been determined, Robertson et al. stated that this may be due to the loss of the ability of fetuses with permanent end-diastolic flow loss after betamethasone to induce a vascular response to corticosteroids.

Miller et al.[24] demonstrated in a sheep experiment that administration of betamethasone in fetal growth restriction may be associated with impaired neuronal integrity and increased cell death in the brain due to increased cerebral oxidative stress. In the review by Vidaeff et al.[11] published a review examining the benefits and harms of antenatal corticosteroids in fetuses with FGR. In their study, they recommended close fetal monitoring to fetuses with severe FGR until 48-72 hours after antenatal corticosteroids due to the effects of antenatal corticosteroid on umbilical and placental blood flow. In our study, we observed a temporary worsening of UA Doppler parameters in fetuses with FGR compared to fetuses without FGR, 24 hours after the last dose of betamethasone administration, and we found that this situation improved after 48 hours. Therefore, in our

study, similar to Vidaeff et al., we recommend close fetal monitoring for fetuses with FGR during the first 72 hours after antenatal corticosteroid administration.

In our study, the the transiently higher umbilical artery Doppler parameters 24 hours after treatment in the FGR group than in the non-FGR group may be due to the difficulty of fetuses with FGR in meeting the physiological and metabolic demands imposed on the fetus by antenatal corticosteroid treatment. In the fetus group with FGR, all pregnant women whose umbilical artery Doppler parameters were found to be normal, flow loss, and reverse flow before treatment were included in our study. We do not know whether the results of our study would have been different from the present if only pregnant women with loss of flow or reverse flow in the umbilical artery were included.

The strength of our study is its prospective nature and the fact that it evaluated the post-corticosteroid Doppler parameters of the FGR as well as the non-FGR group. An important limitation of our study is that we stopped Doppler measurement after evaluating 48 hours after the last corticosterone dose and did not follow up with long-term Doppler. One of the limitations is that we did not separately evaluate the pregnant women with normal umbilical artery Doppler parameters and flow loss and reverse flow in the umbilical artery before antennal corticosteroid treatment in the FGR group. Another limitation is that we only evaluated the Doppler parameters of the UA and did not evaluate the Doppler parameters of other vessels such as MCA, DV, and descending aorta.

In conclusion, it was observed that antenatal corticosteroid did not permanently affect umbilical artery Doppler parameters in pregnancies complicated with FGR. In pregnancies with FGR, umbilical artery Doppler parameters are transiently higher (i.e., worse) 24 hours after the last corticosteroid dose than in pregnancies uncomplicated with FGR, but this resolves after 48 hours. Therefore, if corticosterone is administered to mothers of fetuses with FGR, close monitoring for 72 hours after the first dose may be beneficial.

Compliance with Ethical Standards

This cross-sectional study was approved by Marmara University Clinical Research Ethics Committee on 02.02.2018 with protocol code 09.2018.161.

Informed Consent

Informed consent was obtained from study subjects.

Conflict of Interest

No conflict of interest is declared by the authors.

Author Contributions

Data Collection or Processing: S.Z., S.S.; Analysis or Interpretation: S.Z., S.S.; Literature Search: Writing: S.Z., M.D.

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HENOCH-SCHÖNLEİN PURPURALI ÇOCUKLARIN SİSTEM TUTULUMLARININ KLİNİK DEĞERLENDİRİLMESİ

CLINICAL EVALUATION OF SYSTEM INVOLVEMENT OF CHILDREN WITH HENOCH-SCHÖNLEIN PURPURA INTRODUCTION

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ÖZ

Amaç: Henoch-Schönlein purpurası/İmmunoglobulin A vasküliti (HSP-IgAV) çocukluk döneminde en sık görülen vaskülittir. Etyolojide bazı enfeksiyonlar, ilaçlar, aşılar, genetik faktörler, AAA (ailevi Akdeniz ateşi) birlikteliği suçlanmaktadır. Ana özelliği palpabl cilt döküntüleri olup, eklem ve diğer birçok sistem etkilemektedir. Başlangıçta gastrointestinal komplikasyonlar görülürken daha sonraki izleminde böbrek tutulumu önem kazanmaktadır. Çalışmamızda kliniğimizde yatarak izlenen HSP-IgAV tanılı çocukların demografik özelliklerini, sistem tutulumlarının dağılımını, etyolojik faktörleri ve AAA ile ilişkisini değerlendirmeyi amaçladık.

Yöntem: Tanı kriteri olarak; palpabl purpuraya eşlik eden karın ağrısı, artrit/artralji, renal tutulum veya biyopside IgA depolanması bulgularından birinin varlığı kabul edildi. Hastanemizde yatarak izlenen HSP-IgAV tanısı almış çocukların yaş, cinsiyet, vücut ağırlığı, boy, hastalığın ortaya çıkış tarihi-mevsimi, ilk semptom, fizik muayene ve laboratuvar bulguları kaydedildi.

Bulgular: Tanı kriterlerine uyan toplam 52 hasta çalışmaya alındı. Yaş ortalaması 7,5±3,6 (4-13) olup olguların %40'ı 7-10 yaş arasındaydı. Cinsiyete göre dağılımda hastaların 32'si (%61,5) erkek, erkek/kız oranı 1,6 bulundu. İlk şikayet %80,7 (n=42) oranında döküntü idi. Olguların 21'inde (%40,3) eklem tutulumu, 21'inde (%40,3) karın ağrısı, 21'inde (% 40,3) anormal idrar bulgusu, 13'ünde (%25) yüksek serum IgA, 2'sinde (%3,8) AAA birlikteliği saptandı. Erken dönemde dokuz hastada (%17,3) gastrointestinal sistem kanaması ve 2 hastada (%3,8) invajinasyon gelişti. İzlemde üçüncü ay kontrollerinde 6 olguda (%11,5) idrar bulguları devam etmekteydi. Renal tutulum kızlarda (%25) ve 3-6 yaş arası (%25) ile 10 yaş üzerinde (%28) yoğunlaşmaktaydı.

Sonuç: HSP-IgAV olgularının değerlendirilmesinde prognoz renal tutuluma bağlı olduğundan hastalık sırasında ve uzun süreli izleminde idrar tetkiki kontrolü yapılmalıdır. Gastrointestinal tutulumun hastalığın akut döneminde kanama ve invajinasyona neden olabileceği ve HSP/IgAV tanısı alan vakalarda AAA ile birlikteliği akılda tutulmalıdır.

Anahtar Kelimeler: Henoch-Schönlein purpurası, İmmunoglobulin A vasküliti, renal tutulum, ailevi Akdeniz ateşi

ABSTRACT

Objective: Henoch-Schönlein purpura/Immunoglobulin A vasculitis (HSP/IgAV) is a common vasculitis in childhood. Infections, drugs, vaccines, genetic factors and familial Mediterranean fever (FMF) have been shown to be associated with HSP. Skin (palpable purpura), joints, and many other systems are affected. While gastrointestinal complications are seen initially, kidney involvement gains importance in later periods. This study aimed to evaluate children with HSP/IgAV according to the demographic characteristics, the distribution of system involvement, etiological factors, and their relationship with FMF.

Methods: Purpura and accompanying one of the following four criteria were accepted as diagnostic criteria; abdominal pain, arthritis/arthralgia, renal involvement, and demonstration of IgA storage in the skin biopsy. The age, sex, body weight, height, date of the onset of the disease, first symptom, physical examination, and laboratory findings of children diagnosed with HSP/IgAV were recorded in our hospital.

Results: A total of 52 patients who met the diagnostic criteria were included in the study. The average age was 7.5±3.6 (4-13) years and 40% of the cases were between 7-10 years old. There were 32 boys (61.5%) and 20 girls. The boy/girl ratio was 1.6. The first complaint was rash with a rate of 80.7% (n=42). In others, the rash appeared to occur after edema and arthralgia. Joint involvement in 21 (40.3%) patients, gastrointestinal involvement in 21 (40.3%), abnormal urine findings in 21 (40.3%), high serum IgA in 13 (25%), and FMF in 10 (19.2%) patients were detected. In the early period, nine (17.3 %) patients had gastrointestinal bleeding and two (3.8%) had invagination. In the follow-up, urine findings persisted in 6 cases (11.5%) for 3 months. Renal involvement was concentrated in girls (%25), ages 3-6 (25%) and over 10 years of age (28%).

Conclusion: Since the prognosis of patients with HSP/IgAV depends on renal involvement, it is important to carry out urine tests during the disease and long-term follow-up. Gastrointestinal involvement may cause bleeding and invagination in the acute period of HSP/IgAV and its association with FMF should be kept in mind.

Keywords: Henoch-Schönlein purpura, immunoglobulin A vasculitis, renal involvement, familial mediterranean fever

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Giriş

Küçük çaplı damar duvarında IgA içeren immün komplekslerin birikimi ile seyreden Henoch-Schönlein Purpurası (HSP) çocukluk çağının en sık rastlanan vasküliti olup genellikle selim seyretmektedir. Yıllık insidansı 10-20/100.000 dolaylarında bildirilmekle birlikte kendini sınırlayan bir hastalık olduğu için sayının daha fazla olabileceği de ileri sürülmüştür.[1-4] Etyoloji net olmamakla birlikte tanıdan 1-3 hafta öncesinde geçirilmiş üst solunum yolu enfeksiyonu (ÜSYE), diğer enfeksiyonlar (bakteriyel, viral, paraziter), ilaç kullanımı, aşılar, allerji, böcek ısırığı, besinler ve genetik yatkınlık sorumlu tutulmaktadır.[2] Cilt tutulumu ile birlikte eklem, gastrointestinal sistem, böbrek tutulumu olabilmektedir. Nadiren de olsa beyin, akciğer tutulumu ve orşit görülebilmektedir. Gastrointestinal tutulum hastalığın akut döneminde kanama ve invajinasyona neden olabilmektedir. Uzun dönem izlemde gelişebilecek son dönem böbrek hastalığı nedeniyle böbrek fonksiyonları ve idrar bulguları yakın takip edilmelidir.[1,9-11] Böbrek tutulumunun başlangıcı haftalar ya da aylar içinde ortaya çıkabilmektedir. Ayrıca özellikle ülkemizde HSP'nin ailevi Akdeniz ateşi (AAA) ile birlikteliği de vurgulanmaktadır.[2,5-7] Çalışmamızda kliniğimizde izlenen HSP/IgAV tanılı çocukların demografik özelliklerini, sistem tutulumlarının dağılımını, etyolojik faktörleri ve AAA ile ilişkisini değerlendirmeyi amaçladık.

Yöntem

Bağcılar Eğitim ve Araştırma Hastanesi Çocuk Sağlığı ve Hastalıkları Kliniği'nde 2010-2013 tarihleri arasında HSP tanısıyla yatarak izlenen 52 hastanın kayıtları retrospektif olarak değerlendirildi. HSP-IgAV tanısı için EULAR/PReS HSP tanı kriterleri kullanıldı.[8] Bu kriterler; yaygın karın ağrısı, etkilenen dokudan alınan biyopside baskın IgA birikimi gösteren lökositoklastik vaskülit veya proliferatif glomerülonefrit, herhangi bir eklemi tutan akut artrit veya artralji, hematüri ve/veya proteinüri ile saptanan böbrek tutulumudur. Bu kriterlere göre zorunlu kriter olarak palpabl purpura olmak üzere diğer dört bulgudan birinin varlığı tanı için gerekli idi. HSP-IgAV tanısı alan hastaların dosya bilgileri geriye dönük olarak tarandı.

On sekiz yaş altında olan ve tanı kriterlerini karşılayan hastalar çalışmaya dahil edildi. Trombositopeni ya da kanama bozukluğuna bağlı purpura olması, diğer vaskülitleri düşündürecek semptom ve bulguların olması dışlama kriteri olarak kabul edildi. Hastaneye yatışı anındaki yaş, cinsiyet, vücut ağırlığı, boy, kan basıncı değerleri kaydedildi. Hastalığın ortaya çıktığı ay, mevsim, yakın zamanda geçirilen ÜSYE ya da diğer enfeksiyonların olup olmadığı, kullanılan antibiyotik veya diğer ilaçlar, aşılama, allerjenler, ilk semptom ve bulgular, semptom ve bulguların ortaya çıkış sırası yatış dosyası ve hastane elektronik verilerinden elde edildi. Klinik olarak eklem tutulumu için belirgin ağrı varsa artralji, ek olarak şişlik hareket kısıtlılığı varsa artrit kabul edildi. Ciddi karın ağrısı ya da gaitada gizli kan pozitifliği ile kanama varlığı gastrointestinal tutulum olarak kabul edildi. Başlangıçta ve izlemde idrar testinde eritrosit ve/veya protein varlığı ile böbrek fonksiyonlarında bozulma böbrek tutulumu için kabul edildi. Diğer sistemlere ait bulgu varsa kaydedildi. Başvuru sırasındaki laboratuvar bulgularından hemogram sonuçları, C-reaktif protein (CRP), eritrosit sedimentasyon hızı (ESR), koagülasyon testleri, üre, kreatinin, elektrolit, alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), serum IgA değerleri, tam idrar testi sonuçları, etyolojiye yönelik mevcut şartlarda yapılabilen viral seroloji testleri (Hepatit A, Hepatit B, CMV, HIV) kaydedildi. AAA birlikteliğini değerlendirmek için 52 olgunun MEFV gen analizi verileri kaydedildi. Gen analizi pozitif gelen hastalara çocuk romatoloji kliniği tarafından Yalçınkaya ve Özen pediatrik ailevi Akdeniz ateşi kriterlerine uygun olarak AAA tanısı konuldu.9 Eklem ağrısı yanında şişlik ve/veya hareket kısıtlılığı varlığı ile artrit tanısı; karın ağrısı, invajinasyon bulgusu ya da melena, hematemez gibi belirgin kanama varlığı gasrointestinal tutulum göstergesi kabul edildi. İdrarda mikroskobik ya da makroskobik hematüri olması, proteinüri saptanması, böbrek fonksiyonlarının bozulması ise renal tutulum olarak değerlendirildi. En az 12 hafta sonra gelişen yeni döküntü ya da HSP-IgAV ilişkili semptom ya da bulgu ortaya çıkması rekürrens olarak nitelendirildi.

Çalışmada elde edilen bulgular değerlendirilirken, istatistiksel analizler için NCSS 8 DATA Statistical Software (Utah, USA) programı kullanıldı. Sonuçlar %95'lik güven aralığında, $p>0,05$ istatistiksel olarak anlamsız, $p<0,05$ istatistiksel olarak anlamlı, $p<0,01$ istatistiksel olarak çok anlamlı olarak değerlendirildi. Tanımlayıcı istatistikler ortalama ± standart sapma (minimum-maksimum) olarak gösterildi. Kategorize edilen değişkenlerin değerlendirmesinde univaryant analiz ve Ki-Kare testi kullanıldı.

Bulgular

Hastanemizde yatarak izlenen toplam 52 HSP-IgAV tanılı hasta geriye dönük olarak değerlendirildi. Çalışmaya alınan hastalar 4-13 yaşları arasında olup, ortalama yaş 7,5±3,62 idi. Hastaların 20'si kız (%38,4), 32'si erkek (%61,6) olup; erkekler çoğunlukta idi. Erkek/kız oranı 1,6 idi. Hastaların yaşa göre dağılımına bakıldığında ise 0-2 yaş arasında 8 olgu (%15,3), 3-6 yaş arasında 16 olgu (%30,7), 7-10 yaş arasında 21 olgu (%40,3), 10 üzerinde 7 olgu (%13,4) mevcuttu.

Hastaneye ilk başvuru tarihine göre değerlendirildiğinde en çok aralık ayında (n=10, %19,2) başvuru görüldü. Mevsimsel dağılımda ise 24 olgu (%46,1) kışın, 12 olgu (%23,1) ilkbaharda, 10 olgu (%19,2) yazın, 6 olgu (%11,5) sonbaharda başvurmuştu. Tetikleyici faktör bakımından ele alındığında 26 (%50) olguda ÜSYE, 2 olguda (%3,8) üriner enfeksiyon, 2 olguda (%3,8) gastroenterit öyküsü saptandı.

Mutlak tanı kriteri olan deride palpabl purpura ilk başvuruda 42 hastada (%80,7) tek semptom olup, 10 olguda (%19,3) başvuru anında döküntü olmayıp izlemde ortaya çıkmıştı. İlk başvuru sırasında sıklık sırasına göre 4

olguda (%80,7) döküntü, 21 olguda (%40,3) karın ağrısı, 19 olguda (%36,5) ödem, 8 olguda (%15,3) artrit, 3 olguda (%5,7) kas ağrısı mevcuttu. Olguların %25'inde 3 ya da daha fazla şikâyet vardı. Eklem tutulumu 21 olguda (%40,3) mevcut olup cinsiyete göre dağılımı anlamlı değildi (p=0,258). Gastrointestinal tutulumda ağırlıklı olan semptom karın ağrısı (n= 21, %40,3) idi. Gaitada gizli kan 21 olguda (%40,3) pozitifti. Bağırsak tutulumunun cinsiyete göre dağılımındaki farklılıklar istatistiksel olarak anlamlı değildi (p=0,46). Gastrointestinal tutulumu olan 9 olguya oral steroid tedavisi başlandı. İki olgu invajinasyon nedeniyle opere edildi. Oguların 21'inde anormal idrar bulgusu mevcuttu. 19 hastada (%36,5) mikroskobik hematüri, 2 hastada (%0,3) makroskobik hematüri saptandı. Kız çocuklarda böbrek tutulumu görülme sıklığının erkek çocuklara göre yüksek olması istatistiksel olarak anlamlı kabul edildi (p=0,03). Olgularda ciddi böbrek fonksiyon bozukluğu görülmedi. Laboratuvar tetkiklerinde 7 olguda (%13,4) anemi, 9 olguda (%17,3) lökositoz, 18 olguda (%34,6) trombositoz, 32 olguda (%61,5) ESR artışı ve 42 olguda (%84,6) CRP düzeylerinde artış saptandı. Olguların kan elektrolit düzeylerinde değişiklik ve AST, ALT değerlerinde artış görülmedi. Serum IgA düzeyi 13 (%25) olguda yüksek saptandı. 6 olguda (anti CMV IgG dört olguda, anti Hepatit A IgG iki olguda) (%12) viral seoloji pozitifti. 52 hastanın MEFV gen analizi incelemesinde iki olguda (M694V) homozigot mutasyon saptandı ve çocuk romatoloji kliniğindeki izleminde AAA tanısı aldılar. HSP-IgAV tanısıyla izlenen 52 hastadan 1'inde ilk ataktan 108 gün sonra rekürrens görüldü. Rekürrens esnasında hastada döküntü ve karın ağrısı saptandı.

Tartışma

HSP/IgAV palpabl purpurik döküntü, artrit/artralji, böbrek tutulumu, gastrointestinal semptomlarla karakterize ve diğer sistemleri de nadiren tutabilen bir vaskülittir. Akut hastalık döneminde görülebilen gastrointestinal komplikasyonlar ve daha sonra ortaya çıkabilen böbrek tutulumu hayati önem arz etmektedir.[1,5,10-14]

Hastalık en sık 5-15 yaş arasında olmakla birlikte 2 yaş altında ve erişkin dönemde de görülmektedir.[6,7,14] Olgularımızın yaş ortalaması 7,5±3,5 olup dağılım aralığı 4-13 yaş idi. Cinsiyete bakıldığında ise literatüre benzer şekilde erkek/kız oranı 1,6 idi. Ülkemizde Ece ve ark.[3] izledikleri 214 çocuğun ortalama yaşının 9±3,2 (2-16) ve hastaların %57'sinin erkek olduğunu; İnal ve ark.[4] ise izledikleri 77 olgunun ortalama yaşının 7,1±3,3 (2-14) ve erkek/kız oranın1,48 olduğunu bildirmişlerdir. Calvino ve ark.[15] ile Garcia ve ark.[16] ise HSP/IgAV'nın kızlarda daha sık olduğunu bildirmişlerdir. Shim ve ark.'nın[1] Kore'den topladıkları 10 yıllık sağlık verilerine göre HSP/IgAV'nın yıllık insidansı 55,9/100.000 bulunmuştur. Yine aynı çalışmada ortalama başlangıç yaşı 5; erkek/kız oranı ise 1/1,1 olup kızlarda biraz sık rastlanmıştır. Buna karşın O Chen ve ark.'nın[2] Çin'de yaptıkları çalışmada 120 HSP/IgAV tanılı çocuğun ortalama yaşı 6,6±1,6, 1,9/1 saptanmıştır. İtalya'da Trapani ve ark.'nın[11] 151 çocukta yaptıkları çalışmada ortalama yaş 6,1±2,7, erkek/kız oranı 1,8/1 bulunmuştur. Yunanistan'da Fretzayas ve ark.'nın[17] 74 olgudan oluşan çalışmasında da erkek olguların daha fazla sayıda olduğu (41/74) bildirilmiştir.

Etyolojide çoğu olguda neden bulunamamaktadır. Ancak tüm çalışmalarda 1-3 hafta önce geçirilmiş ÜSYE varlığı vurgulanmaktadır. Hwang ve ark.[2] çocuklarda HSP/IgAV ile enfeksiyon hastalıkları arasındaki ilişkiyi araştırmıştır ve çalışmalarında 8 solunum yolu enfeksiyon ajanı (adenovirus, RSV, parainfluenza, influenza, corona virus, rinovirus, bocavirus, metapnömovirus) ile 4 enterik virus ajanı (rotavirus, norovirus, enterik adenovirus, astrovirus) araştırmışlardır. İki yaş altı grupta önemli korelasyon saptanmazken, küçük çocuk (2-5 yaş) grubunda RSV, influenza ve norovirus ile anlamlı ilişki bulunmuştur. Diğer gruplar ise 6-11 yaş arasında influenza ve norovirus; adölesanlarda (12-18 yaş) ise bocavirus ve rotavirus ile anlamlı ilişki saptanmıştır.

İnal ve ark.[4] 40 (%52) olguda enfeksiyon saptadıklarını ve bunların 28'inin ÜSYE, 7'sinin odağı bilinmeyen ateş, 3'ünün gastroenterit, 2'sinin impetigo olduğunu bildirmişlerdir. Çalışmamızda 52 olgunun 26'sında (%50) ÜSYE geçirme öyküsü, 2'sinde (%3,8) üriner enfeksiyon, 2'sinde (%3,8) gastroenterit saptandı. Otuz hastada (%42,4) sorumlu herhangi bir neden bulunamadı. Yine ülkemizde Ece ve ark.'nın[3] yaptığı 214 hastadan oluşan çalışmada hastaların 108'inde (%50,5) ÜSYE, 13'ünde (%6) piyodermi, 15'inde (%7,1) başka bir enfeksiyon saptamışlardır ve enfeksiyonun döküntüden ortalama 10,1±6,2 gün (2-30 gün) önce olduğunu bildirmişlerdir. Bizim verilerimizde de son 15 gün dikkate alınmış ancak net gün belirtilmemiştir.

HSP/IgAV; enfeksiyon sonrası gelişmesi ile ilişkili olarak en sık kış aylarında görüldüğü öne sürülmektedir. Ülkemizdeki çalışmaların bir kısmı hastalığın en sık ilkbaharda bir kısmı ise bizim çalışmamızda olduğu gibi en sık kışın görüldüğünü bildirmiştir.[3,4,12] Bu durumun bölgesel farklılıklarla ilgili olabileceği düşünülebilir.

Böbrek tutulumuyla ilgili farklı sonuçlar bildirilmektedir. Bu farklılıkların ırk, genetik, coğrafi özellikler, yapılan çalışmanın prospektif ya da retrospektif oluşu, olgu sayısı, değerlendirme kriterleri ilemlakalı olabileceği savunulmaktadır. HSP/IgAV'daki böbrek tutulumu yani HSP/IgAV nefriti (HSPN/IgAVN); mikroskopik ya da makroskopik hematüri, nefrotik düzeye kadar ulaşabilecek proteinüri veya azalmış böbrek glomeruler fonksiyonu ile kendini göstermektedir.[19] Özellikle çocukluktaki HSP/IgAV'de böbrek tutulumu çoğunlukla kendini sınırlayabilmekte ve iyi prognoz göstermektedir. Bununla birlikte %1-7'sinde son dönem böbrek yetmezliğine ilerleyebileceği de gösterilmiştir. Tek başına hematüri veya hafif proteinüri ile birlikte oluşu iyi prognozla ilişkili iken ciddi ve ilerleyici özellikte proteinürinin varlığı kötü prognozu göstermektedir.[19] Bizim çalışmamızda 21 olguda (%40,3) anormal idrar bulgusu vardı ve bunların 3 aylık izlem sonunda 6'sında bulgular devam etmekteydi. Ancak hiçbirinde ciddi böbrek hastalığı gözlenmedi.

Birçok araştırmacı HSP/IgAV ile AAA ilişkisni öne sürmüştür. AAA tanılı hastalarda HSP/IgAV'nın AAA olmayanlara göre daha şiddetli ve uzun (2-9 hafta) bir seyir izlediğini göstermişlerdir.7 AAA olan hastaların tekrarlayan HSP/IgAV atakları geçirdiğini, bazı hastaların HSP/IgAV atağından sonra AAA tanısı aldığını bildirmişlerdir. Ülkemizde yapılan bir çalışmada 207 AAA tanılı hastanın 15'inde (%7) HSP/IgAV atağı saptanmış ve 9'unda HSP/IgAV atağından sonra AAA tanısı konulduğu bildirmiştir. Ayrıca HSP/IgAV geçiren ve önce veya sonrasında AAA tanısı alan olgularda döküntülerin alt ekstremite dışında yüz ve gövdeye de dağıldığına, tekrarlama eğiliminde olduğuna dikkat çekilmiştir.7 Ülkemizde IgA vasküliti ve AAA birlikteliği sık olabilir bu nedenle hastalar ailevi Akdeniz ateşi semptomları açısından dikkatli sorgulanmalı ve yüksek klinik şüphe varlığında genetik analiz önerilmelidir. Çalışmamızda gen analizi bakılan 52 olgudan 2'sinde (M694V) homozigot mutasyon saptanmış ve daha sonra bu hastalar AAA tanısı almıştır.20 İki hasta çocuk romatoloji bölümü tarafından takibe alındı ve hastalar daha sonradan AAA tanısı aldı.

Çalışmamızın geriye dönük olması, dosya verilerinin yeterli açıklamalar açısından eksik oluşu, tek merkezli oluşu ve sayıca az olgu içermesi kısıtlılıkları olup, poliklinikten izlenen daha geniş alandan toplanan, uzun süreli izlemi de kapsayan prospektif çalışmalar gerekmektedir.

Sonuç olarak, HSP-IgAV selim bir vaskülit olmakla birlikte gastrointestinal tutulumun hastalığın akut döneminde kanama ve invajinasyona neden unutulmalıdır. Böbrek tutulumu açısından hastalık sırasında ve uzun süreli izleminde idrar tetkiki kontrolü yapılmalıdır. HSP/IgAV tanısı alan vakalarda AAA ile birlikteliği akılda tutulmalıdır.

Açıklama

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Çıkar Çatışması Beyanı

'Henoch-Schönlein Purpuralı Çocukların Sistem Tutulumlarının Klinik Değerlendirilmesi' isimli makalemiz ile ilgili herhangi bir kurum, kuruluş, kişi ile mali çıkar çatışması yoktur ve yazarlar arasında çıkar çatışması yoktur.

Yazar Katkısı

Yazarlar eşit oranda katkı sağlamışlardır.

Kaynaklar

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Research Article | Araştırma Makalesi

FRAGMENTED QRS PATTERN PREDICTS POOR PROGNOSIS IN SEPSIS AND SEPTIC SHOCK

FRAGMENTE QRS PATERNİ SEPSİS VE SEPTİK ŞOKTA KÖTÜ PROGNOZU ÖNGÖRÜR

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ABSTRACT

Objective: Myocardial dysfunction due to sepsis is a clinical condition associated with a high rate of mortality. Fragmented QRS (fQRS) is a marker that is associated with the function of the myocardium. This study aimed to determine whether fQRS patterns were associated with short-term overall survival (OS).

Methods: 76 patients with sepsis and 68 patients with septic shock were included in the study. SOFA score and APACHE II score were calculated, and the fQRS pattern has been assessed.

Results: In the septic shock group, APACHE II score, SOFA score and mortality were significantly higher [38 (%50) vs 45 (%65), respectively; p<0.01]. Duration of mechanical ventilation, the fQRS pattern, and hospitalization time predicted the mortality. The fQRS pattern's presence was linked to a decreased short-term survival in both groups

Conclusion: fQRS pattern and mortality were significantly higher especially septic shocks. However, in both groups, the presence of the fQRS has been found to have a correlation with mortality and independently predicted worse OS. Thus, we propose that the fQRS pattern could serve as a novel prognostic indicator for septic patients.

Keywords: Sepsis, septic shock, electrocardiography, fragmented QRS

ÖZ

Amaç: Sepsise bağlı miyokardiyal disfonksiyon, yüksek mortalite oranı ile ilişkili klinik bir durumdur. Parçalanmış QRS (fQRS) miyokardın fonksiyonu ile ilişkili bir belirteçtir. Bu çalışmanın amacı fQRS paternlerinin kısa dönem genel sağkalım (OS) ile ilişkili olup olmadığını belirlemektir.

Yöntem: Çalışmaya 76 sepsis ve 68 septik şok hastası dahil edildi. SOFA skoru ve APACHE II skoru hesaplandı ve fQRS paterni değerlendirildi.

Bulgular: Septik şok grubunda APACHE II skoru, SOFA skoru ve mortalite anlamlı olarak daha yüksekti [sırasıyla 38 (%50) vs 45 (%65); p<0,01]. Mekanik ventilasyon süresi, fQRS paterni ve hastanede yatış süresi mortaliteyi öngörmüştür. FQRS paterninin varlığı her iki grupta da kısa dönem sağkalımda azalma ile ilişkilendirilmiştir

Sonuç: fQRS paterni ve mortalite özellikle septik şoklarda anlamlı olarak daha yüksekti. Bununla birlikte, her iki grupta da fQRS varlığının mortalite ile korelasyon gösterdiği ve bağımsız olarak daha kötü OS'yi öngördüğü bulunmuştur. Bu nedenle, fQRS paterninin septik hastalar için yeni bir prognostik gösterge olabileceğini düşünüyoruz.

Anahtar Kelimeler: Sepsis, septik şok, elektrokardiyografi, fragmente QRS

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Introduction

Sepsis is a severe condition characterized by organ failure due to dysregulated immune responses to infection. Septic shock, a more complex clinical condition than sepsis, is associated with higher mortality rates. Myocardial dysfunction, a hallmark of septic patients, is a critical component of multi-organ failure.[1-4] Recent studies have reported cardiac dysfunction in septic patients.[5,6] Cardiac dysfunction has been well-documented in the literature for patients with sepsis. Studies suggest that significant changes in Purkinje fibers following myocardial ischemia or fibrosis may disrupt the QRS complex morphology, leading to fragmented QRS (fQRS) patterns.[7,8] The fQRS pattern indicates irregular ventricular activation and unsynchronized contraction caused by myocardial ischemia or myocardial scarring.[9] This marker reflects pathology arising from impaired and fibrotic electrical conduction in the myocardium.[10,11] In patients with dilated cardiomyopathy, the presence of fQRS has been associated with poor prognosis. Myocardial dysfunction is observed in more than 40% of septic cases, significantly contributing to mortality and morbidity.[12] Despite the investigation of many biomarkers, no specific marker has been identified for evaluating cardiac function in septic patients. This study aims to determine the relationship between fQRS patterns and short-term overall survival (OS) in septic patients.

Methods

Study Population

This study was conducted between 2016 and 2017 in the intensive care unit of Isparta City Hospital. Ethical committee approval and the necessary permissions were obtained prior to the commencement of the study. The study included a total of 144 patients diagnosed with sepsis and septic shock according to Sepsis-3 guidelines[1] with 76 patients diagnosed with sepsis and 68 with septic shock. Patients on medication or with electrolyte imbalances that could affect ECG results, as well as those with a history of cardiac, hematological diseases, or malignancy, were excluded. Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated within the first 24 hours.

ECG Measurements

Standard 12-lead ECGs (9022-K; Nihon Kohden Corp., Tokyo, Japan) were obtained using a filter range of 0.5-150 Hz and an AC filter of 60 Hz, with a paper speed of 25 mm/s and an amplitude of 1 mV/cm while patients were in a supine position following their diagnosis. The ECGs were interpreted by two experienced cardiologists who were blinded to the study, ensuring no significant discrepancies between the investigators. All graphs were manually scrutinized to eliminate any possible technical glitches that could have affected the accuracy of the device's readings.

Measuring QRS Fragmentation

Fragmentation was defined as having different RSR patterns with different QRS complex morphologies.[13] The presence of fQRS was identified by typical bundle branch block patterns in the absence of, or with, a normal QRS duration. A coronary pattern was defined as the presence of an RSR pattern in at least two consecutive leads corresponding to the arterial blood supply area, and/or the presence of notching in the R and S waves.

Statistical Analysis

Data were analyzed using IBM SPSS v.23.0 (IBM Corp., Armonk, NY, USA) software. Continuous variables were presented as means ± standard deviation, or medians with 25^{th}-75^{th} percentile values for normally and non-normally distributed data, respectively. Continuous variables were compared using either the Student's t-test or the Mann-Whitney U test, as appropriate. Categorical variables were compared using the chi-squared test. Technical term abbreviations were explained upon their first use. Kaplan-Meier analyses were employed to generate cumulative survival curves, and the log-rank test was used to compare groups. Univariate and multivariate analyses using Cox's proportional hazards model were conducted to identify differences in survival. Overall survival was calculated from the time of diagnosis until either the date of death from any cause or the date of the final follow-up. Statistical significance was defined as a p-value less than 0.05.

Results

The baseline characteristics of the two groups are presented in Table 1. Age and gender distributions were comparable between the groups.

Clinical Features

The incidence of diabetes was significantly higher in the septic shock group (p=0.02). In addition, SOFA scores (8.5±2.5 vs. 11.0±3.3; p<0.01) and APACHE II scores (20.0±5.7 vs. 23.0±6.0, respectively; p<0.01) were significantly elevated in patients with septic shock. The need for mechanical ventilation (MV) was significantly higher in patients with septic shock compared to those with sepsis (43 [57%] vs. 59 [87%]; p<0.01). Moreover, MV duration was significantly longer in septic shock patients (11.1 [0-57] vs 17.2 [0-62] days; p<0.01]. The fQRS pattern was more common in septic shock patients (27 [36%] vs. 42 [62%], respectively; p<0.01) (Table 1).

Biochemical Analysis Findings

Except for creatinine levels, biochemical tests showed comparable results between the two groups (p=0.08) (Table 2). Additionally, serum platelet levels were significantly lower in the septic shock group (p=0.04). Serum C-reactive protein levels were remarkably higher

in the septic shock group (13.1 [4-52] vs. 17.3 [4-52] mg/L, respectively; p<0.01). More importantly, serum high-sensitivity troponin T levels tended to be higher in septic shock patients compared to sepsis patients (0.17 [0-3.04] vs. 0.40 [0-3.51], respectively; p=0.06) (Table 2). Lactate levels were significantly higher in patients with septic shock (1.88±0.98 vs. 3.04±1.60 mmol/L; p<0.01). No significant differences were observed in other blood gas parameters between the two groups (Table 2).

Table 1. Change in demographic, clinical and laboratory parameters among patients with sepsis and septic shock.

	Sepsis n= 76	Septic Shock n= 68	P value
Mean age, year	76±10	75±8	0.24
Male / Female, n/n	47/29	35/33	0.24
Hypertension, n(%)	24 (%41)	30 (%57)	0.12
Diabetes, n(%)	18 (%20)	41 (%40)	**0.02**
Mechanical Ventilator, n(%)	43 (%56)	59 (%87)	**<0.01**
Mechanical Ventilator duration, day	11.1 (0-57)	17.2 (0-62)	**<0.01**
Hospitalized time, day	20 (5-61)	22 (5-62)	0.43
SOFA score, n	8.5±2.5	11±3.3	**<0.01**
APACHE score, n	20±.5.7	23±6.0	**<0.01**
Atrial fibrilation, n(%)	9 (%12)	20 (%33)	**<0.01**
QRS Fragmantation, n(%)	27 (%35)	42 (%61)	**<0.01**
Mortalite, n(%)	38 (%50)	45 (%65)	**0.03**
Glucose, mg/dl	148±45	145±61	0.75
Creatinine, mg/dl	1.36 (0.3-9)	1.49 (0.2-4)	**0.08**
Sodium, mg/dl	140±5.2	141±5.5	0.46
Potassium, mg/dl	3.9±0.7	3.9±0.8	0.58
Hemoglobin, g/dl	11±2.4	10.2±2.2	0.23
Platelet, x10³/mm³	226 (42-567)	195 (42-516)	**0.04**
Eosinophyl, x103/mL	0.13 (0.01-1.11)	0.58 (0.01-15.3)	0.13
WBC, x103/mL	12.6±6	13.1±6	0.62
C-Reactive Protein, mg/L	13.1 (4-52)	17.3 (4-52)	**<0.01**
Procalsitonin, ng/ml	4,86 (0,03-68,2)	6,71 (0,11-94,1)	0,36
Hs TnT, pg/dl	0.17 (0-3.04)	0.40 (0-3.51)	**0.06**
Lactate, mmol/L	1.88±0.98	3.04±1.60	**<0.01**

APACHE: Acute Physiology and Chronic Health Evaluation, **Hs TnT:** High Sensitive Troponin T, **SOFA:** Sequential Organ Failure Assessment Score, **WBC:** White Blood Cells.

Table 2. Results of Univariate and Multivariate Cox's Proportional Hazard Models Regarding OS.

Characteristics	Univariate Analysis		*Multivariate Analysis*	
	OS HR (95% CI)	P Value	*OS HR (95% CI)*	*P Value*
MV	0.120 (0.029-0.492)	<0.001	0.005 (0.001-0.026)	<0.001
MV duration	0.964 (0.949-0.981)	<0.001	0.849 (0.802-0.877)	<0.001
SOFA score	1.070 (1.002-1.147)	0.03		
APACHE score	1.046 (1.010-1.084)	0.01		
WBC	1.080 (1.040-1.122)	<0.001	1.043 (0.994-1.094)	0.088
Eosinophil count	1.67 (1.07-2.67)	<0.001	0.154 (0.021-1.127)	0.065
QRS Fragmantation	0.360 (0.216-0.601)	<0.001	0.228 (0.123-0.421)	<0.001

APACHE: Acute Physiology and Chronic Health Evaluation, **MV:** mechanical Ventilator **NLR**: neutrophil/lymphocyte ratio **SOFA:** Sequential Organ Failure Assessment Score. **WBC**: White Blood Cell.

Survival and Prognostic Factors

At the final follow-up, 85 patients (59%) had died. The mean survival time for all patients was 20.9 (range: 5 to 62) days, with a mean survival time of 23.2 (range: 5 to 62) days for patients who died. Mortality was significantly higher in septic shock patients compared to sepsis patients (38 [50%] vs. 45 [66%], respectively; p=0.03) (Table 1). However, OS was similar between sepsis and septic shock patients (31.3 vs. 27.4 days; HR: 1.23, 95% CI: 0.80-1.88, p=0.34). Additionally, OS was significantly

shorter in patients with fQRS compared to those without (45.5 [35.3-55.7] vs. 40.1 [32.0-48.3] days; p<0.001) (Figure 1).

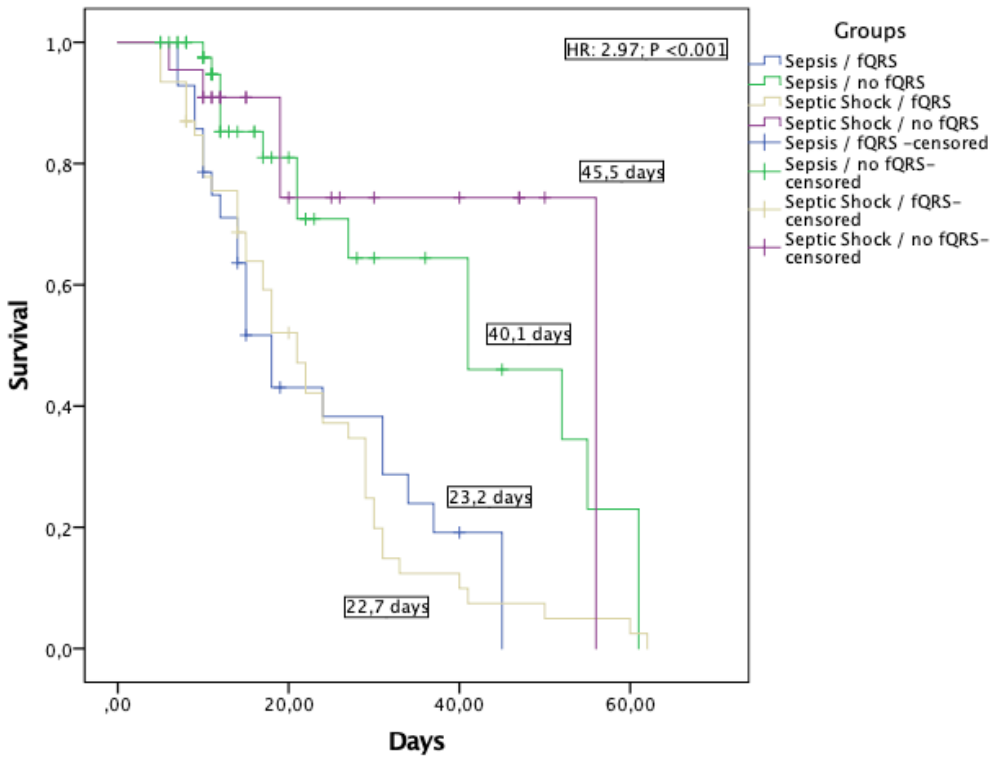

Figure 1. Survival analysis. Kaplan-Meier curves reflecting the difference in survival rates between sepsis and septic shock patients with or without fQRS.

Prognostic Analysis

Prognostic factors and the presence of fQRS patterns were initially evaluated through univariate analysis. MV, MV duration, SOFA score, APACHE II score, white blood cell count, eosinophil count, and the presence of fQRS were significantly associated with OS. Subsequently, all significant prognostic factors and the presence of fQRS were assessed via multivariate analysis using Cox's proportional hazards model. The presence of fQRS (HR: 0.228, 95% CI: 0.123-0.421, p<0.001), the need for MV (HR: 0.005, 95% CI: 0.001-0.026, p<0.001), and MV duration (HR: 0.849, 95% CI: 0.802-0.877, p<0.001) independently predicted worse OS. Detailed results of univariate and multivariate survival analyses are presented in Table 3.

Table 3. Results of Univariate and Multivariate Cox's Proportional Hazard Models Regarding OS.

	Univariate Analysis		***Multivariate Analysis***	
Characteristics	***OS HR (95% CI)***	**P Value**	***OS HR (95% CI)***	***P Value***
MV	0.120 (0.029-0.492)	<0.001	0.005 (0.001-0.026)	<0.001
MV duration	0.964 (0.949-0.981)	<0.001	0.849 (0.802-0.877)	<0.001
SOFA score	1.070 (1.002-1.147)	0.03		
APACHE score	1.046 (1.010-1.084)	0.01		
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APACHE: Acute Physiology and Chronic Health Evaluation, **MV**: mechanical Ventilator **NLR**: neutrophil/lymphocyte ratio **SOFA:** Sequential Organ Failure Assessment Score. **WBC:** White Blood Cell.

Discussion

This study aimed to investigate the association between the presence of fQRS patterns and short-term survival among sepsis and septic shock patients receiving intensive care. Our findings revealed a significantly higher prevalence of fQRS patterns and mortality among patients with septic shock, correlating with shorter survival times. Importantly, the need for MV, MV

duration, and the presence of fQRS patterns independently predicted worse OS in septic patients.

The APACHE II and SOFA scoring systems are standard tools in the assessment of septic patients, where elevated SOFA scores have been consistently linked to increased mortality risks.[14,15] In our cohort, the SOFA and APACHE II scores were notably higher among septic shock patients. Subgroup analyses further underscored the association between higher SOFA scores and increased mortality in patients with septic shock

The APACHE II and SOFA scoring systems are routinely used in the assessment of septic patients, where elevated SOFA scores have been consistently linked to increased mortality risks.[14,15] In our study, the SOFA and APACHE II scores were significantly higher among septic shock patients. Subgroup analyses further underscored the association between higher SOFA scores and increased mortality in patients with septic shock.

Recent literature has documented various forms of cardiac dysfunction in septic patients, including impaired contractile function, diastolic dysfunction, reduced cardiac index, and ejection fraction.[5,6] In particular, patients with septic shock frequently exhibit not only vasoplegia but also myocardial depression.[16] A post-mortem study has additionally identified myocardial injury in a significant proportion of septic shock patients.[17] In the current study, levels of serum cardiac high-sensitive troponin T were observed to be higher in patients with septic shock compared to those with sepsis. Thus, consistent with prior research, our findings suggest that myocardial dysfunction is prevalent among patients with septic shock.

Fragmented QRS patterns in patients with coronary artery disease are associated with myocardial damage and increased risk of adverse cardiac events. Moreover, studies have consistently linked fQRS patterns to elevated rates of ventricular arrhythmias, sudden cardiac death, and recurrent cardiac events in both ischemic and non-ischemic cardiomyopathy.[9-11, 18-20] Mahenthiran et al. argued that the presence of fQRS patterns may be an indicator of myocardial perfusion in coronary artery disease.[21]. In patients with hypertension, fQRS patterns have been shown to be a predictor of major adverse cardiovascular and cerebrovascular events.[22,23] Furthermore, fQRS patterns have been observed more frequently in patients suspected of cardiac involvement due to COVID-19 and are associated with increased mortality.[24]

To the best of our knowledge, there have been no previous studies investigating fQRS patterns in patients with sepsis or septic shock. In our study, although there was no significant difference in survival rates between the groups, we observed significantly higher incidences of fQRS patterns and mortality in the septic shock group. More importantly, the presence of fQRS patterns, along with the requirement for MV and MV duration, independently predicted worse OS in sepsis and septic shock patients. These findings suggest that fQRS patterns may serve as a novel prognostic marker in these patient populations.

Several limitations of our study should be acknowledged. First, the relatively small sample size due to stringent exclusion criteria limited our statistical power to detect small differences. Second, the intensive care unit-based follow-up may not capture longer-term patient outcomes. Finally, echocardiography was not utilized to assess left ventricular parameters related to myocardial scar and ischemia.

Conclusion

The current investigation identified a significant association between mortality rates and the presence of fQRS patterns, particularly in patients with septic shock. While survival rates did not vary significantly between the groups, the presence of fQRS independently predicted poorer OS in both cohorts. In conclusion, the fQRS pattern emerges as a potential novel prognostic marker for patients with sepsis or septic shock.

Description

This study was presented orally at the "23. International Intensive Care Symposium" (19-22 May 2021, Online).

Compliance with Ethical Standards

The study was approved by the Suleyman Demirel University Clinical Research Ethics Committee (21.09.2023/181).

Conflict of Interest

The authors disclose that they have no conflict of interest to declare

Author Contribution

PK: Concept; OP, YK: Data Collection and/or Processing; PK: Analysis and/or Interpretation; OP, YK, MK: Literature Review; PK, MK: Writing the Article.

Financial Disclosure

None of the authors of this manuscript had any financial relationships with other individuals or organizations.

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Research Article | Araştırma Makalesi

ASSOCIATION OF EOSINOPHIL AND TOTAL IGE VALUES WITH ALLERGY TEST RESULTS IN PATIENTS WITH ATOPIC DERMATITIS

ATOPİK DERMATİT TANILI HASTALARIN EOZİNOFİL VE TOTAL IgE DEĞERLERİNİN ALERJİ TEST SONUÇLARIYLA İLİŞKİSİ

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ABSTRACT

Objective: Atopic dermatitis (AD) is a chronic, recurrent, allergic inflammatory skin disease that may have a genetic predisposition. Eosinophilia is a common finding in patients with atopy. In our study, we aimed to investigate the relationship between eosinophil and IgE levels and allergy test results in patients with atopic dermatitis.

Methods: In this descriptive study, the files of patients diagnosed with atopic dermatitis and followed up in our Pediatric Allergy and Immunology Clinic between January 2021 and December 2022 were retrospectively reviewed. Age, gender, eosinophil, total IgE and specific IgE (for food and inhaled allergens) values were analyzed in the study. Skin prick test (SPT) was also performed in patients who had negative results for allergen-specific IgE.

Results: The absolute eosinophil count, eosinophil (%) and total IgE values of patients sensitized to at least one of the food and aeroallergens were significantly higher than those without allergen sensitization. The cut-off point of total IgE in predicting allergen sensitization was found to be 91.5 by ROC analysis. The sensitivity and specificity values for the cut-off point of total IgE were 73.3% and 72.9%.

Conclusion: In this study, allergen sensitization was detected in 3 out of every 4 AD patients with total IgE and eosinophil values above the cut-off point we analyzed. Accordingly, we think that total IgE and eosinophil values are successful in predicting allergen sensitization. In clinics where specific IgE or SPT cannot be performed, eosinophil and total IgE values in whole blood will be useful for preliminary diagnosis.

Keywords: Total IgE, eosinophils, ROC analysis

ÖZ

Amaç: Atopik dermatit (AD), genetik yatkınlık gösterebilen kronik, tekrarlayan, alerjik inflamatuar bir cilt hastalığıdır. Eozinofili, atopi hastalarında yaygın bir bulgudur. Çalışmamızda, atopik dermatit tanılı hastalarda eozinofil ve IgE değerlerinin alerji test sonuçları ile olan ilişkisini incelemeyi amaçladık.

Yöntem: Tanımlayıcı tipte olan çalışmada; Ocak 2021- Aralık 2022 tarihleri arasında atopik dermatit tanısı olan ve Çocuk Alerji ve İmmünoloji Kliniği'mizde takipli hastaların dosyaları retrospektif olarak incelendi. Çalışmada yaş, cinsiyet, eozinofil, total IgE ve spesifik IgE (gıda ve inhaler alerjenler için) değerleri analiz edilmiştir. Hastalarda gıda ve aeroallerjenleri tespit etmek için alerjen spesifik IgE testi yapılmıştır. Alerjen spesifik IgE testi negatif olan hastalara deri prick testi de uygulanmıştır.

Bulgular: Besin ve aeroalerjenlerin en az birisi için duyarlılığı olan hastaların mutlak eozinofil sayısı, eozinofil (%) ve total IgE değerleri alerjen duyarlılığı olmayanlara göre anlamlı olarak daha yüksekti. Hastaların total IgE değerlerinin alerjen duyarlılığını öngörme kapasitesi için ROC analizi yapıldı. Yapılan ROC analizi ile total IgE'nin alerjen duyarlılığını öngörmedeki kesim noktası 91,5 olarak bulundu. Total IgE'nin kesim noktası için sensitivite ve spesifite değerleri %73,3 ve %72,9 bulundu.

Sonuç: Çalışmada, total IgE ve eozinofil değerleri analiz ettiğimiz kesim noktasının üzerinde olan yaklaşık her 4 AD hastasının 3'ünün (%72,2) alerjen duyarlılığı tespit edildi. Buna göre total IgE ve eozinofil değerlerinin alerjen duyarlılığını öngörmede başarılı olduğunu düşünmekteyiz. Spesifik IgE veya deri prick testi bakılamayan kliniklerde ön tanı açısından tam kanda bakılan eozinofil ve total IgE değerleri faydalı olacaktır.

Anahtar Kelimeler: Total IgE, eozinofil, ROC analizi

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Introduction

Atopic disease pathogenesis involves the participation of basophils and eosinophils, along with Immunoglobulin E (IgE). Effector cells engaged in allergic inflammation can be activated by IgE, a fundamental molecule.[5] In the acute phase of atopic disease, particularly at the onset or during exacerbation, IgE is excessively produced due to an increased generation of T helper 2 cytokines in the majority of patients with atopic dermatitis (AD).[6] During the development of related diseases, eosinophils migrate to the affected sites and perform their cellular functions under the influence of the local microenvironment. It has been documented that eosinophils play a crucial role in allergic diseases and combating parasitic infections.[7] Recent studies have explored inflammatory cytokines and biomarkers in several disease contexts.[8, 9]

Our study aimed to explore the correlation between IgE levels, eosinophil counts, and allergy test outcomes in individuals diagnosed with atopic dermatitis. Eosinophils are a cell type that is frequently increased in allergic reactions and total IgE levels are generally accepted as a marker reflecting allergic sensitization. In this context, understanding how eosinophil and IgE levels correlate with allergy test results in patients with AD may be effective in both the pathophysiology of the disease and the development of patient-specific treatment strategies.

Methods

Study type and design

In this retrospective analysis, the records of patients diagnosed with atopic dermatitis and under the care of our Pediatric Allergy and Immunology Clinic from January 2021 to December 2022 were comprehensively examined. During this period, patients with available records and diagnosed with atopic dermatitis were included in the study. The diagnosis of atopic dermatitis was made according to the Hanifin-Rajka diagnostic criteria. Approval for the study's conduct was secured from the Ethics Committee of Umraniye Training and Research Hospital on January 26, 2023, with decision number 24.

Measures

The study involved the analysis of age, gender, eosinophil count, total IgE and specific IgE values (for both food and inhaler allergens). A specific IgE test for allergens, including food and inhalers, was conducted. The patients' IgE and eosinophil values were measured during their initial visit when they had complaints. The eosinophil count was determined from the peripheral blood smear or counter and values higher than 4% were considered eosinophilia. Allergen specific IgE measurements, ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) was used. Specific IgE levels were measured for inhaled allergens (house dust mite, cat epithelium, pollen), food allergens (cow's milk, egg white and food mix (milk, egg white, wheat, peanut, soya, fish) Specific IgE values, equal to or greater than 0,35 kU/L, were considered as positive. Epidermal SPT were performed with the use of allergen extracts (ALK-Abello, Madrid, Spain) along with a positive control (10 mg/dl of histamine phosphate) and a negative control (0.9% sterile saline). Horizontal and vertical measurements were performed for the indurations. Indurations were considered positive, if the average diameter at least 3 mm greater than the negative control. Patients who underwent SPT were tested for inhaled allergens including house dust mite, cat epithelium, tree mix, cockroach, alternaria and cladosporium. Among food allergens, cow's milk, egg white, egg yolk, wheat, peanut, hazelnut, walnut, chicken, red meat were tested. SPT were administered to patients with negative allergen-specific IgE results. Allergen positivity was defined as a positive outcome in either the allergen-specific IgE test or the SPT.

Statistical Analysis

The SPSS for Windows 25.0 program was employed for the statistical analysis and record of data. Descriptive results, including median, interquartile range (IQR), numbers (n), and percentages (%), were presented. The normal distribution was assessed using both visual (graphics) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). In cases of non-normally distributed data, the Mann–Whitney U test was applied to compare two independent variables. The Chi-square test was utilized to compare categorical data. Receiver Operating Characteristics (ROC) curve analysis was conducted to evaluate the predictive capacity of total IgE, eosinophils (absolute), and eosinophils (%) values for specific IgE test positivity concerning food and aeroallergens. Sensitivity and specificity values were computed for cut-off points. A significance level of $p<0,05$ was considered for statistical significance.

Results

The study assessed data from 486 pediatric patients diagnosed with AD. Among the patients, 53.9% (n=262) were male. The median age of the participants was 2.0 years (1.0-5.0). Table 1 presents the median values for absolute eosinophils, eosinophils (%), and total IgE, which were 290.0 10^3/uL (180.0-500.0), 3.4% (2.1-5.4), and 77.0 IU/ml (17.0-241.0), respectively. Aeroallergen sensitisation was present in 71 (14.6%) patients, food allergen sensitisation was present in 99 (20.3%) patients, and both food and aeroallergen sensitisation were present in 17 (3.5%) patients. While 129 (26.5%) of the patients had signs of allergic rhinitis (AR), 65 patients (13.3%) had asthma symptoms and 113 (23.2%) had food allergy symptoms (Table 1).

Patients with sensitization to at least one food or aeroallergen exhibited significantly elevated values in absolute eosinophil count, eosinophil (%), and total IgE compared to those without allergen sensitization ($p<0.001$). No notable correlation was observed between

age and the presence of allergen sensitization (p=0.195) (Table 2).

Tablo 1. Demographic and obstetric data of the participants

	n (%)
Gender	
-Male	262 (53.9)
-Female	224 (46.1)
.	**Median (IQR)**
Age (years)	2.0 (1.0-5.0)
Eosinophils (absolute) (10^3/Ul)	290.0 (180.0-500.0)
Eosinophils (%)	3.4 (2.1-5.4)
IgE (IU/ml)	77.0 (17.0-241.0)
	n (%)
Aeroallergen sensitivity	71.0 (14.6)
Food allergen sensitivity	99.0 (20.3)
Both food and aeroallergen sensitivity	17 (3.5)
Symptoms	
-Allergic rhinitis	129 (26.5)
-Asthma	65 (13.3)
-Food allergy	113 (23.2)

IQR:Interquartile range,

Conducting ROC analysis aimed to assess the predictive capability of total IgE values for allergen sensitization. The analysis revealed a cut-off point of 91.5 for total IgE in predicting allergen sensitization. The Area Under the Curve (AUC) (95% CI) was determined as 0.785 (0.772-0.843) (p<0.001) through ROC analysis. Sensitivity and specificity values for the identified cut-off point of total IgE were 73.3% and 72.9%, respectively.

In the ROC analysis for eosinophil values, the AUC was low (0.622 and 0.633, respectively). Therefore, the cut-off points for absolute and % eosinophil values were 500.0 and 5.0%, respectively. Patients with at least one of the absolute eosinophil and eosinophil (%) values above the cut-off point and total IgE above 91.5 were considered criteria positive. Allergen sensitization was detected in 72.2% (n=65) of criteria positive patients (n=90) (p<0.001) (Table 3).

Discussion

Immunoglobulin E, eosinophils, and basophils are involved in the pathogenesis of atopic disease. IgE is a key molecule that can activate effector cells involved in allergic inflammation.[5]

Table 2. Association of allergen sensitization with age, eosinophils and total IgE

	Allergen Sensitization				
	No		**Yes**		**p value**
	Median	IQR	Median	IQR	
Age (years)	2.0	1.00-5.00	2.0	1.0-6.0	0.195
Eosinophils (absolute)(10^3/uL)	250.0	170.0-430.0	380.0	210.0-630.0	<0.001
Eosinophils (%)	2.9	1.9-4.6	4.0	2.6-7.4	<0.001
Total IgE (IU/ml)	33.0	9.0-100.0	194.0	87.0-479.0	<0.001

IQR:Interquartile range

In our investigation, patients exhibiting sensitization to at least one food or aeroallergen displayed significantly elevated absolute eosinophil count, eosinophil (%), and total IgE values compared to those without allergen sensitization. A study by Özkars in the literature indicated higher eosinophil count and IgE levels in atopic dermatitis patients with food allergy compared to those without food allergy.[10]

Table 3. Association between criteria positivity and allergen sensitization

	Allergen sensitization n (%)		**P value**
Criteria positivity* n (%)	No	Yes	
Yes	25 (27.8)	65 (72.2)	<0.001
No	274 (69.2)	122 (30.8)	

* Absolute eosinophils >500, eosinophils (%) >5, total IgE >91.5

In contrast, İlhan et al.'s study on patients with atopic dermatitis found no parallel correlation between food sensitivity and IgE.[11] Lee et al. suggested that AD patients with concomitant allergic rhinitis were more likely to have higher serum IgE levels, attributing it to the proportional relationship between allergen-specific IgE levels and total serum IgE levels.[12] Saglam et al. discovered that median values of eosinophils (both absolute and %) and total IgE were higher in atopic disease patients with positive skin test and positive specific IgE test results compared to those with negative results.[13] Altaş et al.'s study on atopic dermatitis patients revealed higher total IgE levels in those with positive allergy test results compared to those without positive results.[14] Our study corroborates the efficacy of IgE level and eosinophil values as reliable markers for predicting allergies.

Eosinophils are believed to contribute to tissue damage in the pathogenesis of AD by releasing reactive oxygen metabolites and cytotoxic granules.[15] Borres et al.[16] indicated a connection between eosinophilia in peripheral blood and the presence of atopic disease or the likelihood of subsequent development. Our study examined absolute eosinophil count, eosinophils (%), and median values of total IgE. In Jenerowicz et al.'s study on atopic dermatitis patients, the absolute eosinophil count

was reported as 290.0 ± 205.7, eosinophil percentage as 6.3 ± 5.6%, and IgE and absolute eosinophil count were higher in patients with positive SPT compared to those with negative results.[17] Similar findings were observed in our study, with these values occurring at comparable rates.

Our study found a cut-off point of 91.5 in the ROC analysis, assessing the predictive capacity of total IgE values for allergen sensitization. The Area Under the Curve (AUC) was determined as 0.785, with sensitivity and specificity values of 73.3% and 72.9%, respectively. Jenerowicz et al., in their study, employed ROC curve analysis to assess the predictability of measured parameters in distinguishing AD patients from healthy individuals, finding an AUC value of 0.678 for peripheral blood eosinophilia determined by absolute eosinophil count.[17] Saglam et al. conducted ROC curve analysis to assess the predictive capability of eosinophil (absolute), eosinophil (%), and total IgE values for test positivity, which included results from both the SPT and/or specific IgE positivity. The total IgE cut-off point was 104.5 for all patients (AUC: 0.789), with sensitivity and specificity at 72.0% and 71.9%, respectively. In patients with atopic dermatitis, the total IgE cut-off point was 86.5. Notably, our study observed a higher total IgE cut-off point in atopic dermatitis patients compared to Saglam et al.'s study, although sensitivity and specificity rates were comparable.[13]

In conclusion, our findings suggest that total IgE and eosinophil values serve as reliable indicators of atopy in patients showing signs of allergy based on history and physical examination. This allows for patient monitoring until access to more detailed and costly tests, such as specific IgE and SPT, becomes feasible.

Result and Recommendations

In the study, allergen sensitization was detected in 3 out of every 4 AD patients (72.2%) with total IgE and eosinophil values above the cut-off point we analyzed. This suggests that total IgE and eosinophil values effectively predict allergen sensitization. The effectiveness of these laboratory parameters should be acknowledged in both the diagnosis and ongoing monitoring of atopic dermatitis. The risk of developing food allergy is lower in mild atopic dermatitis. Therefore, in mild AD patients, eosinophil and total IgE values in whole blood can be interpreted in terms of allergy in clinics that do not have access to allergy test or specific IgE. Since our patients were generally in the young age group, we did not differentiate by age group, but further studies can be performed with a larger patient group.

Compliance with Ethical Standards

Approval for the study's conduct was secured from the Ethics Committee of Umraniye Training and Research Hospital on January 26, 2023, with decision number 24.

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

All the authors equally contributed to this work.

Financial Disclosure

None

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Research Article | Araştırma Makalesi

APICAL SET-BACK, A SIMPLE MODIFICATION OF MUSTARDÉ-FURNAS TECHNIQUE OTOPLASTY

APICAL SET-BACK, MUSTARDÉ-FURNAS OTOPLASTİ TEKNİĞİNİN BASİT BİR MODİFİKASYONU

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ABSTRACT

Objective: To introduce an alternative way to perform otoplasy, a modified Mustarde-Furnas technique that decreases significantly the revision surgery.

Methods: Between 2015 and 2021, a total of 43 consecutive patients underwent otoplasty using a modified Mustarde-Furnas techique. All patients were followed-up for a period of 3 years after surgery.

Results: Reoperation was needed in just 2.5% of our patients. Some minor complications like suture extrusion(%12.5) and infection (%2.5) were encountered in the late postoperative period. No major complications occured in any of our patients.

Conclusion: Otoplasty is one of the most performed aestethic surgeries in younger patients. Based on our clinical experience, we observed that the results of our modified technique were more successful than those of standart techniques.

Keywords: Otoplasty, apical set back suture, Mustardé-Furnas technique

ÖZ

Amaç: Otoplasti ameliyatını yapmanın alternatif bir yöntemini anlatmak.

Yöntem: 2015-2021 yılları arasında yeni modifiye Mustardé- Furnas tekniği kullanarak opere edilen 43 primer hasta çalışmaya alınmıştır. Tüm hastalar en az 3 yıl süreyle takip edilmiştir.

Bulgular: Hastalarımızın sadece %2,5'inde yeniden operasyon gerekti. Geç postoperatif dönemde sütür atması (%12,5) ve enfeksiyon (%2,5) gibi bazı minör komplikasyonlarla karşılaşıldı. Hiçbir hastamızda majör komplikasyon gelişmedi.

Sonuç: Otoplasti genç hastalarda en çok uygulanan estetik ameliyatlardan biridir. Klinik tecrübemize dayanarak modifiye tekniğimizin sonuçlarının standart tekniklere göre daha başarılı olduğunu gözlemledik.

Anahtar Kelimeler: Otoplasti, apical-set back sütürü, Mustardé-Furnas tekniği

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Introduction

A prominent ear is a common deformity, affecting approximately 5% of the population. The two main causes of this deformity, which is commonly known to be autosomal dominant in inheritance, are a deep cavum concha and the absence of an antihelix curve.[1,2] These factors, individually or together, increase the concha-mastoid angle to more than 30 degrees, thereby increasing the distance between the auricle and the head. In the early 1990s, a prominent ear was described as an ear with a helical-mastoid distance of 20 mm or more.[3]

Although this deformity does not cause functional problems, it can have psychological effects on patients. Consequently, over the years, otoplasty has become one of the most commonly performed aesthetic procedures in children and adolescents.[4]

Various otoplasty techniques, categorized as either cartilage-sparing or cartilage-destructive methods, have been described since the early 20th century. Cartilage-destructive methods are primarily based on longitudinal incisions of the cartilage, resulting in a sharp contour of the antihelix.[5] We believe that cartilage-sparing techniques provide a more natural result.

We have observed that cases with unsatisfactory results are often those showing some laxity in the apical part of the auricle. It appears that permanent stability is lacking in this area.

Herein, we present our ten-year experience with a simple yet noteworthy modification of the Mustardé-Furnas technique.

Methods

This eight-year retrospective study was approved by the Antalya Education and Research Hospital Ethics Board (Decision No. 15/15, 11.08.2022). Informed consent for participation in the study was obtained from all patients. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

A total of 43 patients (29 women, 14 men) underwent surgery between 2015 and 2021. The patients' ages ranged from 6 to 45 years (mean age, 17 years). In total, 42 bilateral otoplasties and one unilateral primary otoplasty were performed. All patients underwent primary surgery using a modified Mustardé-Furnas technique. Patients with isolated deep cavum concha who did not require antihelix intervention were excluded from the study.

Surgical Technique

All adult patients were operated on under local anesthesia accompanied by IV sedation, while pediatric patients were operated on under general anesthesia. Prophylaxis included preoperative antibiotics.

Preoperative preparation of both ears was performed to ensure that both could be equally viewed to allow intraoperative comparison. Before the local anesthetic was injected, the neo-antihelix position was planned and marked (Figure 1a). Then, the distance between the pinna and mastoid was measured in the apical, middle, and lower positions.

On the posterior face of the auricle, an "8" shape or sandglass shape (Figure 1b) was drawn to mark the course for skin excision. Then, local anesthetics (Figure 1c), including lidocaine and 1:200,000 epinephrine, were injected into the postauricular area, mastoid tip, and temporal fascia region. Next, the skin was excised. The perichondrium was then carefully dissected and exposed (Figure 2a). Skin excision allowed easy access to the conchal bowl if cartilage excision was required, and simultaneously allowed the surgeon to work freely in the area where the antihelix was planned. Careful excision of the mid-auricular skin avoids the risk of postoperative sulcus synechiae. Moreover, it is important to have realistic expectations regarding the impact of skin excision on the final shape due to its elastic structure. Nevertheless, skin excision was necessary because if not performed, skin redundancy would have been apparent at the end of the operation.

At this stage, we exposed the mastoid tip and the temporal fascia but did not cut or touch the posterior auricular muscle in any way to maintain existing stability. If conchal bowl reduction was needed, we performed it at this stage.

Next, Mustardé sutures were placed using the suture guide points. Three 4.0 round white Prolene sutures were applied using the horizontal mattress suture technique (Figure 2b). We applied and simultaneously tied all sutures to ensure equal tension and to create a natural antihelical curve. Immediately thereafter, the setback suture, commonly known as the Furnas suture, was stitched between the conchal bowl and the mastoid tip.

At this stage of the operation, we realized that the apex of the auricle could not be aligned exactly as planned in a large proportion of patients. Even in patients where we achieved exact alignment as planned, laxity occurred in the late postoperative period. Based on our clinical experience, we were convinced that additional fixation would be needed for the auricular apex.

As the final step before skin closure, we placed an additional setback suture parallel to the original Furnas suture. This critical suture bonds the apex of the auricle to the temporal fascia (Figure 3a, b), ensuring stability in a region prone to postoperative laxity. The temporal fascia, known for its robust and resilient nature, provides a strong anchor point for the auricular apex. By incorporating this extra layer of fixation, we aimed to maintain the desired alignment and contour of the auricle over the long term, addressing the common issue of late postoperative laxity observed in many patients. This additional apical setback suture was instrumental in achieving the structural integrity and aesthetic outcomes we sought, highlighting the importance of comprehensive fixation in otoplasty procedures.

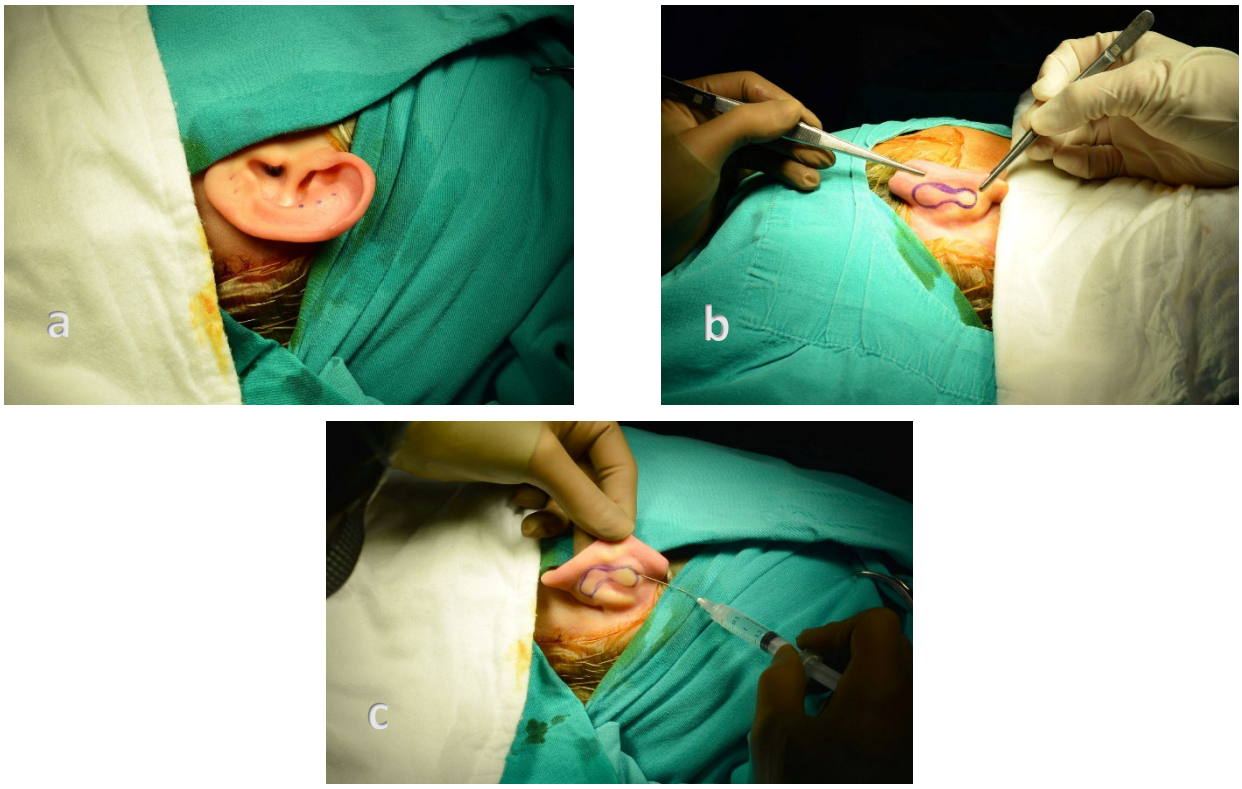

Figure 1. The neo-antihelix position was planned and marked in advance (a), In the posterior face of the auriclen an "8" shape/sandglass shape is drown (b), local anaesthetic application (c)

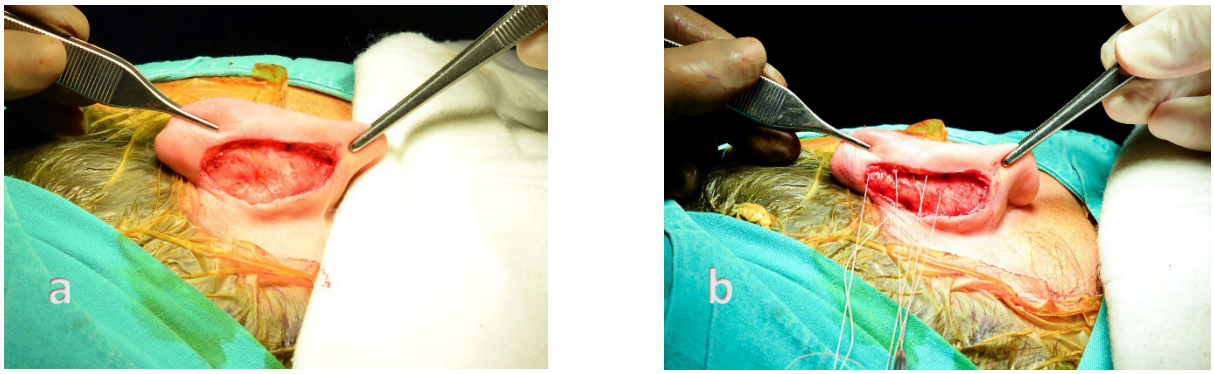

Figure 2. Postauricular skin excision and pericondrium exposition is performed (a), a minimum of 3 Mustardé sutures is placed using the suture guide points (b)

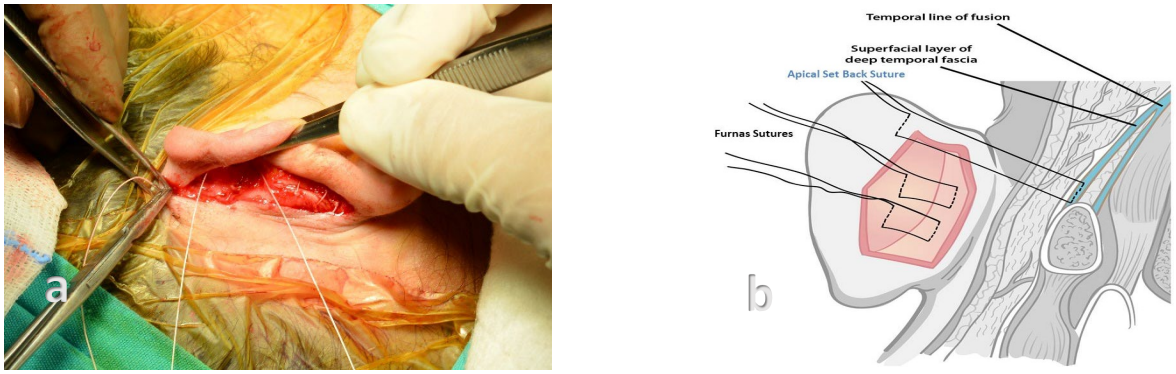

Figure 3. Demonstration of apical set back suture (a) and its schematic drawing (b)

Finally, the skin was closed with 5.0 Prolene sutures (Figure 4a). Postoperatively, we placed a cotton ball with antibiotic ointment within the newly created contours of the ears and then wrapped the head. This ensured that the ears maintained their new shape and were protected from infection. Patients were instructed to wear a headband continuously for 24 hours a day during the first week to support the ears in their new position and minimize movement. After the first week, patients were advised to wear the headband only at night for the following two weeks to ensure continued stability and optimal healing.

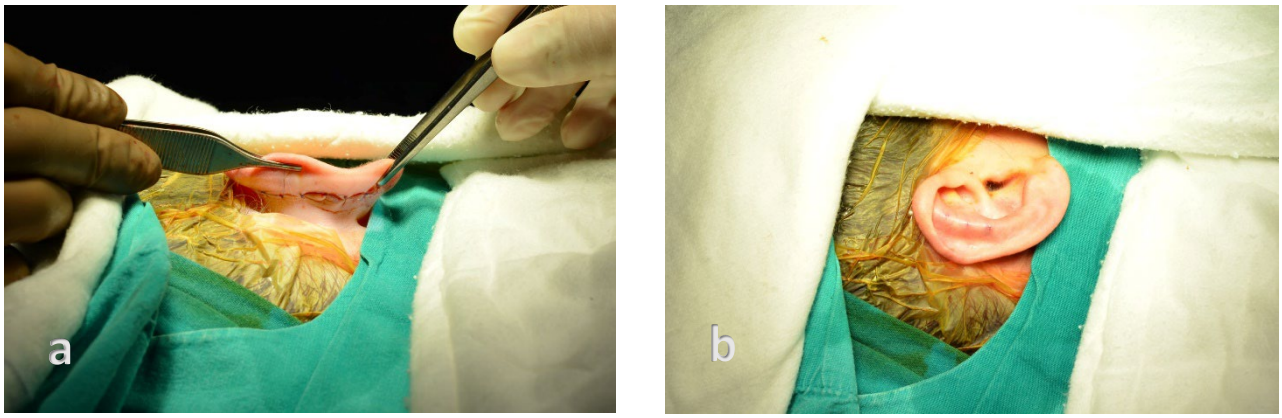

Figure 4. The closed incision is shown (a) , The anterior view of the ear at the end of the operation (b)

Results

In this study, the follow-up period ranged from 6 to 36 months after surgery. The postoperative clinical evaluation included inspection and photographic documentation at 1 month (Figure 5), 6 months (Figure 6), and annually thereafter (Figure 7). No notable complications, such as hematoma, perichondritis, cellulitis, or skin/cartilage necrosis, were observed. Some minor complications were encountered in the late postoperative period: suture extrusion in 5 patients (12.5%) and infection in one patient (2.5%). One patient, who underwent unilateral otoplasty, required reoperation due to partial relapse one year postoperatively.

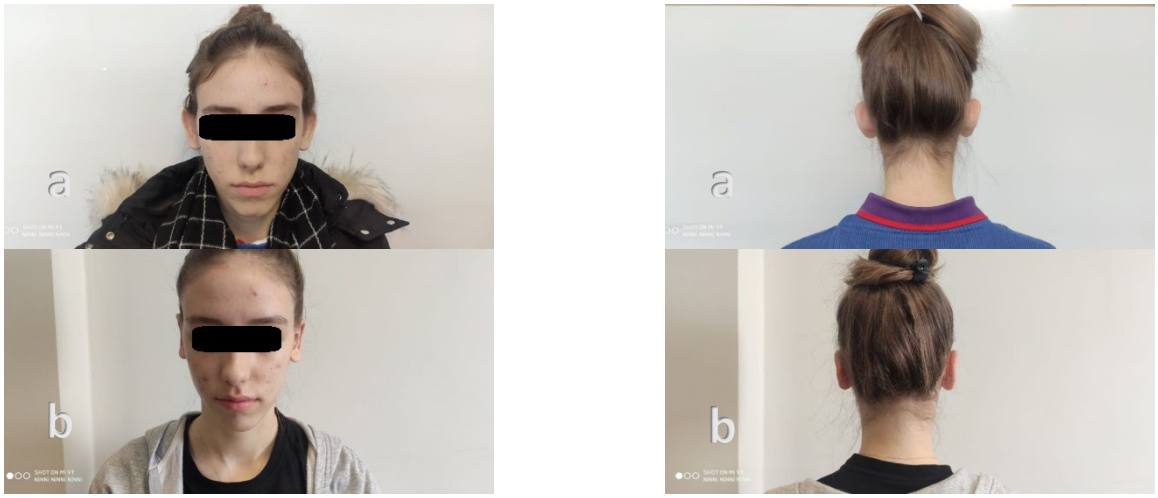

Figure 5. Preoperative (a) and 1 month postoperative (b) photo

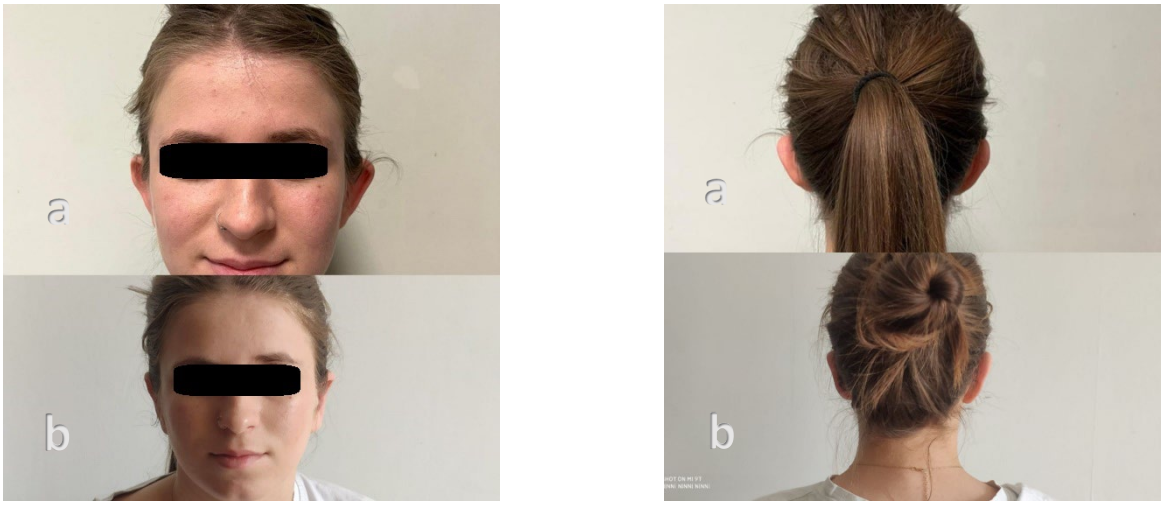

Figure 6. Preoperative (a) and postoperative 6 month (b) photo

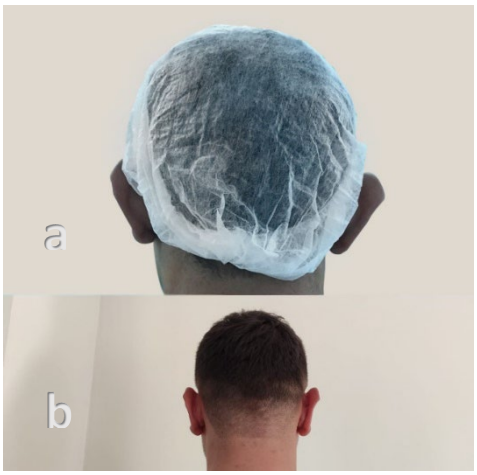

Figure 7. Preoperative (a) and 3 year postoperative (b) photo

Discussion

At present, otoplasty is one of the most commonly performed aesthetic surgeries in younger patients. Although it can be performed at any point in the lifespan, it is known that as patients age, cartilage motility is reduced, which may lead to less successful outcomes. Mustardé et al., in their 10-year otoplasty follow-up study[4], reported that 1.8% of patients operated on before age 6 showed relapse, whereas approximately 30% of patients who underwent the operation at an older age showed relapse. In our study, the median age of patients was 17 years.

It is important to note that in the literature, cases reported as failures are mostly due to undercorrection, particularly in the upper third of the auricle.[6] In our study, we introduced a technique that effectively addresses this issue. After the posterior skin was excised and undermined, we applied at least three horizontal mattress sutures, as recommended by Mustardé.[7] We then placed at least two Furnas sutures, followed by a critical innovation: the apical setback suture. This apical setback suture, stitched between the auricular apex and the temporal fascia, plays a pivotal role in maintaining the planned position of the ear and ensuring long-term stability. By anchoring the auricular apex securely to the strong temporal fascia, this technique not only reinforces the contour but also reduces the need for excessive Mustardé sutures, which can sometimes lead to complications or overcorrection. Our apical setback modification has proven highly effective, meeting the goals outlined in the literature[8] and demonstrating its value in preventing relapse and improving aesthetic outcomes. No infections or other complications were observed during the follow-up period, underscoring the success of this approach.

Although a prominent ear does not typically have significant physiological effects, it can have a profound psychological impact on patients.[9] The appearance of the ear can significantly affect self-esteem and social interactions, making it a sensitive issue for many individuals. Therefore, no surgeon wants to repeat a surgery on a deformity that is such a sensitive topic for the patient. Addressing the issue effectively in the initial procedure is crucial to avoid the need for revision surgery and to ensure the best possible outcome for the patient's psychological well-being.

Conclusion

Based on our clinical experience, we observed that the results of our modified technique were more successful compared to standard techniques. However, it is important to note that this is a preliminary study. We believe that as more patients undergo the surgery and the follow-up periods extend, a more comprehensive evaluation of the results will be possible. Continued assessment will help to validate the efficacy of our modified technique and provide further insights into its long-term outcomes.

Compliance with Ethical Standards

This article does not contain any studies with animals performed by the authors.

All procedures performed in the study involving human participants were in accordance with the ethical standarts of the institutional and/or national research comittee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standarts.

Informed consent was obtained from all individual participants involved in the study.

Conflict of Interest

The author declares that they have no conflicts of interes.

Author Contribution

The study was designed and written by the author. We feel it would be important to emphasise that all the patients were operated by one surgeon, who is the author herself.

Financial Disclosure

The author did not get any grant support from any institution

Main Points:

Prominent ear is a commonly seen deformity that has important phsycological effects on patients.

In the last century numerous otoplasty techniques have been described. As already has been mentioned in the literature the majority of cases showing unsatisfactory results are often the ones showing some laxing in the apical part of the auricula. It seems that we lack permanent stability in this part of the auricle.

We present a simple and effective modification of common otoplasty techniques. In our patients we placed an apical setback suture parallel to the original Furnas suture, bonding the apex of the auricle to the temporal fascia, which is a very strong connective tissue.

This additional suture seems to give us the missing fixation.

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Araştırma Makalesi | Research Article

TIP FAKÜLTESİ ÖĞRENCİLERİNİN HUMAN PAPİLLOMA VİRÜS ENFEKSİYONU VE AŞISI KONUSUNDA BİLGİ TUTUM VE DAVRANIŞLARININ BELİRLENMESİ

DETERMINATION OF THE INFORMATION, ATTITUDES AND BEHAVIORS OF MEDICAL FACULTY STUDENTS TOWARDS HUMAN PAPILLOMA VIRUS INFECTION AND ITS VACCINE

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ÖZ

Amaç: Toplumda aşılanma oranının yükseltilmesinde doktorların tavsiyesinin büyük rolü olduğu için çalışmamızda tıp fakültesinde öğrenim gören öğrencilerin HPV enfeksiyonu ve aşısına ilişkin bilgi tutum ve davranışlarının değerlendirilmesi ve farkındalığın arttırılmasını amaçlamaktayız.

Yöntem: Gaziantep Üniversitesi Tıp Fakültesi'nde 2023 yılında eğitim öğretim döneminde öğrenim gören tıp fakültesi öğrencileri üzerinde yapıldı. Çalışma Haziran 2023-Kasım 2023 tarihleri arasında yürütüldü. Bu çalışma, tanımlayıcı tipte kesitsel bir çalışmadır. Çalışma, katılımcıların HPV enfeksiyonu ve aşısı konusunda bilgi, tutum ve davranışlarını değerlendirmek amacıyla anket çalışması yapılarak uygulandı.

Bulgular: Çalışmaya katılan 328 öğrencinin %47,9'u kadın, %52,1'i erkek olup, yaş aralıkları 17 ile 34 arasında değişmekteydi. Öğrencilerin %42,4'ü HPV enfeksiyonu hakkında bilgi düzeyini yeterli buluyorken, %31,1'i HPV aşısı hakkında bilgi düzeyini yeterli bulmaktaydı. Katılımcıların %8,8'i HPV aşılarından birini yaptırdığını, %81,1'i HPV aşılarından birini yakınlarına önereceğini belirtti. Cinsiyete ve medeni duruma göre HPV enfeksiyonu ve aşısı hakkında bilgi düzeyi puanları benzer olarak saptandı (p>0,05). Öğrencilerin son üç sınıftaki HPV enfeksiyonu ve aşısı hakkında bilgi düzeyi puanları, ilk üç sınıfa göre anlamlı olarak yüksek bulundu (p<0,05). Katılımcıların %64,6'sı HPV aşısını ücretli olarak alıp yaptıracaklarını, %76,8'i sosyal güvence kapsamında karşılanırsa yaptıracaklarını belirtti.

Sonuç: Tıp fakültesi öğrencilerinde HPV enfeksiyonu ve aşısı hakkında bilgi düzeyinde yetersizlik söz konusudur. Öğrenciler arasında HPV aşısı yaptırma oranı da düşüktür. HPV aşısı ulusal bağışıklama programına eklenirse aşılanma oranının artacağı düşünülmektedir.

Anahtar Kelimeler: HPV, HPV aşısı, öğrenci, bilgi, tutum

ABSTRACT

Objective: Since the recommendation of doctors plays a significant role in increasing vaccination rates in society, our study aims to evaluate the knowledge, attitudes, and behaviors of medical faculty students regarding HPV infection and vaccine, and to increase awareness.

Method: The study was conducted on medical faculty students studying at Gaziantep University Faculty of Medicine during the 2023 academic year. The study was carried out between June 2023 and November 2023. This study is a descriptive cross-sectional study. A survey was conducted to assess the knowledge, attitudes, and behaviors of the participants regarding HPV infection and vaccine.

Results: Of the 328 students participating in the study, 47.9% were female and 52.1% were male, with ages ranging from 17 to 34. While 42.4% of the students found their knowledge level sufficient about HPV infection, 31.1% found their knowledge level sufficient about HPV vaccine. 8.8% of the participants stated that they had received one of the HPV vaccines, and 81.1% stated that they would recommend one of the HPV vaccines to their relatives. Knowledge level scores regarding HPV infection and vaccine were found to be similar according to gender and marital status (p>0.05). Students' knowledge level scores about HPV infection and vaccine in the last three grades were found to be significantly higher than those in the first three grades (p<0.05). 64.6% of the participants stated that they would pay for and receive the HPV vaccine, while 76.8% stated that they would receive it if covered by social security.

Conclusion: There is insufficient knowledge about HPV infection and vaccine among medical faculty students. The rate of HPV vaccination among students is also low. It is thought that the vaccination rate will increase if the HPV vaccine is added to the national immunization program.

Keywords: HPV, HPV vaccine, student, knowledge, attitude

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Giriş

Serviks kanserinden sorumlu tutulan Human Papilloma Virus (HPV), cinsel aktif kadınlarda en sık görülen cinsel yolla bulaşan hastalık etkenidir.[1] HPV'nin düşük riskli benign özellikte ve yüksek riskli malign özellikte kutanöz veya mukozal lezyonlara neden olan 200'ü aşkın HPV genotipi tanımlanmıştır. Düşük riskli HPV tipleri anogenital bölgede siğiller ve kondilom ile ilişkiliyken, yüksek riskli HPV tipleri serviks, anüs, penis, vajen, vulva ve orofarenks kanserler ile ilişkilidir.[2] Servikal kanserlerin gelişiminden HPV tip 16 ve 18 sorumlu iken, genital siğillerin gelişiminden HPV tip 6 ve 11 sorumludur.[3]

HPV'nin en önemli bulaş yolu cinsel yoldur. Ayrıca ciltteki lezyonlardan, kontamine yüzeylerden, doğum kanalından direkt ve indirekt yollarla bulaşabilmektedir.[4] HPV enfeksiyonu için çeşitli risk faktörleri bulunmaktadır. Cinsel hayatın erken yaşta başlaması, korunmasız cinsel ilişki ve cinsel partner sayısının fazla olması HPV ile temas riskini arttırmakta iken; kondom kullanımı, HPV bulaş riskini azalttığı için serviks kanseri oluşma riskini de azaltmaktadır.[5-7]

Servikal kanserler büyük çoğunlukla HPV enfeksiyonu ile ilişkilidir.[6] Serviks kanseri, önlenebilir olmasına rağmen, kadınlarda kanser ölümlerinin önde gelen nedenidir. Dünyada kadınlarda en sık teşhis edilen dördüncü kanserdir. Kanserden ölümlerin de en sık dördüncü sebebidir. 2022'de dünya çapında 661.044 kadın serviks kanseri tanısı almış ve yaklaşık 348.186 kadın da serviks kanseri nedeniyle hayatını kaybetmiştir.[8] Vakaların çoğu aşılama ve tarama programlarının uygulanmadığı düşük ve orta gelirli ülkelerde görülmektedir.[9]

Google Akademik ve Medline'deki veri tabanlarından yola çıkılarak yapılan bir çalışmada Amerika Birleşik Devletleri ve Avrupa'da partneri kadın olan erkeklerde HPV enfeksiyonu prevalansı %12,9 ile %86 arasında değişkenlik göstermekte olup toplam prevalans %49.1 olarak bulunmuştur.[10] Erkekler arasında HPV enfeksiyonu prevalansının incelendiği bir çalışmada, prevalansın %1,3 ile %72,9 arasında değişkenlik gösterdiği bulunmuş ve oranlardaki bu farklılığın hasta popülasyonu ve alınma tekniğindeki farklılıktan kaynaklandığı bulunmuştur.[11,12]

HPV'den primer korunma, risk faktörlerinin ortadan kaldırılmasını ve profilaktik aşı uygulamalarını kapsamaktadır.[13] HPV'nin yol açtığı enfeksiyonlara karşı korumada aşılar geliştirilmiştir. 3 adet HPV aşısı bulunmaktadır. Bivalan aşı HPV tip 16 ve 18'e karşı geliştirilmiştir. Kuadrivalan aşı HPV tip 6, 11, 16 ve 18'e karşı koruma sağlamaktadır. Nanovalan aşı ise HPV tip 6, 11, 16, 18, 31, 33, 45, 52 ve 58'e karşı koruma sağlamaktadır.[14] Bivalan aşı 9-25 yaş arası kadınlarda, kuadrivalan aşı 9-26 yaş arası kadın ve erkeklerde, nanovalan aşı ise 9-45 yaş arası kadın ve erkeklerde kullanımı onay almıştır.[14] 2009'dan bu yana Dünya Sağlık Örgütü (DSÖ), HPV aşısının ulusal bağışıklık programına dahil edilmesini tavsiye etmektedir; fakat ülkemizde hala dahil edilmemiştir. 117 ülkede, Mart 2022 itibariyle HPV aşısı ulusal bağışıklama programlarına dahil edilmiştir.[15]

Bu çalışmada tıp fakültesi öğrencilerinin HPV enfeksiyonu ve aşısı ile ilgili bilgi, tutum ve davranışlarını belirlemek amaçlandı.

Yöntem

Etik Kurul ve İzinler

Bu çalışma Gaziantep Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 10.05.2023 tarih ve 2023/155 sayılı karar ile bilimsel ve etik açıdan uygun görülmüştür. Anket çalışma izni, Gaziantep Üniversitesi Tıp Fakültesi Dekanlığı tarafından 03.05.2023 tarihli ve 323970 sayılı karar ile uygun görülmüştür. Bu çalışma; Gaziantep Üniversitesi Tıp Fakültesi'nde 2023 yılında eğitim öğretim döneminde öğrenim gören tıp fakültesi öğrencileri üzerinde yapıldı.

Araştırmanın Tipi

Bu çalışma, kesitsel tipte gözlemsel bir çalışmadır. Çalışma, katılımcıların HPV enfeksiyonu ve aşısı konusunda bilgi, tutum ve davranışlarını değerlendirmek amacıyla anket çalışması yapılarak uygulandı.

Araştırmanın Evreni

Araştırmanın evrenini; Gaziantep Üniversitesi Tıp Fakültesi'nde 2023 yılında öğrenim görmekte olan tıp fakültesi öğrencileri oluşturdu. Dönem 1'de 423, dönem 2'de 319, dönem 3'te 249, dönem 4'te 273, dönem 5'te 271, dönem 6'da 207 öğrenci olup toplam öğrenci sayısı 1742'dir. Araştırmaya 328 kişi katıldı.

Araştırmaya Dahil Olma Kriterleri

Gaziantep Üniversitesi Tıp Fakültesi'nde 2023 yılında öğrenim görmekte olan 1742 tıp fakültesi öğrencisinden çalışmaya katılmayı kabul edip onam veren öğrenciler dahil edildi.

Araştırmadan Dışlama Kriterleri

Gaziantep Üniversitesi Tıp Fakültesi'nde 2023 yılında öğrenim görmekte olan tıp fakültesi öğrencilerinden araştırmaya katılmaya kabul etmeyenler ve anket sorularına eksik cevap verenler çalışma dışı bırakıldı.

Araştırmanın Örneklem Büyüklüğü

2023 yılında Gaziantep Üniversitesi Tıp Fakültesi'nde öğrenim gören 1742 tıp fakültesi öğrenci bulunmakta ve %95 güven aralığında %5 hata payı ile minimum örneklem büyüklüğü 315 olarak hesaplandı.

Verilerin Toplanması

Verilerin toplanması için bir çevrimiçi anket formu hazırlandı. Anket formu hem yüz yüze hem de Google forms yöntemi kullanılarak link olarak, tıp fakültesindeki öğrencilerin mail adreslerine veya telefon numaralarına mesaj yoluyla iletilmiştir. Literatür taranması ve daha önce yapılan çalışmaların incelenmesi sonucunda 57 sorudan oluşan anket formu hazırlandı. İlk bölüm 16 sorudan oluşmakta olup katılımcıların sosyodemografik özellikleri (yaş, cinsiyet, medeni durum, eğitim, ekonomik durum) sorgulandı. İkinci bölüm ise 41 sorudan oluşmakta olup

katılımcıların HPV enfeksiyonu ve aşısı konusunda bilgi, tutum ve davranışları sorgulandı. İkinci bölümde "Katılıyorum-Katılmıyorum-Fikrim yok" şeklinde seçenekler sunularak birini seçmeleri istendi. Likert tipi derecelendirme yapılan bu sorularda her doğru cevap için 1 puan, yanlış ve bilmiyorum şeklindeki cevaplar 0 puan olarak değerlendirildi. HPV enfeksiyonu ile ilgili alınacak en yüksek puan 20 iken, en düşük puan 0 idi. HPV aşısı ile ilgili alınacak en yüksek puan 16 iken, en düşük puan 0 idi. Yüksek puan alınması, bireyin HPV ve aşısı konusunda bilgisinin yüksek olduğunu göstermektedir. Bilgi düzeyi değerlendirilmesinde kesme değeri belirlenmedi. Veriler gönüllülük esasına uygun olarak, katılmayı kabul eden tıp fakültesi öğrencilerinden toplandı. Bilgilendirilmiş onam formuna onay verenler (Google form) ve imzalayanlar (yüz yüze anket) çalışmada yer aldı**.**

İstatiksel Analiz

Araştırmada elde edilen veriler SPSS 22.0 yazılımı kullanılarak analiz edilmiştir. Verilerin dağılımı tanımlayıcı analiz parametreleri (frekans, ortalama ve standart sapma) ile gösterilmiştir. Sayısal değişkenlerin normal dağılıma uygunluğu Shaphiro Wilk testi ile değerlendirildi. Normal dağılmayan değişkenlerin ikili karşılaştırılmasında Mann Whitney U testi, üç ve üzeri grup karşılaştırılmasında Kruskal Wallis ve Dunn testleri kullanıldı. Sayısal değişkenler arasındaki ilişkiler Spearman rank korelasyon katsayısı ile, kategorik değişkenler arasındaki ilişkiler kikare testi ile test edildi. $p<0,05$ olduğu durumlar istatiksel anlamlı kabul edildi.

Bulgular

Katılımcıların %47,9'u kadın (n=157), %52,1'i erkek (n=171) olup, yaş aralığı 17-34 olarak saptandı. Öğrencilerin %97'si (n=318) bekar olduğunu, %57,6'sı (n=189) ailelerinin ekonomik durumlarının orta düzeyde olduğunu, %37,8'i (n=124) sigara kullandığını belirtti.

Katılımcıların %42,4'ü (n=139) HPV enfeksiyonu hakkında bilgi düzeyini yeterli, %57,6'sı (n=189) yetersiz bulduğunu ifade etti. Öğrencilerin %5,2'si (n=17) fakülte öncesi eğitim sürecinde, %64'ü (n=210) fakültedeki eğitim sürecinde, %7,6'sı (n=25) yazılı veya görsel basın aracılığıyla, %19,8'i (n=65) internet aracılığıyla ve %3,4'ü (n=11) sağlık çalışanları vasıtasıyla bilgi sahibi olduklarını belirtti.

Katılımcıların %31,1'i (n=102) HPV aşıları hakkında bilgi düzeyini yeterli, %68,9'u (n=226) yetersiz bulduğunu ifade etti. Öğrencilerin %5,8'i (n=19) fakülte öncesi eğitim sürecinde, %61'i (n=200) fakültedeki eğitim sürecinde, %7,3'ü (n=24) yazılı veya görsel basın aracılığıyla, %21'i (n=69) internet aracılığıyla, %0,6'sı (n=2) kongrelerde ve %4,3'ü (n=14) sağlık çalışanları vasıtasıyla bilgi sahibi olduklarını belirtti.

HPV enfeksiyonu ve aşıları ile ilgili eğitim almak ister misiniz? Sorusuna öğrencilerin %94,8'i (n=311) 'evet', %5,2'si (n=17) 'hayır' şeklinde yanıt verdi. Evet diyenlerin %48'si (n=150) fakültede eğitim müfredatı ile, %31,8'i (n=99) kongrelerde, %7,1'i (n=22) öğrenci toplulukları ile, %12,9'u (n=40) internet aracılığıyla bilgilendirme yapılmasını istediklerini belirttiler.

Daha önce cinsel birliktelik yaşama durumuna öğrencilerin %23,2'si (n=76) evet, %67,7'si (n=222) hayır, %9,1'inin (n=30) belirtmek istemiyorum şeklinde yanıt verdi. Daha önce cinsel deneyimi olan öğrencilerin %22,4'ünün (n=17) HPV aşılarından birini yaptırdığı; %77,6'sının (n=59) HPV aşısı yaptırmadığı belirtti. HPV aşısı yaptırma oranı; daha önce cinsel birliktelik yaşayan kişilerde, cinsel birliktelik yaşamayanlara göre anlamlı oranda yüksek bulundu (p=0,001). Katılımcıların cinsel deneyimleri ve HPV aşısı yaptırma bilgileri tabloda sunuldu (Tablo 1).

Daha önce cinsel deneyimi olan öğrencilerin %89,5'inin (n=68) HPV aşılarından birini yakınlarına önerdiği; %10,5'inin (n=8) önermediği tespit edildi. Katılımcıların cinsel deneyimlerine göre yakınlarına HPV aşılarından birini önermeleri arasında istatistiksel olarak anlamlı bir farklılık saptanmadı (p=0,102).

Tablo 1. Cinsel Deneyim ile HPV Aşısı Yaptırma ve Önerme Arasındaki İlişki

		Daha önce cinsel birlikteliğiniz oldu mu?			
		Evet	Hayır	Belirtmek istemiyorum	
		n (%)	n (%)	n (%)	p
HPV aşılarından birini yaptırdınız mı?	**Evet**	17 (22,4)	10 (4,5)	2 (6,7)	0,001*
	Hayır	59 (77,6)	212 (95,5)	28 (93,3)	
HPV aşılarından birini yakınlarınıza önerir misiniz?	**Evet**	68 (89,5)	174 (78,4)	24 (80)	0,10
	Hayır	8 (10,5)	48 (21,6)	6 (20)	

*p<0,05 düzeyinde anlamlı, Ki-kare testi *n: Öğrenci sayısı

Öğrencilerin HPV enfeksiyonunun bulaş yolları ile ilgili sorulara doğru cevap verme oranları; 'Cinsel yolla bulaşır' doğru önermesine %90,9 (n=298), 'Solunum yolu ile bulaşır' yanlış önermesine %77,4 (n=254), 'Kan ve kan ürünleri bulaşır' yanlış önermesine %26,5 (n=87) olarak belirlendi.

Katılımcıların HPV enfeksiyonunun neden olduğu hastalıklara yönelik sorulara verdikleri doğru cevap oranları; 'HPV genital siğile neden olur' önermesi %86,3 (n=283), 'HPV servikal kansere neden olur' önermesi %82,9 (n=272), 'HPV oral kansere neden olur' önermesi %57,3 (n=188), 'HPV anal kansere neden olur' önermesi %47,6 (n=156), 'HPV penis kanserine neden olur' önermesi %45,4 (n=149) olarak belirlendi (Tablo 2).

HPV enfeksiyonu ile ilgili diğer sorulara verilen doğru cevap oranları; 'HPV'nin yol açtığı enfeksiyon önlenebilir' ifadesi için %72,6 (n=238), 'HPV'nin yol açtığı enfeksiyon tedavi edilebilir' ifadesi için %59,8 (n=196), 'HPV bulaşını önlemek için kullanılan en güvenilir yöntem kondom kullanımıdır' ifadesi için %54,0 (n=177) olarak saptandı.

Katılımcılarca 'HPV sadece kadınlarda enfeksiyona yol açar' ifadesi %78,4 (n=257), 'HPV sadece erkeklerde enfeksiyona yol açar' ifadesi %84,1 (n=276), 'HPV hem kadında hem erkekte enfeksiyona yol açar' ifadesi %81,7 (n=268), 'HPV'nin yol açtığı enfeksiyonlardan korumada aşı yüzde yüz koruma sağlar' ifadesi %61,3 (n=201) oranında doğru olarak cevap verilmişti. Katılımcıların bilgi sorularına verdikleri yanıtlar tabloda sunuldu (Tablo 2).

Tablo 2. HPV Enfeksiyonu Hakkında Bilgi Düzeyini Ölçen Sorulara Verilen Cevapların Dağılımı

	Katılıyorum	**Katılmıyorum**	**Fikrim yok**
	n (%)	**n (%)**	**n (%)**
HPV cinsel yolla bulaşır.	***298 (90,9)***	9 (2,7)	21 (6,4)
HPV solunum yoluyla bulaşır.	25 (7,6)	***254 (77,4)***	49 (14,9)
HPV kan ve kan ürünleri ile bulaşır.	191 (58,2)	***87 (26,5)***	50 (15,2)
HPV genital siğillere neden olur.	***283 (86,3)***	6 (1,8)	39 (11,9)
HPV servikal kansere neden olur.	***272 (82,9)***	13 (4,0)	43 (13,1)
HPV oral kansere neden olur.	***188 (57,3)***	44 (13,4)	96 (29,3)
HPV anal kansere neden olur.	***156 (47,6)***	56 (17,1)	116 (35,4)
HPV penis kanserine neden olur.	***149 (45,4)***	70 (21,3)	109 (33,2)
HPV'nin yol açtığı enfeksiyonlar genellikle semptomatiktir.	133 (40,5)	***107 (32,6)***	88 (26,8)
HPV'nin yol açtığı enfeksiyon önlenebilir.	***238 (72,6)***	33 (10,1)	57 (17,4)
HPV'nin yol açtığı enfeksiyon tedavi edilebilir.	***196 (59,8)***	55 (16,8)	77 (23,5)
HPV bulaşını önlemek için kullanılan en güvenilir yöntem kondom kullanımıdır.	***177 (54,0)***	66 (20,1)	85 (25,9)
HPV sadece kadınlarda enfeksiyona yol açar.	28 (8,5)	***257 (78,4)***	43 (13,1)
HPV sadece erkeklerde enfeksiyona yol açar.	11 (3,4)	***276 (84,1)***	41 (12,5)
HPV hem erkekte hem kadında enfeksiyona yol açar.	***268 (81,7)***	18 (5,5)	42 (12,8)
HPV'nin yol açtığı tüm enfeksiyonlardan korunmada aşı yüzde yüz koruma sağlar.	40 (12,2)	***201 (61,3)***	87 (26,5)
HPV tarama testi 30-65 yaş aralığında yapılmaktadır.	***168 (51,2)***	65 (19,8)	95 (29,0)
HPV tarama testi sadece kadınlara yapılır.	***77 (23,5)***	195 (59,5)	56 (17,1)
HPV tarama testi sadece erkeklere yapılır.	6 (1,8)	***258 (78,7)***	64 (19,5)
HPV tarama testi hem erkeklere hem kadınlara yapılır.	201 (61,3)	***62 (18,9)***	65 (19,8)

*n= Öğrenci sayısı. Doğru cevaplar tabloda bold ve italik olarak gösterilmiştir.

Kadınların HPV enfeksiyonu konusunda bilgi düzeyi puanı ortalaması 12,24 ± 4,17; erkeklerinki 11,99 ± 4,57 olarak bulundu. Kadın ve erkeklerin HPV konusundaki bilgi düzeyi puanı arasında anlamlı fark olmadığı belirlendi (p>0,05). Sınıflara göre HPV konusunda bilgi düzeyi puanları arasında istatistiksel olarak anlamlı bir fark olduğu saptandı (p=0,001). Bu anlamlı farklılığa sebep olan grupları belirlemek için yapılan ikili karşılaştırmalarda; 1. sınıf öğrencilerin puanları 3. sınıf (p=0,001), 4. sınıf (p=0,001), 5. sınıf (p=0,001) ve 6. sınıf (p=0,001) öğrencilerinden düşük bulundu (p<0,05). 2. sınıf öğrencilerin puanları 3. sınıf (p=0,001), 4. sınıf (p=0,001), 5. sınıf (p=0,001) ve 6. sınıf (p=0,001) öğrencilerinden düşük olduğu tespit edildi (p<0,05). 3. sınıf öğrencilerin puanları 4. sınıf (p=0,013) ve 6. sınıf (p=0,015) öğrencilerinden düşük olduğu görüldü (p<0,05) (Kruskall Wallis testi). Diğer ikili karşılaştırmalarda sınıflara göre bilgi düzeyi puanı benzer bulundu (p>0,05). Medeni durumuna göre HPV enfeksiyonu konusunda bilgi düzeyi puanları benzer olarak bulundu (p>0,05). Daha önce cinsel deneyimi olan öğrencilerin HPV enfeksiyonu bilgi düzeyi puanı ortalaması 14,05±3,22 olup istatistiksel olarak anlamlı farklılık saptandı (p=0,001). Bu anlamlı farklılığa sebep olan grupları belirlemek için yapılan ikili karşılaştırmalarda; cinsel deneyimini belirtmek istemeyenlerin puanının, hayır (p=0,003) ve evet (p=0,001) diyen öğrencilere göre düşük olduğu görüldü (p<0,05). Daha önce cinsel deneyimi olmayan öğrencilerin bilgi puanlarının, cinsel deneyimi olan öğrencilere göre anlamlı oranda düşük olduğu tespit edildi (p<0,05). Katılımcıların bilgi düzeyi puanları tabloda sunuldu (Tablo 3).

Kadınların %44,1'i (n=45), erkeklerin %55,9'u (n=57) HPV aşıları hakkında bilgi düzeyini yeterli bulduklarını belirtti. Cinsiyete ve medeni duruma göre HPV aşıları hakkında bilgi düzeyini yeterli bulma durumu benzer saptandı (p>0,05). 4., 5. ve 6. sınıfların HPV aşıları hakkında bilgi düzeyini yeterli bulma durumu 1., 2. ve 3. sınıflara göre anlamlı olarak yüksek bulundu (p=0,001). Cinsel deneyime göre HPV aşıları hakkında bilgi düzeyini yeterli bulma durumu istatistiksel olarak anlamlı bulundu (p=0,001).

Öğrencilerin HPV aşısı hakkında bilgi düzeyini ölçen sorulara verdikleri cevaplara bakıldığında 'HPV aşısı ülkemizde rutin aşı takviminde yer almaktadır' ifadesine %66,5 (n=218), 'HPV aşısı canlı aşıdır' ifadesine %22,9 (n=75), 'Ülkemizde kullanılan 3 tip HPV aşısı vardır' ifadesine %43,3 (n=142), 'HPV aşısı sadece kadınlara

yapılır' ifadesine %69,5 (n=228), 'HPV aşısı sadece erkeklere yapılır' ifadesine %79,9 (n=262), 'HPV aşısı hem kadınlara hem erkeklere yapılır' ifadesine %69,8 (n=229), 'HPV aşısı enfeksiyonu geçirdikten sonra yapılırsa da koruma sağlar' ifadesine %44,2 (n=145), 'HPV aşıları servikal kansere karşı koruma sağlar' ifadesi %77,1 (n=253) oranında doğru cevap verdikleri görüldü. Öğrencilerin HPV aşısı konusunda bilgi sorularına verdikleri yanıtlar tabloda sunuldu (Tablo 4).

Tablo 3. HPV enfeksiyonu bilgi düzeyi puanlarının demografik özelliklere göre değerlendirilmesi

		HPV Enfeksiyonu Bilgi Düzeyi Puanı		
		n	**Ort ± Ss**	**p**
Cinsiyet	Kadın	157	12,24 ± 4,17	0,816†
	Erkek	171	11,99 ± 4,57	
Sınıf	1	55	8,09 ± 5,07	0,001*ǂ
	2	51	9,67 ± 4,21	
	3	52	12,6 ± 2,89	
	4	48	14,17 ± 3,59	
	5	54	13,5 ± 3,29	
	6	68	14,26 ± 2,89	
Ailenin Ekonomik Durumu	İyi	113	13,58 ± 3,32	0,001*ǂ
	Orta	189	11,68 ± 4,57	
	Kötü	26	8,85 ± 4,69	
Medeni Durum	Evli	5	12,4 ± 6,11	0,834ǂ
	Bekar	318	12,11 ± 4,33	
	Boşanmış	5	11,6 ± 6,66	
Cinsel Deneyim	Evet	76	14,05 ± 3,22	0,001*ǂ
	Hayır	222	11,91 ± 4,22	
	Belirtmek istemiyorum	30	8,7 ± 5,58	

*Ss: Standart sapma, Ort: Ortalama, n: Öğrenci sayısı.
*p<0,05 düzeyinde anlamlı, †Mann Whitney U testi, ǂKruskal Wallis ve Dunn testi.

Tablo 4. HPV aşıları hakkında bilgi düzeyini ölçen sorulara verilen cevapların dağılımı

	Katılıyorum	**Katılmıyorum**	**Fikrim yok**
	n (%)	**n (%)**	**n (%)**
HPV aşısı ülkemizde rutin aşı takviminde yer almaktadır.	33 (10,1)	***218 (66,5)***	77 (23,5)
HPV aşısı canlı aşıdır.	91 (27,7)	***75 (22,9)***	162 (49,4)
Ülkemizde kullanılan 3 tip HPV aşısı vardır.	***142 (43,3)***	29 (8,8)	157 (47,9)
HPV aşısı sadece kadınlara yapılır.	40 (12,2)	***228 (69,5)***	60 (18,3)
HPV aşısı sadece erkeklere yapılır.	8 (2,4)	***262 (79,9)***	58 (17,7)
HPV aşısı hem kadınlara hem erkeklere yapılır.	***229 (69,8)***	31 (9,5)	68 (20,7)
HPV aşısı enfeksiyonu geçirdikten sonra yapılırsa da koruma sağlar.	62 (18,9)	***145 (44,2)***	121 (36,9)
HPV aşısının baş ağrısı, aşı yerinde ağrı gibi yan etkilerin dışında ciddi yan etkileri de bulunmaktadır.	129 (39,3)	***66 (20,1)***	133 (40,5)
HPV aşısı 9-26 yaş aralığında yapılır.	***173 (52,7)***	44 (13,4)	111 (33,8)
HPV aşılarının dozu valan tipine göre değişir.	153 (46,6)	***41 (12,5)***	134 (40,9)
HPV aşılarının dozu yaş aralığına göre değişir.	***149 (45,4)***	60 (18,3)	119 (36,3)
HPV aşıları valan tipine göre genital siğile karşı koruma sağlar.	***220 (67,1)***	12 (3,7)	96 (29,3)
HPV aşıları servikal kansere karşı koruma sağlar.	***253 (77,1)***	8 (2,4)	67 (20,4)
Gebelikte HPV aşısı önerilir.	23 (7,0)	***157 (47,9)***	148 (45,1)
Gebeyken HPV aşısı yapılması durumunda gebelik sonlandırılır.	28 (8,5)	***104 (31,7)***	196 (59,8)
Emzirme döneminde HPV aşısı yapılmasının zararı yoktur.	***68 (20,7)***	66 (20,1)	194 (59,1)

*n= Öğrenci sayısı

Kadınlarda HPV aşısı hakkında bilgi düzeyi puanı ortalaması 7,83 ± 3,71, erkeklerde 7,60 ± 3,94 olduğu saptandı. Kadın ve erkeklerde HPV aşısı bilgi düzeyinin benzer olduğu görüldü (p>0,05). Evli olanlarda HPV aşısı bilgi düzeyi puan ortalaması 7,40±4,34; bekarlarda 7,73±3,82; boşananlarda 6,80 ±3,96 olup istatistiksel olarak anlamlı farklılık saptanmadı (p>0,05). Sınıflara göre HPV aşısı hakkında bilgi düzeyi puanları arasında istatistiksel olarak anlamlı farklılık saptandı (p=0,001). Bu anlamlı farklılığa sebep olan grupları belirlemek için

yapılan ikili karşılaştırmalarda; 1. sınıf öğrencilerin puanları, 2. (p=0,005), 3. (p=0,001), 4. (p=0,001), 5. (p=0,001) ve 6. sınıf (p=0,001) öğrencilerinden düşük bulundu (p<0,05). 2. sınıf öğrencilerin puanları, 3. (p=0,014), 4. (p=0,001), 5. (p=0,002) ve 6. sınıf (p=0,001) öğrencilerinden düşük olduğu görüldü (p<0,05). 3. sınıf öğrencilerin puanları, 4. sınıf (p=0,027) öğrencilerinden düşük saptandı (p<0,05) (Kruskall Wallis testi). Diğer ikili karşılaştırmalarda istatistiksel olarak anlamlı bir fark olmadığı görüldü (p>0,05). Cinsel deneyime göre HPV aşısı hakkında bilgi düzeyi puanları arasında istatistiksel olarak anlamlı fark olduğu saptandı (p=0,001). Bu anlamlı farklılığa sebep olan grupları belirlemek için yapılan ikili karşılaştırmalarda; cinsel deneyimini belirtmek istemeyenlerin puanları hayır (p=0,001) ve evet (p=0,001) diyen öğrencilere göre, daha önce cinsel deneyimi olmayan öğrencilerin puanları evet (p=0,037) diyen öğrencilere göre daha düşük olarak bulundu (p<0,05). Öğrencilerin bilgi düzeyleri tabloda sunuldu (Tablo 5).

Tablo 5. HPV aşıları hakkında bilgi düzeyi puanlarının demografik özelliklere göre değerlendirilmesi

		HPV Aşısı Hakkında Bilgi Düzeyi Puanı		
		n	**Ort ± Ss**	**p**
Cinsiyet	Kadın	157	7,83 ± 3,71	0,588†
	Erkek	171	7,6 ± 3,94	
Sınıf	1	55	3,98 ± 3,51	0,001*‡‡
	2	51	6,41 ± 3,63	
	3	52	8,31 ± 3,08	
	4	48	9,92 ± 3,17	
	5	54	8,76 ± 3,23	
	6	68	8,87 ± 3,22	
Ailenin Ekonomik Durumu	İyi	113	9,4 ± 3,02	0,001*‡‡
	Orta	189	7,04 ± 3,82	
	Kötü	26	5,27 ± 4,29	
Medeni Durum	Evli	5	7,4 ± 4,34	0,883‡‡
	Bekar	318	7,73 ± 3,82	
	Boşanmış	5	6,8 ± 3,96	
Cinsel Deneyim	Evet	76	8,89 ± 2,78	0,001*‡‡
	Hayır	222	7,68 ± 3,93	
	Belirtmek istemiyorum	30	5 ± 3,95	

Ss: Standart sapma, Ort: Ortalama, n: Öğrenci sayısı. *p<0,05 düzeyinde anlamlı, †Mann Whitney U testi, ‡‡Kruskal Wallis ve Dunn testi.

Öğrencilerin %8,8'i (n=29) HPV aşılarından birini yaptırdığını, %91,2'si (n=299) yaptırmadığını belirtti. %81,1'i (n=266) HPV aşılarından birini yakınlarına önerirken, %18,9'u (n=62) önermeyeceğini ifade etti.

Öğrencilerin HPV aşısı ile ilgili tutum sorularından 'HPV aşısını ücretli olarak alıp yaptırırım' ifadesine %64,6 (n=212) oranında katılıyorum, %16,8 (n=55) oranında katılmıyorum, %18,6 (n=61) oranında fikrim yok şeklinde cevap verdikleri görüldü. 'HPV aşısı sosyal güvence kapsamında karşılanırsa yaptırırım' ifadesine %76,8 (n=252) oranında katılıyorum, %7,6 (n=25) oranında katılmıyorum, %15,5 (n=51) oranında fikrim yok şeklinde yanıtladılar. 'Kız çocuğum olsa ona HPV aşısı yaptırırım' sorusuna %79,0 (n=259) oranında katılıyorum, %4,9 (n=16), %16,2 (n=53) oranında fikrim yok şeklinde cevap verdiler. 'Erkek çocuğum olsa ona HPV aşısı yaptırırım' ifadesine %62,8 (n=206) oranında katılıyorum, %15,2 (n=50) oranında katılmıyorum, %22,0 (n=72) oranında fikrim yok şeklinde yanıt verdikleri görüldü.

Tartışma

Çalışmamızda öğrencilerin HPV enfeksiyonu ve aşısı hakkında bilgi düzeyini yeterli bulma oranı sırasıyla %42,4 ve %31,1 olarak bulundu. Tıp fakültesi öğrencilerinde yapılan bir çalışmada, HPV enfeksiyonu hakkında bilgi düzeyini yeterli bulma %63,5, HPV aşısı hakkında bilgi düzeyini yeterli bulma oranı %48,2 olarak bulunmuştur.[16] Bizim çalışmamızdakine benzer olarak bu çalışmada da HPV aşısı hakkında bilgi düzeyini yeterli bulma durumu, HPV enfeksiyonuna göre daha düşük düzeydedir. Tıp fakültesi öğrencilerinin bu konuda kendini yetersiz hissetmesi önemli bir bulgudur. Tıp fakültesi öğrencilerinin ve sağlık çalışanlarının bilgi düzeyleri toplumu bilinçlendirme, erken tanı ve tedavi amacıyla yönlendirme ve koruyucu önlemlerin alınması açısından önemli olduğundan dolayı HPV enfeksiyonu ve aşısı hakkında bilgilendirmeler için tıp fakültesindeki eğitim önem arz etmektedir.

Katılımcılar HPV enfeksiyonu ve aşısı hakkında bilgi kaynağı olarak en sık fakültedeki eğitim sürecini (%64) ve interneti (%19,8) belirtmiştir. Bir tıp fakültesinde 340 tıp fakültesi öğrencisinde yapılan bir çalışmada, HPV enfeksiyonu hakkında bilgi kaynağı olarak öğrencilerin %58,3 fakültedeki eğitim sürecini belirtmiştir.[16] Shetty ve ark.'nın Hindistan'da yaptıkları bir çalışmada tıp, diş hekimliği ve hemşirelik fakültesi öğrencileri HPV aşısı ile ilgili bilgiye ulaşım kaynakları olarak %42,1'i üniversite eğitimini ve %12,1'i interneti belirtmişlerdir.[17] Emre ve arkadaşlarının 780 tıp fakültesi öğrencisi ile yaptıkları çalışmada öğrencilerin HPV enfeksiyonu ve aşısı hakkında bilgiyi %79,6'sı derslerden ve %30'u internetten öğrendiğini ifade etmiştir.[18] Literatürde tıp öğrencilerinin HPV enfeksiyonu konusunda bilgiyi tıp eğitiminden ve

internetten öğrendiği açıktır. Bu sebeple öğrencilerin müfredatında daha bilgilendirici eğitim ve uygulamaların yapılması gerekmektedir.

Çalışmamızda öğrencilerin %90,9'u HPV'nin cinsel yolla bulaştığını belirtti. Emre ve arkadaşlarının çalışmasında Tıp Fakültesi öğrencilerinin %85,4 HPV'nin cinsel yolla bulaştığını belirtmiştir.[18] 2019 yılında Güney Hindistan'da tıp, diş hekimliği ve hemşirelik fakültelerinden 988 öğrencinin katıldığı bir çalışmada %78 oranında HPV'nin cinsel yolla bulaştığını belirtmişlerdir.[17] Bizim çalışmamızda olduğu gibi bu çalışmalarda da HPV'nin cinsel yolla bulaştığını bilme konusundaki oranın yüksek olmasının sebebi, HPV enfeksiyonunun cinsel yolla bulaşan hastalıkların başında gelmesi olarak düşünülebilinir.

Çalışmamızda HPV bulaşını önlemek için en güvenilir yöntemin kondom kullanımı olduğunu bilenlerin oranı %54'tür. Tıp Fakültesi'nde 174 öğrenci üzerinde yapılan bir başka çalışmada kondom kullanımının HPV enfeksiyonundan koruduğu ifadesine öğrencilerin %50,6'sının katıldığı ve %18,4'ünün kesinlikle katıldığı bulunmuştur.[19] İntern öğrenciler üzerinde yapılan bir çalışmada kondomun HPV enfeksiyonu üzerinde koruyucu olduğunu bilenlerin oranı %76,1 olarak bulunmuştur.[20] Cinsel ilişkide kondom kullanımının HPV enfeksiyonundan koruduğuna yönelik eğitimler verilmesi koruyucu cinsel sağlık açısından önemlidir.

Çalışmamızda öğrencilerin %8,8'i HPV aşısı yaptırdığını belirtti. Swarnapriya ve arkadaşlarının Hindistan'da yaptıkları bir çalışmada medikal ve paramedikal öğrencilerde HPV'ye karşı aşılanma oranı %6,8 olarak bulunmuştur.[21] Çeşmeci ve arkadaşlarını çalışmasında internlerde aşılanma oranı %5,3 olarak bulunmuştur.[22] 2016 yılında Hong Kong'da üniversite öğrencilerinde yapılan bir çalışmada HPV aşısını yaptırma oranı %13,3 olarak bulunmuştur.[23] 2009 yılında Birleşik krallık ve Portekiz'de üniversite öğrencilerinde yapılan çalışmada HPV aşısı yaptırma oranları sırasıyla %81 ve %80 olarak kaydedilmiştir.[24] Literatürdeki benzer çalışmalara bakıldığında HPV aşısı olma oranın genel olarak yeterli düzeyde olmadığı gözlenmektedir. Bunun sebebi olarak HPV aşısının ulusal aşı programında olmaması, aşı maliyetinin yüksek olması, öğrencilerde HPV aşısının yan etkileri ile ilgili endişelerinin olması ve bilgi eksikliğinden kaynaklı olduğu düşünülebilinir. Portekiz ve Birleşik Krallık'ta aşı yaptırma oranının yüksek olmasının sebebi, aşının ücretsiz olması ve ulusal aşı programında yer alması olabilir.

Bu çalışmada daha önce cinsel birliktelik yaşayan öğrencilerin oranı %23,2 olarak belirlendi. Ankara Üniversitesi Tıp Fakültesi'nde intern öğrencilerde yapılan çalışmada öğrencilerin %41,5'inin daha önce cinsel deneyimi olduğu bulunmuştur.[20]

Öğrencilerde cinsel deneyim oranlarını göz önünde bulundurursak, HPV'ye karşı aşılamanın erken dönemde başlanılması önem arz etmektedir. Çalışmamızda daha önce cinsel deneyimi olan öğrencilerin %22,4'ü HPV aşılarından birini yaptırmışken, daha önce cinsel deneyimi olmayan öğrencilerin %4,5'i HPV aşılarından birini yaptırmıştır. Yine daha önce cinsel deneyimi olan öğrencilerin %89,5'i HPV aşılarından birini yakınlarına önerirken, daha önce cinsel deneyimi olmayan öğrencilerin %78,4'ü HPV aşılarından birini yakınlarına önermektedir. HPV aşısı yaptırma durumunun tıp öğrencilerinde yetersiz olduğu açıkça gözükmektedir.

Cinsel deneyimi olan öğrencilerin, cinsel deneyimi olmayan öğrencilere göre HPV aşısını yaptırma ve önerme oranının daha yüksek olma sebebinin, HPV'nin cinsel yolla bulaşan bir enfeksiyon olduğunu bilmeleri sebebiyle gerçekleştiğini düşünmekteyiz. Aynı zamanda cinsel deneyimi olan öğrenciler sağlık konusunda daha dikkatli davranmakta olup, koruyucu önlem alma konusunda daha bilinçlidirler ve çevrelerine HPV enfeksiyonu ve aşısı hakkında önerilerde bulunmaktadırlar.

Çalışmamızda HPV enfeksiyonu konusunda bilgi düzeyi puanları kadın ve erkeklerde benzer olarak bulundu. Maksimum alınabilecek puan 20 iken, kadınlarda 12,24; erkeklerde 11,99 puan alındı. Şanlıurfa'da tıp fakültesi öğrencilerinin HPV enfeksiyonu ve aşısı hakkında bilgi düzeyleri kadın ve erkeklerde benzer olarak saptanmıştır. Bu çalışmada bilgi düzeyi puanı 100 puan üzerinden değerlendirilmiş olup erkeklerde 58,65; kadınlarda 60,57 puan olarak bulunmuştur. [25] Tıp öğrencilerinde bilgi düzeyinin kadın ve erkeklerde benzer olması fakülte eğitiminde cinsiyet ayrımı olmaksızın herkesin bilgiye eşit derecede ulaşmalarından kaynaklandığını düşünmekteyiz.

Çalışmamızda 1., 2. ve 3. sınıftaki öğrencilerin HPV enfeksiyonu bilgi düzeyi puanları 4., 5. ve 6. sınıftaki öğrencilerden düşük saptandı. Ayazöz'ün tıp fakültesi öğrencilerinde yaptığı çalışmada da sınıflara göre bilgi düzeyi puanı karşılaştırması bizimkine benzer olarak bulunmuştur.[25] Tıp fakültesi öğrencilerinde yapılan bir çalışmada HPV enfeksiyonu bilgi düzeyi puanı 4., 5. ve 6. sınıflarda, ilk üç sınıfa göre daha yüksek olarak saptanmıştır.[26] Bunun sebebinin tıp fakültesinde son 3 yılda öğrencilerin klinik anlamda daha fazla bilgi ve deneyim sahibi olmaları sebebiyle gerçekleştiğini düşünmekteyiz.

Bu çalışmada medeni duruma göre HPV enfeksiyonu bilgi düzeyi puanları arasında farklılık saptanmadı. Ayazöz'ün tıp fakültesi öğrencileri üzerinde yaptığı çalışmada evli öğrencilerde HPV enfeksiyonu bilgi düzeyi, bekarlara göre anlamlı olarak yüksek bulunmuştur.[25] Yıldız'ın 2021 yılında üniversite öğrencilerinde yaptığı çalışmada bekarların genel HPV enfeksiyonu bilgisinin, evlilerden daha yüksek olduğu saptanmış.[27] Yapılan çalışmalarda farklı sonuçlar olmakla birlikte, çalışmaların katılımcı örüntüsü, toplumsal değer ve sosyodemografik değişkenler buna etki etmiş olabilir.

Çalışmamızda daha önce cinsel deneyimi olan öğrencilerin HPV enfeksiyonu bilgi düzeyi puanları, cinsel deneyimi olmayanlara göre yüksek bulundu. Bunun cinsel deneyimi olan öğrencilerin, cinsel yolla bulaşan hastalıklar açısından daha fazla araştırma yapmaları ve bilgi sahibi olmalarından kaynaklandığı düşünülebilinir.

Tıp fakültesi öğrencilerinim HPV enfeksiyonu ve aşısı konusunda bilgi eksikliği olduğu belirlendi. Kadın ve erkeklerin bilgi düzeyi benzerdi. HPV aşısı yaptırma durumu düşük seviyedeydi. HPV enfeksiyonu ve aşısı

konusunda bilgi düzeyi klinik dönem öğrencilerinde, preklinik döneme göre anlamlı seviyede yüksekti. Öğrencilerin önemli bir kısmı HPV enfeksiyonu ve aşısı konusunda bilgi düzeyini yeterli bulmaktaydı. Bilgi kaynağı olarak tıp fakültesi eğitimi ve internet ön plandaydı. HPV enfeksiyonu ve aşısı konusunda öğrenciler yüksek oranda eğitim almayı istemekteydi. Daha önce cinsel birlikteliği olan öğrencilerin HPV enfeksiyonu ve aşısı hakkında bilgi düzeyi puanı anlamlı olarak daha yüksek bulundu.

Etik Standartlara Uygunluk

Herhangi bir yerde özet metni yayınlanmamış ve tam metni hiçbir dergiye gönderilmemiştir. Çalışma için Helsinki bildirgesi kapsamında etik kurul izni alınmıştır. Bu çalışma Gaziantep Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 10.05.2023 tarih ve 2023/155 sayılı karar ile bilimsel ve etik açıdan uygun görülmüştür. Anket çalışma izni, Gaziantep Üniversitesi Tıp Fakültesi Dekanlığı tarafından 03.05.2023 tarihli ve 323970 sayılı karar ile uygun görülmüştür.

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Yazar Katkısı

MS: Fikir, veri toplama, tasarım, denetleme; HSK: Denetleme, fikir; GGD: Tasarım, denetleme.

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Research Article | Araştırma Makalesi

EVALUATION OF INSULIN RESISTANCE AND METABOLIC SYNDROME COMPONENTS IN PATIENTS WITH SEBORRHEIC DERMATITIS

SEBOREIK DERMATITLI HASTALARDA İNSÜLIN DIRENCI VE METABOLIK SENDROM PARAMETRELERININ DEĞERLENDIRILMESI

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ABSTRACT

Objective: Seborrheic dermatitis (SD) is a common inflammatory skin disorder. There is scarce data regarding SD and metabolic syndrome. We aimed to investigate the prevalence of metabolic syndrome in patients with seborrheic dermatitis.

Methods: Sixty-six patients with seborrheic dermatitis and 52 healthy controls were enrolled. Subjects' height, weight, waist circumference, smoking status, and comorbidities were recorded. Blood pressure, fasting blood glucose, triglyceride, HDL, total cholesterol, and insulin levels were measured. Seborrheic dermatitis area and severity index (SASI) score, body mass index (BMI), and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) were calculated. The presence of metabolic syndrome was evaluated.

Results: BMI, waist circumference, glucose, HOMA-IR, and C-reactive protein (C-RP) were significantly higher in the SD group. The prevalence of hypertension and type II diabetes was significantly higher in the SD group than in the controls. There was no difference between the patient and control groups regarding metabolic syndrome. However, the duration of SD was significantly higher in SD with metabolic syndrome than those of SD without metabolic syndrome. There were no significant differences in age and SASI score between seborrheic dermatitis patients with and without metabolic syndrome.

Conclusion: SD patients may have an increased risk of metabolic syndrome development and may have higher inflammation and insulin resistance status compared with controls.

Key words: Metabolic syndrome; Seborrheic Dermatitis, Insulin resistance, Hypertension

ÖZ

Amaç: Seboreik dermatit (SD) yaygın görülen inflamatuar bir deri hastalığıdır. SD ve metabolik sendrom ile ilgili az veri vardır. Bu çalışmada seboreik dermatitli hastalarda metabolik sendrom prevalansını araştırmayı amaçladık.

Yöntem: Çalışmaya seboreik dermatit tanılı 66 hasta ve 52 sağlıklı kontrol dahil edildi. Olguların boyları, kiloları, bel çevreleri, sigara içme durumları ve ek hastalıkları kaydedildi. Kan basıncı, açlık kan şekeri, trigliserit, HDL ve total kolesterol, insülin düzeyleri ölçüldü. Seboreik dermatit alan ve şiddet indeksi (SASI) skoru, vücut kitle indeksi (VKİ) ve Homeostatik Model Değerlendirme-İnsülin Direnci (HOMA-IR) hesaplandı. Metabolik sendrom varlığı değerlendirildi.

Bulgular: VKİ, bel çevresi, glukoz, HOMA-IR ve C-Reaktif Protein (C-RP) SD grubunda anlamlı olarak yüksekti. Hipertansiyon ve tip II diyabet prevalansı SD grubunda kontrol grubuna göre anlamlı derecede yüksekti. Metabolik sendrom varlığı açısından gruplar arasında fark izlenmedi Ancak metabolik sendromlu SD'de SD süresi, metabolik sendromu olmayan SD'ye göre anlamlı olarak daha yüksekti. Metabolik sendromu olan ve olmayan seboreik dermatitli hastalar arasında yaş ve SASI skoru açısından anlamlı fark yoktu.

Sonuç: SD hastalarında metabolik sendrom gelişme riski artmış olabilir. Seboreik dermatit (SD) hastalarında kontrollere kıyasla daha yüksek inflamasyon ve insülin direnci durumu olabileceğini belirledik.

Anahtar Kelimeler : Metabolik sendrom, Seboreik dermatit, İnsülin direnci, Hipertansiyon

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Introduction

Seborrheic dermatitis (SD) is a common inflammatory skin disorder that affects 1-3% of immunocompetent adults.[1,2] Although the exact etiology is still unknown, there are some hypotheses including chronic Malassezia species infestation, hyperproliferative theory, and immunologic mechanisms.[1,3] It is thought that there are increased cell turnover and inflammation in the epidermis of SD patients similar to psoriasis.[1,2]

Seborrheic dermatitis manifests as recurrent erythematous scaling plaques like psoriasis. It is usually localized in the sebum-rich areas such as scalp, nasolabial folds, postauricular area, beard and anterior chest.[2,4] Because clinical appearance of SD and psoriasis lesions are quite similar, differential diagnosis sometimes may be difficult even with histopathologic examination of the scalp lesions.[1,2,5] Several lines of evidence have shown that psoriasis is a systemic inflammatory disorder with an increased risk of metabolic syndrome, atherosclerosis, and cardiovascular disease development.[6-8] Likewise, there is a cross-sectional study reporting the significantly increased prevalence of hypertension among patients with seborrheic dermatitis. Close similarities in the pathophysiology and increased prevalence of hypertension imply that SD might also be associated with increased systemic inflammation and its clinical results, i.e. hypertension, metabolic syndrome and cardiovascular disease.[9] Moreover, to our knowledge, there is only few studies reporting the association between seborrheic dermatitis and insulin resistance.[10] Despite its prevalence, studies investigating the status of systemic inflammation in SD are scarce compared to those in psoriasis.

Thus, the primary objective of this study was to investigate the prevalence and determinants of metabolic syndrome and insulin resistance in patients with SD.

Methods

This cross-sectional case-control study was conducted in the dermatology outpatient clinics in a university-affiliated teaching hospital. Sixty-six patients with seborrheic dermatitis and 52 healthy controls over 18 years old were enrolled in the study. Patients who have been receiving any systemic treatment for SD, chlorpromazine, cimetidine, and methyldopa within the last six months, patients with active infection, malignancy, parkinson's disease, and cutaneous inflammatory diseases such as psoriasis, atopic dermatitis, and acanthosis nigricans were excluded. The study was started after receiving ethics committee approval (E-16214662-050.01.04-175) and was carried out by the rules stated in the Declaration of Helsinki. All participants gave written informed consent.

Height, weight, waist circumference, smoking status, and comorbidities of the patients were recorded. Patients who were receiving anti-hypertensive medications were recorded. Fasting blood glucose, triglyceride, total cholesterol, HDL-cholesterol, and serum insulin levels of the subjects were measured by auto-analyzer. Duration and severity of seborrheic dermatitis were recorded. The disease severity of seborrheic dermatitis was assessed via seborrheic dermatitis area and severity index (SASI) score. The SASI score ranges between 0 and 48.[11] Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared for all subjects. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) was calculated by multiplying fasting plasma insulin (μIU/ml) by fasting plasma glucose (mmol/l), then dividing by the constant 22.5.[12]

Metabolic syndrome was diagnosed with the American Heart Association & National Heart, Lung, and Blood Institute's update of the National Cholesterol Education Program-Adult Treatment Panel III (ATP III) definition.[13] According to this definition, the diagnosis was established when a subject meets three or more of the following five criteria: 1) central obesity (waist circumference ≥ 102 cm for men, ≥ 88 cm for women); 2) raised triglycerides (≥ 150 mg/dL or use of fibrates); 3) raised blood pressure (BP ≥ 130/ ≥ 85 mm Hg or receiving pharmacological treatment for hypertension); 4) raised fasting blood glucose (≥ 100 mg/dL or presence of previous diagnosis of type 2 diabetes); and 5) reduced HDL cholesterol (< 40 mg/dL for men, <50 mg/dL for women or use of fibrates).

Statistical Analysis

Statistical analyses were performed using statistical software (IBM SPSS Statistics 20, SPSS Inc. an IBM Corp., Armonk, NY). Comparisons between the groups were performed with the Chi-square or Fisher's exact test. Analysis of the normality of the continuous variables was performed with Kolmogorov-Smirnov test. Independent samples t-test was used for normally distributed continuous variables, and the Mann-Whitney U test for not normally distributed variables. Pearson correlation analysis was performed to evaluate the correlation between continuous variables. Normally distributed variables were presented as mean ± standard deviation and not normally distributed variables were presented as medium (range). P value ≤ 0.05 was deemed as statistically significant.

Results

The demographic features of the patient and control groups are shown in Table 1. There was no significant difference between the groups in terms of age and sex (p= 0.126 and 0.121, respectively). Concomitant hypertension and type-2 diabetes mellitus were significantly more frequent in the seborrheic dermatitis group than those in controls. No difference was determined between the patient and control groups regarding metabolic syndrome (p=0.142) (Table 1). The mean SASI score of the patients with seborrheic dermatitis was 16.3±7.3. BMI, waist circumference,

glucose, HOMA-IR, and C-RP were significantly higher in seborrheic dermatitis patients than those in controls (Table 2).

Table 1. Comparison of demographic features, comorbidities of seborrheic dermatitis patients and healthy controls

	Seborrheic dermatitis group (n= 66)	Control group (n= 52)	P value
Sex (N)			
Female	19 (28.8%)	23 (44.2%)	0.121
Male	47 (71.2%)	29 (55.8%)	
Smoking	17 (25.8%)	8 (15.4%)	0.171
Frequency of metabolic syndrome (N)	16 (24.2%)	7 (13.5%)	0.142
Age (year)	38.8±13.7	42.1±9.3	0.126
BMI (kg/m^2)	27.8±6.9	25.5±3.5	**0.022**
Waist circumference (cm)	93.7±12.6	88.9±12.8	**0.045**
Frequency of hypertension (N)	11 (16.7%)	2 (3.8%)	**0.037**
Frequency of type II Diabetes (n)	9 (13.6%)	1 (1.9%)	**0.041**

Table 2. Comparison of laboratory values of seborrheic dermatitis patients and healthy controls

	Seborrheic dermatitis group (n= 66)	Control group (n= 52)	P value
Glucose (mg/dL)*	96 (75-343)	92 (66-117)	**0.004**
HDL (mg/dL)	43.4±9.2	46.4±11.1	0.119
Triglycerides (mg/dL)*	117 (60-721)	116 (46-283)	0.260
Total cholesterol (mg/dL)	198.1±44.7	184.6±30.4	0.055
Insulin*	8.0 (1.8-31.7)	7.1 (1.1-19.7)	0.106
HOMA-IR*	1.98 (0.38-9.40)	1.56 (0.26-4.13)	**0.022**
C-RP (mg/L)	3.7±1.7	2.7±0.7	**<0.001**

* Median value and range of these parameters were given because of not normal distribution of them. BMI: body mass index, HOMA-IR: The homeostasis model assessment of insulin resistance C-RP: C reactive protein.

A comparison of the age, SASI score, and duration of the disease between seborrheic dermatitis patients with and without metabolic syndrome is shown in Table 3. Duration of disease was significantly higher in seborrheic dermatitis with metabolic syndrome than in seborrheic dermatitis without metabolic syndrome (p=0.007). There were no significant differences regarding age and SASI between seborrheic dermatitis patients with and without metabolic syndrome. C-RP was significantly higher in the seborrheic dermatitis group with metabolic syndrome than those without metabolic syndrome (p= 0.004).

Significant correlations of CRP were observed with waist circumference (p= 0.007, r=0.330), insulin level (p=0.006, r=0.336), and HOMA-IR p=0.005, r= 0.341) in the Pearson Correlation test.

Table 3. Comparison of the age, SASI score, and duration of disease of seborrheic dermatitis patients with and without metabolic syndrome

	Seborrheic dermatitis with metabolic syndrome (n= 16)	Seborrheic dermatitis without metabolic syndrome (n= 50)	P value
Age (year)	44.3±9.6	36.9±14.4	0.060
SASI score	16.5±7.8	16.3±7.2	0.941
C-RP (mg/L)	5.3±2.3	3.3±1.0	**0.004**
Duration of disease (month)*	50 (20-208)	24 (1-96)	**0.007**

*Median value and range of these parameters were given because of not normal distribution of them. SASI: Seborrheic dermatitis area and severity index.

Discussion

There are several salient findings of this study. First, BMI, waist circumference, glucose, HOMA-IR, and C-RP were significantly higher in patients with seborrheic dermatitis than in controls. Second, the prevalence of hypertension and type II diabetes was significantly higher in the seborrheic dermatitis group than in controls. Third, the duration of disease was significantly higher in seborrheic dermatitis patients who had metabolic syndrome than those in seborrheic dermatitis patients without metabolic syndrome.

Since SD and psoriasis share some features such as increased cell turnover and inflammation in the epidermis, the high prevalence of metabolic syndrome and related conditions might be owing to the same pathophysiologic pathways in both diseases.[1,2] However, although there are many studies regarding the association of psoriasis with metabolic syndrome, insulin resistance, and cardiovascular diseases, the exact mechanisms of these associations have yet to be elucidated.

Watanabe et al. reported that variable levels of interleukin 6 and 8, and tumor necrosis factor-alpha in the supernatants increased in response to Malassezia yeasts lending support to the notion that Malassezia may stimulate cytokine production by keratinocytes.[14] In SD, these cytokines might have been playing a role in increased inflammation.

In the study presented, none of the participants had very high levels of C-RP which may be associated with infection. However, C-RP levels were significantly higher in the seborrheic dermatitis group with metabolic syndrome than those without metabolic syndrome. These results are also in concurrence with the study presented by Tosun et al.[15]

A previous cross-sectional study found that the prevalence of hypertension was significantly higher in patients with seborrheic dermatitis.[9] The authors suggested that this association can be explained by several factors such as genetic predisposition, psychological conditions, lipid abnormalities, and chronic inflammation of the skin with an accompanying change

in cytokine balance like the case in psoriasis. They found that SD patients were more likely to be obese and of higher socioeconomic status, but less likely to be current smokers or diabetics. Moreover, SD patients had a higher prevalence of psoriasis in that study. We did not include the patients who have cutaneous inflammatory diseases such as psoriasis and atopic dermatitis in the current study. In line with the previous study, we found that BMI, waist circumference, and prevalence of hypertension were significantly higher in the seborrheic dermatitis group than those of controls. In contrast, we also determined that glucose, HOMA-IR, and prevalence of Type II diabetes were significantly higher in the seborrheic dermatitis group than those of controls.

The studies in the literature on this topic reported different results between seborrheic dermatitis and serum insulin levels. The authors reported that the mean fasting serum insulin levels in patients with seborrheic dermatitis were not significantly different from that of the control group.[10] Erdogan et al. determined the levels of fasting plasma insulin and HOMA-IR were also significantly higher in the SD group than in the healthy control group. Consistent with Erdogan et al., we found that both glucose and HOMA-IR levels were significantly higher in the seborrheic dermatitis group than those of controls but not serum insulin levels as Dowlati et al.[10,16] In support of our finding, seborrheic dermatitis accompanying a case of Alström syndrome, which is one of the rare causes of insulin resistance, is mentioned.[17]

We did not find any significant differences in terms of age and SASI between seborrheic dermatitis patients with and without metabolic syndrome. However, the duration of the disease was significantly higher in seborrheic dermatitis patients with metabolic syndrome. It seems that the duration of SD is more significant for the development of metabolic syndrome compared with the severity of SD. This may be explained by chronic exposure to inflammation.

Study Limitations

There are some limitations of this study. The sample size is relatively small. It would be ideal to measure some markers of atherosclerosis such as carotid intima media thickness.

Conclusion

The results of this study indicate that SD patients may be at increased risk for metabolic syndrome development. Further observational studies with more patients are needed to clarify this association.

Compliance with Ethical Standards

Sakarya University Ethics Comittee approved this study (E-16214662-050.01.04-175). Informed consent was obtained from all participants.

Conflicts of interest: None declared.

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Research Article | Araştırma Makalesi

FROM REQUEST TO RESULT: THE CLINICAL SIGNIFICANCE OF EEG IN A TERTIARY HOSPITAL IN TURKEY

İSTEMDEN SONUCA: TÜRKİYE'DE ÜÇÜNCÜ BASAMAK BİR HASTANEDE EEG'NİN KLİNİK ÖNEMİ

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ABSTRACT

Objective: This study aimed to assess the clinical and demographic profiles of patients undergoing electroencephalography (EEG) over one year, evaluate EEG's impact on patient management, and ascertain the appropriateness of EEG requests.

Methods: A retrospective analysis was conducted in the neurophysiology department at Harran University Faculty of Medicine over 12 months from October 2022 to October 2023. The study included the EEG request to the neurophysiology department; pediatrics and the cases that had artifacts or technical issues were excluded. Age, gender, imaging characteristics, EEG findings, and the reasons for EEG requests were recorded. The contribution of EEG to patient management was analyzed.

Results: The study included 1217 patients with a mean age of 34.4±16.5 years, ranging from 18 to 90 years. Of these, 656 were female and 561 male. A history of epilepsy was reported in 821 patients. Neurology outpatient clinics, inpatient wards, and intensive care units requested the majority of EEGs. Epilepsy (48%), presyncope/syncope (13%), and psychogenic non-epileptic seizures (PNES) (5%) were the top reasons for EEG requests. Among the newly diagnosed epilepsy patients, 13 (65%) showed abnormal EEG results. Out of the 202 patients with magnetic resonance imaging (MRI) abnormalities, 39 exhibited focal epileptiform anomalies on EEG, with 32 of these cases (82%) showing concordance between MRI and EEG results. The EEG influenced patient management in 577 (46.5%) cases by confirming diagnoses, guiding drug adjustments, directing further diagnostics, and differentiating between epilepsy and PNES, highlighting its crucial role in clinical decision-making.

Conclusion: This study emphasizes the critical role of EEG in patient management, noting that although it is effective for confirming epilepsy, it is not reliable for excluding the diagnosis, thus stressing the need for careful clinical evaluation and prudent use of EEG in diagnostics

Keywords: Electroencephalography (EEG), epilepsy, seizure, patient management, diagnostic utility

ÖZ

Amaç: Bu çalışmanın amacı, bir yıl boyunca elektroensefalografi (EEG) çekilen hastaların klinik ve demografik verilerini incelemek, EEG'nin hasta yönetimi üzerindeki etkisini ve EEG taleplerinin uygunluğunu değerlendirmektir.

Yöntem: Bu çalışma Ekim 2022 ve Ekim 2023 tarihleri arasında Harran Üniversitesi Tıp Fakültesi'nde retrospektif olarak gerçekleştirildi. Çalışmada, nörofizyoloji laboratuvarına gelen EEG talepleri tarandı ve pediatrik vakalara ek olarak artefakt veya teknik sorunlar nedeniyle değerlendirilemeyen EEG'ler çalışmadan dışlandı. Hastaların yaşı, cinsiyeti, görüntüleme özellikleri, EEG bulguları ve EEG taleplerinin nedenleri kaydedildi. EEG'nin hasta yönetimine katkısı analiz edildi.

Bulgular: Çalışmaya yaş ortalaması 34,4±16,5 yıl olan ve yaşları 18 ile 90 arasında değişen 1217 hasta dahil edildi. Bunların 656'sı kadın ve 561'i erkekti. Hastaların 821'inde epilepsi öyküsü mevcuttu. EEG'lerin çoğu nöroloji poliklinikleri, nöroloji yatan hasta servisleri ve yoğun bakım ünitelerinden istenmişti. Epilepsi (%48), presenkop/senkop (%13) ve psikojenik epileptik olmayan nöbetler (PNES) (%5) EEG taleplerinin en önemli nedenleriydi. Yeni tanı konulan 20 epilepsi hastasının 13'ünde (%65) anormal EEG bulguları saptandı. MR görüntüleme anormallikleri olan 202 hastanın 39'unda EEG'de fokal epileptiform anormallikler görüldü ve bu vakaların 32'sinde (%82) MR ve EEG sonuçları arasında uyum bulundu. EEG, 577 vakada (%46,5) tanıları doğrulayarak, ilaç doz ayarına rehberlik ederek, ileri tanılara yönlendirerek ve epilepsi ve PNES arasında ayırıcı tanıya katkı yaparak hasta yönetimini etkilemiş ve klinik karar verme sürecindeki önemli rolünü vurgulamıştır.

Sonuç: EEG'nin özellikle epilepsi ve diğer nörolojik hastalıklarda tanıya katkısı önemini korumaktadır. Uygun EEG taleplerinin teşvik edilmesi ve hekimlerin nöbetler hakkındaki bilgilerinin artırılması hasta bakımını ve kaynak kullanımını optimize edebilir.

Anahtar Kelimeler: Elektroensefalografi (EEG), epilepsi, nöbet, hasta yönetimi, tanısal yarar

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Introduction

Electroencephalography (EEG) is an essential tool in diagnosing and following neurological disorders affecting the brain, especially epilepsy. It is used to diagnose, classify, and characterize interictal epileptiform abnormalities in people with epilepsy. Besides epilepsy, it is very supportive in the differential diagnosis, especially in confusional states, metabolic or toxic encephalopathies, or nonconvulsive status epilepticus. It is also valuable in central nervous system infections such as Creutzfeldt-Jacobs disease, Herpes simplex encephalitis, and subacute sclerosing panencephalitis.[1,2] In addition to all these diseases, its use is frequent in psychiatric practice to differentiate neurological disorders from psychiatric disorders, as well.[3]

In addition to the misdiagnosis of epilepsy, the abuse of EEG has been an issue for years. In a community-based investigation specifically targeting the adult population, the misdiagnosis rate was identified as 23%. Furthermore, it was discerned that among the cases referred to an adult neurologist for "refractory epilepsy," 26% were erroneously diagnosed, revealing the absence of epilepsy.[4,5] In another study, only 22% of performed EEGs were considered 'useful', meaning they confirmed diagnoses or influenced the management of patients, all of which were requested by only neurologists.[6]

In the present study, our objective was to assess the clinical and demographic profiles of patients who underwent EEG over the past year, along with evaluating the impact of EEG on patient management. Furthermore, we aimed to determine the appropriateness of EEG requests

Methods

The present study consisted of a retrospective analysis spanning one year, conducted at Harran University Faculty of Medicine between October 2022 and October 2023. All cases being requested EEG from the Department of Neurology were screened for inclusion. EEGs that were affected due to artifacts or technical issues were excluded from the analysis. Pediatric EEGs were omitted from the study. All EEG assessments were conducted ensuring a minimum duration of 20 minutes, and scalp electrodes adhered to the international 10-20 system, including T1 and T2 electrodes. Standard activation protocols, encompassing eye opening-closing, hyperventilation, and photic stimulation, were administered, unless medically contraindicated.

Parameters such as age, gender, clinical manifestations, imaging characteristics, and EEG findings and types were recorded. The EEGs were reviewed by the same neurologist experienced in interpreting EEGs and epilepsy. Each patient's reason for requesting EEG, final neurological diagnosis, and department for EEG requisition were documented. EEG outcomes were categorized into the following groups: normal, focal epileptiform activity, generalized epileptiform activity, and non-epileptiform abnormalities (such as slow activity). Normal variants were encompassed within the normal EEG classification. The rationale for the EEG request was categorized into several classifications, including epilepsy, patients with a first seizure, syncope/pre-syncope, psychogenic non-epileptic seizures (PNES), altered consciousness, transient global amnesia (TGA), central nervous system (CNS) infection, sleep disorders, movement disorders, and others. In instances where patients underwent multiple EEGs, each recording was documented separately if the reasons for EEG demands varied. The EEG's contribution to the patient's management was deemed significant if it resulted in a modification to the patient's diagnosis or treatment regimen. This included instances where the EEG confirmed or modified diagnoses, guided treatment adjustments such as the adaptation of antiepileptic drug regimens, directed further diagnostic investigations, particularly when MRI abnormalities were present, or differentiated between epileptic and non-epileptic events such as psychogenic non-epileptic seizures (PNES), thus guiding appropriate therapies.

The MRI scans were performed using a standard 1.5 Tesla MRI machine at the Radiology Department of Harran University Faculty of Medicine. The MRI results were evaluated by an experienced radiologist who interpreted the images in a blinded manner. The MRI abnormalities identified in our cohort encompassed gliotic changes, mesial temporal sclerosis, focal cortical dysplasias, intracranial masses (including meningiomas and other tumors), cerebrovascular diseases such as intracranial hemorrhages, infarcts, subdural and subarachnoid hemorrhages, as well as sequelae of previous cerebrovascular events, EEG and MRI were deemed concordant if both detected pathological abnormalities in matching areas.

Ethical approval for this study was granted by the local ethics committee of Harran University Medical Faculty in 2024, with the assigned protocol number being HRÜ/24.02.01.

Data acquisition was facilitated through Microsoft Excel, while statistical analyses were conducted utilizing SPSS statistical software version 25 (IBM Inc., NC, USA). Categorical variables were expressed as frequencies with accompanying percentages, with statistical comparisons performed using the Pearson Chi-square and Fisher exact tests. A significance threshold of $p<0.05$ was applied to determine statistical significance.

Results

During the study period, 1626 patients underwent EEG. After excluding EEGs with artifacts or repetitive EEGs, 1217 patients were included in the study. 656 EEGs were obtained from female patients, whereas 561 were from male patients. The mean age was 34.4±16.5 years and ranged from 18 to 90 years. EEGs were performed as routine, sleep deprived, and sleep EEGs. 1103 were routine EEGs, 32 were sleep-deprived and 82 were sleep

EEGs. While 936 EEGs were reported as normal and 83 as slow, 136 had focal, and 62 had generalized epileptiform activity. 821 patients had a history of epilepsy. A total of 1217 EEGs, including 1053 from neurology outpatient clinics, 75 from neurology inpatient wards, and 39 from neurology intensive care units, were directly requested by the neurology department. 13 EEGs were requested from the emergency department, 25 from other inpatient wards, and 12 from other intensive care units (Table 1).

When the reasons for requesting EEG were analyzed, the 3 most common reasons were epilepsy, presentation with presyncope/syncope, and PNES respectively (Table 2). Of the 43 patients who had a first seizure, 18 were diagnosed with symptomatic seizure and 20 were diagnosed with epilepsy in the following period. 4 patients were diagnosed with PNES and 1 patient with presyncope/syncope. Of the 20 patients newly diagnosed with epilepsy, 7 had normal EEG and abnormal EEG results were detected in 13 patients.

Table 1. Comparative Analysis of EEG Patterns by EEG Type, Epilepsy History, and Requesting Departments

		EEG Patterns			
		Normal	**Non Epileptiform Abnormalities**	**Focal Epileptiform Activity**	**Generalized Epileptiform Activity**
EEG Type	**Routine**	845	82	120	56
	Sleep Deprived	24	0	5	3
	Sleep	67	1	11	3
Epilepsy History		580	57	130	54
The Department EEG was Requested	**Neurology Outpatient Clinic**	841	55	113	44
	Neurology Inpatient Clinic	43	13	13	6
	Neurology ICU	17	7	8	7
	Emergency Department	10	1	0	2
	Other Inpatient Clinic	16	5	2	2
	Other ICU	9	2	0	1

EEG: Electroencephalography, ICU: Intensive Care Unit

Table 2. EEG Patterns According to the Reason for Requesting an EEG

		EEG Results			
		Normal	**Non Epileptiform Abnormalities**	**Focal Epileptiform Activity**	**Generalized Epileptiform Activity**
The Reason for Requesting an EEG	**Epilepsy patients**	579	57	130	56
	Patients with a first seizure	28	8	3	4
	Syncope/Pre-syncope	158	3	0	0
	PNES	57	0	1	0
	Altered consciousness	17	5	0	1
	TGA	35	1	0	0
	CNS Infection	4	3	1	1
	Sleep Disorders	10	1	0	0
	Movement Disorders	28	0	0	0
	Other	20	5	1	0

CNS: Central Nervous System, EEG: Electroencephalography, PNES: Psychogenic Non-Epileptic Seizures, TGA: Transient Global Amnesia

Out of the 202 patients with MRI abnormalities, 39 exhibited focal epileptiform anomalies on EEG, with 32 of these cases (82%) showing concordance between MRI and EEG results. 577 (46.5%) of EEG's contribution to patient management was deemed significant. EEGs from the neurology service influenced the clinical course of patients in 70.7% of cases, while those from the neurology intensive care unit impacted 69.2%. In the emergency department, EEGs contributed to patient management in 53.8% of instances. However, EEGs from the neurology outpatient clinic had a lower contribution rate, at 44.3%. The most common final diagnosis was epilepsy (Figure 1).

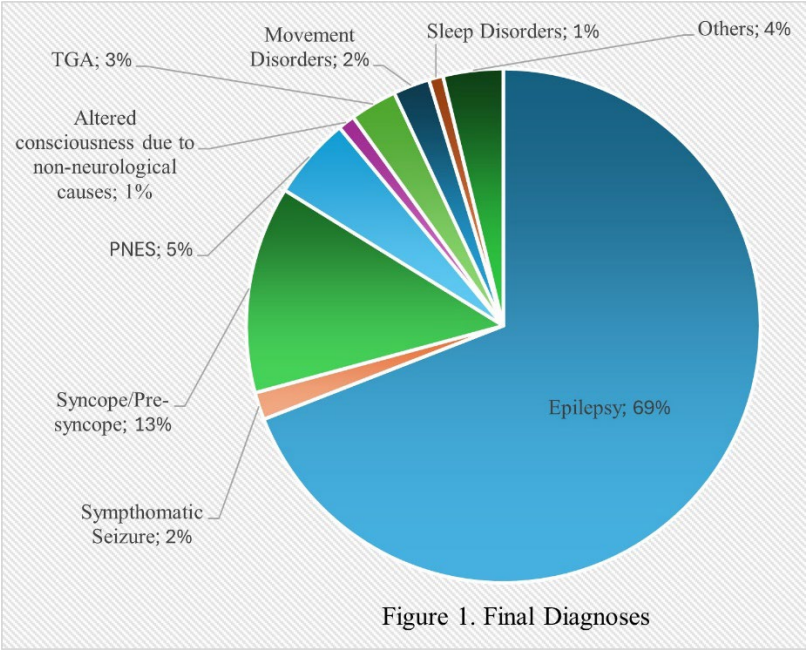

Figure 1. Final Diagnoses
PNES: Psychogenic Non-Epileptic Seizures, TGA: Transient Global Amnesia

Discussion

Over eight decades since its discovery, the standard EEG has persisted as a secure, non-invasive, cost-effective, and bedside method for assessing neurological function. In the clinical management of epilepsy, the timing for conducting a standard EEG is vital for ensuring superior patient care. Although advancements in neuroimaging have enhanced the detection of structural abnormalities within the central nervous system, EEG remains indispensable in offering crucial diagnostic information that influences therapeutic decisions.[2] EEG plays a pivotal role in diagnosing epilepsy, guiding the selection of antiepileptic medications, assessing treatment efficacy, conducting initial evaluations for alternative invasive therapies, and gauging seizure recurrence risk following medication cessation.[7-10] Epilepsy patients constitute the most commonly evaluated group in routine EEG laboratories.[11,12] In the present study, epilepsy was the leading cause of EEG requests.

In our investigation, the majority of referrals originated from neurologists, particularly those from outpatient clinics. Our study revealed that only 15% of routine neurology outpatient EEGs exhibited epileptiform changes. These results are consistent with those reported by Monif et al.[13] In routine EEG laboratories, roughly half or sometimes even more of the EEG recordings may yield normal results.[12] In this investigation, 77% of EEGs exhibited normal findings. This aligns with Monif et al.'s study, which reported that 67% of routine EEGs were normal. The elevated rate of normal EEGs is influenced by patient selection, especially with many referrals for syncope, presyncope, and psychogenic non-epileptic seizures. This occurrence was attributed to the predominant referral of our patients from outpatient clinics, where the available time for each patient was restricted. Additionally, The sensitivity of EEG for diagnosing epilepsy is low, ranging from 25% to 56%, whereas its specificity is much higher, between 78% and 98%. The differences in case selection, EEG recording techniques, antiepileptic drug usage, and definitions of epilepsy account for these varied ranges. Overall, findings from these studies, including ours, suggest that EEG is effective for confirming ("ruling in") but not excluding ("ruling out") the diagnosis of epilepsy.[13] These findings highlight the importance of careful clinical consideration and appropriate EEG requests to enhance diagnostic utility.

EEG has been shown to have prognostic utility in assessing the likelihood of seizure episodes following an initial unprovoked seizure event.[14] In patients newly diagnosed with epilepsy, the initial EEG test revealed that 53% exhibited abnormal brain activity patterns, specifically epileptiform abnormalities. However, it is noteworthy that following a first unprovoked seizure episode, a normal interictal EEG is frequently observed, indicating that a single normal EEG does not rule out the diagnosis of epilepsy.[15] Patients presenting with an unprovoked first seizure have a 21-45% risk of recurrent seizures within the first two years after the first seizure.[2] In our cohort, 65% of patients with newly diagnosed epilepsy had abnormal EEG findings and seizures recurred in the following period in approximately 47% of patients with a first seizure.

Differentiating epileptic seizures from paroxysmal non-epileptic events continues to be a critical and challenging task in the routine clinical practice of neurologists and epileptologists.[16] While the utility of EEG for this patient cohort remains a subject of debate, it is commonly used in clinical practice for differential diagnosis. Azman-İste and colleagues reported that the highest frequencies of normal EEG findings were associated with patients experiencing non-epileptic paroxysmal attacks and individuals referred for EEG due to other conditions,

including sleep disorders, movement disorders, and headaches.[12] Similar to their study, the highest occurrences of normal EEG outcomes were detected in patients for whom EEG testing was conducted based on preliminary diagnoses of movement disorders, PNES, and syncope or presyncope episodes in our research.

Although neuroimaging techniques have become the dominant approach for detecting intracranial lesions, EEG maintains a vital role in the diagnostic toolkit, especially during the presurgical examination of epilepsy patients.[11] In the present study, magnetic resonance imaging (MRI) abnormalities were identified in 202 participants, with concordance between EEG findings and MRI observations present in roughly 82% of the patients with focal epileptiform activity.

Our investigation ascertained that EEG conducted on 46.5% of the subjects played a pivotal role in patient care management. The literature demonstrated that confirmed diagnoses or influenced the management of patients in half of performed EEGs. The notion prevails that the appropriateness of EEG requests would be enhanced if they were exclusively made by neurologists.[6,17] In addition to solutions in the literature, such as publishing guidelines, making it easier for the doctor requesting to communicate with the neurologist, and including sufficient information in EEG request forms[6], we believe that increasing the time allocated per patient in outpatient clinics and the level of knowledge of physicians about seizures will be helpful to prevent unnecessary requests in our country.

In conclusion, the present study demonstrates the essential role of EEG in patient management and underscores the importance of its careful application. Our findings highlight that while EEG is effective for confirming a diagnosis of epilepsy, it is not reliable for excluding it, underscoring the need for careful clinical evaluation and judicious use of EEG in diagnostics.

Compliance with Ethical Standards

The study was approved by the local ethics committee of Harran University Medical Faculty in 2024 (Protocol number: HRÜ/24.02.01).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

DA, MB, TGD, OE: Concept; DA, MB, MC, TGD, OE: Design; MB, MC, AG: Data Collection or Processing; DA, OE: Analysis or Interpretation; DA, TGD, MC, AG: Literature Search; DA, AG, OE: Writing.

Financial Disclosure

Null.

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Research Article | Araştırma Makalesi

THE EFFECTS OF ROSEMARY (ROSMARINUS OFFICINALIS L.) EXTRACT ON THE PROLIFERATION AND APOPTOSIS OF A549 AND H1299 HUMAN LUNG CANCER CELLS

BİBERİYE (ROSMARINUS OFFICINALIS L.) EKSTRESİNİN A549 VE H1299 İNSAN AKCİĞER KANSERİ HÜCRELERİNİN ÇOĞALMASI VE APOPTOZU ÜZERİNDEKİ ETKİLERİ

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ABSTRACT

Objective: Apoptosis resistance and increased proliferation rates are characteristics of cancer cells. The anticancer properties of rosemary (*Rosmarinus officinalis* L.) extract (RE) have been demonstrated in a small number of *in vivo* and *in vitro* animal studies; however, no research has investigated the role of RE in human non-small cell lung cancer (NSCLC) A549 and H1299 cells, and its underlying mechanism of action remains unknown. In the current study, we examined the effects of RE on human non-small cell lung cancer cell proliferation, survival, and apoptosis.

Methods: NSCLC cell lines A549 and H1299 were incubated with (2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, and 12.5 µg/ml) doses of RE for 12, 24, and 48 hours. MTT, Annexin V-PI, and caspase 3/7 assay kit were performed to detect the cell viability, apoptosis and necrosis.

Results: According to MTT analysis, the viability of A549 and H1299 human lung cancer cells was reduced by approximately 49.74% and 47.76%, respectively, for 24 hours by treatment with a dose of 5 µg/ml RE. The results of Annexin V-PI staining and Caspase 3/7 activation showed that RE had a greater effect on inducing cell death and necrosis.

Conclusion: In conclusion, we can say that rosemary extract has both apoptotic and antiproliferative properties on human lung cancer cells. We might propose that additional investigation is necessary to ascertain the therapeutic impacts of rosemary extract.

Keywords: Annexin V-PI, A549, rosemary extract, Caspase 3/7, H1299

ÖZ

Amaç: Apoptoz direnci ve artan proliferasyon oranları kanser hücrelerinin karakteristik özelliğidir. Biberiye (*Rosmarinus officinalis* L.) ekstresinin (BE) antikanser özellikleri, az sayıda *in vivo* ve *in vitro* hayvan çalışmasında gösterilmiştir; ancak BE'nin insandaki Küçük Hücreli Olmayan Akciğer Kanseri (KHDAK) A549 ve H1299 hücrelerindeki rolünü araştıran araştırma yoktur ve bunun altında yatan etki mekanizması belirsizliğini korumaktadır. Bu çalışmada BE'nin insandaki Küçük Hücreli Olmayan Akciğer Kanseri hücre hatlarının çoğalması, canlılığı ve apoptoz üzerindeki etkilerini araştırdık.

Yöntem: KHDAK hücre hatları A549 ve H1299, 12, 24 ve 48 saat süre boyunca (2,5 µg/ml, 5 µg/ml, 7,5 µg/ml, 10 µg/ml ve 12,5 µg/ml) BE dozlarıyla inkübe edildi. Hücre canlılığı, apoptoz ve nekrozu belirlemek için MTT, Annexin V-PI ve kaspaz 3/7 kiti kullanıldı.

Bulgular: MTT analizine göre, A549 ve H1299 insan akciğer kanseri hücrelerinin canlılığı, 5 µg/ml BE doz tedavisiyle 24 saat boyunca yaklaşık sırasıyla %49,74 ve %47,76 oranında azaldı. Annexin V-PI ve Kaspaz 3/7 aktivasyonunun sonuçları, BE'nin hücre ölümünü ve nekrozu indüklemede büyük bir etkiye sahip olduğunu gösterdi.

Sonuç: Sonuç olarak biberiye ekstresinin insan akciğer kanseri hücreleri üzerinde hem apoptotik hem de antiproliferatif özelliklere sahip olduğunu söyleyebiliriz. Biberiye ekstresinin terapötik etkilerini belirlemek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Annexin V-PI, A549, biberiye ekstresi, Caspase 3/7, H1299

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Introduction

The majority of cancer-related fatalities are caused by lung cancer.[1] Lung cancer includes non–small-cell lung cancer (NSCLC; approximately 85%) and small-cell lung cancer (SCLC; approximately 15% of all lung cancers).[2] Adenocarcinoma, large cell carcinoma, and squamous cell carcinoma are the three primary subtypes of NSCLC.[3] Despite the use of powerful chemo- and radiation therapy, less than 20% of people with NSCLC survive for five years. This disease is particularly aggressive. Research into novel therapeutic options for this aggressive kind of cancer is required because the resistance of NSCLC to existing medicines is a rising concern.[4]

According to Hanahan and Weinberg, cancer has six distinct characteristics: it continues to exhibit proliferative signals, avoids growth suppressors, resists cell death, permits replicative immortality, stimulates angiogenesis, and initiates invasion and metastasis.[5]

Plant-based natural materials have been screened to find numerous potential medicinal compounds. Certain medications, such as etoposide, which is derived from the mandrake plant and Queen Anne's lace, and docetaxel and paclitaxel, which are derived from the bark and wood of the Nyssaceae tree, are presently being effectively used in the treatment of cancer.[6]

The Mediterranean region is home to the plant *Rosmarinus officinalis* L., a member of the Lamiaceae (mint) family with a diversity of medicinal and culinary uses. The primary polyphenols present in rosemary extract (RE) are rosmarinic acid (RA), carnosol (CN), and carnosic acid (CA).[7]

Diterpenic compounds of the plant are recognized to have a broad range of biological activities, containing antifungal,[8] antibacterial,[9] anticancer,[10] antioxidant,[11] antiangiogenic,[12] anti-inflammatory,[13] and chemoprotective.[14] Additionally, rosmarinic acid has a broad range of biological properties, the most notable of which are anti-inflammatory,[15] anti-oxidative,[16, 17] anti-apoptotic,[18] antifibrotic,[19] and neuroprotective.[20]

Among other health advantages, plants high in polyphenols have drawn a lot of interest for their anticancer capabilities. The investigation of novel and potentially useful mechanisms of action, in addition to new chemical classes of anticancer drugs, is possible through the study of natural products.[21]

It has been discovered that RE and a few of its polyphenol constituents, such as CA, RA, and CN, have strong anticancer properties. Research employing cancer cells from the breast,[22-24] pancreas,[25] prostate,[26] and liver[24,27] has demonstrated that treatment with RE inhibits the growth and viability of cancer cells and induces apoptosis.

A small number of research have investigated how RE administration affects tumor growth *in vivo* in animals. Inhibitory effects have been reported in myeloid leukemia, colon, prostate, and skin cancer models.[26, 28-31]

In this study, we aim to investigate the effects of rosemary extract on human non-small cell lung cancer (NSCLC) A549 and H1299 cell proliferation, survival, and apoptosis.

Methods

Plant Material

The aerial parts of *Rosmarinus officinalis* were used in the study. Plant samples were collected during the flowering stage from Türkiye. Plant samples were kept in the shadow and dried. A 70% ethanolic extract was prepared from the plant material by sonication. The extract was then filtered and concentrated. The dried extract was weighed and stored at +4°C until further study.

Cell Culture

Human non-small-cell lung cancer cell lines H1299 (CRL-5803) and A549 (CCL-185) were provided by the ATCC (Manassas, VA, USA). The A549 and H1299 cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Thermo Fisher Scientific, USA) and RPMI-1640 medium (Thermo Fisher Scientific, USA), respectively. The cultures were then placed in an incubator at 37°C in 5% CO_2 and supplemented with 1% penicillin- (Capricorn Scientific, Germany) and 10% fetal bovine serum (Capricorn Scientific, Germany). The cells were placed in ninety-six-well plates and six-well plates for cell viability and flow cytometry, respectively, after being trypsinized with 0.25% trypsin at 80-90% confluence.

An Assessment of Cell Viability Using the MTT Assay

The effects of various doses (2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, and 12.5 µg/ml) of rosemary extract [32] on human lung cancer cells H1299 and A549 were assessed over 12, 24, and 48 hours using the MTT cell viability test. H1299 and A549 cell lines ($5x10^3$ cells/well) were seeded into 96-well plates including medium and incubated overnight. After incubation, RE at different concentrations was diluted in cell culture medium and added to the dishes. Plates were incubated for 12, 24, and 48 hours at 37 °C in 5% CO_2. The fresh medium and 15 µl of MTT solutions were added and incubated for four hours under the same circumstances. Then, 100 µl isopropanol–HCl to dissolve the formed dark blue formazan crystals was applied and incubated for half an hour in a dark condition. The untreated cells served as the control. Epoch Microplate Reader (Winooski, USA) was used to read the wells at 570 nm. Each assay was performed three times. We used GraphPad Prism software (San Diego, CA, USA) to analyze the data and determine the inhibitor doses (IC_{50}) required to achieve 50% inhibition of cell viability.

Flow Cytometry

Annexin V-PI Analysis

A549 and H1299 cells adhered to the plate were detached with trypsin-EDTA (Capricorn Scientific, Germany). Then, all cells were collected, and washed with phosphate-buffered saline (PBS), and the concentration was set to 1 x 10^5 cells in 100 µl. The cell

solution was put into 12 x 75 mm polystyrene tubes, and 1X Annexin Binding Buffer, 5 µl of Annexin V-FITC, and propidium iodide (PI) were added. After an incubation period of 15 minutes at rt, the cells were examined using an ACEA NovoCyte (USA) flow cytometry device.

Caspase 3/7 Activity Assay

In 6-well plates, H1299 and A549 cell lines were first seeded at a density of 5 x 10^5 cells per well and left to culture for the entire night. After that, the cells were treated with rosemary extract and incubated at 37 °C for 24 hours. After incubation, cells were collected in 0.5 ml of warm medium and incubated with a caspase 3/7 detection reagent for 1 hour at 37 °C. The cells were then rinsed and suspended in 0.5 milliliters of assay buffer. In the cells, caspase 3/7 activity was assessed using the NovoCyte D3000 flow cytometry instrument (USA). The process was used to determine the activation levels of caspases 3/7, which are indicators of the induction of apoptosis in the treated cells.

Statistical Analysis

The statistical analysis was assessed using version 8.4.2 of GraphPad Prism (San Diego, CA, USA). Two-way ANOVA and Tukey's test for the multiple comparisons of means were used to assess the significance of the data between the groups. The statistical significance level was accepted as $p < 0.05$.

Results

Cytotoxic Effects of RE on A549 and H1299 Human Lung Cancer Cells

The effects of rosemary extract on cell viability at various concentrations and durations are demonstrated in Figure 1. Rosemary extract was treated at 0, 2.5, 5, 7.5, 10, and 12.5 µg/ml doses for H1299 and A549 human lung cancer cells. The incubation times were 12, 24, and 48 h. As shown in Figure 1 rosemary extract demonstrated potent cytotoxicity against human lung cancer cell lines, A549 and H1299 with IC_{50} being at 5.041 µg/ml and 4.151 µg/ml respectively for 24 h (Figure 1). Furthermore, according to MTT analysis, the viability of A549 and H1299 human lung cancer cells was reduced by approximately 49.74% and 47.76%, respectively, for 24 hours by treatment with a dose of 5 µg/ml RE.

The Impact of RE on Cell Viability, Apoptosis, and Necrosis in A549 and H1299 Cells

The Annexin V-PI assay was carried out following rosemary extract administration to determine which stage of apoptosis is mostly caused by the extract. A549 and H1299 human cell lines were treated with 4 µg/ml and 5 µg/ml rosemary extract for 24 hours. As a control, untreated cells were used. It was found in both A549 and H1299 cell lines treated with 5 µg/ml RE, reduced cell viability, induced apoptosis and necrosis compared to the control and 4 µg/ml RE dose (Figure 2).

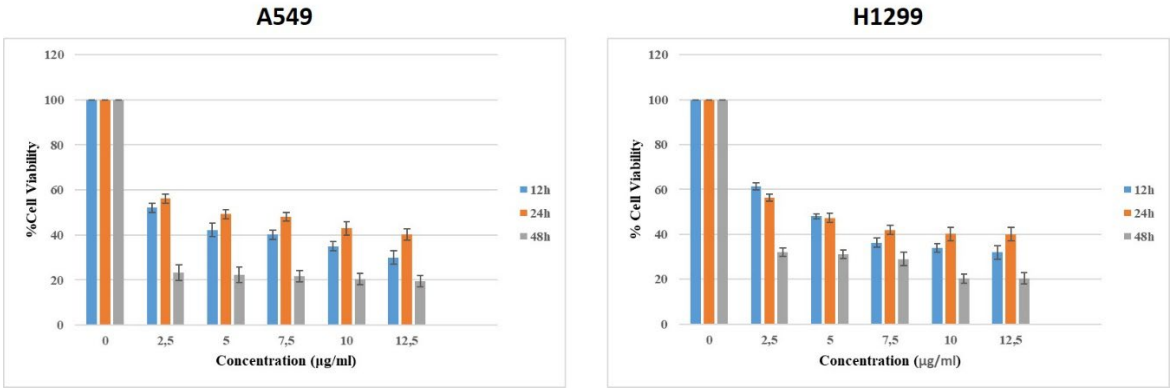

Figure 1. The effect of different concentrations of rosemary extract on the cell viability of A549 and H1299 human lung cancer cells. RE reduces cell viability in A549 and H1299 human lung cancer cells. All experiments were performed three times.

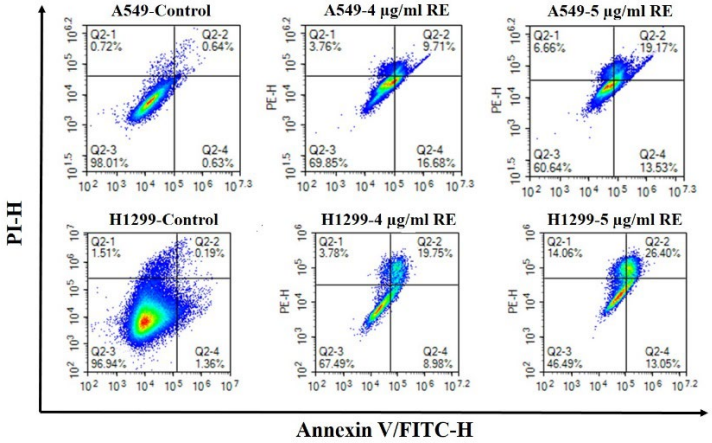

Figure 2. The effect of RE on apoptosis, and cell viability in A549 and H1299 lung cancer cells. The percentages of early and late apoptotic cells, necrotic cells, and cell viability were assessed by flow cytometry. Annexin V-PI staining was applied following RE treatment in A549 and H1299 lung cancer cell lines

Percentages of necrotic cells, late and early apoptotic cells, and cell viability were assessed by flow cytometry. Annexin V-PI staining was performed after RE treatment at 4 µg/ml and 5 µg/ml in H1299 and A549 cells. As a control, untreated cells were used. The four groups (Q2-1, Q2-2, Q2-3, and Q2-4) represent necrosis (Annexin V-negative/PI-positive), late apoptosis (Annexin V-positive/PI-positive), cell viability (Annexin V-negative/PI-negative), and early apoptosis (Annexin V-positive/PI-negative) that are identified by flow cytometry.

In comparison with the control group, it was determined that cell viability gradually reduced as the dose increased and there was a decrease, especially in the groups given 5 µg/ml RE both in A549 and H1299 cell lines**.** Furthermore, the apoptotic and necrotic cells were increased with the treatment of 5 µg/ml RE compared to the control and 4 µg/ml dose of RE (Figure 3).

Assessment of Caspase 3/7 assay

One accurate indicator of apoptosis is the activation of caspase 3/7. This assay measures caspase 3/7 activity to determine the composition and percentage of cells in various phases of apoptosis. A549 and H1299 lung cancer cell lines, including both 4 µg/ml and 5 µg/ml RE treated and untreated control cells, were subjected to caspase 3/7 assessment.

Similar to Annexin V results especially at doses of 4 µg/ml and 5µg/ml rosemary extract increased caspase activation compared to the control group at 24 hours in A549 and H1299 cells. (Figure 4).

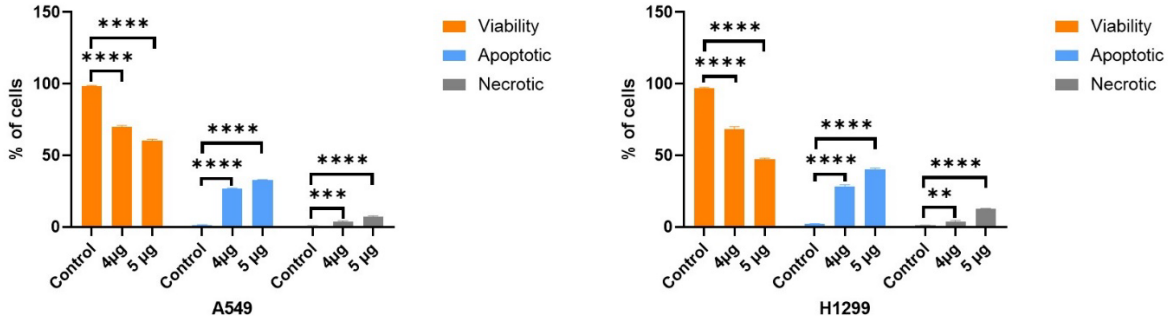

Figure 3. RE stimulates apoptosis in A549 and H1299 cell lines. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

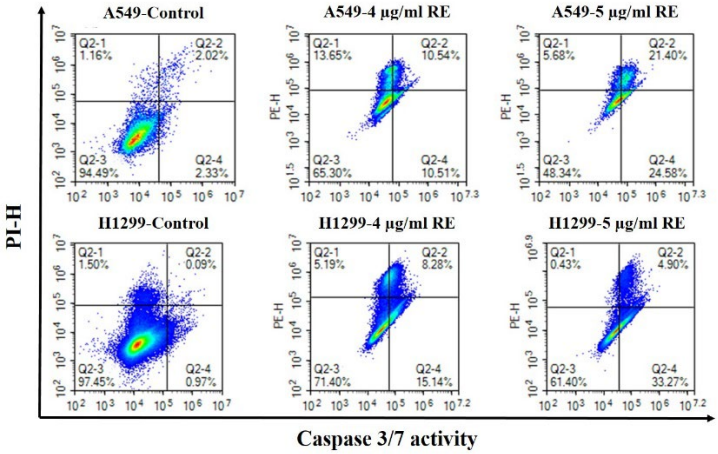

Figure 4. Caspase 3/7 activation as an apoptotic marker in human lung cancer cell lines A549 and H1299 Caspase 3/7 activities in these cells were evaluated by flow cytometry.

Compared to control group, it was determined that apoptosis or caspase 3/7 activation was increased according to doses. It was determined that 5 µg/ml rosemary extract treatment has the highest caspase 3/7 activation in both H1299 and A549 cells (Figure 5).

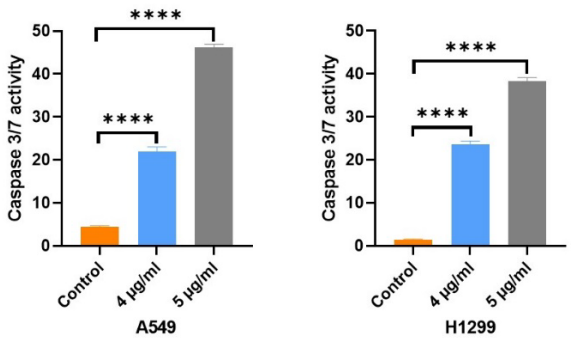

Figure 5. Rosemary affects caspase 3/7 activities in A549 and H1299 cells. RE affects caspase 3/7 activities significantly in two cancer cell lines. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

Discussion

Plants are the source of a number of drugs used in the treatment of cancer.[33,34] High polyphenolic plant-derived extracts have also demonstrated anticancer properties, such as green tea,[35] rosemary extract,[36] and specific polyphenols like quercetin,[37] oleuropein,[38] etc.

Following foodomics techniques, the antiproliferative action of polyphenol-rich extracts from rosemary has been shown in a variety of cancer cell lines in recent years.[39]

Rosemary's phytochemical components, which include phenolic acids (e.g., chrologenic acid caffeic acid, rosmarinic acid, gallic acid), triterpenes (e.g., oleanolic acid, ursolic acid, betulinic acid), and flavonoids (e.g., salvigenin, genkwanin, apigenin, scutellarein, cirsimaritin) are responsible for these health-promoting qualities.[40] The anti-neurodegenerative, antioxidant, antibacterial, anti-inflammatory, hypolipidemic, hypoglycemic, hypotensive, antiatherosclerotic, anticancer, and antimutagenic qualities of rosemary have been shown in both *in vivo* and *in vitro* studies.[41]

Rosemary (*Rosmarinus officinalis*) is a well-liked herb in both culinary and traditional medicine. It possesses pharmacologic properties for cancer treatment and chemoprevention, according to recent studies. Tai et al. assessed whether RE and its three primary active ingredients carnasol (CS), rosmarinic acid (RA), and carnosic acid (CA) could increase the antiproliferation activity of cisplatin (CDDP), and assess the antiproliferative activity of RE against human ovarian cancer cells. Utilizing human ovarian cancer A2780 and its CDDP-resistant daughter cell line A2780CP70, they demonstrated that RE had strong antiproliferation action, with IC_{50} (50 percent inhibitory concentration) measured at 1/1000 and 1/400 dilutions, respectively. With CDDP, RE improved the antiproliferation effect on A2780 and A2780CP70 cell lines. They have shown that by altering the cell cycle at several stages, RE suppressed the growth of ovarian cancer cells.[42]

The antiproliferative ability of rosemary cell lines against human HT-29 colorectal cancer cell line has been examined by Urquiza-López et al. Three rosemary cell line cultures were established: green (RoG), yellow (RoY), and white (RoW). Cell aggregates were sorted based on color. After 48 hours of treatment with the RoW extract (IC_{50} of 49.63 µg/ml), the antiproliferative activity test against HT-29 colon cancer cells using the MTT assay showed that the viability of the HT-29 cells was impaired.[43]

The most prevalent cancer diagnosed in men in North America is prostate cancer, which is usually categorized as androgen receptor positive or negative based on androgen receptor (AR) expression. Hormone therapy is a treatment option for AR-positive prostate cancer; however, AR-negative prostate cancer is aggressive and has no response to hormone therapy. The research in the literature has indicated that RE possesses anti-inflammatory, anti-cancer, and antioxidant properties. Jaglanian et al. discovered that administering RE to the androgen-insensitive PC-3 prostate cancer cell led to a notable suppression of Akt, mTOR signaling, migration, proliferation, and survival. These results imply that RE has strong anti-prostate cancer properties. Aside from cell proliferation, RE treatment resulted in a dose-dependent inhibition of cell survival with IC_{50} values of 4.17 µg/ml and 2.43 µg/ml for 22RV1 and PC-3 cell lines, respectively. [44]

The most prevalent malignancy in women to be diagnosed is breast cancer. Chemotherapy agents have been established in part because of compounds originating from plants. The impacts of rosemary extract on TN MDA-MB-231 cells survival/apoptosis, proliferation, mTOR, and Akt signaling were investigated by Jaglanian and Tsiani. In a dose-dependent manner, RE impeded the proliferation and survival of the MDA-MB-231 cells. Moreover, RE promoted the cleavage of PARP, a hallmark of apoptosis, and reduced the phosphorylation/activation of Akt and mTOR. According to their research, RE affects important signaling molecules contained in cell proliferation and survival and possesses strong anticancer capabilities against TN breast cancer.[45]

The spread of melanoma skin tumors is fast expanding worldwide, and their high resistance to cytotoxic agents contributes to their malignancy. Therefore, novel cytotoxic medication treatments would be highly beneficial in improving the prognosis of melanoma. The impact of a rosemary hydroalcoholic extract on the survival of the human melanoma A375 cell was examined by Cattaneo et al. Using MTT and Trypan blue tests, the impact of the crude extract or purified components on the growth of cancer cells was examined. Cell growth was hindered by rosemary extract in a time- and dose-dependent manner. Extract dilutions at ratios of 1:120, 1:240, and 1:480 significantly decreased cellular metabolic activity. The anti-proliferative impact was visible as early as 24 hours and was strengthened at 48 and 72 hours. After a 72-hour incubation period, the estimated IC_{50} was 1:480. [46]

As mentioned above, there is some research in the literature about the anticancer effects of rosemary extract on several cancer cell lines however, there was a limited study focused on lung cancer cells. In the current study, we examined the possible effects of RE on human lung cancer cell lines A549 and H1299. Firstly to select the inhibition concentrations of rosemary extract, an MTT assay was used to verify the cell survival of H1299 and A549 human lung cancer cells after being treated with 2.5, 5, 7.5, 10, and 12.5 µg/ml of rosemary extract. The durations of incubation were 12, 24, and 48 hours. The strong cytotoxicity of rosemary extracts against the human lung cancer cell lines A549 and H1299 with IC_{50} values of 5.041 µg/ml and 4.151 µg/ml, respectively, over a 24-hour period.

Apoptosis is a normal physiological mechanism of cell death that removes undesired cells while preserving tissue equilibrium. Additionally, pathological circumstances and extreme stress can cause it to happen.[47]

Two natural extracts, high in carnosic acid and rich in curcuminoid compounds, turmeric root extract (TE) and rosemary leaf extract (RE) were tested *in vitro* by using Annexin V and caspase 3/7 experimental protocols. Using the identical extract quantities and experimental setup, the researchers discovered that TE alone was a more potent cell therapy than RE alone. Caspase 3/7 activation and Annexin V staining demonstrated that TE had a higher effect on triggering cell apoptosis and a similar result was obtained from the combination treatment with just half the concentration of each extract.[48]

To ascertain which stage of apoptosis the rosemary extract mostly causes, the Annexin V assay and caspase 3/7 assays were performed after the extract was administered. It was discovered that A549 cells treated with 5 µg/ml rosemary extract induced apoptosis and necrosis also reduced cell viability. In the current study, we found a dose-dependent inhibition of A549 and H1299 human lung cancer cell proliferation with rosemary extract treatment.

It has been reported that rosemary extract decreases Akt/mTOR/p70S6K activation and inhibits the survival and proliferation of A549 human lung cancer cells. [21] Similarly an experimental study has shown that inhibition of non-small cell lung cancer survival and proliferation by rosemary extract is related to the activation of AMPK and ERK.[49] In the present study, we observed that rosemary extract has both apoptotic and antiproliferative properties on human lung cancer cells. Further research is required in the future to completely elucidate the molecular mechanisms.

In an experimental study, MG-63 bone osteosarcoma cell line viability was importantly reduced with increasing concentration of analyzed extract (beyond 300 µg/mL for rosemary dry extract). Their findings indicated that apoptosis is one of the basic mechanisms included in the cytotoxic properties of the analyzed extract.[50] In another study in CT-26 mouse colorectal cancer cells, the authors indicated that ginger and rosemary could induce cell death by early apoptosis.[51] In the study by Jang et al., the findings demonstrated that rosmarinic acid (RA) mainly boosted the number of cells in late apoptosis and necrosis, while suberoylanilide hydroxamic acid (SAHA) mainly boosted the number of cells in early apoptosis in the DU-145 cell line. Also, in the PC-3 cell line, RA boosted the number of early apoptotic cells, and SAHA boosted the number of late apoptotic cells compared to NC (DMSO).[52] Likewise the literature, we also examined the effects of rosemary extract on H1299 and A549 human lung cancer cells and found the early and late apoptotic index by using flow cytometry.

In summary, we may say that rosemary extract has both antiproliferative and apoptotic effects on human lung cancer cells. More research is needed to fully understand the medicinal potential of rosemary extract. Furthermore, the effect of RE noticed in human lung cancer cells brings out the possibility of using rosemary as a supplement/addition to existing treatments in high-grade tumors, which have limited medical treatment but can be treated with surgical intervention. We suggest that further research (such as *in vivo* studies) is needed to evaluate rosemary extract's therapeutic effects.

Compliance with Ethical Standards

No ethical approval was required, as this was an *in vitro* cell line study.

Conflict of Interest

There are no relevant conflicts of interest for the authors of this article.

Author Contribution

EA, BK, AFC: Study idea, study design, hypothesis; EA, BK: Literature Search; EA, TOS, SAE: Material preparation, data collection, and analysis; EA, TOS: Cell culture experiments; EA, BK, TOS: Writing the first draft of the article; EA, SAE: Reviewing and Editing. All the authors read and approved the final manuscript.

Financial Disclosure

Financial disclosure none.

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Research Article | Araştırma Makalesi

FREQUENCY, TYPES, AND RISK FACTORS OF ANEMIA IN PEDIATRIC INFLAMMATORY BOWEL DISEASE

ÇOCUKLUK ÇAĞI İNFLAMATUAR BAĞIRSAK HASTALIKLARINDA ANEMİNİN SIKLIĞI, TÜRLERİ VE RİSK FAKTÖRLERİ

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ABSTRACT

Objective: Inflammatory bowel disease patients are prone to be anemic at diagnosis and follow-up. As it is a common extra-intestinal manifestation, its early identification and treatment are essential. We aimed to evaluate the frequency, types, and predictors of anemia and its treatment in pediatric inflammatory bowel disease patients.

Methods: The electronic records of pediatric IBD patients who attended our outpatient clinics between 1 April 2018 and 01 May 2019 were retrospectively evaluated. Patients who had the results of hemoglobin, hematocrit, mean corpuscular volume, iron indices, vitamin B12 level, folic acid level, reticulocyte count, C-reactive protein, and erythrocyte sedimentation rate at least once on a single day were included in the study. Laboratory results associated with anemia and disease activity index scores at three- and six-months follow-ups were recorded. Anemia was diagnosed according to WHO criteria in childhood. Anemia, risk factors, and management of anemia were determined.

Results: Forty patients were included in the study. At first evaluation, anemia was observed in 38.1% of Crohn's disease patients and 57.9% of ulcerative colitis patients. Iron deficiency anemia was the main type of anemia in both groups. The rate of anemia decreased at follow-up. Out of 40 patients, 21 had treatment at the initial evaluation. Active disease was the only predictor of iron deficiency anemia.

Conclusion: Anemia was common in pediatric inflammatory bowel disease patients, ranging between 25-47.5% during the 6-month follow-up in our study. Iron deficiency anemia was the main type of anemia. Having active disease was the only risk factor for anemia. The treatment of anemia and iron deficiency without anemia should be kept in mind in parallel with anti-inflammatory treatment.

Keywords: Anemia, inflammatory bowel disease, children, iron deficiency

ÖZ

Amaç: İnflamatuvar bağırsak hastalığı olan hastalar tanı ve takip sırasında anemik olmaya eğilimlidir. Anemi, yaygın bir ekstra-intestinal bulgu olduğundan, erken tanınması ve tedavisi önemlidir. Bu çalışmada pediatrik inflamatuar bağırsak hastalığı olan hastalarda aneminin sıklığını, tipini, risk faktörlerini ve tedavisini değerlendirmeyi amaçladık.

Yöntem: 1 Nisan 2018 ile 01 Mayıs 2019 tarihleri arasında polikliniğimize başvuran pediatrik İBH hastalarının elektronik kayıtları retrospektif olarak değerlendirildi. Hemoglobin, hematokrit, ortalama korpüsküler hacim, demirle ilgili belirteçler, B12 vitamini düzeyi, folik asit düzeyi, retikülosit sayısı, C-reaktif protein ve eritrosit sedimentasyon hızı sonuçlarına aynı gün içerisinde en az bir kez bakılmış olan hastalar çalışmaya dahil edildi. Üç ve altı aylık takiplerde anemi ile ilişkili laboratuvar sonuçları ve hastalık aktivite indeksi skorları kaydedildi. Çocukluk çağında anemi tanısı WHO kriterlerine göre konuldu. Anemi, risk faktörleri ve anemi tedavisi belirlendi.

Bulgular: Kırk hasta çalışmaya dahil edildi. İlk değerlendirmede Crohn hastalarının %38,1'inde, ülseratif kolit hastalarının ise %57,9'unda anemi gözlendi. Her iki grupta da ana anemi tipi demir eksikliği anemisiydi. Takiplerde anemi oranının azaldığı görüldü. Kırk hastadan 21'i ilk değerlendirmede tedavi aldı. Aktif hastalığa sahip olma demir eksikliği anemisinin tek risk faktörüydü.

Sonuç: Pediatrik inflamatuvar bağırsak hastalığı olan hastalarda anemi, 6 aylık takipte %25-47,5 arasında değişmekte olup, sık görülmektedir. Demir eksikliği anemisi aneminin ana tipiydi. Aktif hastalığa sahip olmak anemi için tek risk faktörüydü. Anti-inflamatuvar tedaviye paralel olarak anemi ve anemi olmaksızın demir eksikliğinin tedavisi de çocukluk çağı inflamatuvar bağırsak hastalığında akılda tutulmalıdır.

Anahtar Kelimeler: Anemi, inflamatuvar barsak hastalığı, çocuklar, demir eksikliği

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Introduction

Anemia is a frequently seen extra-intestinal manifestation of inflammatory bowel disease (IBD). Its prevalence in adult studies varies between 6% and 74%.[1,2] Studies evaluating the prevalence of anemia in pediatric IBD are scarce, but children are more likely to have anemia than adult IBD patients. This can be explained by a more extensive disease course and the tendency of children to anemia in general.[3,4]

In IBD patients, the main types of anemia are iron deficiency anemia (IDA) and anemia of chronic disease (ACD). Vitamin B12 deficiency, folic acid deficiency, drug-induced anemia, and hemolysis are other etiologic factors.[5,6]

It is not surprising to find a pediatric IBD patient anemic at diagnosis. However, anemia is reported during follow-up visits even during remission of pediatric IBD.[7] As IBD-related anemia is not found to be correlated with remission, it shouldn't be underestimated. Determining the etiology of anemia and starting the appropriate treatment is crucial as it affects growth and quality of life.[8]

This study aimed to examine the types of anemia, assess the connection between anemia and disease severity, and evaluate the treatment response to anemia in pediatric IBD.

Methods

The study was approved by the ethics committee of Kocaeli University Hospital (KÜ GOKAEK 2019/225) and were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments. The electronic records of pediatric IBD patients who attended our outpatient clinics between 1 April 2018 and 01 May 2019 were retrospectively reviewed. Patients who had hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), iron indices, vitamin B12 level, folic acid level, reticulocyte count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) obtained at least once on a single day were included in the study. The first time including all these data was accepted as the initial evaluation. If the patients didn't have all these parameters on a single day, they were excluded from the study. Hb, hct, MCV, iron indices, CRP, and ESR levels of these patients were recorded at three-month and six-month follow-ups, if accessible. Patients with hemoglobinopathy disorders and those who had been administered blood transfusions were excluded from the study.

Clinical data (age, gender, disease type, age at diagnosis, disease duration, disease activity, disease location) and data associated with the therapy (current medications used for the treatment of IBD, medications used for treatment of anemic patients, and laboratory response to the treatment) were collected from the electronic medical records of patients. To evaluate disease activity, the Pediatric Crohn's Disease Activity Index (PCDAI) or the Pediatric Ulcerative Colitis Activity Index (PUCAI) scores of patients were calculated retrospectively from the electronic records at the initial evaluation, at three-month and six-month follow-ups.

Anemia was defined according to the World Health Organization's (WHO) criteria in childhood.[9] The level of inflammation was determining factor to define iron deficiency and anemia of chronic disease. A ferritin level of <30 μg/L, when CRP was <10 mg/L, or a ferritin level of <100 μg/L when CRP was ≥10 mg/L, was consistent with iron deficiency. Anemia of chronic disease was defined as a ferritin level of >100 μg/L and transferrin saturation (TfS) <%20 in the presence of biochemical (CRP ≥10 mg/L) or clinical evidence of inflammation.[10] B12 deficiency was defined as a level of <191 ng/L and folate deficiency as a level of serum folate <3.8 μg/L.

Iron sucrose infusion was given as intravenous therapy. IV iron requirement was calculated according to Ganzoni's formula.[11] For IV ferrous sulfate, the following calculation was used to determine the total iron deficit for initial repletion: total cumulative dose (mg) = [target Hb (12 g/dL) − actual Hb] × weight (kg) × 0,24 + [15 × weight (kg)]. To prevent adverse reactions, the maximum daily dose of iron sucrose was limited to 200 mg/day or 4mg/kg/day. It was diluted in 100 mL of normal saline and administered for 1 hour on each day.[12] Ferrous sulfate was the preferred oral iron formulation. Response to anemia treatment was evaluated by the change in hemoglobin levels three months and six months after the initial evaluation in those treated for more than one month.

Statistical Analysis

IBM SPSS 20.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses. The Shapiro-Wilk test was used to examine if a variable was normally distributed. Numerical variables were quoted as mean values±, standard deviation, and median (25th-75th percentile). Categorical variables were expressed as frequencies (percentages). Differences based on the evaluation time were evaluated depending on normality. When normal distribution for the variable was provided, one-way ANOVA was used for repeated measurements. If the normality wasn't provided, the variables were evaluated with Friedman's two-way analysis of variance. Binary logistic regression analysis was performed in order to determine risk factors for iron deficiency anemia. The relationship between categorical variables was evaluated by chi-squared and McNemar analyses. P values of <0.05 were considered significant for two-tailed tests.

Results

The number of IBD patients who met the criteria was 40. Of these patients, 21 (52.5%) were diagnosed with Crohn's disease and 19 (47.5%) with ulcerative colitis. The overview of the study is shown in Figure 1. The characteristics of the patients are given in Table 1. A

statistically significant difference was observed in gender between the two groups (p<0.001). There was a female predominance in patients with UC. Active disease according to PCDAI and PUCAI was higher in UC (57.9%) than in CD (42.1%) (p=0.350). Upper gastrointestinal system endoscopy was performed in 90.5% of CD patients. Of these patients, 7 (33.3%) had upper GI involvement. Among CD patients, 5 (23.8%) had colonic involvement, and 16 (76.2%) had ileocolonic involvement. CRP was significantly higher in patients with CD than in UC (p=0.036).

Anemia was observed in 8 (38.1%) of CD patients and 11 (57.9%) of UC patients at initial evaluation (Table 2). Anemia type mainly consisted of iron deficiency anemia in both groups. Mean hemoglobin levels were measured 12.5 ± 1.9 g/dl in CD and 11.2 ± 1.9 g/dl in UC at the beginning (p=0.04). There was a statistically significant difference between the groups in terms of ferritin (p=0.003). B12 was deficient in 4 of the CD patients and 1 of the UC patients. These patients had no anemia.

Table 3 shows the data related to anemia and disease activity for all IBD patients initially, at three months, and six months after the initial evaluation. The rate of anemia decreased gradually. Iron deficiency anemia was the primary type of anemia in each assessment. According to PCDAI or PUCAI, patients who have active disease decreased during follow-up.

Regarding possible risk factors of iron deficiency anemia, the diagnosis, the duration of the disease, the activity of the disease, having anti-TNF treatment, and early-onset IBD were evaluated. In the multivariate binomial logistic regression analysis, the activity of the disease was the only predictor of iron deficiency anemia (Table 4).

The management of anemia and iron deficiency was evaluated. Out of 40 patients, 21 had treatment at the first evaluation. This treatment consisted of oral iron (18/21, 85.7%), intravenous (iv) iron (2/21, 9.5%), combination therapy of oral iron, and B12 (1/21, 4.8%). At three months, 13 of the total patients (40.6%) had treatment for anemia or iron deficiency. Of these patients, 10 (76.9%) had oral iron therapy, and 3 (23%) had iv iron therapy.

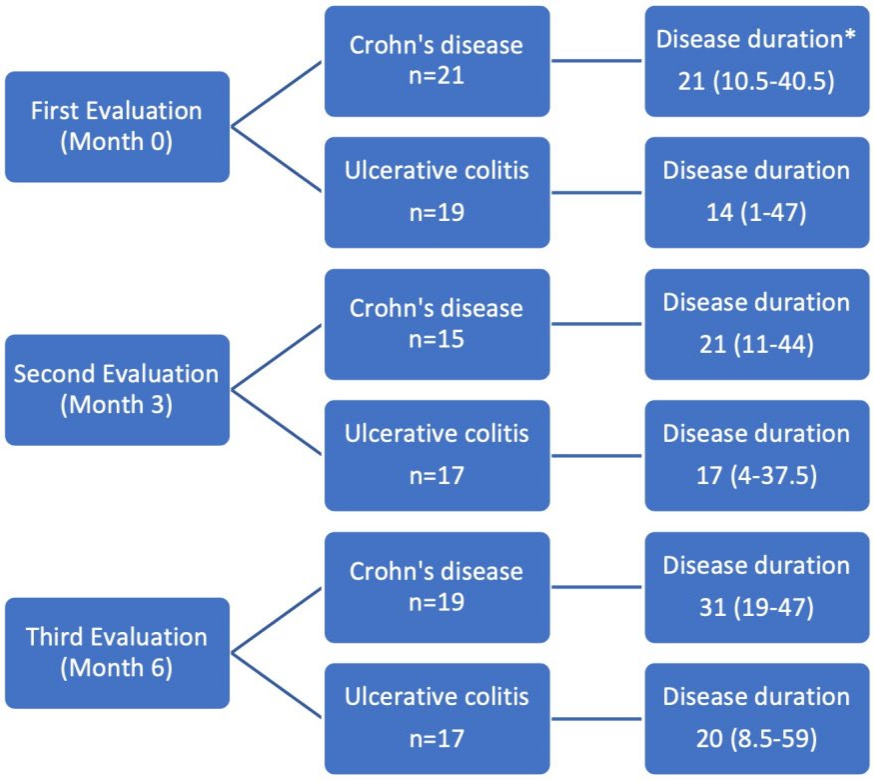

Figure 1. Overview of the study. *Median (Interquartile range)

Table 1. Characteristics of pediatric IBD patients at first evaluation

	Crohn's disease (n=21)	Ulcerative colitis (n=19)	P-value
Age, months; median (IQR)	188 (145.5-208.5)	188 (130-197)	0.537
Gender, females; n (%)	3 (15.8)	16 (84.2)	**<0.001**
Age at diagnosis, months; median (IQR)	163 (127.5-180.5)	170 (76-190)	0.830
Disease duration, months; median (IQR)	21 (10.5-40.5)	14 (1-47)	0.390
Height z-score, mean ± SD	0.55 ± 0.85	0.43 ± 1.18	0.137
Weight z-score, mean ± SD	0.71 ± 1.17	0.49 ± 1.33	0.580
BMI z-score, mean ± SD	-0.89 ± 1.42	-0.87±1.22	0.953
Disease activity			
	PCDAI	PUCAI	
Remission; n (%)	13 (61.9)	8 (42.1)	
Mild; n (%)	3 (14.3)	5 (26.3)	
Moderate; n (%)	5 (23.8)	3 (15.8)	
Severe; n (%)		3 (15.8)	
Active disease	8 (38.1)	11 (57.9)	0.350
Treatment			
5-aminosalicylic acid; n (%)		11 (57.9)	
Systemic corticosteroids; n (%)	2 (9.5)	2 (10.5)	
Azathioprine; n (%)	10 (47.6)	1 (5.3)	
Methotrexate; n (%)	2 (9.5)		
Anti-TNF; n (%)	5 (23.8)		
Exclusive enteral nutrition; n (%)	2 (9.5)		
Combination therapy*; n (%)	-	4 (21)	
None; n (%)	-	1 (5.3)	
C-reactive protein (mg/L), median (IQR)	7.49 (0.9-61.1)	1.1 (0.3-3.6)	**0.036**
Sedimentation (mm/h), median (IQR)	9 (5.5-32.5)	20 (7-35)	0.668
Albumin (g/dl), median (IQR)	4.1 (3.5-4.3)	4.1 (3.6-4.4)	0.688

BMI: Body mass index; IQR: Interquartile range; PCDAI: Pediatric Crohn's disease activity index; PUCAI: Pediatric ulcerative colitis activity index. Systemic corticosteroids consisted of methylprednisolone.
*Combination therapy consisted of 5-aminosalicylic acid and azathioprine, systemic corticosteroid and azathioprine, systemic corticosteroid and 5-aminosalicylic acid. Anti-tumor necrosis factor agent was infliximab.

Table 2. Characteristics and laboratory parameters of pediatric IBD patients related to anemia at first evaluation

	Crohn's disease (n=21)	Ulcerative colitis (n=19)	P-value
Anemia; n (%)	8 (38.1)	11 (57.9)	0.35
Hemoglobin (g/dl); mean ± SD	12.5 ± 1.9	11.2 ± 1.9	**0.04**
Hematocrit (%); median (IQR)	37.1 (33.5-41.7)	35.1 (31-37.4)	**0.041**
Mean corpuscular volume (fl); mean ± SD	76.4 ± 10.7	76 ± 7.86	0.79
Red cell distribution with, %; median (IQR)	15.3 (14-18.2)	16 (13.9-20)	0.573
Reticulocyte count (10^6/µL); median (IQR)	0.053 (0.038-0.070)	0.055 (0.045-0.070)	0.491
White blood cell count (10^3/ µL); median (IQR)	7.5 (6.35-9.97)	8.37 (6.88-11.5)	0.247
Platelet cell count (10^3/ µL); median (IQR)	384.4 ± 114.4	392.48 ± 143.91	0.846
Serum iron (µg/dl); median (IQR)	40 (19-65)	32 (17-59)	0.452
Ferritin (ng/ml); median (IQR)	29.3 (13.4-38)	9.9 (7.3-25.4)	**0.003**
TIBC (µg/dl); mean ± SD	336.6 ± 62.3	368.73 ± 50.7	0.086
Transferrin saturation (%); median (IQR)	13 (6-16.5)	8 (4-17)	0.196
Vitamin B_{12} (pg/ml); median (IQR)	273 (215.5-338)	310 (267-439)	0.078
Folate (ng/ml); median (IQR)	10.4 (6.8-13.8)	6.65 (5-8.2)	**0.027**
Anemia type			
Iron deficiency anemia (IDA); n (%)	6/8 (75)	10/11 (90.9)	
Anemia of chronic disease (ACD); n (%)	-	-	
IDA+ACD; n (%)	2/8 (25)	1/11 (9.1)	
B12/folate deficiency anemia	-	-	
Thiopurines	-	-	
Iron deficiency; n (%)	19/21 (90.5)	17/19 (89.5)	

IQR: Interquartile range, SD: Standard deviation, TIBC: Total iron binding capacity

Table 3. Characteristics and laboratory parameters related to anemia at first evaluation and at follow-up

	First evaluation	Three months later	Six months later	P-value
Anemia; n (%)	19/40 (47.5)	10/32 (31.3)	10/36 (25)	
Anemia type				
Iron deficiency anemia (IDA); n (%)	16/19 (84.2)	10/10 (100)	6/10 (60)	
Anemia of chronic disease (ACD); n (%)			2/10 (20)	
IDA+ACD; n (%)	3/19 (15.8)		2/10 (20)	
Iron deficiency; n (%)	36/40 (90)	23/32 (57.5)	29/36 (80.6)	
Hemoglobin (g/dl); mean ± SD	11.89 ± 1.99	12 ± 1.59	12.5 ± 1.74	0.056
Hematocrit (%); mean ± SD	35.89 ± 5.19	35.92 ± 4.16	37.74 ± 4.56	**0.018**
Mean corpuscular volume (fl); mean ± SD	76 ± 9.37	77.65 ± 9.2	79.56 ± 9.21	**0.005**
Serum iron (µg/dl); median (IQR)	33.5 (18.25-59.75)	52.5 (23.75-97.25)	54 (34-73.75)	0.288
Ferritin (ng/ml); median (IQR)	20.9 (8.6-36)	15.95 (10.67-30.3)	24.45 (13.8-36.8)	0.361
TIBC (µg/dl); mean ± SD	351.87 ± 59	337.69 ± 42.5	343.11 ± 53.29	0.541
Transferrin saturation (%); median (IQR)	8.5 (6-16.75)	14.5 (7-27)	17 (9.25-23.75)	**0.025**
ESR; median (IQR)	18.5 (6.25-32.75)	18 (8-23.25)	17 (8-22.75)	1
CRP; median (IQR)	2.6 (0.7-10.3)	2.58 (0.67-11.52)	2 (0.64-5.5)	0.547
Active disease; n (%)	19/40 (47.5)	14/32 (43.7)	11/36 (30.5)	

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, IQR: Interquartile range, SD: Standard deviation, TIBC: Total iron binding capacity

Table 4. Simultaneous Multivariate Binary Logistic Regression Analysis of Factors Predictive of Iron Deficiency Anemia Among Pediatric IBD Patients

	Odds ratio	95% CI	P-value
Diagnosis (vs. Crohn's disease)	1.832	0.336-9.998	0.484
Duration	0.980	0.947-1.014	0.244
Activity (vs. inactive disease)	11.553	2.289-58.295	**0.003**
Anti-TNF (vs. no anti-TNF)	0.850	0.055-13.242	0.908
Age group (vs. >120 months)	1.691	0.247-11.576	0.593

Anti-TNF: Anti-tumor necrosis factor

Discussion

In this study, the rate of anemia was 47.5% at the initial evaluation, decreasing to 31.3% at three months and 25% at six months. These rates were consistent with other studies evaluating anemia during follow-up in pediatric IBD.[13-18]

In these studies, the prevalence of anemia at diagnosis ranged between 54.9-77%.[14-17] One or two years after the diagnosis; the range was between 27.8-65%. Goodhand et al.[18] evaluated consecutive patients with IBD attending pediatric, adolescent, and adult outpatient clinics in April 2009. The prevalence of anemia was 70% in children and 42% in adolescents.[18] Unfortunately, because of the missing data, we weren't able to evaluate the prevalence of anemia at diagnosis.

Iron deficiency anemia was the most common type of anemia in each evaluation, which is compatible with previous studies.[15,17,18] Iron deficiency without anemia was even more common than any kind of anemia. These findings might be explained by poor iron intake, gastrointestinal bleeding, and increased disease activity. Anemia prevalence declined gradually at follow-up, probably due to the treatment of anemia and lower disease activity.

In this study, we also compared the characteristics of patients with ulcerative colitis and Crohn's disease. There wasn't a statistically significant difference in the anemia rate between groups. On the other hand, hemoglobin, hematocrit, and ferritin levels were significantly lower in ulcerative colitis patients, probably owing to higher disease activity indices and significant blood loss in UC. In a study by Aljomah et al.[13], ferritin was found to be lower in UC patients, too.

Iron therapy was given to 21 of the total patients. Of these patients, 15 had iron deficiency anemia, and 6 had iron deficiency without anemia. The percentage of anemic patients receiving iron therapy (78.9%) was higher than the percentages found in other studies.[14,15,17,18] But our sample size was smaller than the sample sizes in other studies. The treatment rate of iron-deficient patients without anemia was 30%. This lower rate of treatment made us think that treatment of iron deficiency was overlooked more than iron deficiency anemia. As pediatric gastroenterologists, we are probably more involved in putting the patient into remission. Besides, there might be a misconception that treating the patient with anti-inflammatory drugs will correct anemia simultaneously. There are other concerns mentioned in other studies. Goodhand et al.[18] suggested that there was no published evidence showing that oral iron improved the quality of life in pediatric IBD. Moreover, the side effects of oral iron and the risk of exacerbation caused by oral iron were other drawbacks mentioned in the literature.[1,15,18]

In our study, the therapy mainly consisted of oral iron. Only two patients had intravenous therapy at the first evaluation. They both had active inflammation. One of these patients had severe anemia. The other patient had anemia unresponsive to oral iron therapy. At three months, ten patients had oral iron, and three patients had iv iron therapy. These three patients had moderate anemia, which was unresponsive to oral iron. Iron supplementation and the decrease in disease activity might be accounted for the improvement in the anemia rate at follow-up. Both oral and iv iron therapy were well tolerated. No side effects were reported. Gisbert et al.[19]

reported in their study that iron treatment was safe and well-tolerated, and a beneficial impact on hemoglobin concentration and quality of life was observed in adult patients.

Simultaneous binary logistic regression analysis showed that active disease was the only factor predictive of anemia. It wasn't surprising as similar results have been reported in other studies.[16-18] Goodhand et al.[18] reported that the key determinants were the activity of the disease and being a pediatric patient as they compared the anemia prevalence between different age groups. Gerasimidis et al.[16] found that active disease was the strongest determinant of anemia at diagnosis and after one year of follow-up. In terms of disease activity, our study revealed that activity indices of IBH (PCDAI and PUCAI) were predictors of anemia. Interestingly, Aljomah et al.[13] reported that PCDAI and PUCAI were poor predictors of anemia, and the degree of anemia was consistent with inflammatory markers in their study.

Our study had some limitations. Because the study was retrospective, we weren't able to collect all the necessary data. We had to choose the first time, including information about hematologic parameters, inflammatory markers, and activity indices as the initial evaluation, which made our number of patients relatively small.

The key strength of this study is that it contains data describing the type of anemia and the relationship between activity indices and anemia. Additionally, it gives information about the treatment of anemia and anemia rate during follow-up visits.

In conclusion, this study confirms that anemia (especially iron deficiency anemia) and iron deficiency without anemia have high rates in each evaluation of pediatric IBD patients. Having a higher disease activity index was found to be a risk factor for anemia. As these results were consistent with other studies, pediatric gastroenterologists should raise their awareness of anemia and iron deficiency without anemia in patients with IBD. Anemia screening tests should be kept in mind each visit and should be done if needed. To provide a better quality of life, the treatment of anemia should be considered in parallel with anti-inflammatory treatment. However, further studies are required to figure out the treatment of anemia.

Ethical Approval

The study was approved by the ethics committee of Kocaeli University Hospital (KÜ GOKAEK 2019/225) and were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

NUA, EZ, AU: Concept-Design; EZ, AU: Supervision; NUA: Data Collection and/or Processing; NUA, AU: Analysis and/or Interpretation; NUA, EZ: Literature Review; NUA: Writer; EZ, AU: Critical Review

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Research Article | Araştırma Makalesi

EXPLORING THE PSYCHOLOGICAL BURDEN ON CAREGIVERS OF MULTIPLE SCLEROSIS PATIENTS

MULTIPL SKLEROZ HASTALARININ BAKIM VERENLERİ ÜZERİNDEKİ PSİKOLOJİK YÜKÜN ARAŞTIRILMASI

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ABSTRACT

Objective: Multiple sclerosis is a chronic, progressive disease, leading to significant psychological impacts on patients and their caregivers. This study focuses on the psychological impact on caregivers of Multiple Sclerosis patients, particularly examining the correlation between the severity of disability and depression, caregiver burden and self-stigma.

Methods: The study included 65 Multiple Sclerosis patients and their caregivers. Caregivers were assessed using the Beck Depression Inventory, ZARIT Burden Interview, Self-Stigma of Depression Scale and patients were evaluated using the Expanded Disability Status Scale. Statistical analysis was performed using IBM SPSS Statistics 17.

Results: The study found higher scores in Beck Depression Inventory, Self-Stigma of Depression Scale and ZARIT Burden Interview among female caregivers, indicating a greater burden. Unemployment and lower educational backgrounds were significantly correlated with increased caregiver burden. A positive correlation was observed between the severity of the patient's disability and caregiver burden. Caregivers with mental illnesses reported higher levels of burden and depression.

Conclusion: The study underscores the multifaceted impact of Multiple Sclerosis on caregivers, highlighting the need for comprehensive care approaches that include psychological support, education, and socio-economic assistance for caregivers. This holistic approach is essential for improving the overall management of Multiple Sclerosis, benefiting both patients and their caregivers.

Keywords: Multiple sclerosis, caregiver burden, depression

ÖZ

Amaç: Multipl Skleroz, hastalar ve onların bakım verenleri üzerinde önemli psikolojik etkilere yol açan kronik, ilerleyici bir hastalıktır. Bu çalışmanın amacı, Multipl Skleroz hastalarının bakım verenleri üzerindeki psikolojik etkiye odaklanarak, özellikle engelliliğin şiddeti ile depresyon, bakım verenin yükü ve kendini damgalama arasındaki ilişkiyi incelemektedir.

Yöntem: Çalışmaya 65 Multipl Skleroz hastası ve onların bakım verenleri dahil edilmiştir. Bakım verenler Beck Depresyon Envanteri, ZARIT Yük Ölçeği, Kendini Damgalama Ölçeği; hastalar ise Genişletilmiş Engellilik Durum Ölçeği kullanılarak değerlendirilmiştir. İstatistiksel analiz IBM SPSS İstatistik 17 kullanılarak yapılmıştır.

Bulgular: Araştırmada Beck Depresyon Envanteri, Kendini Damgalama Ölçeği ve ZARIT Yük Ölçeği'nde kadın bakım verenlerde daha yüksek puanlar elde edilmiştir. İşsizlik ve düşük eğitim düzeyleri artan bakım veren yükü ile anlamlı bir şekilde ilişkilendirilmiştir. Hastanın engelliliği ile bakım veren yükü arasında pozitif bir ilişki gözlemlenmiştir.

Sonuç: Çalışmamız, Multipl Skleroz'un bakım verenler üzerindeki çok yönlü etkilerini vurgulayarak, bakım veren için psikolojik destek, eğitim ve sosyo-ekonomik yardımı içeren kapsamlı yaklaşımlarının gerekliliğini vurgulamaktadır. Bu bütünsel yaklaşım, hem hastaların hem de bakım verenlerin genel yönetimini geliştirmek için esastır.

Anahtar Kelimeler: Multipl skleroz, bakım veren yükü, depresyon

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating progressive disease in the central nervous system. Progressiveness leads to neurological disabilities. The global median prevalence of MS is 33 in every 100,000. Incidence and prevalence rates continue to rise worldwide.[1]

The clinical presentation of MS is highly diverse. All kinds of neurological symptoms may occur as a result of involvement of any part of the central nervous system from the spinal cord to the cerebral cortex. The most common symptoms and signs include vision loss, sensory complaints, muscle weakness, ataxia, nystagmus, bladder dysfunction, fatigue and cognitive involvement. In addition to physical and cognitive signs, psychiatric findings are also frequent in MS patients. The lifetime risk of major depression in MS patients can reach 50%. Depression presumably affects the frontotemporal networks due to MS lesions and also develops secondary to a chronic disease.[2]

While the physical and cognitive impacts on patients are well-documented, MS also significantly affects caregivers, necessitating a comprehensive treatment approach that includes their well-being. MS is thought to affect both the patient physically, cognitively and psychologically and the caregiver as the disability increases.[3] Thus, MS treatment must be more than a patient-centered approach and include the caregiver as well.[4]

This study aims to bridge the gap in literature regarding the psychological impact on caregivers of MS patients, focusing on the severity of disability and its correlation with depression, caregiver burden, and self-stigma.

Methods

The study includes 65 patients and 65 caregivers having applied to Kocaeli University Multiple Sclerosis Outpatient Clinic in the July 2021-January 2022 period, met the exact Multiple Sclerosis criteria according to the McDonald criteria and agreed to participate in the study. Caregivers were assessed for mental health and daily contact with patients. We conducted neurological examinations using the Expanded Disability Status Scale (EDSS) and administered the Beck Depression Inventory (BDI), ZARIT Burden Interview (ZBI), and Self-Stigma of Depression Scale (SSDS) to caregivers. Ethical approval was obtained, and participants provided informed consent.

Measurement Instruments

Beck Depression Inventory (BDI)

The Inventory consists of 21 questions. The score varies between 0 and 63 and the severity of depression increases as the score rises.

ZARIT Burden Interview (ZBI)

The Interview consists of 22 items including the subgroups of Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations and Economic Burden. The severity of burden increases as the score rises.

Self-Stigma of Depression Scale (SSDS)

The scale is a 14-item measure consisting of Internalized Fear of Stigma and Self-Degradation subgroups. The assessment is performed by a 5-point Likert scale. A form prepared for patient relatives is also used. The higher the score, the higher the self-stigma.

Expanded Disability Status Scale (EDSS)

The Expanded Disability Status Scale is the most widely used disability scale for MS patients. The Scale provides effective and reliable assessment at every stage of the disease. This scale is mainly based on the evaluation of functional systems. The evaluation is made between 0 and 10 points. 0 points indicate normal neurological examination and 10 indicates death due to MS.

Statistical Analysis

We used IBM SPSS Statistics 17 for statistical analysis. We used histogram charts and Kolmogorov-Smirnov test for examining the conformity of the variables to normal distribution. We used mean, standard deviation, median, minimum, and maximum values to present descriptive analyses. In cases where the data did *not* show normal distribution, we evaluated groups of 2 with the Mann Whitney U test and groups of 2+ with the Kruskall Wallis test. We used the Spearman Correlation Test in the analysis of measurement data. The cases with $p \leq 0.05$ were statistically significant results.

Results

The mean age of the patients (54 female, 11 male) is 45.35±10.82. The mean age of the caregivers (26 female, 39 male) is 42.16±16.08. The caregivers are spouses (n=32), sons or daughters (n=22), mothers (n=8), fathers (n=2) and brothers or sisters (n=1). Forty-six of the caregivers are married and 19 single. The mean age of disease onset is 34.95±10.26. The mean of Disease Duration is 10.92±8.07 and the mean of Duration of Care is 8.26±7.59. The mean EDSS score of the patients is 4.28±2.20 (Table 1).

BDI Total, SSDS-social withdrawal, ZBI Total, Mental Tension and Private Life, Irritability and Restriction scores were higher in female caregivers than in males ($p \leq 0.05$). There are no significant differences between the groups in the tests for the degrees of relationship and marital status. SSDS Total, SSDS-social withdrawal, SSDS-inadequacy, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations, and Economic Burden scores are higher in the unemployed caregivers than in the employed ones.

Table 1. Sociodemographic Data

		*n	%
Gender of Caregiver	Female	26	40.00
	Male	39	60.00
Gender of Patient	Female	54	83.08
	Male	11	16.92
Marital Status of Caregiver	Married	46	70.77
	Single	19	29.23
Marital Status of Patient	Married	53	81.54
	Single	12	18.46
Closeness of Caregiver	Spouse	32	49.23
	Brother/Sister	1	1.54
	Mother	8	12.31
	Father	2	3.08
	Son/Daughter	22	33.85
Job Status of Caregiver	No	28	43.75
	Yes	36	56.25
Job Status of Patient	No	43	66.15
	Yes	22	33.85
Caregiver cares for another person	No	59	90.77
	Yes. 1 person.	6	9.23
Self-Care Need of Patient	No	36	55.38
	Yes	29	44.62

*n: Total number

BDI Total, SSDS-social withdrawal, SSDS-inadequacy, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations, Economic Burden, and Dependence scores are higher in the caregivers of the patients in need of self-care than in the caregivers of those with no self-care problems ($p \leq 0.05$). SSDS Total, SSDS-social withdrawal and SSDS-inadequacy scores are in the caregivers of the patients non-adherent to the treatment than in those with adherent patients.

BDI Total and Deterioration in Social Relations scores are higher in the non-adherent patients than in the treatment-compliant patients. We compared physical illnesses of the caregivers and any additional illnesses of the patients with BDI Total, SSDS subscales' total, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations, Economic Burden, and Dependence scores and found no statistically significant results.

Mental Tension and Private Life, Deterioration in Social Relations and Economic Burden scores are higher in the caregivers with mental illnesses than in those with no mental illnesses. BDI Total, SSDS Total, SSDS-social withdrawal, SSDS-inadequacy, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations, Economic Burden, and Dependence scores are higher in the relatives of the caregivers with mental illnesses. There are no significant differences between the caregivers' scores in terms of alcohol and cigarette use.

Considering the scale scores of the patients according to the presence of verbal violence to the caregivers, we found the scores are significantly lower in the relatives of the patients with no verbal violence.

We found an inverse relationship between Caregiver's Educational Background and SSDS Total, SSDS-social withdrawal, SSDS-inadequacy, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations and Economic Burden and Dependence scores ($p \leq 0.05$). There is a positive correlation between Disease Duration and ZBI Total, Mental Tension and Private Life, Irritability and Restriction and Dependence scores. There is a positive correlation between the EDSS score and SSDS Total, SSDS-social withdrawal, SSDS-inadequacy, ZBI Total, Mental Tension and Private Life, Irritability and Restriction, Deterioration in Social Relations, Economic Burden and Dependence scores.

Discussion

MS is a progressive disease that mostly affects the productive young population. Early disability and incapacity to work cause an increase in the burden for both the patient and his/her environment. The studies show that approximately 50% of MS patients will need help in walking, psychological treatment and rehabilitation, and as a result, their economic efficiency will decrease.[5]

With a wide range of findings and functional limitation, MS patients and their families adapt to major lifestyle changes and many constraints in daily life. The patient loses autonomy and begins to need the presence of a caregiver for daily activities as the disease progresses. Thus, the studies that examined the relationship between the characteristics of MS patients and of caregivers and analyzed the effect of this relationship on the quality of the caregiver's life are important for their impact on the prognosis of the disease.[2,6,7]

The findings of this study contribute valuable insights into the psychological and social burdens experienced by caregivers of multiple sclerosis patients. Our results align with existing literature, emphasizing the multifaceted impact of MS not only on patients but also on those who care for them.

The higher scores in Beck Depression Inventory (BDI), Self-Stigma of Depression Scale (SSDS), and ZARIT Burden Interview (ZBI) among female caregivers, as observed in our study, resonate with previous research indicating gender differences in caregiving burden and mental health outcomes.[8,9] This underscores the need for targeted support strategies that address the unique challenges f aced by female caregivers.

Our findings highlight the significant impact of unemployment and lower educational backgrounds on caregiver burden. This is consistent with the broader literature on chronic diseases, which suggests that socioeconomic factors play a crucial role in the wellbeing

of caregivers.[10,11] These factors should be considered in the development of support programs for caregivers.

The positive correlation between disease duration, EDSS scores, and caregiver burden aligns with previous studies.[12,13] As MS progresses, the increasing physical and cognitive impairments in patients intensify the demands on caregivers, leading to greater psychological and social challenges.

Our study also found that caregivers with mental illnesses experienced higher levels of burden and depression, a finding that is echoed in the literature.[14,15] This highlights the importance of mental health support for caregivers, not just as a means of improving their own wellbeing but also as a crucial factor in the quality of care they provide.

The significant impact of verbal violence on caregiver burden is a critical finding, suggesting that the emotional aspects of the caregiver-patient relationship can profoundly affect caregiver wellbeing. This aspect of caregiving in MS has been less explored in the literature and warrants further investigation.

These findings have important implications for the management of MS. They underscore the necessity of adopting a holistic approach to MS treatment, one that extends beyond the patient to include support for caregivers. This approach aligns with the recommendations of Hauser and Cree[16] and Makhani and Tremlett[17], who advocate for comprehensive care strategies in MS that address both physical and psychological needs.

While our study provides important insights, it is not without limitations. The single-center nature and the relatively small sample size may limit the generalizability of the findings. Future research should aim to include a more diverse and larger sample, possibly incorporating longitudinal studies to better understand the evolving nature of caregiver burden over time.

In conclusion, this study highlights the significant burden borne by caregivers of MS patients, influenced by factors such as gender, employment status, educational background, and the severity of the patient's condition. Addressing these challenges requires a multifaceted approach that includes psychological support, education, and socio-economic assistance for caregivers, alongside the medical management of MS patients.

Compliance with Ethical Standards

This study was approved Kocaeli University Ethics Committee (Decision number: 2021/220, Date: 08.07.2021).

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

Author Contribution

The authors contributed equally to this work.

Financial Disclosure

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OPİOİD VE METAMFETAMİN KULLANIM BOZUKLUĞU TANILI HASTALARDA ÖZŞEFKAT, İYİ OLUŞ VE BAĞLANMA ÖZELLİKLERİ

SELF COMPASSION, WELLBEING AND ATTACHMENT STYLES IN PATIENTS WITH OPIOID AND METAMFETAMINE USE DISORDER

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ÖZ

Amaç: Madde kullanım bozukluğu, tüm toplumu ilgilendiren ve çok yönlü ele alınması gereken kronik psikiyatrik bir rahatsızlıktır. Madde kullanım bozukluklarının gelişiminde ve devam etmesinde etkili olabilecek birçok faktör bulunmaktadır. Bu çalışmada, opioid ve metamfetamin kullanan hastalarda öz-şefkat, iyi oluş kavramları incelenecek ve hastaların bağlanma stilleri araştırılacaktır. Ayrıca bu kavramların kontrol grubuyla karşılaştırılması hedeflenmiştir.

Yöntem: Çalışmamızda 30 opioid (OKB), 31 metamfetamin kullanım bozukluğu (MKB) tanılı hasta ile 30 kişiden oluşan kontrol grubu; sosyodemografik veri formu, Öz-Şefkat Ölçeği (ÖŞÖ), Psikolojik İyi Oluş Ölçeği (İOÖ) ve İlişki Ölçekleri Anketi (İÖA) ölçeklerini tamamlamıştır.

Bulgular: OKB grubunda İÖA-saplantılı bağlanma ile ÖŞÖ arasında negatif yönde (r=-0,537, p<0,01); MKB grubunda İÖA-kayıtsız, saplantılı ve güvenli bağlanma ile İOÖ arasında pozitif yönde anlamlı ilişkiler saptanmıştır (r=0,428 – 0,499, p<0,05). Kontrol grubuyla yapılan karşılaştırmada, ÖŞÖ kontrol grubunda metamfetamin grubundan anlamlı olarak farklı (p=0,004), İOÖ kontrol grubunda hem metamfetamin hem de opioid grubundan anlamlı olarak farklı saptanmıştır (p<0,001). Gruplar arasında İÖA alt ölçek puan ortalamaları karşılaştırıldı. Gruplar arasında kayıtsız bağlanma puanları ortalamaları açısından istatistiki açıdan anlamlı fark yalnızca kontrol grubu ile metamfetamin grubu arasında saptandı. Kontrol grubu metamfetamin grubundan anlamlı derecede yüksekti.

Sonuç: Bulgularımız, madde kullanan bireylerde kontrol grubuna göre öz-şefkat ve iyi oluş seviyelerinin anlamlı olarak daha düşük olduğunu göstermiş; madde çeşidine göre iyi oluş ve öz-şefkat, bağlanma stilleri ile farklı ilişkiler kurmuştur. Madde kullanım bozuklukları literatüründe öz-şefkat ve bağlanma kavramları terapi hedefi olarak ele alınmalıdır.

Anahtar Kelimeler: Opiyat, metamfetamin, öz-şefkat, iyi oluş, bağlanma

ABSTRACT

Objective: Substance use disorder is a chronic psychiatric disorder that concerns the whole of society and needs to be assessed as multidimensional. Many factors may be effective in the development and maintanence of substance use disorders. We examined self-compassion, well-being concepts, and the attachment styles of the patients using opioid and methamphetamine.

Method: 30 opioid (OUD), 31 methamphetamine use disorder (MUD) and a control group consisting of 30 people; Sociodemographic Data Form, Self-Compassion Scale (SCS), Psychological Well-Being Scale (PWBS) and Relationship Scales Questionnaire (RSQ) completed the scales.

Results: There were significant correlation between the RSQ-preoccupied and SCS in the OUD group (r=-0.537, p<0.01) and significant correlations between preoccupied, dismissive and secure dimensions of RSQ and PWBS (r=0.428 - 0.499, p<0.05). When we compared the control group with patients' groups, SCS was significantly different from the methamphetamine group (p = 0.004) and PWBS was significantly different from both methamphetamine and opioid groups (p<0.001). The statistically significant difference between the groups in terms of dismissive subdimension of RSQ was determined only between the control group and the methamphetamine group. The control group's score was significantly higher than the methamphetamine group.

Conclusion: Our findings shows that self-compassion and well-being are significantly lower than the control group in the patient groups; According to the type of substance, well-being and self-compassion have established different relationships with attachment styles. In the literature on substance use disorders, the concepts of self-compassion and attachment should be considered as therapy targets.

Keywords: Opioid, methamphetamine, self-compassion, well-being, attachment

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Giriş

Madde kullanım bozukluğu, ataklarla seyreden tekrarlayıcı, kronik bir psikiyatrik bozukluktur. Klinik sonuçlarının yanında bir çok alanda işlevsellik kaybı yaratan bu rahatsızlık giderek toplumun birçok kesimini etkilemekte ve global bir kriz haline gelmektedir.[1] Etkili koruma ve tedavi stratejileri madde kullanım bozukluğu ile mücadelede ayrıntılı şekilde ele alınmalıdır. Madde kullanımını tetikleyen, süreğen hale gelmesine sebep olan ve tedavi sürecinde prognozu olumlu ve olumsuz etkileyen faktörlerin araştırılması önem arz etmektedir.[2]

Öz-şefkat, bireyin yaşadığı acıyı kaçınmayarak kabul etmesi, bu acıyı yaşamın bir parçası olarak görerek kendine anlayışlı ve şefkatli yaklaşması olarak tanımlanabilir.[3] Öz-şefkat çeşitli psikopatolojilerde araştırılmıştır ve ruhsal sağlık için önemli bir yapı olarak kabul edilir.[4] Araştırmacılar öz-şefkatin gelişiminin erken dönem bağlanma tecrübelerinden kaynaklandığını öne sürmektedir.[5, 6] Ayrıca, öz-şefkat, bir bireyin tüm insanlar ile ortak olarak deneyimlediği olumsuz duyguları fark etmesini, anlamasını ve kabul etmesini sağlayan duygu düzenleme tekniğidir.[7] Stres yaratan koşullar altında duygu düzenlemenin madde kullanım bozuklukları üzerine olan etkisi düşünüldüğünde öz-şefkat bağımlılık alanında ayrıca önem arz etmektedir. Literatürdeki çalışmalar alkol kullanımında koruyucu etkisine odaklansa da diğer madde kullanım bozukluklarında da öz-şefkat kavramına odaklanılması önerilmektedir.[8] Shahin ve arkadaşları, farklı madde bağımlılıkları olan 100 hasta ile yaptıkları çalışmada, hastaların orta düzeyde bir öz-şefkate sahip olmalarına rağmen aşermenin öz-şefkat ile negatif bir ilişkisi olduğunuu göstermiştir.[7] Bu çalışmada spiritüel iyi oluş kavramı da incelenmiş ve öz-şefkate benzer şekilde aşerme ile negatif yönde bir ilişki kurduğu gösterilmiştir.

İyi-oluş çeşitli alt başlıklarda incelenen bir pozitif psikoloji kavramıdır.[9] Bu alt başlıklardan biri olan psikolojik iyi oluş, bireyin kendini gerçekçi bir çerçevede tanıyarak gücünü ve sınırlarını bilmesi; kendinden memnun olması olarak tanımlanabilir.[10] Psikolojik iyi oluş teorisine göre bireyin ruhsal sağlığı kişinin yaşam alanlarındaki işlevselliğine bağlıdır.[9] Psikolojik iyi oluş farklı bağımlılık alanlarında kısıtlı da olsa çalışılmış bir kavramdır. Üniversite öğrencilerinde internet bağımlılığı üzerine yapılmış bir çalışmada internet bağımlılığının psikolojik iyi oluşla negatif yönde bir ilişki kurduğu saptanmıştır.[9] Madde bağımlılığı olan erkek bireylerin kontrol grubuyla karşılaştırıldığı başka bir çalışmada psikolojik iyi oluşun hasta grubunda anlamlı olarak daha düşük olduğu gösterilmiştir.[11] Alkol ve madde kullanımı olan ergen bireylerde yapılan bir çalışmada ise psikolojik iyi oluş madde kullanımı olan hastalarda alkol kullanımı olanlara göre daha düşük saptanmıştır.[12] Ancak ülkemizde bu alanda madde kullanım bozukluğu olan bireylerle yapılmış bir çalışma olmadığı görülmüştür.

Bağlanma stilleri de, uyuşturucu bağımlılığını ve tekrarlanan tüketimini etkileyen ana faktörler arasındadır.[13] Bartholomew bağlanma modeli, bireyin kendi ve diğerlerinin benliğinde yapmış olduğu içsel pozitif ve negatif değerlendirmelerden temel alan dört bağlanma stilinden oluşmaktadır. Bartholomew modeli ile madde kullanımı ilişkisini araştıran çalışmalara bakıldığında daha çok alkol kullanımının klinik olmayan örneklemlerde araştırıldığı görülmüştür.[14] McNally ve arkadaşlarının üniversite öğrencilerinde yaptığı çalışmada kaygılı bağlanma problemli alkol kullanımı ile ilişkili bulunmuştur.[15] Magai benzer şekilde alkol kullanımını araştırdığı çalışmada kaygılı bağlanmanın daha çok olumsuz duygulanımı azaltmak için olduğunu ve içme sıklığı ile pozitif yönde ilişkili olduğunu saptamıştır.[16] Schindler ve arkadaşları ise 2005 yılında klinik grupla kontrol grubunu karşılaştırarak korkulu bağlanmanın madde bağımlılığı olan ergen bireylerde ön plana çıktığını belirtmiştir.[14]

Çalışmamızda bu faktörlerden öz-şefkat, psikolojik iyi oluş ve bağlanma stilleri incelenecektir. Çalışmanın ilerleyen kısmında eroin kullanan hastaları ifade etmek için opioid terimi kullanılacaktır. Metamfetamin kullanan hastaların profili incelendiğinde, diğer maddeleri kullanan hastaların profilleri ile karşılaştırılığında daha ileri yaşta ortaya çıkması, yalnız yaşama oranının yüksek olması, düşük gelir gibi farklılıklar olduğu görülmektedir.[17] Bu farklılıkların, öz-şefkat, psikolojik iyi oluş ve bağlanma gibi parametreleri de etkileyebileceği düşünülerek örneklem belirlenmiştir. Çalışmamızda opioid ve metamfetamin kullanım bozukluğu tanılı hastaları kontrol grubuyla karşılaştırarak Bartholomew bağlanma stilleri ve bağımlılık literatürüne katkı sağlanması amaçlanmaktadır. Çalışmamızın hipotezleri:

- Opioid kullanan hastalarda öz-şefkat skorları kontrol grubuna göre daha düşüktür.
- Opioid kullanan hastalarda psikolojik iyi oluş skorları kontrol grubuna göre daha düşüktür.
- Metamfetamin kullanan hastalarda öz-şefkat skorları kontrol grubuna göre daha düşüktür.
- Metamfetamin kullanan hastalarda psikolojik iyi oluş skorları kontrol grubuna göre daha düşüktür.
- Metamfetamin kullanan hastalarda bağlanma stilleri ile öz-şefkat ve psikolojik iyi oluş skorları ilişkilidir.
- Opioid kullanan hastalarda bağlanma stilleri ile öz-şefkat ve psikolojik iyi oluş skorları ilişkilidir.
- Kayıtsız ve korkulu bağlanma stilleri opioid ve metamfetamin kullanan hastalarda daha yüksektir.

Yöntem

Çalışmaya Ankara Eğitim ve Araştırma Hastanesi AMATEM Kliniği'ne başvuran Ruhsal Bozuklukların Tanısal ve Sayımsal El kitabı (DSM-5) tanı kriterlerine göre 30 opioid kullanım bozukluğu (OKB), 31 metamfetamin kullanım bozukluğu (MKB) tanılı hasta ve benzer sosyokültürel özelliklere sahip ancak madde kullanım bozukluğu ve ek psikiyatrik rahatsızlığı olmayan 30 sağlıklı kontrol dahil edilmiştir. Sağlıklı kontrol grubu hastanede çalışan sağlık çalışanlarından oluşmaktadır. Araştırmanın dışlama kriterleri hem hasta hem kontrol grubu için epilepsi,

demans, deliryum benzeri organik bir durumun varlığı, anlıksal yetiyitimi varlığı, son 6 ay içinde EKT almış olmak, psikoz ve bipolar afektif bozukluk tanılarının olması olarak belirlenmiştir. Çalışmanın konusu ve amacı anlatıldıktan sonra çalışmaya katılmayı kabul eden katılımcılarda aydınlatılmış onam formu alınmıştır. Katılımcılar sosyodemografik veri formu ile Özşefkat Ölçeği, Psikolojik İyi Oluş Ölçeği ve İlişki Ölçekleri Anketi'ni doldurmuştur. Çalışma için hastanemizin etik kurulundan onay alınmıştır (tarih: 26/07/2023 no: E-23-1347). Tüm prosedürler, kurumsal ve/veya ulusal araştırma komitesinin etik standartlarına ve 1964 Helsinki Bildirgesi'ne uygun olarak gerçekleştirilmiştir.

Katılımcıların yaş, cinsiyet, medeni durum gibi demografik bilgileri ile kullandığı madde, maddeyi ilk kullandığı yaş gibi klinik bilgileri sosyodemografik veri formu ile toplanmıştır. Katılımcıların öz-şefkat seviyeleri Neff (2003) tarafından oluşturulan; Deniz ve arkadaşları tarafından Türkçe'ye uyarlanan Öz-Şefkat Ölçeği (ÖŞÖ) ile belirlenmiştir.[3, 18] Orijinal formunda 26 sorudan oluşan ölçek, Türkçe uyarlama çalışmasında 1. ve 22. Maddelerin madde toplam korelasyon değeri 0.30'dan düşük olduğu için çıkarılarak 24 soruya indirilmiştir. 5'li Likert tipinde olan ölçek tek boyuttan oluşmaktadır. Ölçekten alınan yüksek puanlar yüksek öz-şefkat seviyesine işaret etmektedir.

Psikolojik İyi Oluş Ölçeği (İOÖ) Diener ve arkadaşları tarafından geliştirilen 5'li Likert tipindedir ve toplamda 8 sorudan oluşmaktadır.[19] Tek bir boyuttan oluşan bu ölçekte bireylerin amaçlı, anlamlı ve yeterli bir hayat yaşamaya dair düşünceleri değerlendirilmektedir. Ölçekten alınan yüksek puanlar daha iyi bir psikolojik iyi oluşa işaret etmektedir. Ölçeği Türkçe geçerlilik ve güvenilirlik çalışması Telef (2013) tarafından yapılmıştır.[20]

Katılımcıların bağlanma stillerini araştırmak amacıyla İlişki Ölçekleri Anketi (İÖA) kullanılmıştır. Griffin ve Bartholomew tarafından geliştirilen ve güvenli, kayıtsız, saplantılı ve korkulu olmak üzere dört tip bağlanma stilini araştıran bu ölçek 7'li Likert tipindedir ve 17 maddeden oluşmaktadır.[21, 22] Ölçeğin orijinalinde 30 soru bulunmaktadır ancak Sümer ve Güngör tarafından yapılan Türkçe uyarlama çalışmasında madde sayısı 17'ye indirilmiştir. İlişki ölçekleri anketinin alt boyut puanları, her bir boyut için belirlenen maddelerin toplamının madde sayısına bölünmesi ile elde edilir. Ölçeğin literatürle uyumlu biçimde yapı geçerliliği ve güvenilirliği yüksektir.

Araştırma verisi SPSS (Statistical Package For Social Sciences for Windows v.22,0, SPSS Inc. Chicago, IL) aracılığıyla değerlendirildi. Tanımlayıcı istatistikler ortalama (±) standart sapma, frekans dağılımı ve yüzde olarak sunuldu. Verilerin dağılımının normalliği Shapiro-Wilk's testi ve histogram grafikleri kullanılarak değerlendirildi. Gruplar arasındaki karşılaştırmalarda kategorik değişkenler için Ki-Kare testi kullanıldı. Sürekli değişkenler için ise değişkenler normal dağılım gösterdiğinde One-Way Anova testi, normal dağılım göstermediğinde Mann-Whitney U tesi ve Kruskal-Wallis testi kullanıldı. 3 grup arasındaki anlamlı bulunan değerlerde farkın hangi gruplardan kaynaklandığının tespiti için post hoc analiz yapıldı. Değişkenler arasındaki ilişkinin değerlendirilmesi için değişkenler normal dağıldığında Pearson korelasyon analizi değişkenler normal dağılmadığında Spearman korelasyon analizi uygulandı. İstatistiksel anlamlılık düzeyi $p<0,05$ olarak kabul edildi.

Bulgular

Çalışma 91 katılımcıyla gerçekleştirildi. Katılımcıların 30 (%33) tanesi opioid kullanıcısı, 31 (%34,1) tanesi metamfetamin kullanıcısı ve 30'u (%33) da sağlıklı kontrol idi. Katılımcıların ortalama yaşı 31,3 idi. Katılımcıların %78'i (n=71) erkek, %22'si (n=20) kadın idi ve 42 (%46,2) tanesi evli, 49 (%53,8) tanesi bekar ya da boşanmış idi. Katılımcıların ortalama eğitim süresi 12,1 yıl idi.

Gruplar arasında yaş ve cinsiyet dağılımı benzerdi. Medeni durum bakımından gruplar arasında anlamlı fark vardı, kontrol grubunda evli olanların oranı, opioid ve metamfetamin grubunda ise bekar ya da boşanmış olanların oranı fazla idi. Eğitim durumu açısından gruplar arasında anlamlı fark vardı. Kontrol grubunun eğitim süresi opioid ve metamfetamin kullananların eğitim süresine göre anlamlı oranda fazlaydı. Opioid kullanan hastalar, metamfetamin kullanan hastalar ve kontrol grubunun çeşitli sosyodemografik verileri Tablo 1'de gösterilmiştir.

Tablo 1. Opioid kullanan hastalar, metamfetamin kullanan hastalar ve kontrol grubunun çeşitli sosyodemografik verileri

n=91	Opioid (n=30) Ort ± Ss / n (%)	Metamfetamin (n=31) Ort ± Ss / n (%)	Kontrol (n=30) Ort ± Ss / n (%)	*p*
Yaş	30,6±5	30,0±7	33,4±6	0,073[a]
Cinsiyet /erkek	26 (86,7)	25 (80,6)	20 (66,7)	0,158[b]
Medeni durum/evli	12 (40)	8 (25,8)	22 (73,3)	0,001[b]
Eğitim süresi-yıl	9,8±3	10,4±3	16,2±3	<0,0001[a]
İlk madde yaşı	17,9±4	19,2±3,6	-	0,760[c]

a: Kruskall Wallis testi, b: Chi-Square, c: Mann-Whitney U testi, n:sayı, ort:ortalama, Ss: standart sapma

Opioid, metamfetamin ve kontrol gruplarının her birisi için ölçek puanları arasındaki ilişkinin incelenmesi amacıyla korelasyon analizi yapıldı. Opioid grubunda; öz-şefkat ölçeği toplam puanı (ÖŞÖ-T) ile ilişki ölçekleri anketinin (İÖA) saplantılı bağlanma alt saplantılı bağlanma arasında, İÖA-korkulu bağlanma puanı ile kayıtsız ve saplantılı bağlanma puanları arasında, İÖA-kayıtsız bağlanma ile korkulu, saplantılı ve güvenli bağlanma arasında, İÖA-

güvenli bağlanma ile kayıtsız ve saplantılı bağlanma arasında anlamlı ilişki var idi (Tablo 2).

Metamfetamin grubunda, iyi oluş ölçeği (İOÖ) ortalama puanı ile İÖA-kayıtsız, saplantılı ve güvenli bağlanma arasında, İÖA-korkulu bağlanma ile İOÖ, kayıtsız, saplantılı ve güvenli bağlanma arasında, İÖA-kayıtsız bağlanma ile İOÖ, korkulu bağlanma arasında, İÖA-saplantılı bağlanma ile İOÖ, kayıtsız bağlanma ve korkulu bağlanma arasında, İÖA-güvenli bağlanma ile İOÖ, korkulu ve kayıtsız bağlanma arasında anlamlı ilişki vardı (Tablo 2).

Kontrol grubunda; ÖŞÖ-T ile İOÖ arasında, İÖA-korkulu bağlanma ile kayıtsız ve saplantılı bağlanma arasında, İÖA-kayıtsız bağlanma ile korkulu ve saplantılı bağlanma arasında, İÖA-saplantılı bağlanma ile korkulu ve kayıtsız bağlanma arasında anlamlı ilişki vardı (Tablo 2). Çalışma gruplarındaki korelasyon analizi sonuçları Tablo 2'de gösterilmiştir.

Tablo 2. Opioid, metamfetamin ve kontrol gruplarının her birisi için ölçek puanları arasındaki korelasyon analizi

n=91			1	2	3	4	5	6
Opioid (n=30)	1-ÖŞÖ-Toplam	r	1					
	2-İOÖ-Toplam	r	0,252	1				
	3-İÖA- Korkulu Bağlanma	r	-0,287	0,317	1			
	4-İÖA- Kayıtsız bağlanma	r	-0,197	0,243	0,491**	1		
	5-İÖA-Saplantılı bağlanma	r	-0,537**	-0,080	0,498**	0,429*	1	
	6-İÖA- Güvenli bağlanma	r	-0,148	0,000	0,159	0,411*	0,478**	1
Metamfetamin (n:31)	1-ÖŞÖ-Toplam	r	1					
	2-İOÖ-Toplam	r	0,225	1				
	3-İÖA- Korkulu Bağlanma	r	-0,235	0,347	1			
	4-İÖA- Kayıtsız bağlanma	r	-0,102	0,441*	0,653**	1		
	5-İÖA-Saplantılı bağlanma	r	-0,307	0,428*	0,586**	0,467**	1	
	6-İÖA- Güvenli bağlanma	r	0,062	0,499**	0,420*	0,570**	0,115	1
Kontrol (n:30)	1-ÖŞÖ-Toplam	r	1					
	2-İOÖ-Toplam	r	0,455*	1				
	3-İÖA- Korkulu Bağlanma	r	-0,147	0,092	1			
	4-İÖA- Kayıtsız bağlanma	r	0,042	0,187	0,630**	1		
	5-İÖA-Saplantılı bağlanma	r	-0,225	0,224	0,425*	0,434*	1	
	6-İÖA- Güvenli bağlanma	r	0,306	0,119	-0,306	-0,031	-0,238	1

r: Correlation coefficient, *: p<0,05 **:p<0,01, ÖŞÖ: Öz şefkat ölçeği, İOÖ: İyi oluş ölçeği, ÖİA: İlişki ölçekleri anketi

Katılımcıların öz şefkat ölçeği puanları karşılaştırıldığında gruplar arasında anlamlı fark vardı (p=0,004). Opioid grubu ve kontrol grubunun ortalama puanı, metamfetamin grubu ortalama puanından anlamlı derecede yüksekti. Opioid ve kontrol grubundaki katılımcıların puan ortalamaları benzerdi. Gruplar arasında iyi oluş ölçeği ortalama puanları arasında da anlamlı fark vardı (p<0,001). Opioid ve metamfetamin grubu puan ortalaması kontrol grubuna göre anlamlı derecede daha düşük idi. Metamfetamin ve opioid grubu puan ortalaması benzerdi (Tablo 3).

Gruplar arasında İÖA alt ölçek puan ortalamaları karşılaştırıldı. Gruplar arasında kayıtsız bağlanma puanları ortalamaları açısından istatistiki açıdan anlamlı fark yalnızca kontrol grubu ile metamfetamin grubu arasında saptandı. Kontrol grubu metamfetamin grubundan anlamlı derecede yüksekti (Tablo 3).

Gruplar arasında korkulu bağlanma, saplantılı bağlanmalı ve güvenli bağlanma puanları ortalamaları benzerdi (sırasıyla; p=0,092, p=0,171, p=0,965) (Tablo 3). Grupların ölçek puan ortalamalarının karşılaştırılması Tablo 3'te sunulmuştur.

Öğrencilerin %8,8'i (n=29) HPV aşılarından birini yaptırdığını, %91,2'si (n=299) yaptırmadığını belirtti. %81,1'i (n=266) HPV aşılarından birini yakınlarına önerirken, %18,9'u (n=62) önermeyeceğini ifade etti.

Öğrencilerin HPV aşısı ile ilgili tutum sorularından 'HPV aşısını ücretli olarak alıp yaptırırım' ifadesine %64,6 (n=212) oranında katılıyorum, %16,8 (n=55) oranında katılmıyorum, %18,6 (n=61) oranında fikrim yok şeklinde cevap verdikleri görüldü. 'HPV aşısı sosyal güvence kapsamında karşılanırsa yaptırırım' ifadesine %76,8 (n=252) oranında katılıyorum, %7,6 (n=25) oranında katılmıyorum, %15,5 (n=51) oranında fikrim yok şeklinde yanıtladılar. 'Kız çocuğum olsa ona HPV aşısı yaptırırım' sorusuna %79,0 (n=259) oranında katılıyorum, %4,9 (n=16), %16,2 (n=53) oranında fikrim yok şeklinde cevap verdiler. 'Erkek çocuğum olsa ona HPV aşısı yaptırırım' ifadesine %62,8 (n=206) oranında katılıyorum, %15,2 (n=50) oranında katılmıyorum, %22,0 (n=72) oranında fikrim yok şeklinde yanıt verdikleri görüldü.

Tablo 3. Gruplar arasındaki ÖŞÖ, İOÖ ve İÖA puan ortalamalarının karşılaştırılması

n=91	Opioid (n=30) Ort ± Ss / n (%)	Metamfetamin (n=31) Ort ± Ss / n (%)	Kontrol(n=30) Ort±Ss/n (%)	*p*
ÖŞÖ-Toplam	78,9±13	67,7±13	76,9±13	0.004[a]
İOÖ-Toplam	28,4±7	24,3±7	43,9±7	<0.001[b]
İÖA- Korkulu Bağlanma	17,6±6	14,3±5	16,1±5	0.092[a]
İÖA- Kayıtsız bağlanma	23,0±5	20,5±4	23,4±3	0.038[a]
İÖA-Saplantılı bağlanma	11,8±5	12,7±4	10,4±4	0.171[a]
İÖA- Güvenli bağlanma	21,1±4	20,7±4	20,8±5	0.965[a]

a: One- Way Anova, b: Kruskall -Wallis testi, post hoc: ÖŞÖ-T: opioid=kontrol> metamfetamin, İOÖ-T: opioid =metamfetamin<kontrol, İÖA- Kayıtsız bağlanma: kontrol> metamfetamin, kontrol=opioid, opioid=metamfetamin, n:sayı, ort:ortalama, Ss: standart sapma

Tartışma

Bu çalışmada opioid ve metamfetamin kullanan hastalar kontrol grubuyla karşılaştırılarak öz-şefkat, psikolojik iyi oluş ve bağlanma özelliklerinin incelenmesi hedeflenmiştir. OKB olan bireylerde öz-şefkat düzeyi ve saplantılı bağlanma arasında negatif yönde korelasyon saptanırken MKB grubunda psikolojik iyi oluş düzeyi ve kayıtsız, saplantılı ve güvenli bağlanma biçimleri arasında pozitif yönde korelasyon saptanmıştır. Kontrol grubunda ise yalnızca öz-şefkat düzeyleri ile psikolojik iyi oluş arasında anlamlı pozitif bir ilişki mevcuttur. Ayrıca sonuçlarımız öz-şefkat, psikolojik iyi oluş ve kayıtsız bağlanma alt boyutunda kontrol grubunun iki hasta grubundan da daha yüksek puanlar aldığını göstermektedir. Bildiğimiz kadarıyla çalışmamız, ülkemizde bu üç kavramı, opioid ve metamfetamin kullanan hasta grupları ve kontrol grubuyla karşılaştırarak inceleyen ilk çalışmadır.

Literatüre bakıldığında öz-şefkat ve bağlanma biçimlerini araştıran çalışmaların daha çok sağlıklı örneklemlerde yapıldığı görülmektedir. Neff ve McGehee'nin (2010) ergen ve genç erişkinlerde yaptıkları çalışmada saplantılı ya da kaygılı bağlanma stili olanlarda öz-şefkat düzeyi düşük saptanmıştır.[5] Düşük öz-şefkat, ayrıca endişeli ve kaçıngan bağlanma ile de anlamlı bir şekilde ilişkili bulunmuştur.[23, 24] Yüksek risk altındaki gençlerde yapılan bir başka çalışmada güvensiz bağlanma ile öz-şefkat arasında ters yönde bir ilişki olduğu saptanmıştır.[25] Güvensiz bağlanma kavramı ise korkulu, saplantılı ve kaçıngan bağlanma stillerini kapsayan çatı bir kavram olarak ele alınmaktadır.[26] Her ne kadar madde kullanım bozukluğu alanında yeterli bir literatür olmasa da çalışmamızda opioid kullanan bireylerde saplantılı bağlanma ile öz-şefkat arasında bulunan olumsuz ilişki geçmiş çalışmalarla uyumlu görünmektedir.

Metamfetamin kullanım bozukluğu olan bireylerde ise bağlanma stilleri ile öz-şefkat düzeyi arasında bir ilişki saptanmazken psikolojik iyi oluş düzeyi ile kayıtsız, saplantılı ve güvenli bağlanma arasında aynı yönde bir ilişki saptanmıştır. Aynı anda hem güvensiz hem de güvenli bağlanma stili ile iyi oluş arasında ilişki olması şaşırtıcıdır. Delvecchio ve arkadaşlarının yatarak takip edilen madde kullanan hastalarda yapılan bir çalışmada, hastaların daha çok güvensiz bağlanma stillerini kullandığı ve duygusal kaynaklarını kullanmakta zorluklar yaşayarak daha fonksiyonel olmayan bir yaşam sürdükleri görülmüştür.[27] Sigara bağımlısı üniversite öğrencilerinde yapılmış bir çalışmada ise, içen grubun içmeyen gruba göre daha fazla kaygılı bağlandığı ve psikolojik problemler yaşadığı görülmüştür.[28] Bu çalışmalarda psikolojik iyi oluş kavramını ölçmek için "genel sağlık anketi" kullanmış olmaları dikkat çekicidir. Metamfetamin kullanan grupta hem güvenli ve hem güvensiz bağlanma stilleri ile iyi oluş arasında görülen ilişki her ne kadar literatürle uyumsuz görünse de kişilik özellikleri ve benzeri bu çalışmada incelenmemiş bir takım kavramlar bağlanma stilleri ve iyi oluş ilişkisinde aracı ve belirleyici rol oynuyor olabilir.[29]

Öz-şefkat ve psikolojik iyi oluş düzeyleri çalışmamızda geçmiş çalışmalarla uyumlu biçimde kontrol grubunda madde kullanan hastalara göre anlamlı düzeyde yüksek saptanmıştır.[11, 12] Öz-şefkatin hem gençlerde hem de erişkinlerde koruyucu bir faktör olduğu bilinmektedir.[30] Hem hasta hem de sağlıklı kontrollerden oluşan erişkin gruplarda öz-şefkatin daha düşük seviyede psikopatoloji ile ilişkili olduğu gösterilmiştir.[4] Yüksek seviyede öz-şefkat ise daha iyi bir özgüven, iyi oluş ve yaşam kalitesi ile ilişkilidir.[31-33] Bu bağlamda, çalışmamızda kontrol grubunda öz-şefkat ile iyi oluş arasında saptanan olumlu ilişki beklenen bir sonuçtur. Alkol kullanım bozukluğu olan hastalarda öz-şefkatin potansiyel bir koruyucu faktör olabileceğine dair yapılan çalışmada özellikle farkındalık alt başlığı alkol kullanımına karşı koruyucu bir faktör olarak saptanmıştır.[8] Opioid, metamfetamin benzeri bağımlılık yapıcı maddeler kullanan bireylerde patolojinin devam etmesinin en önemli sebeplerinden biri olan aşerme de farklı tanı gruplarında çalışılarak öz-şefkat ile ilişkili saptanmıştır.[7] Hem madde kullanım bozukluğunun gelişimi, hem de aşerme ya da duygusal kaynakları kullanamama veya duygu düzenleme problemleri sebebiyle bozukluğun sürmesinde öz-şefkat önemli bir rol oynamaktadır. Sonuç olarak olarak kendinden memnun olmayan ve yaşam alanlarında işlevsellikte problemler yaşayan bireyler karşımıza çıkmaktadır.[10]

Gruplar arasında bağlanma stilleri açısından bakıldığında, kayıtsız bağlanma stilinin kontrol grubunda opioid ve metamfetamin grubuna göre daha fazla kullanıldığı görülmüştür. Literatürde kayıtsız bağlanma ile ilgili çelişkili sonuçlar bulunmaktadır. Çalışmalar kaygılı ve korkulu bağlanma stillerinin alkol ve madde kullanan bireylerde daha fazla kullanıldığını göstermektedir.[14, 16] Kayıtsız bağlanmanın ise madde kullanım şiddeti ile ters

yönde bir ilişki kurduğu gösterilmiştir.[14] Benzer şekilde McNally ve arkadaşları (2003) güvensiz bağlanma stillerinden biri olan kayıtsız bağlanmanın alkol kullanımı ile ilişki olmadığını göstermiştir.[15] Ek olarak Magai ve arkadaşları kayıtsız bağlananların pozitif duygularını daha da artırmak amacıyla alkol kullanabildiğini öne sürmüştür.[16] Ayrıca kayıtsız bağlanma stilini kullanan bireylerde reddetme ve inkar davranışı daha fazla olacağından çalışmamızda madde kullanan bireylere göre kontrol grubunda daha fazla saptanmış olabilir.[34] Çalışmamızda kontrol grubunun hasta grubu ile benzer sosyokültürel çevreden gelmiş olması da diğer bağlanma stilleri arasında anlamlı bir farklılık bulunmamasını açıklayabilir.

Çalışmamızın bazı kısıtlılıkları bulunmaktadır. Öncelikle çalışmanın tek merkezde yürütülmesi ve veri toplama araçlarının öz-bildirim tarzında olması kısıtlılık olarak sayılabilir. Ayrıca çalışmamız her ne kadar kontrol grubuyla uyum içinde olsa da ağırlıklı olarak erkek bireylerden oluşmakta bu da genellenebilirliğini kısıtlamaktadır. Çalışmamızda opioid ve metamfetamin kullanan bireylerin psikoz ve bipolar bozukluk gibi ciddi psikiyatrik hastalığa sahip olup olmadıkları değerlendirilse de psikolojik iyi oluş seviyelerini etkileyebilecek depresyon ve anksiyete bozukluğu değerlendirilmemiştir. Ayrıca opioid ve metamfetamin kullanan bireyler dışında diğer madde çeşitleri çalışmamıza dahil edilmemiştir.

Sonuç olarak, çalışmamız ülkemizde yapılmış ve madde kullanan bireyleri kontrol grubuyla karşılaştırarak öz-şefkat, psikolojik iyi oluş ve bağlanma stillerini araştıran ilk çalışmadır. Bulgularımız, madde kullanan bireylerde kontrol grubuna göre öz-şefkat ve iyi oluş seviyelerinin anlamlı olarak daha düşük olduğunu göstermiştir. Ayrıca madde çeşidine göre iyi oluş ve öz-şefkat bağlanma stilleri ile farklı korelasyonel ilişkiler kurmuştur. Öz-şefkatin özellikle son dönemde gittikçe artan bir teorik arkaplan ile terapi hedefi olarak ele alındığı düşünüldüğünde çalışmamızın kıymeti artmaktadır [35]. Gelecek çalışmalarda, daha geniş örneklemlerde, farklı madde çeşitlerinin de dahil edilerek kişilik özellikleri/dayanıklılık/çocukluk çağı yaşantıları gibi kavramların bağlanma üzerine rolünün araştırılması önerilir. Sonuçlarımızın genellenebilirliğinin artırılması açısından farklı örneklemlerde bulgularımızın test edilmesi önemlidir.

Etik Standartlara Uygunluk:

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Yazar Katkısı

KSK, GZİ, MD: Fikir/kavram; KSK, GZİ: Tasarım; KSK, GZİ Veri Toplama; KSK, GZİ: Veri İşleme; KSK, GZİ, MD: Analiz/Yorum; KSK, GZİ, MD: Literatür taraması; KSK, GZİ: Yazma.

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RETROSPECTIVE EVALUATION OF PATIENTS WHO RECEIVED SURGERY AS THE FIRST OPTION IN THE TREATMENT OF GREEN TYPE 3-4 TRIGGER FINGER

GREEN TİP 3-4 TETİK PARMAK TEDAVİSİNDE İLK SEÇENEK OLARAK CERRAHİ TEDAVİ UYGULANAN HASTALARIN RETROSPEKTİF DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: To evaluate the clinical and functional outcomes of the patients with trigger finger patients who are treated by open surgery method without applying conservative treatment modalities.

Methods: Open A1 pulley surgical release under local anesthesia was applied to 67 trigger finger patients (45 female, 22 male, mean age 53,82, range 11-81). The mean follow-up was 38,37 months (range 4-76 months). As assosiated pathologies, there were type 2 DM in 14 and chronic renal failure in 1 patients.

Results: In 67 patients pain and triggering were found to be treated by open surgical A1 pulley release. No recurrence seen in triggering nodularity. Postoperatively there were no significant neurovascular complications noted.

Conclusion: As similar to literature, we believe that in the treatment of trigger finger with open surgery method is a safe and effective method and also supplies fast return to daily life. We think that the surgery may be the first choice in selected cases.

Keywords: Trigger finger, stenosing tenosynovitis, A1 pulley, hand surgery

ÖZ

Amaç: Konservatif tedavi önerilen fakat hasta uyumsuzluğu nedeniyle konservatif tedavi uygulanmadan açık cerrahi teknik uygulanan tetik parmaklı hastaların klinik ve fonksiyonel sonuçlarını değerlendirmektir.

Yöntem: Tetik parmak tanılı 67 hastanın (45 kadın, 22 erkek; ort. yaş 53,82; dağılım 11-81) parmağına açık insizyonla A1 pulley gevşetme cerrahi tedavisi uygulandı. Hastalar ortalama 38,37 ay (dağılım 4-76 ay) izlendi. İlave patolojiler bakımından, 14 hastada tip 2 Diabetes Mellitus (DM) ve 2 hastada kronik böbrek yetmezliği bulunmaktaydı

Bulgular: Açık cerrahi ile A1 pulley gevşetme uygulanan 67 parmaktaki ağrı ve takılmanın tamamen geçtiği görüldü. Hiçbir hastada tetiklenme veya nodül oluşumu tekrarlamadı. Olgularda postoperatif nörovasküler komplikasyona rastlanılmadı.

Sonuç: Literatüre benzer olarak tetik parmağın tedavisinde açık cerrahi ile elde edilen gevşetmelerin güvenli, etkili ve günlük yaşama dönmeyi hızlandıran bir yöntem olduğu kanısındayız. Cerrahinin seçili vakalarda ilk tercih olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Tetik parmak, stenozan tenosinovit, A1 pulley, el cerrahisi

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Introduction

The flexor tendon dysfunction which occurs in association with the tenosynovitis developing under the A1 flexor tendon pulley is called the "Trigger Finger Disease" (stenosing tenosynovitis). It frequently occurs at the age 45 and above, especially in women. Its prevalence corresponds to nearly 3% of the general population. Although its etiology is uncertain, diabetes mellitus, presence of renal disease, collagen tissue diseases, hypothyroidism, history of carpal tunnel syndrome surgery and De Quervain's disease are predisposing factors associated with the trigger finger.[1-4] As a result of the stenosing tenosynovitis at the level of A1 pulley, pain, locking, and loss of function occurs in the affected finger. Furthermore, joint contracture may develop as a result of repetitive forceful finger movements. The disease may impact a single finger or multiple fingers.[5] Initial treatments include local and systemic anti-inflammatory agents, local anesthetic and steroid injections, hot-cold applications, splinting and paraffin baths. Generally, the surgical therapy option is applied in subjects where such implementations fail.
In our study, we evaluated the clinical and functional results of trigger finger patients who underwent open surgical techniques without the administration of conservative therapy due to patient non-compliance.

Methods

Sixty-seven patients, who applied to the Orthopedics and Traumatology polyclinic of Izmit SEKA State Hospital of Kocaeli (Turkey) between June 2012 - June 2018, suffered from painful nodules due to triggering and were diagnosed with trigger finger, underwent A1 pulley mini open surgery under wide awake local anesthesia (mixture of 2 cc prilocaine and 1 cc isotonic 0.9% sodium chloride) without tourniquet hemostasis (WALANT) and without administration of a conservative treatment due to patient non-compliance. A transverse incision was made over the A1 pulley for the 1st finger, while a longitudinal incision was applied on the other fingers. All patients underwent a perioperative active movement control. Active and passive flexion-extension exercises were initiated on the 3rd post-operative day.
The patients' time return to daily activities, pulp-to-palm distance (PPD), elimination of pain and locking in the fingers, extension restriction, surgery site scar, development of reflex sympathetic dystrophy and deformity, nodule formation in the incision site and development of infections were evaluated in the clinical evaluation.
Written informed consent was obtained from patients or their legal caregivers. All procedures were performed following the ethical standards specified in the Declaration of Helsinki (2008). The study was approved by İnstutional Review Board of Kocaeli University Ethics Committee (year 2024, No: E-80418770-020-591348).

Results

Forty-five patients were female patients, while 22 were male patients and the average age was 53.82 years (age distribution varied between 11-81 years). The patients were followed up for 38.37 months on average (at a distribution of 4-76 months). Among the trigger fingers, 33 (49.2%) were in the thumb, 10 (14.9%) were in the second finger, 9 (13.4%) were in the third finger, 10 (14.9%) were in the fourth finger and 5 (7.45%) were in the fifth finger. 58 patients (86.5%) were grade 3 and 9 patients (13.5%) were grade 4 according to Green's classification. In terms of comorbidities, 14 (20.8%) patients had type 2 diabetes mellitus (DM) and 2 (0.29%) patient had chronic renal failure.
No active or passive movement restriction developed in the subjects. The PPD values of the patients were below 1 cm (good result). Trigger and nodule formation did not occur again in any patient. No neurovascular damage or spontane flexor tendon rupture were observed in the patients. Eighteen patients (26.8%) continued to suffer from pain until the end of the 2nd month and these complaints disappeared in the follow-ups. No reflex sympathetic dystrophy developed in any patient. Superficial infection developed in 1 diabetic patient (0.14%) and it improved in the 3rd week with the 1st generation cephalosporin treatment administered daily with medical dressing. Scar sensitivity was observed in the scar site in 4 patients (0.59%). NSAID medical treatment was initiated along with massage therapy. These sensitivities disappeared in the 3rd month in 1 patient and in the 4th month in 3 patients.

Table 1. Results of the trigger patients. (P= patients)

Total	67 p
Age (average)	53,82
Age (range)	11-81
Gender	45 Female, 22 male
Grade of patient (Green's Classification)	Grade 3- 58 p Grade 4- 9 p
Affected fingers **1 F** **2 F** **3 F** **4 F** **5 F**	 33 p (49.2%) 10 p (14.9%) 9 p (13.4%) 10 p (14.9%) 5 p (7.45%)
Related diseases	DM (14 p) (20.8%) Cr. Renal Failure (2 p) (0.29%)
Early complications	Pain (18 p) (26.8%) Superficial infection (1 p) (0.14%) Scar sensitivity (4 p) (0.59%)

Discussion

Surgical and relaxation of A1 pulley at the metacarpal head is recommended in the surgical treatment of trigger fingers.[4] The conventional surgical method involves the open release of A1 pulley achieved via longitudinal or

palmar transverse incision performed by giving importance to interphalangeal and palmar creases, with a success rate of approximately 97%.[1] Complications such as infections, scar development and neurovascular wounding may occur in open surgery.[6,7] It is observed that percutaneous release has been standing out recently.[8,9] There are trials indicating that this is a preferable method as it is safe , effective and is easily applied, has a low cost and has a minimum complication risk if performed carefully.[10,11] However, they reported that a section of nearly 15% could remain unreleased at the pulley distal in percutaneous releases.[12] In a study conducted by Yalçınkaya et al., it was demonstrated that open surgery was successful but that the recurrence rates were higher in patients with systemic diseases (especially diabetes mellitus).[13]

There are studies showing that, despite open surgical release, triggering may persist due to tendons hooking on the transverse fibers of palmar aponeurosis or failure to achieve a full release.[1] It may be verified whether locking continues by active flexion and extension movements of the finger prior to wound closure as a superiority of the surgeries performed under local anesthesia compared to those conducted under general anesthesia. Furthermore, it was reported that the complication rates in open surgeries performed under general anesthesia or sedation were higher.[14] All patients in our series were operated under local anesthesia and all patients underwent a perioperative active movement control.

The role of therapeutic steroid injections in the treatment of trigger finger is still debatable. There are publications which report that steroid injection is a preferable therapeutic option with acceptable side effects in the first phase of treatment especially in non-diabetic patients.[1,4,13,14] The efficacy of single-dose steroid injection is reported to vary between 35-60%, while there is literature reporting that the success rate rises to 82% with additional injections.[1,4,13,14] Moreover, single-dose injection was demonstrated to be more successful in female patients in whom trigger finger was observed for the first time.[16] However, in the study conducted by Ng WKY et al., it was necessary to wait for at least 80 days before surgery following steroid injection to reduce infection risk.[17]

In addition to patients who undergo steroid injection, the infection risk following A1 pulley open surgery increases in smokers, co-administration of epinephrine with lidocaine for local anesthesia (epinephrine-related), elderly patients and in the use of antibiotics prior to surgery.[17] There are studies indicating that the risk will increase in diabetic patients, while there are also studies which show that the risk does not change.[18] Superficial infection was observed in 1 diabetic patient (1.4%) in our patient group, but as this percentage was insignificant, we may state that there is no difference between diabetics and nondiabetics.

In conclusion, full visibility of all anatomic structures, starting with A1 pulley in trigger finger subjects who underwent open surgery, ensures minimum neurovascular complications. Generally, although surgical treatment is recommended in subjects who cannot achieve a response following conservative treatment in the literature, open surgery may be considered primarily as an effective and safe method especially in patients with locking complaint and suffering from severe pain (Green's Classification grade 3 or 4) due to high postoperative patient satisfaction.[19] Making the patient do finger movements at an early phase following surgery minimizes scar development and enables the patient to return to his/her daily activities at an earlier phase. It should be remembered that the infection risk will increase in subjects with concomitant systemic diseases starting with diabetes mellitus and in surgeries performed at an early phase following steroid injection.

Compliance with Ethical Standards

The study was approved by İnstutional Review Board of Kocaeli University Ethics Committee (year 2024, No: E-80418770-020-591348).

Conflict of Interest

The authors have indicated they have no potential conflicts of interest to disclose.

Author Contribution

ÜG, ÖS: Designed the study and reviewed the manuscript; ÜG: Collected data, carried out the analyses and drafted the initial manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Research Article | Araştırma Makalesi

KIDNEY HEALTH CONSEQUENCES OF PATIENTS WITH GLOMERULONEPHRITIS; BEFORE AND AFTER SARS-COV2 INFECTION

SARS-COV2 ENFEKSİYONUNDAN ÖNCE VE SONRASI GLOMERÜLONEFRİTLİ HASTALARIN BÖBREK SAĞLIĞI

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ABSTRACT

Objective: The virus that causes severe acute respiratory syndrome (SARS-CoV-2) was first identified in Wuhan, China, in December 2019. Recent studies have proven that SARS-CoV-2 is also a nephrotrophic virus.

Methods: Our study aimed to evaluate kidney function and general kidney health of patients with previously diagnosed glomerular diseases and follow-up after SARS-CoV-2 infection. For this purpose, the data of 36 patients who were diagnosed with and routinely followed up for glomerulonephritis and had SARS-CoV-2 infection at the Kocaeli University Faculty of Medicine Hospital nephrology outpatient clinics between January 2020 and January 2022 were examined before and after the infection.

Results: No significant differences were observed in serum creatinine, estimated glomerular filtration rate, and 24-hour urine protein values after infection. There was an increase in platelet and albumin levels following the SARS-CoV-2 infection. A significant decrease was detected in 24-hour urine creatinine values.

Conclusion: The results of the study showed that kidney function and general kidney health of patients with SARS-CoV-2 infection diagnosed with glomerulonephritis were not different when compared to their condition before SARS-CoV-2 infection.

Keywords: COVID-19 infection, glomerulonephritis, chronic kidney disease, vaccine

ÖZ

Amaç: Şiddetli akut solunum yolu sendromuna neden olan virüs (SARS-CoV-2) ilk olarak Aralık 2019'da Çin'in Wuhan kentinde tanımlandı. Son çalışmalar SARS-CoV-2'nin de nefrotrofik bir virüs olduğunu kanıtlamıştır.

Yöntem: Çalışmamızın amacı daha önce glomerüler hastalık tanısı konmuş hastaların böbrek fonksiyonlarını ve genel böbrek sağlıklarını değerlendirmek ve SARS-CoV-2 enfeksiyonundan sonra böbrek sağlığı kontrolunü yapmaktır. Bu amaçla Ocak 2020 ile Ocak 2022 arasında Kocaeli Üniversitesi Tıp Fakültesi Hastanesi nefroloji polikliniklerinde glomerülonefrit tanısı konulan ve rutin olarak takibi yapılan, SARS-CoV-2 enfeksiyonu olan 36 hastanın verileri enfeksiyondan öncesi ve sonrası olarak incelendi.

Bulgular: Enfeksiyondan sonra serum kreatinin, tahmini glomerüler filtrasyon hızı ve 24 saatlik idrar protein değerlerinde anlamlı bir fark gözlenmedi. SARS-CoV-2 enfeksiyonundan sonra trombosit ve albümin seviyelerinde artış görüldü. 24 saatlik idrar kreatinin değerlerinde anlamlı bir düşüş tespit edildi.

Sonuç: Çalışmanın sonuçları, glomerülonefrit tanısı konulan SARS-CoV-2 enfeksiyonlu hastaların böbrek fonksiyonlarının ve genel böbrek sağlıklarının, SARS-CoV-2 enfeksiyonundan önceki durumlarıyla karşılaştırıldığında farklı olmadığını gösterdi.

Anahtar Kelimeler: COVID-19 enfeksiyonu, glomerülonefrit, kronik böbrek hastalığı, aşı

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Introduction

The virus that causes severe acute respiratory syndrome (SARS-CoV-2) was first identified in December 2019. Within months, the virus spread throughout the world and was declared a pandemic at the beginning of March 2020. SARS-CoV-2, viral pneumonia, and multi-organ involvement.[1] Chronic kidney disease has also been identified as a risk factor because it causes immune deficiency.[2]

Possible targets and facilitating factors for the virus to cause infection in human cells are cell membrane-bound receptor Angiotensin Converting Enzyme 2 (ACE2), Transmembrane Serine Protease 2 (TMPRSS2), Furin and Basigin (CD147). The expression of ACE2, TMPRSS2, Furin, and CD147 is not specific to the lung, and their expression in various tissues, including the brain, intestine, and kidney, may expose these organs to SARS-CoV-2 infection.[3] Renal parenchymal cells, specifically proximal tubular cells, secrete high levels of ACE2. The expression of genes related to injury, inflammation, and fibrosis is increased in kidney tubule cells and podocytes in which SARS-CoV-2 RNA was found.[4] Many studies have reported that SARS-CoV-2 RNA and protein were detected using electron microscopy in the kidneys of COVID-19 patients.[5]

In a post-mortem renal histopathologic study of SARS-CoV-2 patients; It was shown that 85% of patients had AKI and 74% had acute glomerular damage.[6] In another study conducted in living patients, biopsies were performed. The pathological conditions identified included acute tubular damage, minimal change disease, membranous glomerulonephritis, anti-GBM disease, and lupus nephritis. SARS-CoV-2 infection has been reported to have the potential to affect innate or adaptive immune responses, triggering new glomerular diseases or exacerbating pre-existing autoimmune conditions.

Thus, further attention should be focused to the long-term impact of SARS-CoV-2 infection and kidney function monitoring. This study aimed to evaluate the kidney function and general kidney health of patients with previously diagnosed glomerular diseases and follow-up after SARS-CoV-2 infection.

Methods

This study included 36 patients diagnosed with glomerulonephritis who had SARS-CoV-2 infection and were followed up at Kocaeli University Faculty of Medicine Hospital Nephrology Outpatient clinics between January 2020 and January 2022.

During the data collection process, all patients who were diagnosed with glomerulonephritis after renal biopsy using the hospital automation system retrospectively and who had COVID-19 were included in the study. In light of the fact that the antiviral medication molnupiravir was effective against SARS-CoV-2 and was available in our country from 2022 onwards, none of the patients received such treatment. However, all inpatients were administered low molecular weight heparin and oral or intravenous prednol treatment.

Patient age, sex, comorbidities, laboratory values (hemoglobin, hematocrit, platelet, urea, BUN, creatinine, eGFR, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, electrolyte values, 24-hour urine protein, creatinine, albumin, C-reactive protein, low-density lipoprotein, and uric acid), and pathology results were collected from the hospital electronic data system. Patients with a history of malignancy and pregnant women were excluded from this study. Patients with an average follow-up of two months (1-3mos) after SARS-CoV-2 infection were included in the study. None of the patients received any antiviral treatment for SARS-CoV-2 as there was no effective treatment available at that time.

This study complied with the principles of the Declaration of Helsinki. Ethical approval for the study was obtained from the ethics committee of Kocaeli University Hospital (GOKAEK-2021/170).

Statistical Analysis

Data analysis was performed using IBM SPSS 20.0 program. The Shapiro-Wilk test was used to assess conformity with a normal distribution. Normally distributed variables were expressed as mean standard deviation and non-normally distributed variables as medians. Frequencies were used to represent categorical variables. Differences between the dependent samples were examined using the Wilcoxon signed-rank test. The Mann-Whitney U test was used to determine the differences between the groups. The chi-squared test was used to analyse the relationships between categorical variables. To evaluate the two-way hypotheses, we considered the differences in biochemical parameters to be statistically significant at $p<0.05$.

Results

We retrospectively scanned the data of 36 patients who had SARS-CoV-2 infection with a previous diagnosis of glomerulonephritis and were followed up between January 2020 and January 2022, using the hospital information registry data system and patient files. Seventeen patients were female (47.2%) and 19 were male (52.8%). The mean age of the patients was 43 years (range: 19–68 years). 44% of the patients had been diagnosed with glomerulonephritis for more than five years.

As for the comorbid conditions, 20 (55.5%) patients had comorbidities namely: diabetes mellitus (9/25%), hypertension (16/44.4%), hypothyroidism (4/11.1%), and hyperlipidaemia (8/22.2%).

Regarding the subtypes of glomerulonephritis according to the kidney biopsy results, nine (25%) patients were diagnosed with IgA nephropathy, seven (19.4%) with FSGS, six (16.7%) with membranous nephropathy, four (11.1%) with membranoproliferative glomerulonephritis,

three (8.3%) with minimal change disease, two (5.6%) with chronic glomerulonephritis, two (5.6%) with lupus nephritis, two (5.6%) with mesangioproliferative glomerulonephritis, and one (1%) with tubulointerstitial nephritis 2.8).

The cases were grouped according to the COVID-19 dates: 18 people (50%) had contracted SARS-CoV-2 in 2020 and 18 (50%) in 2021. Twenty-eight of 36 cases (77.8%) recovered simultaneously, while 8 (22.2%) required hospitalization. Thoracic tomography was not necessary in 24 patients (66.6%). Among the 12 patients who underwent the procedure, seven (58.3%) exhibited pneumonic infiltrations, while five (41.6%) did not.

Four people did not receive the COVID-19 vaccine throughout the pandemic and 32 were vaccinated. Of the 32 patients, 19 (59.3%) had received the vaccine prior to being infected with SARS-CoV-2, while 13 (40.6%) had received the vaccine afterwards. Of the eight hospitalized patients, six (75%) did not receive the vaccine. The 24-hour urine albumin and protein levels of people who were hospitalized due to SARS-CoV-2 infection were found to be significantly higher than those of people who were not hospitalized (Table 3).

There was a substantial increase in platelet and albumin levels following SARS-CoV-2 infection (Table 1, Figure 1 and 2). A significant decrease was detected in 24-hour urine creatinine values (Figure 3) and spot urine erythrocyte values ($p < 0.05$). No significant changes were observed in other laboratory values (Tables 1 and 2).

The disease activity spectrum of the patients was as follows: 25 (69.4%) were in remission without taking immunosuppressives, 9 (25%) were in remission with ongoing immunosuppressive therapy, and 2 (5.6%) were not in remission despite immunosuppressive agents. Immunosuppressant agents included corticosteroids (3 patients), cyclosporine (1 patient), corticosteroids+CSI (2 patients), corticosteroids+ tacrolimus (3 patients), corticosteroids+cyclophosphamide (1 patient), and corticosteroids+MMF (1 patient).

Table 1. The change in laboratory values before and after SARS CoV-2 infection

Parameter	Before SARS-CoV-2 infection Median (IQR)	After SARS-CoV-2 infection Median (IQR)	p value
Hemoglobin, g/dL	13.7 (13.1-14.9)	13.8 (12.9-14.9)	0.414
Hematocrit, %	41.2 (38.6-43.8)	40.3 (37.8-43.2)	0.385
Platelet, μ/L	255,000 (216,625-314,500)	272,800 (215,500-314,500)	**0.044**
Urea, mg/dl	30 (24.3-43.7)	30.4 (24.9-45.3)	0.437
BUN, mg/dl	14.7 (11.8-23.6)	14 (11.9-21)	0.120
Creatine, mg/dl	0.82 (0.71-1.16)	0.82 (0.74-1.18)	0.566
GFR, ml/dk	101.5 (69.1-111.2)	95.7 (66.9-114.1)	0.623
AST, U/L	20.3 (16-25)	19.5 (14.2-25.7)	0.329
ALT, U/L	22 (14.2-33.2)	21.4 (13.2-27.7)	0.966
Total protein, g/dl	7.1 (6.7-7.4)	7 (6.7-7.5)	0.622
Albumin, g/dl	4.3 (3.9-4.4)	4.4 (4-4.5)	**0.048**
Sodium, mEq/L	139.9 (138.1-141)	139 (138-140.4)	0.214
CRP, mg/dl	2.4 (1.1-5)	2.1 (1.2-4.5)	0.981
LDL, mg/dl	126 (105-173)	114 (95-144)	0.128
24-hour urine protein mg/day	683 (284.6-2.105.8)	921.3 (320.2-1.737.4)	0.937
24-hour urine albumin	375.9 (56.9-1492.3)	360.7 (70.2-1107.4)	0.789
Spot urine erythrocyte	1.5 (0-7.7)	1 (0-3)	**0.026**

BUN: Blood Urea Nitrogen AST: Aspartate Aminotransaminase ALT: Alanine Transaminase CRP: C reactive protein
IQR: Interquartile range

Table 2. The change in laboratory values before and after SARS CoV-2 infection

Parameter	Before SARS-CoV-2 infection Mean ± SD	After SARS-CoV-2 infection Mean ± SD	p value
Chloride, mEq/L	102.5 ± 3.5	101.8 ± 2.5	0.215
Potassium, mg /dL	4.4 ± 0.4	4.6 ± 0.42	0.086
Phosphorus, mg/dL	3.5 ± 0.6	3.6 ± 0.7	0.262
Uric acid, mg/dL	6.1 ± 1.53	5.8 ± 1.54	0.212

SD: Standard deviation

Table 3. Values before and after SARS-CoV-2 infection of hospitalized patients and out-patients

Parameter 1/2	Hospitalization Median (IQR)	No hospitalization Median (IQR)	p* value
Urea 1, mg/dl	36.5 (26.8-59.9)	29 (21.6-42.7)	0.168
Urea 2, mg/dl	43.9 (23.8-41.6)	28.4 (29.2-70.7)	**0.044**
Urea difference	3.2 (-4.5-5)	-2.5 (-5.5-3.6)	0.358
p value**	0.674	0.269	-
BUN 1, mg/dl	20.4 (14.5-28.9)	14 (11.1-20)	0.107
BUN 2, mg/dl	20.3 (13.5-32.9)	13.2 (11-19.8)	**0.044**
BUN difference	0.0 (-9.4-2.3)	-1.6 (-3.7-1.5)	0.780
p value**	0.833	0.113	-
K 1, mmol/L	4.6 (3.9-4.9)	4.5 (4.2-4.7)	0.955
K 2, mmol/L	4.8 (4.7-5.2)	4.5 (4.2-4.7)	**0.012**
K difference	0.19 (0.09-0.76)	0.11 (-0.35-0.41)	0.193
p value**	**0.018**	0.487	-
P 1, mg/dl	3.2 (3-3.8)	3.5 (3.2-3.9)	0.339
P 2, mg/dl	3.9 (3.3-4.5)	3.4 (3-4.1)	0.221
P difference	-0.7 (-0.8 – (-0.5))	-0.005 (-0.53-0.5)	**0.015**
p value**	0.123	0.973	-
24-hour urine albumin 1, mg/dl	1703.2 (586.5-3097.6)	199.1 (40.9- 857.8)	**0.006**
24-hour urine albumin 2, mg/dl	1611.4 (685.2-2248.1)	212.8 (56.2-1037.1)	**0.012**
24-hour urine albumin difference	-183.9 (-1109.4-550.2)	16.1 (-38.9-157.3)	0.466
p value**	0.575	0.327	-
24-hour urine protein 1, mg/dl	2398.4 (893.1-4197)	476.8 (213-1399.2)	**0.009**
24-hour urine protein 2, mg/dl	2000.4 (1099.7-2697.7)	485.8 (310.2-1605.4)	**0.044**
24-hour urine protein difference	-215.6 (-2369.4-466.5)	20.1 (-101.9-221.7)	0.489
p value**	0.401	0.569	-

BUN: Blood Urea Nitrogen, K: Potassium, Parameter 1: Before SARS-CoV-2 infection, Parameter 2: After SARS-CoV-2 infection, IQR: Interquartile range

*p value between group analysis, **p value within group analysis

Table 4. Values before and after SARS-CoV-2 infection of hospitalized patients and outpatients

Parameter 1/2	Hospitalization Median (IQR)	No hospitalization Median (IQR)	p* value
P 1, mg/dl	3.2 (3-3.8)	3.5 (3.2-3.9)	0.339
P 2, mg/dl	3.9 (3.3-4.5)	3.4 (3-4.1)	0.221
P difference	-0.7 (-0.8 – (-0.5))	-0.005 (-0.53-0.5)	**0.015**
p value**	0.123	0.973	-
24-hour urine albumin 1, mg/dl	1703.2 (586.5-3097.6)	199.1 (40.9- 857.8)	**0.006**
24-hour urine albumin 2, mg/dl	1611.4 (685.2-2248.1)	212.8 (56.2-1037.1)	**0.012**
24-hour urine albumin difference	-183.9 (-1109.4-550.2)	16.1 (-38.9-157.3)	0.466
p value**	0.575	0.327	-
24-hour urine protein 1, mg/dl	2398.4 (893.1-4197)	476.8 (213-1399.2)	**0.009**
24-hour urine protein 2, mg/dl	2000.4 (1099.7-2697.7)	485.8 (310.2-1605.4)	**0.044**
24-hour urine protein difference	-215.6 (-2369.4-466.5)	20.1 (-101.9-221.7)	0.489
p value**	0.401	0.569	-

P: Phosphorus, Parameter 1: Before SARS-CoV-2 infection, Parameter 2: After SARS-CoV-2 infection, IQR: Interquartile range

*p value between group analysis, **p value within group analysis

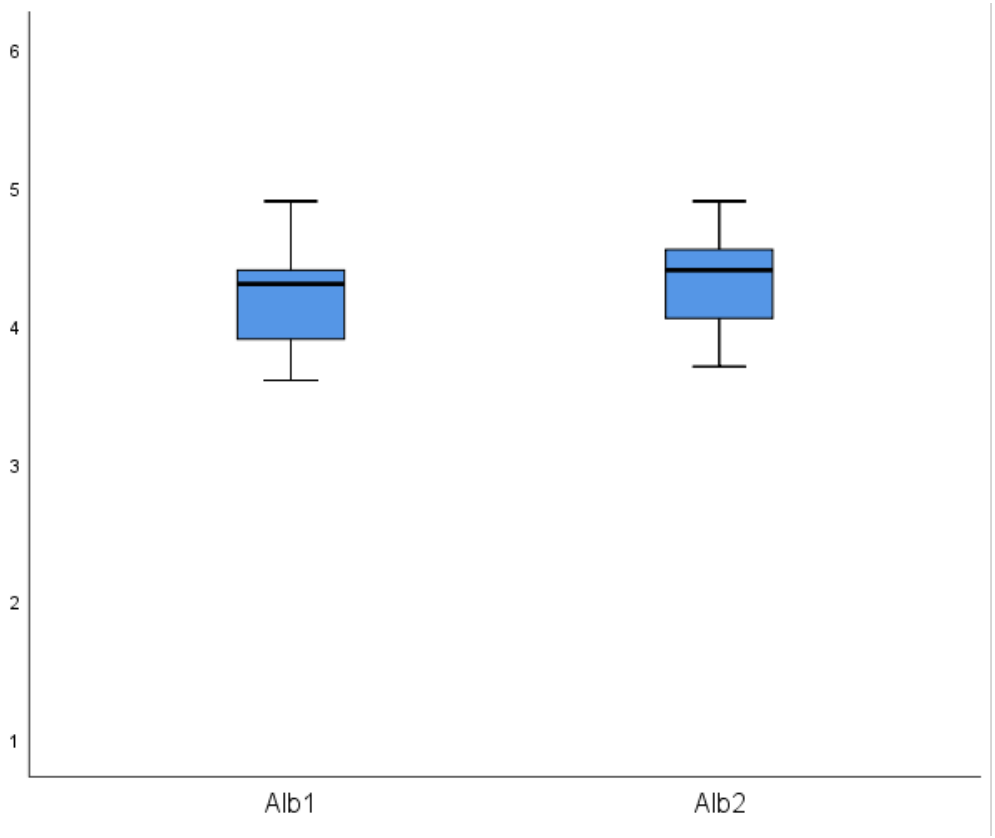

Figure 1. Change in serum albumin levels after COVID-19 infection

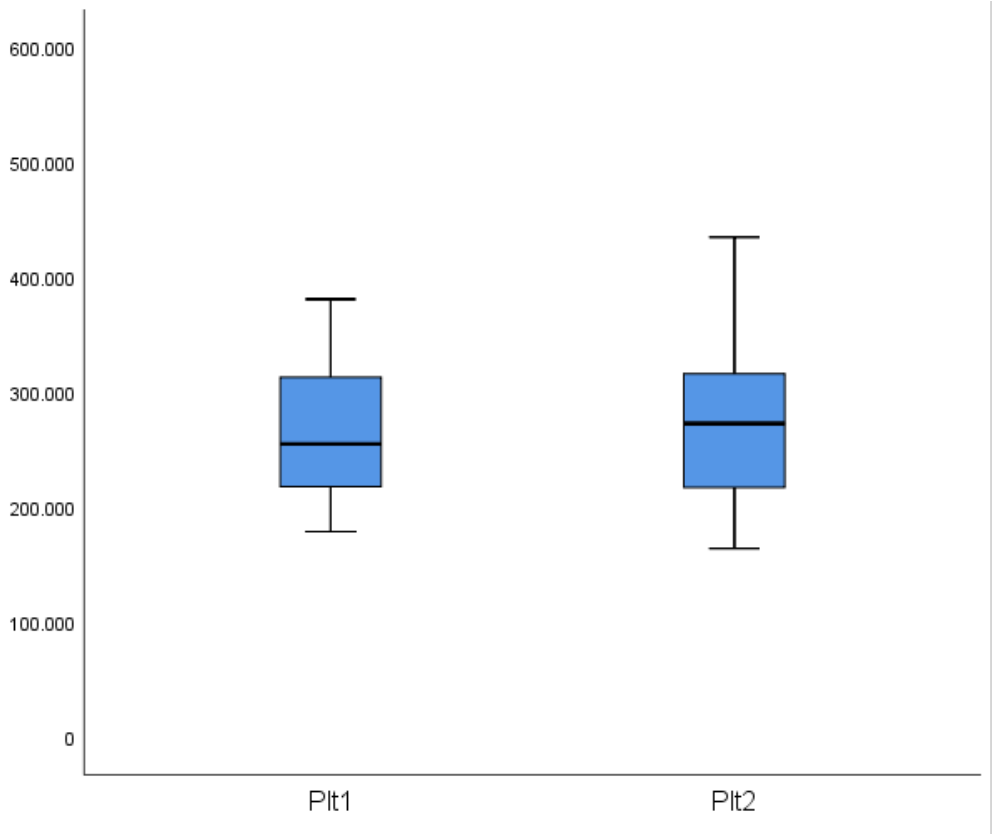

Figure 2. Changes in thrombocyte number after COVID- 19 infection

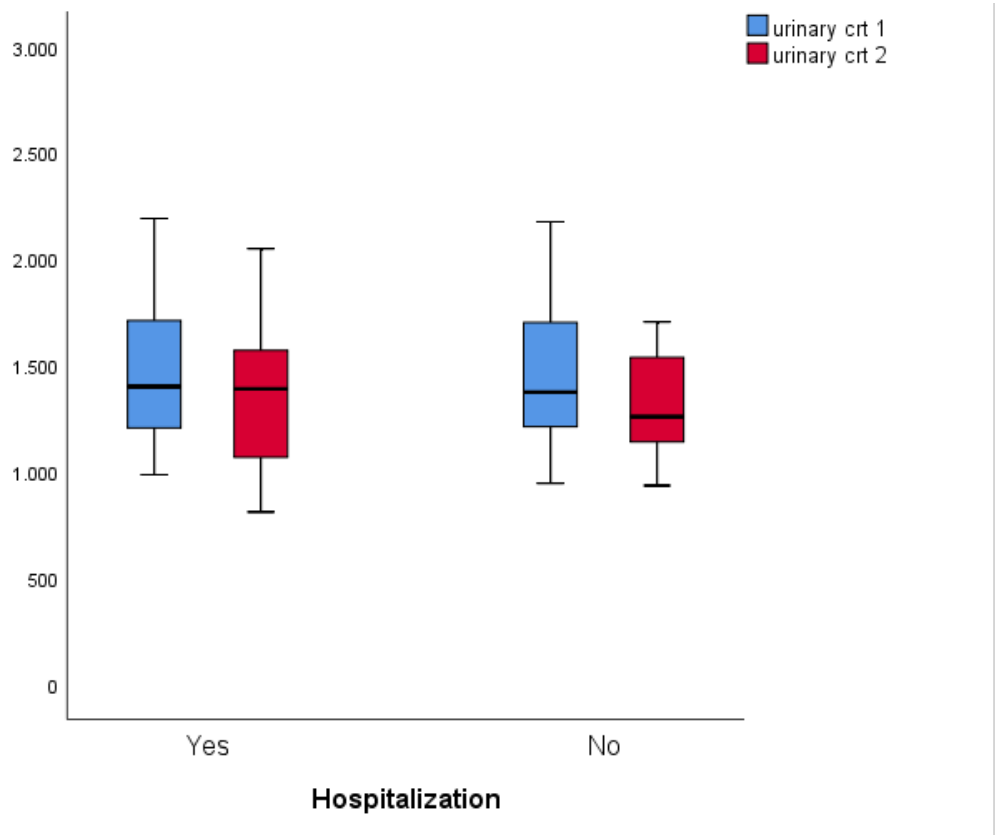

Figure 3. Decrease in 24-hour urine creatinine secretion after COVID-19 infection in hospitalized and non-hospitalized patients

Discussion

Unlike other studies on COVID-19 and glomerular diseases, our study focused on a younger population.[7] Studies have shown that kidney cells secrete proteins that can induce SARS-CoV-2 infection. However, how often kidney cells are infected and whether this infection contributes to kidney disease are not clearly delineated.[8] A study on kidney biopsy results in patients infected with SARS-CoV-2 showed that although acute tubular damage was more common, both glomerular and tubular damage developed.[7] Another study examining postmortem biopsy pathologies showed that glomeruli were preserved in most kidneys and that AKI occurring in most of the deceased was associated with acute tubular damage.[9]

In our study, no significant differences were observed in the proteinuria values of the patients diagnosed with glomerulonephritis. A study has shown that ACE 2, the functional receptor of SARS-CoV-2 infection, is highly expressed in tubules rather than glomeruli in the kidney. Accordingly, the main effect of SARS-CoV-2 infection on the kidney is thought to be tubular damage. The observation that the tubulointerstitial infiltration by inflammatory cells like macrophages during acute tubular damage while the glomeruli are usually preserved confirms this "***primarily tubular***" hypothesis.[10] This might also explain why a significant change in the magnitude of proteinuria in our patients did not occur following SARS-CoV-2 infection. Additionally, the patients included in the study had SARS-CoV-2 infection without hospitalization or serious organ failure. Epidemiologically, they were infected with SARS-CoV-2 in the second wave of the pandemic, during which milder cases were more prevalent than in the initial days of the pandemic. This may have contributed to the more favourable clinical presentation of the patients.

Philipp et al. demonstrated that out of 59 individuals with a history of immune-mediated glomerular illness and SARS-CoV-2 infection, 16.9% experienced acute kidney injury.[11] In our study, we compared pre- and post-COVID serum creatinine levels in our patients and observed no significant differences. Likewise, the average pre/post-COVID eGFR values were not significantly different, despite a slight increase following SARS-CoV-2 infection. It is important to emphasize that retrospectively assessing the values in our study, without considering them individually, may lead to a misleading understanding of AKI.

Although many studies have mainly reported tubular involvement with SARS-CoV-2 infection, it has also been shown that AKI may develop secondary to glomerular involvement with heavy proteinuria and podocyte damage.[12] It has been shown that advanced age, obesity, hypertension and diabetes are among the risk factors for AKI to develop. Most studies in the literature included hospitalized COVID-19 patients with a relatively higher mean age.[13] The reason for the insignificant correlation between creatinine level and eGFR changes in our study was attributed to the younger age of the patients and the mild nature of the infection without hospitalization.

In a study by Wang et al., the risk of worsening glomerulonephritis after COVID-19 was found to be 35%. It has also been shown that patients diagnosed with glomerulonephritis and vaccinated with the COVID-19 vaccine had lower rates of worsening. In our study, we discovered that the vaccination rate among hospitalized patients was relatively low at 75%, and there was no noticeable protective effect associated with vaccines. The reason behind this variation is believed to be due to the fact that our study's cases primarily experienced the infection during the second wave. Studies in the literature have shown that the development of AKI is less common in patients treated in the intensive care unit in the second wave of COVID-19 compared with first-wave patients.[14]

In our study, the mean platelet count following SARS CoV2 infection significantly increased compared with that before infection. However, studies reporting the opposite do exist in hospitalized COVID-19 patients with thrombocytopenia and an associated suboptimal prognosis.[14] We believe that the reason for the increase in the platelet count in our patients might be the homeostatic response to platelet consumption. There is still uncertainty and no consensus regarding the normal platelet count in hospitalized COVID-19 patients.[15] The hemoglobin levels in our patients before and after COVID-19 infection were not different.

In our study, no significant differences were detected in AST, ALT, and total protein levels after SARS-CoV-2 infection compared to pre-infection levels. A previous study has shown increased AST and ALT levels, indicating liver damage to the level of 1-2 times the upper limit of normal in the acute phase of SARS-CoV-2 infection. A COVID-19 study found that older people with liver disease, alcohol addiction, and obesity were more likely to suffer from liver damage. The prognosis for COVID-19 patients with liver damage was generally good, and in 95.6% of patients, liver function tests returned to normal within two months of discharge,[16] which might be the reason why no statistically significant difference was observed in transaminase levels after the infection period in our study. Furthermore, none of the patients in the present study developed serious organ failure. Therefore, serum albumin levels were higher after the COVID-19 infection. This finding could be incidental and does not correlate with the findings of previous studies. Previous studies have demonstrated that low serum albumin levels are associated with disease severity, despite the discrepancies in albumin levels among study populations based on factors such as age, inflammation status, sex, and geographical location.[17] This finding could be attributed to the fact that our patients remained relatively healthy when compared with hospitalized and more severely ill patients.

Studies have shown that non-COVID-19 viral infections play a role in the recurrence of nephrotic syndrome and IgA nephropathy in pediatric patients.[17] Although the mechanism underlying disease exacerbation is not fully understood, a dysregulated immune response to infection has been reported. It has been shown that none of the patients in these reports developed serious disease, and all responded to glucocorticoid therapy without serious complications. In contrast, some studies have suggested that lymphopenia caused by SARS-CoV-2 may have a protective effect against the progression of glomerular diseases such as drugs that suppress the immune system. Studies conducted with glucocorticoids have shown that dexamethasone reduces mortality in ARDS due to SARS-CoV-2, but has no positive or negative effect on moderately severe infection.[18] In a single-center study, cyclosporine use reduced mortality. The use of immunosuppressive agents such as rituximab, cyclophosphamide, and mycophenolate has been shown to result in worse outcomes in patients with COVID-19.[19]

No significant difference was observed in LDL levels before and after SARS-CoV-2 infection in patients with glomerulonephritis. Researchers have demonstrated that dyslipidemia, especially when accompanied by obesity, exacerbates the severity and progression of COVID-19.[19] However, there are no studies in the literature on the effects of SARS-CoV-2 infection on LDL levels.

The limitations of this study were the short follow-up period for consequences, limited number and younger age range of patients, and exclusion of more serious forms of COVID-19 infection.

Many patients developed SARS-CoV-2 infections during the second wave of the COVID-19 pandemic. Most patients recover from SARS-CoV-2 infection without hospitalization or ARDS development. Most hospitalized patients were from a group of patients who had not been vaccinated with the COVID-19 vaccine. Considering proteinuria and eGFR values after SARS-CoV-2 infection, there was no change in the disease activity of primary glomerulonephritis.

Ethical Approval

This study complied with the principles of the Declaration of Helsinki. Ethical approval for the study was obtained from the ethics committee of Kocaeli University Hospital (GOKAEK-2021/170).

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

BD, OG, ME, SGB: Concept-Design; SGB, OG: Supervision; BD, ME: Data Collection and/or Processing; BD, ME, SGB, OG: Analysis and/or Interpretation; SGB, BD, OG: Literature Review; BD, OG, SGB, ME: Writer; SGB, OG: Critical Review

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Research Article / Araştırma Makalesi

MORPHOLOGICAL EVALUTION OF CORPUS CALLOSUM ATROPHY OVER TIME IN RELAPSING REMITTING MULTIPLE SCLEROSIS

RELAPSING REMITTING MULTIPLE SKLEROZDA KORPUS KALLOZUM ATROFİSİNİN ZAMAN İÇİNDE MORFOLOJİK DEĞERLENDİRMESİ

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ABSTRACT

Objective: Multiple sclerosis (MS) is a chronic central nervous system (CNS) disease that generally affects young adults and is marked by inflammation, demyelination, and neurodegeneration. Magnetic resonance imaging (MRI) is widely used diagnosis tool for relapsing remitting MS (RRMS). Corpus callosum (CC), the largest commissural tract in brain which is associated with both cognitive and physical impairment by atrophy in MS. Our study aimed to evaluate CC in RRMS patients using MR images and compare it to measurements from healthy controls within the same age

Methods: We manually measured changes in CC thickness in T1 brain MR images of RRMS patients in 2017, 2019, and 2022.

Results: Our results showed that control group had greater thickness, length, and index values in all CC sections compared to patient group. Additionally, a significant difference was observed in thickness of genu and splenium sections and CC index between patient and control groups. However, no significant difference was detected in truncus part of CC or overall CC length. CC measurements in patient group decreased over time, with 1st MRI showing greater values than 2nd and 3rd MRI scans. Furthermore, there was a statistically significant difference in thickness of truncus part of CC and volume values of subcortical areas between 2nd-3rd and 1st-3rd MRI measurements.

Conclusion: As a result of these findings, our study provides important information about changes in CC measurements for MS patients.

Keywords: Atrophy, Corpus Callosum, Magnetic Resonance Imaging, Relapsing Remitting Multiple Sclerosis

ÖZ

Amaç: Multiple skleroz (MS), genellikle genç yetişkinleri etkileyen ve inflamasyon, demiyelinizasyon ve nörodejenerasyon ile kendini gösteren kronik bir merkezi sinir sistemi (MSS) hastalığıdır. Manyetik rezonans görüntüleme (MRG), relapsing remitting MS (RRMS) için yaygın olarak kullanılan bir tanı aracıdır. Beyindeki en büyük komissural kanal olan korpus kallozum (KK) MS'te atrofiye uğrayarak hem bilişsel hem de fiziksel bozulma ile ilişkilendirilmektedir. Çalışmamızın amacı, MR görüntüleri kullanarak RRMS hastalarında KK'yi değerlendirmek ve aynı yaştaki sağlıklı kontrollerden elde edilen ölçümlerle karşılaştırmaktır.

Yöntem: RRMS hastalarının 2017, 2019 ve 2022 yıllarındaki T1 beyin MR görüntülerinde KK kalınlığındaki değişiklikleri manuel olarak ölçtük.

Bulgular: Sonuçlarımız, kontrol grubunun hasta grubuna kıyasla tüm KK bölümlerinde daha fazla kalınlık, uzunluk ve indeks değerlerine sahip olduğunu gösterdi. Ayrıca, genu ve splenium bölümlerinin kalınlığında ve KK indeksinde hasta ve kontrol grupları arasında anlamlı bir fark gözlendi. Ancak, KK'nin trunkus kısmında veya toplam KK uzunluğunda anlamlı bir fark saptanmadı. Hasta grubundaki CC ölçümleri zaman içinde azaldı ve 1. MRG, 2. ve 3. MRG taramalarından daha yüksek değerler gösterdi. Ayrıca, KK'nin trunkus kısmının kalınlığında ve subkortikal alanların hacim değerlerinde 2.-3. ve 1.-3. MRG ölçümleri arasında istatistiksel olarak anlamlı bir fark vardı.

Sonuç: Elde ettiğimiz bulgular neticesinde araştırmamız MS hastaları için KK ölçümlerindeki değişiklikler hakkında önemli bilgiler sunmaktadır.

Anahtar Kelimeler: Atrofi, Korpus Kallozum, Manyetik Rezonans Görüntüleme, Relapsing Remitting Multiple Skleroz

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Introduction

Multiple Sclerosis (MS) is a chronic, inflammatory and demyelinating disease of the central nervous system (CNS) that usually affects young adults.[1] In MS, demyelination occurs in the cortex particularly in the white matter of the CNS, leading to symptoms caused by chronic inflammation.[2,3] The evaluation of multiple sclerosis (MS) involves the use of four primary spectrums, including relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS).[4] RRMS is the most pre-valent subtype of MS, accounting for 85% of cases.[5] RRMS is a subtype of MS that is characterized by complete or sequential recovery after relapses (attacks) associated with transient neurological dysfunctions.[6]

Magnetic resonance imaging (MRI) is a vital tool for diagnosing MS with identifying lesion location and morphology, as well as assessing disease progression and response to treatment. In order to diagnose, monitor, and treat MS, MRI protocols typically include T2, FLAIR, and T1-weighted imaging with contrast. Axial and sagittal slices need be obtained, and it is recommended to use a 1.5 Tesla or 3 Tesla MRI system.[7] The 2017 McDonald diagnostic criteria have increased the importance of para-clinical evaluations, particularly imaging, in the diagnosis and management of MS.[8]

The corpus callosum (CC), which is the largest commissural tract comprised of thick myelinated fibers, connects the corresponding centers in the right and left cerebral hemispheres. The CC has been found to be the brain region that undergoes the most atrophy in multiple sclerosis (MS), and this atrophy has been linked to both cognitive and physical impairment.[9] Furthermore, the atrophy of the CC has been shown to predict cognitive outcomes in MS, making it a valuable biomarker for monitoring the disease.[10]

Our primary objective is to evaluate the CC from magnetic resonance (MR) images of individuals diagnosed with RRMS and compare it to the measurement values obtained from MR images of healthy control subjects within the same age range. Additionally, our study aims to uncover the changes in corpus callosum thickness measurements over time in MR images of RRMS patients in three different years between 2017 and 2022, and to determine the proportionate effect on affected parameters. Moreover, we aim to establish a volumetric data set for monitoring MS patients and provide a preliminary basis for future studies. The primary target of our investigation is to underscore the changes that occur in the corpus callosum over time, in order to monitor the prognosis of MS disease and identify the causes of clinical symptoms.

Methods

Experimental design

Our research was approved by the Kocaeli University Non-Interventional Clinical Research Ethics Committee, which granted approval number GOKAEK-2022/19.13 and project number 2022/321. The research was executed at Kocaeli University Training and Research Hospital, Department of Radiology and Department of Neurology. Our retrospective investigation was carried out using data from patients who were treated in the Department of Neurology, diagnosed with RRMS, and undergone follow-up. The study included patients with RRMS aged between 20-40 years and healthy control subjects in the same age range who presented to the Department of Neurology with headache complaints. T1 brain MR images taken at Kocaeli University Training and Research Hospital, Department of Radiology, were used to measure corpus callosum thickness and indices of patients diagnosed with RRMS and healthy control subjects. A total of 1317 individuals with T1 brain MRI images who were diagnosed with MS or admitted to the hospital with headache complaints between 2017 and 2022 were retrospectively evaluated. Based on the exclusion criteria, 1217 patients were excluded from the study. We included 50 patients diagnosed with RRMS and 50 healthy control subjects, according to the predetermined inclusion and exclusion criteria.

Participants

The 50 individuals diagnosed with RRMS and the 50 healthy control subjects underwent assessments of CC thicknesses and indices derived from T1 brain MR images. Measurements of volume and thickness were conducted on the T1 brain MR images of the RRMS patient group from 2017, 2019, and 2022. In contrast, the healthy control group's measurements were taken from a single T1 brain MR image. The CC thickness measurements were performed manually. The alterations in thickness observed over time in the MR images from different years were proportional and expressed as percentages. Moreover, immunomodulatory agents were incorporated as a standard component of the drug therapy regimen and routine treatment for all individuals within the RRMS patient group.

Inclusion and exclusion criteria

The criteria for inclusion in our study were as follows: patients who were diagnosed with RRMS at Kocaeli University Training and Research Hospital, Department of Neurology, and underwent T1 brain magnetic resonance imaging (MRI) at three different times between 2017 and 2022 were included in our study. Additionally, patients between ages of 20 and 40 who were diagnosed with RRMS and followed up at XXX University Training and Research Hospital, Department of Neurology, as well as healthy individuals in the same age range who were admitted to Kocaeli University Training and Research Hospital, Department of Neurology with complaint of headache but had no cranial pathology, were included in control group. The criteria for exclusion in our study were as follows: the presence of neoplastic, degenerative, or vascular pathologies that could be mistaken for MS, as well as presence of any cranial pathology in control group,

and individuals who did not have an MRI performed at least three times. These were determined to be necessary conditions for exclusion from our study.

MRI protocol

Measurements of anatomical structures were conducted with utilization of a 3-Tesla MRI scanner (Gyroscan Intera, Philips Medical) in accordance with the imaging protocol, which included sagittal and axial T1-weighted images with a TR/TE of 500/minimum and axial T2-weighted images at 4000/102, with a resolution of 256 x 256 and a slice thickness of 5 mm and a field of view of 220 mm.

Measurement parameters of CC

The thickness of the genu, truncus, splenium, length and index of the CC were measured manually on brain MR images. Genu thickness was measured by transverse width of the genu on line connecting anterior and posterior points of CC in planum midsagittale. Truncus thickness was measured by measuring the maximum thickness of the truncus portion of the CC in the planum midsagittale (Figure 1). Splenium thickness was measured by transverse width of splenium on line connecting anterior and posterior points of CC in planum midsagittale (Figure 1). The length of CC was measured by distance between anterior and posterior most prominent points of CC on planum midsagittale (Figure 1). The index of CC was obtained by summing thickness of genu, corpus, splenium in planum midsagittale and dividing by anteroposterior length of the CC (Figure 2).

Statistical analysis

The analysis of statistical data was carried out using the IBM SPSS 20.0 program (IBM Corp., Armonk, NY, USA). To assess compatibility of obtained data with a normal distribution, Kolmogorov-Smirnov and Shapiro-Wilk tests were employed. Since assumption of normal distribution was not met, numerical variables are presented as median (25th-75th percentile). The categorical variables are presented as frequency (percentage). To determine difference between groups, the Mann-Whitney U test was used, and the Friedman two-way analysis of variance was applied for dependent group comparisons. Multiple comparisons were made using Dunn's test. To examine relationships between categorical variables, chi-square analysis was employed. In hypothesis testing, a $p<0.05$ was considered to indicate statistical significance.

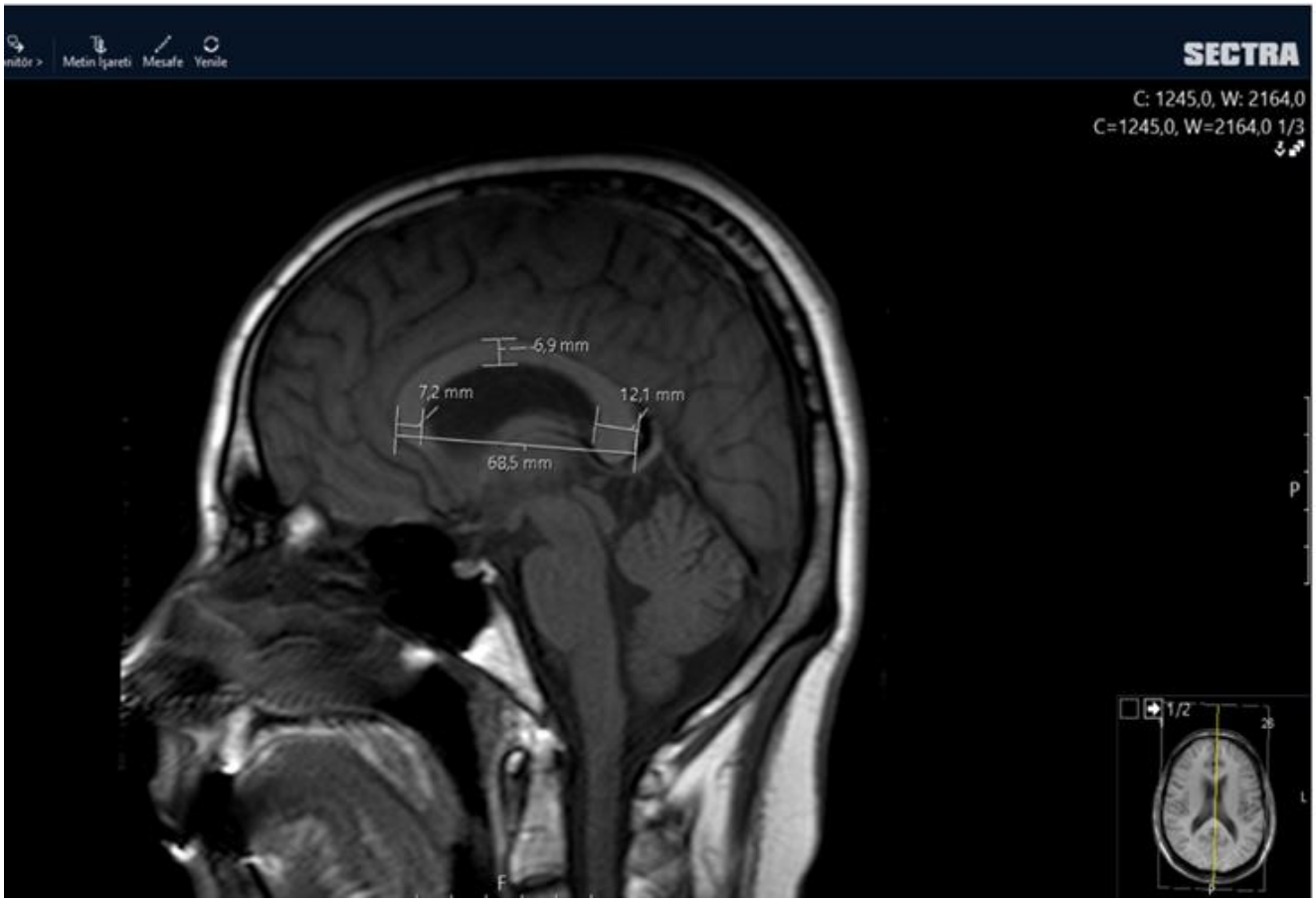

Figure 1. Corpus callosum measurements on midsagittal T1-weighted MRI.

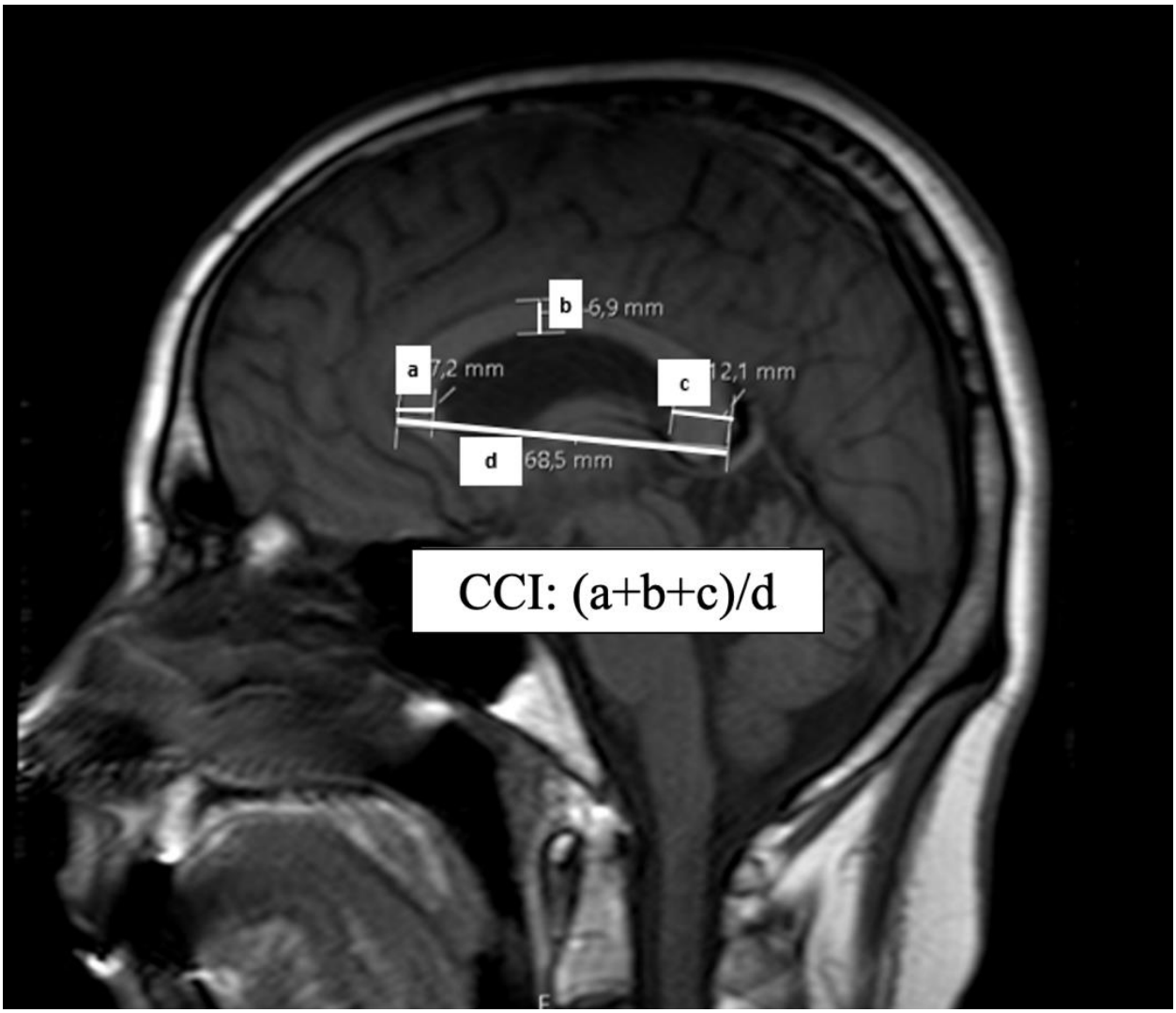

Figure 2. Corpus Callosum Index Measurement (CCI): Corpus Callosum Index, **a.** Genu thickness; **b.** Truncus thickness; **c.** Splenium thickness; **d.** Total anteroposterior length of the corpus callosum.

Results

The study population comprised of 16 male participants (32%) and 34 female participants, while the healthy control group consisted of 18 male participants (36%) and 32 female participants. The median age of the patient group was 33.50 years (25.75-38.00), whereas the median age of the healthy control group was 38.00 years (29.75-40.00).
We revealed that the genu, truncus, and splenium sections of the control group exhibited greater thickness, while the corpus callosum was longer and displayed higher indexes compared to the patient group. Additionally, there was a statistically significant difference was observed in the genu and splenium sections and corpus callosum index, when comparing the thickness measurements of the corpus callosum sections between the individuals in the patient and control groups ($p<0.05$). However, no significant difference was detected in the truncus section of the corpus callosum or the corpus callosum length ($p>0.05$) (Table 1).

Table 1. Comparison of Thickness Measurements of Corpus Callosum Sections

	Control Median (25^{th}-75^{th})	Patient Median (25^{th}-75^{th})	P value
Corpus Callosum			
Genu (mm)	9.15 (8.02-10.04)	8.45 (7.77-9.30)	**0.043**
Truncus (mm)	5.90 (5.35-6.72)	5.65 (4.97-6.45)	0.173
Splenium (mm)	10.02 (9.27-10.82)	8.55 (7.47-9.62)	**<0.001**
Length (mm)	67.00 (63.70-70.40)	66.75 (63.47-69.92)	0.677
Indexes	0.38 (0.32-0.42)	0.34 (0.30-0.37)	**0.004**

*The corpus callosum thickness measurement unit in the table was mm.

The corpus callosum measurements of the patient group were evaluated in terms of time-dependent changes. The thickness measurements, length, and index values of the genu, truncus, and splenium sections of the corpus callosum were measured in the 1st-2nd MRI, 2nd-3rd MRI, and 1st-3rd MRI pairwise comparisons, and these comparisons showed a decrease in percentage values (Table 2).

In the assessment of corpus callosum thickness measurements in the patient group across the 1st, 2nd, and 3rd MRI images, it was determined that the genu, truncus, splenium, length, and index values in the 1st MRI were greater than those in the 2nd and 3rd MRI. Furthermore, the values for the truncus, splenium, length, and index in the 2nd MRI were also greater than those in the 3rd MRI. When comparing the values between the 1st MRI and 3rd MRI, all values, except for the genu section, were higher in the 1st MRI. In the evaluation of the MRIs of individuals in the patient group at three different time points, a statistically significant difference was observed in the volume values of the subcortical areas in the 2nd-3rd and 1st-3rd MRI measurements. Additionally, a statistically significant difference was observed in the thickness measurement of the truncus section of the corpus callosum ($p<0.05$) (Table 3).

Table 2. Percentage Changes in Corpus Callosum Measurements of the Patient Group

	1st-2nd MRI Percentage Alteration Median (25th-75th)	2nd-3rd MRI Percentage Alteration Median (25th-75th)	1st-3rd MRI Percentage Alteration Median (25th-75th)
Corpus Callosum			
Genu (mm)	-0.47 (-9.84-7.76)	-2.25 (-10.85-11.85)	0.00 (-12.90-16.57)
Truncus (mm)	-4.81 (-14.77-4.97)	-2.51 (-12.11-14.83)	-4.17 (-15.20-8.02)
Splenium (mm)	-4.58 (-13.21-9.24)	-1.55 (-10.26-12.69)	-4.83 (-16.72-17.97)
Length	-0.41 (-3.46-2.51)	-0.80 (-3.42-3.14)	-0.87 (-5.00-4.57)
Indexes	-2.85 (-8.89-2.70)	0.00 (-8.70-6.61)	-3.33 (-14.73-9.61)

* The corpus callosum thickness measurement unit in the table was mm.

Table 3. Multiple Comparison of Measurement Parameters of the Patient Group

	Measurement Parameters			
	1st MRI	**2nd MRI**	**3rd MRI**	**P value**
CC Genu (mm)	8.45 (7.77-9.30)	8.40 (7.40-9.22)	8.55 (7.00- 9.92)	0.618
CC Truncus (mm)	5.65 (4.97-6.45)[a]	5.25 (4.65- 6.02)[a]	5.15 (4.60- 6.02)[b]	**0.016**
CC Splenium (mm)	8.55 (7.47-9.62)	8.40 (7.10-9.72)	8.20 (6.82- 9.52)	0.288
CC Length (mm)	66.75 (63.47-69.92)	66.50 (63.35-69.57)	65.95 (62.25-69.25)	0.379
CC Index	0.34 (0.30-0.37)	0.33 (0.30-0.36)	0.32 (0.29-0.38)	0.112

*The unit for multiple comparison of measurement of the RRMS patients in the tables was mm.
**Different letters (a or b) represent statistical significance between measurements ($p<0.05$).

Discussion

Multiple Sclerosis (MS) is a severe, autoimmune, and demyelinating disease that affects the CNS, primarily occurring in adults aged 20-40 years.[11] Our study focused on patients with RRMS, the most prevalent clinical form of MS, and investigated atrophic changes in CC parts over time. Investigating brain structures in MS patients provides essential information about disease progression and treatment, as well as insights into changes in the disease process. Several studies have explored these alterations.[12] Brain atrophy, a common consequence of MS, has been linked to myelin sheath damage, progressive axonal degeneration, and functional disorders of the nervous system.[13] Notably, brain atrophy in MS is five times more prevalent than that associated with normal aging.[14] In MS, gray matter damage could occur early on and result in irreversible disability and cognitive impair-ment.[15]

CC is a crucial pathway made up of largely myelinated fibers that connect the left and right brain hemispheres, enabling the sharing of information between them.[16] MS patients typically exhibit CC atrophy, which is one of the disease's most widely recognized symptoms. In MS, the CC is adversely affected by both focal lesions and Wallerian degeneration.[17] It is worth noting that CC atrophy increases with the progression of MS, leading to approximately 2% volume loss per year, a rate that is ten times higher than that observed in healthy individuals.[18] It has been reported that CC atrophy mainly affects the genu, posterior part of the truncus, and splenium, and develops in a postero-anterior gradient as the disease progresses.[19] Additionally, it has been noted that approximately 20% of patients with RRMS experience CC atrophy.[19]

Our study revealed a significant difference in the thick-nesses and lengths of the genu, truncus, and splenium, as well as the index values of the CC between individuals in

the patient group and those in the control group (Table 1). Specifically, the genu, splenium, and CC index values showed a statistically significant difference ($p<0.05$) (Table 1). The low CC index values indicated atrophy of the CC, and our results demonstrated that this atrophy was more pronounced in the genu and splenium regions. Previous studies have established that atrophy in these regions is associated with cognitive dysfunction.20 Additionally, CC atrophy in MS is known to cause decreased cognitive function, attention deficits, decreased information processing speed, dysfunction in visual memory, and speech fluency. In our study, we found a significant decrease in the time-dependent percentage decrease in CC as %-median in the truncus section, with values of -2.51 (-12.11-14.83) in the 2nd-3rd MRI and -4.17 (-15.20-8.02) in the 1st-3rd MRI (Table 2). We thought that the fact that time-dependent atrophy was more pronounced in the truncus section of the CC compared to other sections may be due to the fact that the truncus section was more sensitive to atrophy and affected more. We analyzed the MRI values of corpus callosum measurements obtained at 3 different times and found that atrophy occurred in all values over time except the 3rd MRI of the genu section (Table 3). It is considered that the increase in the genu section could be due to the triggering of the remyelinization mechanism by symptomatic treatments as reported in the literature.21 In our analysis of the multiple comparisons of CC measurements in MRI obtained at three different times in the patient group, we found a statistically significant difference in the truncus section of the 2nd-3rd MRI and 1st-3rd MRI CC ($p<0.05$) (Table III). Although there was a decrease in other CC measurement values over time, this decrease was not statistically significant ($p>0.05$) (Table 3).

Our study had notable limitations. First, small sample size resulted from exclusion of patients with cranial pathology or demyelinating symptoms, as well as those with neoplastic, degenerative, or vascular conditions that could be mistaken for MS. Consequently, the factors that could be compared were limited. Second, the participants' information was obtained from the hospital registration system, which has a retrospective nature and prevented us from conducting a cognitive assessment. Lastly, the study included patients with RRMS in the patient group and individuals without any cranial pathology in control group. The consequences of excluding any diseases that are not part of the hospital records could impact the outcomes.

Our investigation has contributed to the understanding of the impact of RRMS on CC. The novel aspect of our research was the calculation of the percentage values of atrophy in these structures over time, achieved by proportioning the MRI values to each other. We believe this approach adds to the existing literature on this subject, and the measurement of affected parameters expressed as percentage values could serve as a data set for monitoring MS patients and preparing for future studies.

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Conflict of Interest
The authors declared no potential conflict of interest.

Author contributions
Study idea/Hypothesis: B.K., A.T.O.; Data preparation: B.K., A.T.O., S.D.B., Ö.Ç.; Analysis: B.K., A.T.O., S.D.B., H.E., Ö.Ç, S.B.; Literature review: B.K., A.T.O., H.E., T.Ç.; Manuscript writing: B.K., A.T.O.; Critical Review: B.K., A.T.O., T.Ç.

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Research Article | Araştırma Makalesi

KERARING IMPLANTATION WITH FEMTOSECOND LASER IN KERATOCONUS TREATMENT AT DIFFERENT STAGES

KERATOKONUS TEDAVİSİNİN FARKLI EVRELERİNDE FEMTOSANİYE LAZERLE KERARING İMPLANTASYONU

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ABSTRACT

Objective: To investigate the reliability and effectiveness of femtosecond laser-assisted KeraRing (Mediphacos, Belo Horizonte, Brazil) implantation in treating keratoconus.

Methods: Intrastromal corneal ring segments (KeraRing, Mediphacos, Brazil) were implanted in 15 eyes of 14 patients unable to tolerate contact lenses. Femtosecond laser (Intralase, 60 Hz) was used for corneal tunnel creation. Based on the distribution of the ecstatic area on the cornea, dual segments were implanted in 9 eyes, while single segments were implanted in 6 eyes. Preoperative and postoperative uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), objective and subjective refraction, and topographic corneal curvature (K) values of the cases were compared using the Wilcoxon test.

Results: The median age of patients was 26 (range: 15-39) years, with a median postoperative follow-up period of 12 (range: 1-24 months). BCVA improved in all eyes during the follow-up period, increasing from a median of 0.4 (range: 0.15-0.6) to 0.5 (range: 0.15-1) ($p<0.001$). BCVA increased in 12 eyes, with a median of 0.1 (range: 0-0.4) ($p=0.003$), remained unchanged in 2 eyes, and decreased by 1 line in 1 eye. Subjective spherical refraction decreased from -3.02±3.8 to -1.43±2.7, and subjective cylindrical refraction decreased from -4.2±1.8 to -1.03±1.1 ($p<0.005$). Mean topographic astigmatism decreased from -5.03±2.0 D to -3.27±2.35 D ($p=0.012$), and the mean K value decreased from 52.6±4.7 D to 50.35±4.4 D ($p<0.005$). During the postoperative period, no complications were observed except for a slight migration of ring segments within the tunnel in one case.

Conclusion: Femtosecond laser-assisted intracorneal ring implantation is an effective and reliable method for visual outcomes in keratoconus.

Keywords: KeraRing, femtosecond laser, keratoconus

ÖZ

Amaç: Femtosaniye lazer destekli KeraRing (Mediphacos, Belo Horizonte, Brezilya) implantasyonunun keratokonus tedavisinde güvenilirliğini ve etkinliğini araştırmak.

Yöntem: Kontakt lens kullanamayan 14 hastanın 15 gözüne intrastromal korneal halka segmentleri (KeraRing, Mediphacos, Brezilya) implante edildi. Korneal tünel oluşturulması için femtosaniye lazer (Intralase, 60 Hz) kullanıldı. Korneadaki ektatik alanın dağılımına göre, 9 göze çift segment, 6 göze tek segment implante edildi. Olguların preoperatif ve postoperatif düzeltilmemiş görme keskinliği (UCVA), en iyi düzeltilmiş görme keskinliği (BCVA), objektif ve subjektif refraksiyon ile topografik korneal eğrilik (K) değerleri Wilcoxon testi kullanılarak karşılaştırıldı.

Bulgular: Hastaların ortalama yaşı 25±7.46 (aralık: 15-39) yıl olup, ortalama postoperatif takip süresi 10.8±7.37 (aralık: 1-24 ay) aydır. BCVA, takip süresi boyunca tüm gözlerde artmış, ortalama 0.12±0.1'den 0.38±0.24'e yükselmiştir ($p<0.001$). BCVA, 12 gözde ortalama 0.40±0.15'ten 0.55±0.23'e yükselmiş ($p=0.003$), 2 gözde değişmemiş ve 1 gözde 1 satır azalmıştır. Subjektif sferik refraksiyon -3.02±3.8'den -1.43±2.7'ye, subjektif silindirik refraksiyon ise -4.2±1.8'den -1.03±1.1'e düşmüştür ($p<0.005$). Ortalama topografik astigmatizma -5.03±2.0 D'den -3.27±2.35 D'ye ($p=0.012$) ve ortalama K değeri 52.6±4.7 D'den 50.35±4.4 D'ye düşmüştür ($p<0.005$). Postoperatif dönemde, bir vakada tünel içinde halka segmentlerinin hafif migrasyonu dışında komplikasyon gözlenmemiştir.

Sonuç: Femtosaniye lazer destekli intrakorneal halka implantasyonu, keratokonusta görsel sonuçlar açısından etkili ve güvenilir bir yöntemdir.

Anahtar Kelimeler: Keraring, femtosaniye lazer, keratokonus

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Introduction

Keratoconus is characterized as a progressive, asymmetric, bilateral corneal ectasia resulting from non-inflammatory thinning of the corneal stroma, leading to a conical shape of the cornea. Corneal thinning can cause a significant reduction in visual acuity due to irregular astigmatism, myopia, and corneal steepening. The visual management of keratoconus generally depends on the disease's severity. In advanced stages, penetrating keratoplasty is required to improve visual acuity due to severe irregular astigmatism, progressive stromal thinning, and apical stromal scar.[1,2] In mild to moderate stages, contact lenses are typically tried as initial treatment, with surgical options becoming necessary for patients intolerant to contact lens wear.[3] Corneal collagen cross-linking is a treatment option that slows the progression of the disease.[4]

Initially described by Barraquer in the mid-1950s for correcting myopia and astigmatism, corneal rings became an effective treatment option for stabilizing keratoconus and other ectasias.[4-6] Intracorneal ring segment implantation flattens the central corneal curvature in transparent corneas with moderate to advanced keratoconus, providing refractive improvement. Its reversible nature and preservation of the central cornea are significant advantages.[7-9] Additionally, it is suggested that progression to keratoplasty be delayed by providing biomechanical support to the ectatic cornea.[8,10] With the advancement of femtosecond laser technology, intracorneal ring implantation has become safer and is described as a minimally invasive technique [9,11] Three main types of intracorneal ring segments, produced from polymethyl methacrylate (PMMA) material and available in different geometric shapes and diameters, include Intacs segments (Addition Technology, CA, USA), Ferrara rings, and the KeraRing (Mediphacos, Belo Horizonte, Brazil) segments used in our study.[6,12]

Methods

Our study retrospectively reviewed the 15 eyes of 14 patients diagnosed with keratoconus. It underwent intracorneal ring segment (KeraRing) implantation by the same surgeon at the Haseki Training and Research Hospital Ophthalmology Clinic. All cases were patients with contact lens intolerance or inability to use contact lenses for various reasons. During the preoperative examination, all patients underwent assessments for uncorrected visual acuity, best corrected visual acuity, subjective and objective refraction, keratometry measurement, computerized corneal topography (Orbscan, Bausch and Lomb, Rochester, NY, USA), biomicroscopy for corneal wound formation and other pathologies, intraocular pressure measurement, and fundus examination. The eligibility criteria for intracorneal ring implantation included transparency of the central cornea, minimum corneal thickness of 400 µm at the site where the ring segment would be placed, absence of other ocular diseases, low visual acuity with glasses, and intolerance to contact lens wear or poor lens fit. Before surgical procedures, the type of ectasia was determined based on the steepest axis of corneal topography for each patient. The recommended segment thickness was determined using the chart provided in nomograms, based on the lowest pachymetry values obtained in the central 6 mm optical zone, and single or double-segment usage was planned. As a pre-tunneling medication, 0.5% proparacaine hydrochloride was instilled, and the periocular area was wiped with povidone-iodine. After the eye was sterilely draped, the central point of Purkinje reflex was marked. A disposable vacuum ring for the femtosecond laser was placed. Tunnel creation was performed using a femtosecond laser (Intra-lase, 60 Hz) at a depth of 80% of the thinnest para-central corneal thickness at a distance of 5-7 mm. The inner diameter of the tunnel was 4.7 mm, and the outer diameter was 5.8 mm. The entry incision was made perpendicular to the axis of topographic astigmatism with a length of 1.3 mm. Intracorneal ring segment implantation was performed under topical anesthesia by the same surgeon one to two hours after tunnel formation. A bandage contact lens was placed on the cornea, and the eye was covered with a bandage. Patients were started on artificial tear drops, nonsteroidal anti-inflammatory drops, and topical antibiotic drops on the day of surgery. After epithelialization of the incision site (within 1-2 days), the contact lens was removed, and topical steroid drops were initiated instead of nonsteroidal anti-inflammatory drops. Topical steroid drops were used thrice a day for 2 weeks and then tapered off gradually over 3 weeks. Topical antibiotic drops were used four times daily for 2 weeks and then discontinued. Artificial tears were used for an average of 3 weeks, depending on the epithelial status of the patients. Postoperative follow-up visits were scheduled for day 1, day 3, day 7, and month 1, and for patients with longer follow-up, at months 3, 6, and 12, and subsequently at 1-year intervals. Uncorrected and corrected visual acuity, objective and subjective refraction, keratometry values, corneal topography, and complications were recorded. Patients with a minimum follow-up period of 1 month were included in the study.

Statistical analyses were performed using SPSS version 13F software (SPSS Inc IBM, Armonk, NY, USA). The normality of variables was assessed using visual and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Preoperative and postoperative data were compared using the Wilcoxon test. P-values less than 0.05 were considered statistically significant.

Results

Of the patients, 8 (57.1%) were male and 6 (42.9%) were female, with a median age of 26 (range: 15-39 years). The median follow-up period was 12 (range: 1-24) months. The minimum follow-up period was 1 month, with only 2

patients having a follow-up of 1 month, 73.3% having at least 6 months, and 53.3% having at least 12 months (Table 1).

Table 1. The preoperative and postoperative ophthalmological findings

Patient No	Age	preop UCVA	postop UCVA	preop BCVA	postop BCVA	preop K ast (D)	postop K ast (D)	preop K min (D)	postop K min (D)	preop K max (D)	postop K max (D)	Follow-up time (month)
1	22	0.05	0.4	0.6	0.7	4.7	1.6	50.5	46.5	56.3	48.1	21
2	39	0.2	0.6	0.5	0.8	4.2	4.5	42.8	41.4	46.9	45.9	12
3	29	0.1	0.4	0.3	0.4	4.9	3.8	60.5	57.2	65.4	61	17
4	26	0.02	0.05	0.4	0.6	8.3	1.2	50.5	49.6	58.7	50.8	4
5	16	0.1	0.4	0.4	0.7	8.1	3.5	46.3	44.25	54.4	47.75	1
6	15	0.05	0.1	0.6	0.5	3.5	1.3	51.5	48.1	55	49.4	24
7	15	0.3	0.9	0.5	1	2.7	1.6	48.6	48	51.3	49.6	12
8	26	0.3	0.7	0.6	0.8	3	2.7	49.6	51	52.6	53.7	20
9	26	0.05	0.15	0.15	0.15	7.3	5.3	57.3	56.3	64.6	61.6	8
10	16	0.2	0.3	0.4	0.5	6.3	1.6	47.9	48.5	54.2	50.1	14
11	31	0.02	0.5	0.5	0.6	1.6	1.3	52.5	50	54.1	51.3	12
12	23	0.1	0.15	0.2	0.2	4	9.7	48.5	47.8	52.5	57.5	7
13	37	0.2	0.5	0.3	0.5	3.9	2.3	46.3	45.7	50.2	48	6
14	26	0.1	0.4	0.3	0.5	6.6	6.1	44.8	43.8	51.4	49.9	3
15	28	0.02	0.1	0.2	0.3	6.3	2.5	52.5	52.6	58.8	55.1	1

*Preop: Preoperative, postop: Postoperative; UCVA: Uncorrected visual acuity; BCVA: Best-corrected visual acuity (with glasses); K ast: Keratometric astigmatism value; K min: Minimum keratometry value; K max: Maximum keratometry value; D: diopter

During biomicroscopic examinations, Vogt's striae were observed in 2 patients, and signs related to atopy were present in 3 patients. Among those with signs of atopy, one patient had pannus in the superior cornea, and the other 2 had lid findings. Intraocular pressure measurements were standard in all patients, and no pathology was detected on fundus examination. For KeraRing implantation, 9 eyes received double segments, and 6 eyes received single segments based on the nomogram provided by the manufacturer and tailored to the ectatic area (Figure 1). Segment thickness ranged from 150 to 300 µm, and the arc length varied from 120° to 160°. Both eyes of one patient underwent KeraRing implantation. The incision site for tunneling was selected as the steepest axis topographically in all cases.

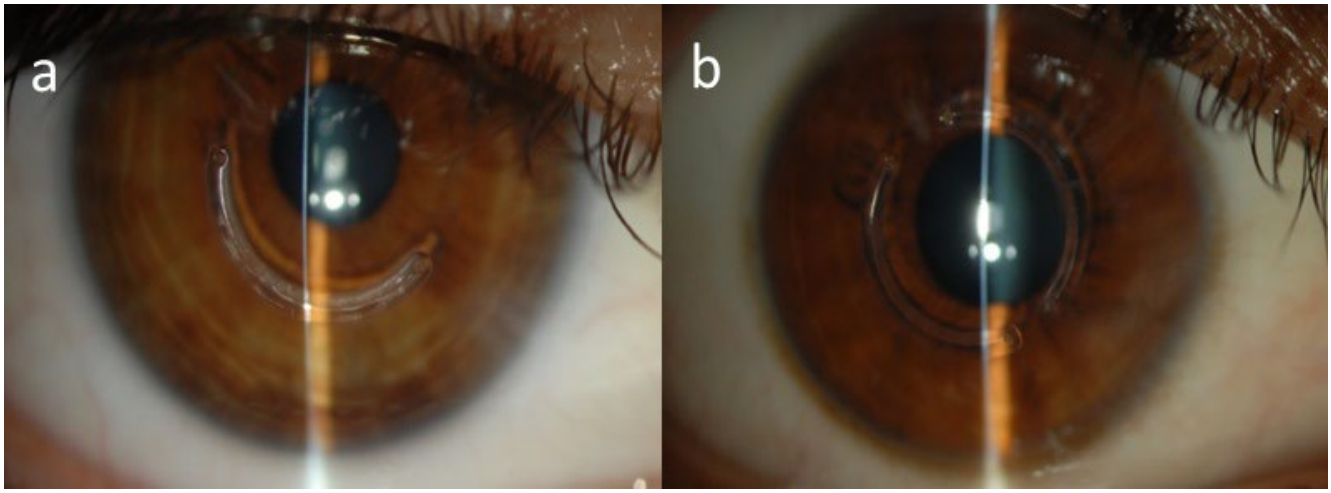

Figure 1. Biomicroscopic view of eyes implanted with a single ring segment (a) (left) and double ring segment (b) (right).

A statistically significant increase was observed in median uncorrected visual acuity (UCVA) (p<0.001). Increased UCVA was noted in all patients, rising from a preoperative median of 0.1 (range: 0.02-0.3) to a postoperative median of 0.4 (range: 0.15-0.6), with an average gain of 2.6 Snellen lines (Figure 2).

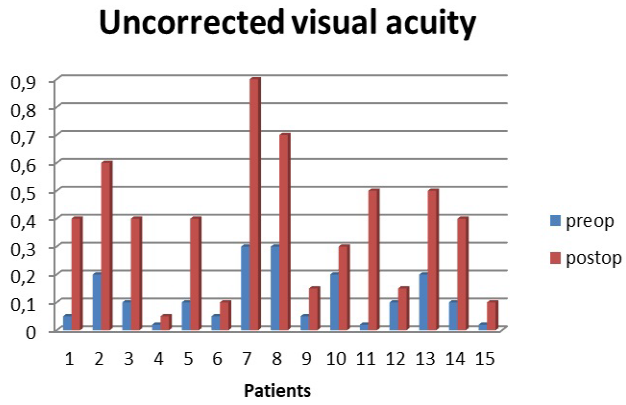

Figure 2. Change in uncorrected VA (visual acuity).

Statistically significant improvement was also observed in best-corrected visual acuity (BCVA) (p=0.003). While BCVA remained stable in two patients, a decrease of 1 Snellen line was observed in one patient. The median BCVA increased from a preoperative value of 0.4 (range: 0.15-0.6) to 0.5 (range: 0.15-1) postoperatively, with an average gain of 1.5 lines (Figure 3).

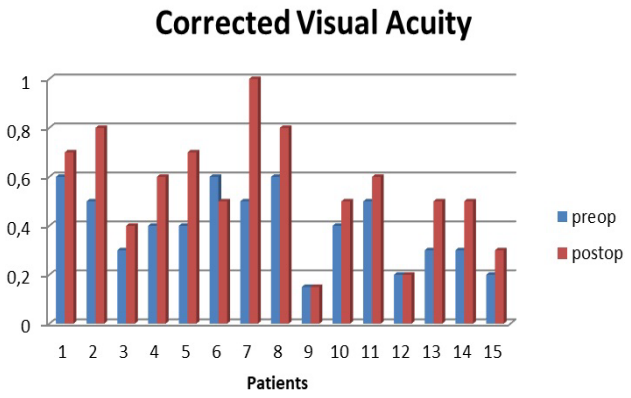

Figure 3. Change in best-corrected VA (visual acuity).

Postoperatively, the mean objective spherical refraction decreased from -4.67±5.4 to -2.88±4 (p=0.234). While it decreased in 10 eyes during follow-up, it remained stable in 2 eyes, and an increase in myopia was observed in 3 patients. The mean subjective spherical refraction decreased from -3.02±3.8 preoperatively to -1.43±2.7 postoperatively (p=0.114). Subjectively, spherical refraction decreased in 6 eyes, increased slightly in 4 eyes, and remained unchanged in 5 eyes. The mean objective cylindrical refraction decreased from -4.53±1.9 to -2.28±1.7 (p=0.002), and the mean subjective cylindrical refraction decreased from -4.20±1.8 to -1.03±1.1 (p=0.001). While objective cylindrical refraction decreased in 10 eyes and increased slightly in 5 eyes, subjective values decreased in all patients.

Following ring implantation, a decrease in minimum, maximum, and median corneal curvature (K value) and corneal flattening were observed (Table 2). The preoperative and postoperative corneal topographies of a patient are shown in Figure 4.

Table 2. The preoperative and postoperative topographic K values.

	preop (D) (median-min-max)	**postop (D) (median-min-max)**	**pre-post (D) (median-min-max)**	**p-value**
Kmax	54.10 (46.90 - 65.40)	51.30 (45.90 - 61.60)	3.0 (-5.0 - 8.2)	p=0,002*
Kmin	49.60 (42.80 - 60.50)	48.10 (41.40 - 57.20)	1.0 (-1.4 - 4.0)	p=0,008*
Kast	4.70 (1.60 - 8.30)	2.50 (1.20 - 9.70)	1.6 (-5.7 - 7.1)	p=0,012*
Kmean	51.10 (44.85 - 62.95)	49.30 (43.65 - 59.10)	1.8 (-2.15 - 6.1)	p=0,004*

*:statistically significant; Preop: Preoperative, postop: Postoperative; K ast: Keratometric astigmatism value; K min: Minimum keratometry value; K max: Maximum keratometry value; D: diopter

No progression was observed in any eye during the follow-up period, and no reoperations were required. One patient, who showed progression in one eye three months after KeraRing implantation and was planning pregnancy, was referred for collagen cross-linking treatment and received cross-linking in both eyes. One patient complained of glare, which spontaneously resolved within a few months. As a complication, a slight migration of ring segments into the tunnel was observed in one patient. The intervention was unnecessary as the migration direction was not towards the incision site.

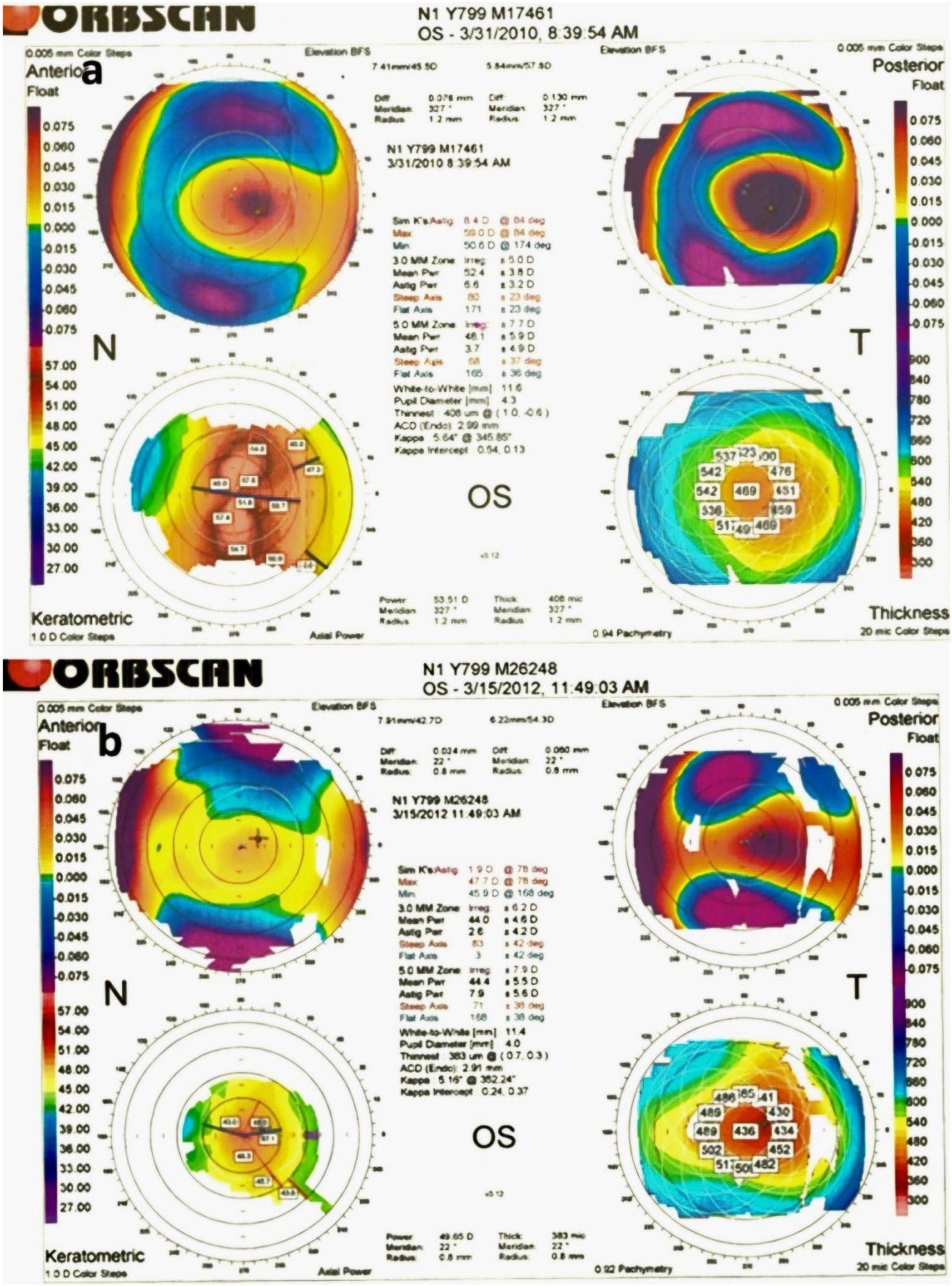

Figure 4. Corneal topography of a patient's preoperative (a) and postoperative (24th month) (b) images.

Discussion

In treating keratoconus, corneal ring implantation is a novel approach mainly applied before the indication for keratoplasty. Colin et al.[13] were the pioneering authors who reported a reduction in astigmatism associated with keratoconus and improved visual acuity using corneal ring (Intacs) segments. KeraRing, developed after Intacs, is a ring segment positioned closer to the optical center of the cornea with various design alternatives. The objective of KeraRing implantation is to reduce refractive errors in patients with contact lens intolerance or those unable to achieve optimal visual acuity with contact lenses/glasses, thereby improving uncorrected visual acuity (UCVA) and corrected distance visual acuity (CDVA) and enabling the use of contact lenses or glasses. Mainly designed for treating corneal ectasias, KeraRing aims to stabilize the cornea and delay the indication for keratoplasty.

Our study also demonstrated improvement in visual acuity, refractive values, and topographic features with KeraRing implantation in patients at different stages of keratoconus. Significant gains were observed with ring implantation in terms of UCVA and CDVA. A more tremendous increase was detected in UCVA compared to CDVA (100% vs. 93.3%). The postoperative mean UCVA was approximately equivalent to the preoperative mean CDVA. The average increase of 2.6 lines in UCVA and 1.5 lines in CDVA persisted without decrement during follow-up. Most patients included in the study experienced increased visual acuity from the first day following KeraRing implantation. The improvement in visual acuity post corneal ring implantation was attributed to corneal flattening and reduction in spherical and astigmatic refractive errors, as evidenced by changes in corneal curvature, topography, and refraction.

Corneal intrastromal rings were initially designed and utilized for the treatment of myopia. Patel et al.[14], in their investigation of the relationship between corneal asphericity and spherical aberrations in myopia correction using corneal intrastromal rings, suggested that the use of wide-diameter and thin rings would have less impact on corneal asphericity and thus would not increase spherical aberrations. According to the authors, a corneal intrastromal ring cannot correct myopia exceeding -4 D without significantly increasing spherical aberrations but may improve outcomes. Subsequently, numerous studies have been published regarding using corneal intrastromal rings for treating keratoconus, yielding successful outcomes. It is well-known that keratoconic corneas are more elastic compared to myopic corneas. Hence, more significant flattening is achieved in keratoconic corneas following ring implantation.

Different perspectives have been reported in the selection of corneal intrastromal ring segments. During their two-year follow-up study, Colin and Malet[15] utilized a standard nomogram, implanting two symmetric Intacs segments in 100 keratoconic patients. Boxer Wachler et al. established a ring segment nomogram based on spherical equivalent. For myopia up to -3.00 D, they implanted a thin segment superiorly and a thicker segment inferiorly. For myopia exceeding -3.00 D, they implanted a thin segment superiorly and a thicker segment inferiorly. This asymmetric ring implantation improved UCVA and CDVA and decreased irregular astigmatism. In both studies, the ring segments were implanted horizontally. Kanellopoulos et al.[16] modified the asymmetric segment placement nomogram, determining segment thicknesses to be implanted superiorly and inferiorly according to five different ranges of myopic values. In their study, the ring center was adjusted to the corneal center, and the position was set between 0.5 and 1.5 mm inferotemporal relative to the corneal geometric center. Colin published a nomogram based on spherical equivalent, corneal localization, and asymmetric astigmatism induced by keratoconus for Intacs segment selection.[13] Swanson, in a study where refractive correction was not specified, reported that in generally 90% of cases, the corneal surface was topographically flattened, curvature was flattened in all cases, and the cone shifted more centrally by placing a thin segment inferiorly and a thick segment superiorly.[17] According to these results, better outcomes were achieved with the asymmetric implantation of two different ring segments, resulting in the uneven flattening of two opposite corneal axes in irregular, asymmetric corneal surfaces and compared to Ferrara's symmetrically implanted corneal intrastromal rings, asymmetric ring implantation yielded more significant improvements in UCVA and CDVA. Conversely, Kwitko and Severo reported significantly better outcomes following symmetric ring implantation in centrally located keratoconic cases.[7] Utilizing KeraRing with different thicknesses and arc lengths asymmetrically allows for a personalized flattening effect on the cornea, thus achieving more effective outcomes. In line with this information, we aimed for effective refractive correction by using segments of different thicknesses and arc lengths based on our study's ecstatic area distribution on the cornea.

Various studies compare mechanical methods with femtosecond laser techniques for creating corneal tunnels. Rabinowitz reported no significant difference in clinical and topographic parameters between the two tunnel creation methods post-ring implantation. [18] Ertan et al.[19], in a retrospective study comparing the two methods, reported better outcomes in uncorrected and corrected visual acuities with tunnels created using the femtosecond laser method. However, this study had limitations that could affect the results: ring implantation was performed using different devices and nomograms at various centers. Finally, Kubaloğlu et al.[11] compared the mechanical method with the femtosecond laser method in a prospective randomized study involving 90 keratoconic patients' 100 eyes implanted with a 160-degree arc length KeraRing segment. They reported no significant difference between the two methods regarding visual and refractive outcomes. However, they observed more intraoperative and postoperative

complications using the mechanical method. Although the mechanical method is less expensive, the femtosecond laser method is reported to be faster, easier, and more comfortable for surgeons and patients. Another advantage of the femtosecond laser is its ability to create tunnels at the desired depth, especially in thin corneas. In our study, we performed tunnel creation using a femtosecond laser (Intralase, 60Hz). Although our sample size was small, the absence of intraoperative complications and the occurrence of only mild migration of ring segments within the tunnel in one patient during the postoperative period demonstrate the reliability of the femtosecond laser method.

Shabayek et al.[5] reported that corrected distance visual acuity (CDVA) was preserved or improved in approximately 95% of patients after KeraRing implantation. Coşkunseven et al. and Kubaloğlu et al. stated this rate as approximately 86% and 95%, respectively.[11,20] Pinero et al.[9] observed a significant increase in CDVA in their series. Alfonso et al.[21] published the results of KeraRing implantation in 219 keratoconic patients and found a substantial rise in CDVA in stage 1 and 2 keratoconus cases. However, there was no significant improvement in visual acuity in stage 3 keratoconus patients; nevertheless, approximately 85% of corrected distance visual acuity was preserved or improved. The same study observed a 3.2% rate of 2 or more lines loss in CDVA on the Snellen chart. The authors attributed this loss to irregular astigmatism that develops after ring implantation, as also mentioned by Ertan et al.[12] In our study, uncorrected visual acuity improved in all our patients (100%), and corrected CDVA improved or was preserved in 93.3% of patients. It was observed that uncorrected CDVA increased by at least 2 lines in 9 eyes (average 2.6 lines), while corrected CDVA increased by at least 2 lines in 7 eyes (average 1.5 lines). It was observed that CDVA decreased by 1 line in 1 patient and remained stable in 2 patients. Although staging was not performed due to the small number of cases, the results are similar to the high success rates reported in the literature.

Many studies have reported that KeraRing implantation is an effective method for correcting corneal shape and reducing astigmatism.[5,6,11,20,21] However, it has been noted that the extent to which astigmatism can be corrected in stage 3 keratoconus cannot be predicted. Pinero et al.[22] reported that the reason for poor outcomes in highly astigmatic eyes is the unpredictable and poor outcome of adding rings in these eyes with highly irregularly arranged corneal lamellae. Our study observed significant K values and astigmatism reductions in moderate and advanced keratoconus patients. Particularly in one patient with advanced keratoconus who had previously waited for keratoplasty at another center, an increase of 3 lines in uncorrected CDVA and 1 line in corrected CDVA was achieved with KeraRing implantation, and it was observed that the average K value decreased from 62.95 D preoperatively to 59.1 D postoperatively.

There has yet to be a consensus on which localization is better for corneal incision placement. Different incision sites have been described in the literature, including temporal position, superior position (12 o'clock), positive cylinder axis not deviating more than 90° from the topographic axis, temporal and 1 o'clock positions above the horizontal axis, and perpendicular to the topographic axis. Theoretically, as most surgeons apply, the ideal position should be on the steepest corneal axis, as this type of incision reduces corneal power along the steepest axis and yields flat keratometric values. However, a significant reduction in astigmatism has been observed with incisions at other locations. In our study, we selected and applied the incision site topographically as the steepest corneal axis, like many surgeons. Considering the more significant difference in Kmax values, we believe the incision on the steep axis also has a relaxing effect.

High degrees of ametropia may occur after KeraRing implantation. This ametropia can be corrected with glasses or contact lenses. In recent years, successful results have been reported in correcting ametropia and residual astigmatism with posterior chamber toric intraocular lens implantation after ring implantation. Although some of our patients developed ametropia, no significant improvement in vision with glasses was observed in these patients. As the follow-up periods were short, none of our patients underwent intraocular lens implantation.

As mentioned earlier, corneal ring implantation does not prevent the progression of keratoconus. Particularly in keratoconic patients showing rapid progression, an increase in mean K values has been observed 6-36 months after ring implantation. This is because ring implantation does not address the structural problem of weakened collagen structure in the disease. Considering the successful visual and refractive outcomes of corneal rings, combined treatment methods have begun to be attempted. With collagen cross-linking therapy, the corneal biomechanical rigidity increases 4-5 times, and the collagen fibril diameter increases. This promotes structural improvement in keratoconus, thus preventing progression. In their studies, Chan et al.[23] reported that combined Intacs implantation with cross-linking increased the corneal flattening effect of Intacs. Combined corneal ring implantation with collagen cross-linking can be used to maintain stability when progression occurs after ring implantation or to maintain the flattening effect of the ring. Our study observed no progression in any patient during the follow-up period. Only one patient was referred for cross-linking therapy due to progression starting in the other eye.

In a study comparing deep anterior lamellar keratoplasty (DALK) and intrastromal corneal ring segments in advanced keratoconus, the authors compared 66 eyes regarding visual, refractive, and topographic K values.[24] They found improved visual acuity and refractive values in advanced keratoconus with DALK. Additionally, they reported that corneal ring segments were an alternative treatment method with fewer complications and sufficient results. [24] Corneal ring implantation should be attempted before keratoplasty in advanced keratoconus

without apical scarring and adequate corneal thickness. [24]

Our study has some limitations. Firstly, it does not include a control group. Selecting an appropriate control group for diseases like this is difficult due to variations in the progression levels of patients, and the small number of patients contributed to this. Moreover, selecting the other eye as the control group will not eliminate the problem because the disease progresses asymmetrically, and each eye's progression rate may differ. Another area for improvement in our study is the small number of patients and, therefore, the inability to group them by stages. Additionally, the follow-up periods were relatively short, especially for some patients. Despite these limitations, our study has shown that corneal ring implantation successfully rehabilitates keratoconus patients who are intolerant to contact lenses or unable to use contact lenses for various reasons. KeraRing are intracorneal ring segments developed for the optical treatment of keratoconus. When used in suitable patients for ring implantation, they significantly improve visual acuity, refractive, keratometric, and topographic outcomes. Although they do not prevent the progression of the disease, obtaining satisfactory visual outcomes and delaying keratoplasty are essential advantages. In our patients, there was a significant increase in both uncorrected ($p<0.001$) and corrected ($p=0.003$) best-corrected visual acuities. This increase is attributed to the corneal flattening effect and the reduction of corneal astigmatism by the rings. A significant decrease in astigmatic refractive values ($p=0.002$) was observed, while the reduction in myopic refractive values ($p=0.234$) was not significant. When looking at topographic characteristics, there was a substantial decrease in median K ast, K min, K max, and K median values due to the corneal flattening effect of KeraRing ($p=0.012$, $p=0.008$, $p=0.002$, $p=0.004$, respectively). No complications were observed except for one patient who migrated the ring segment into the tunnel. In conclusion, KeraRing implantation in keratoconus patients using a femtosecond laser is a safe, low-complication rate, easily applicable, and optically successful method.

Compliance with Ethical Standards

This study was approved by the Institutional Review Board and Ethics Committee of Haseki Training and Research Hospital (date: 23.05.2024 no.31-2024). The methods complied with the principles of the Helsinki Declaration.

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

All the authors equally contributed to this work.

Financial Disclosure

None

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Research Article | Araştırma Makalesi

THE RELATIONSHIP BETWEEN MIR-196A2 POLYMORPHISM AND COLORECTAL CANCER RISK

MIR-196A2 POLİMORFİZMİ İLE KOLOREKTAL KANSER RİSKİ ARASINDAKİ İLİŞKİ

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ABSTRACT

Objective: MicroRNAs are small endogenous, non-coding, single-stranded posttranscriptional RNA molecules. The discovery of microRNAs has made new contributions to cancer diagnosis and treatment. These microRNAs reported as a responsible for colorectal cancer development with several epigenetic changes. In this study, it was aimed to evaluate the relationship between the polymorphism of miR-196a-2 polymorphism rs11614913 and colorectal cancer in Turkish population.

Methods: Two hundred colorectal cancer patient (124 colon cancer and 76 rectal cancer) and 240 health control individuals were included in our study, which was planned as a hospital based retrospective cohort study. MiR-196a2 polymorphism in peripheral blood samples has been determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. Significance of the results has been evaluated by using SPSS (20.0 SPSS Inc., Chicago, IL, USA.) statistical program.

Results: miR-196a2 C / C + C / T genotypes was found to be associated with the risk of colorectal cancer development (p: 0.001; OR: 2.04, 95% CI: 1.293-3.236). The subgroup analysis, showed that the C / C + C / T genotype increased the risk of colon cancer development 2.11 times (p: 0.016; 95% CI: 1.136-3.918) and rectal cancer 2.86 times (p: 0.011; 95% CI:1.242-6.592). The relationship between any clinicopathological features of colorectal cancer and the frequency of the C / C + C / T genotype of miR196a2 was not statistically significant (p> 0.05).

Conclusion: This study supports that miR-196a2's C / C + C / T genotypes is related with increased colorectal cancer development risk.

Keywords: Colorectal cancer, MicroRNA, miRNA-196a2 polymorphism

ÖZ

Amaç: MicroRNA'lar küçük, endojen, kodlanmayan, tek sarmallı posttranskripsiyonel RNA molekülleridir. MikroRNA'ların keşfi, kanser teşhis ve tedavisine yeni katkılar sağlamıştır. Bu miRNA'ların çeşitli epigenetik değişiklerle kolorektal kanser gelişiminden sorumlu olduğu saptanmıştır. Bu çalışmada Türk toplumunda miR-196a2'nin rs11614913 polimorfizmi ile kolorektal kanser arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.

Yöntem: Hastane bazlı retrospektif kohort çalşması olarak planlanan çalışmamıza 200 kolorektal kanser hastası (124 kolon kanseri ve 76 rektum kanseri) ve 240 sağlıklı kontrol bireyler dahil edilmiştir. Periferik kan örneklerinde miR-196a2 polimorfizmi, polimeraz zincir reaksiyonu ve restriksiyon fragman uzunluk polimorfizmi yöntemi ile belirlenmiştir. Sonuçların anlamlılığı SPSS (20.0 SPSS Inc., Chicago, IL, USA.) istatistik programı kullanılarak değerlendirilmiştir.

Bulgular: MiR-196a2 C/C + C/T genotiplerinin kolorektal kanser gelişim riski ile ilişkili olduğu saptanmıştır (p: 0.001; OR: 2.04, %95 CI: 1.293-3.236). Altgrup analizleri ise ise C/C+C/T genotipinin kolon kanseri gelişim riskini 2.11. kat (p: 0.016; %95 CI: 1.136-3.918) ve rektum kanserini ise 2.86 kat (p: 0.011; %95 CI: 1.242-6.592) arttırdığını göstermiştir. Kolorektal kanserin herhangi bir klinikopatolojik özelliği ile miR-196a2'nin C/C+ C/T genotipinin sıklığı arasında istatistiksel olarak anlamlı ilişki saptanmamıştır (p>0.05).

Sonuç: Bu çalışma miR-196a2'nin C / C + C / T genotiplerinin artmış kolorektal kanser gelişim riski ile ilişkili olduğunu desteklemektedir.

Anahtar Kelimeler: Colorectal cancer, MicroRNA, miRNA-196a2 polymorphism

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Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancer which is third most common cancer in men and second most common cancer in women.[1] Although its incidence and mortality rates have increased in recent years, its mobidity and mortality risk has decreased in countries with screening programs.[2]

The etiology of CRC is multifactorial including genetic, epigenetic, lifestyle factors and environmental factors.[3] As one of epigenetic factors, single nucleotide polymorphisms (SNPs) in miRNA's have been reported.[1] MiRNA's are small, non-coding group of RNAs which functions both in tumor supressions and oncogenic activity.[4] These miRNA's act on mRNA's and MiRNA's can play an important role in tumor initiation, proliferation, apoptosis, migration, metastasis, response to chemotherapy and radiotherapy in several cancers.[3,5] SNPs in miR-196a2 in CRC, has been studied in different countries and population groups for the risk of development of CRC. While some studies have shown susceptibility to CRC, some studies have not found any susceptibility.[2,4]

In this study, we aimed to evaluate the relationship between the polymorphism of miR-196a2 and CRC in Turkish population.

Methods

Patients

Two hundred CRC patient (124 colon cancer and 76 rectal cancer), who were diagnosed by endoscopic evaluation, histopathological examination, imaging techniques, and 240 healthy control individuals were included in our study who admitted to Istanbul University, Istanbul Medical Faculty, General Surgery Department which was planned as a hospital based retrospective cohort study. The study protocol was approved by the Biruni University Ethical Committee. This study was funded by Istanbul University Scientific Investigation Projects No: 5821

Genotyping

Blood samples from all CRC patients and healthy control groups were stored in Ethylenediaminetetraacetic acid disodium salt (EDTA) tubes. Genomic DNA was extracted from peripheral whole blood according to salting-out technique. MiR-196a2 polymorphism in peripheral blood samples has been determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method.

Genotyping was performed by the procedures of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using MspI restriction enzyme. PCR was performed with using primers (miR 196a-2 ''F'' 5' CCC CTT CCC TTC TCC TCC AGA TA 3' and ''R'' 5' CGA AAA CCG ACT GAT GTA ACT CCG 3'). The PCR product was then digested using MspI restriction enzyme (Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA) for 16 h at 37˚C. The resulting fragments were separated by electrophoresis on a 3% agarose gel. The CC genotype produced two fragments (125 and 24 bp), the TT homozygote produced one 149 bp fragment and the TC heterozygote produced three fragments (125, 149, and 24 bp).[6]

Statistical analysis

Significance of the results has been evaluated by using SPSS (20.0 SPSS Inc., Chicago, IL, USA.) statistical program.

Statistical analyses were performed using the R (R Core Team, 2017, Vienna, Austria) program. p-Values less than 0.05 were considered to be statistically significant. Statistical analyses were performed using the SPSS software package (revision 20.0 SPSS Inc.,Chicago, IL, USA.). Data are expressed as mean+SD. Differences in clinicopathological characteristics between patients and controls were tested by chi-square test for categorical data and Student's t-test for numerical data. Odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and CRC was computed. A two-sided p-value of less than 0.05 was considered statistically significant.

Results

Characteristics of colorectal cancer patients and control groups

Two hundred CRC patients and 240 controls were studied. Characteristics of CRC patients and control groups were given in Table 1. There was no significant difference in the characteristics between CRC patients and control groups (p>0.05) (Table 1).

MiR-196a2 polymorphism

miR-196a2 C / C + C / T genotypes was found to be associated with the risk of colorectal cancer development (p: 0.001; OR: 2.04, 95% CI: 1.293-3.236). The subgroup analysis, showed that the C / C + C / T genotype increased the risk of colon cancer development 2.11 times (p: 0.016; 95% CI: 1.136-3.918) and rectal cancer 2.86 times (p: 0.011; 95% CI:1.242-6.592). The relationship between any clinicopathological features of colorectal cancer and the frequency of the C / C + C / T genotype of miR196-a2 was not statistically significant (p> 0.05). (Table 2)

Table 1. Characteristics of colorectal cancer patients and control groups

		Colorectal cancer patients (n=200)		Control group(n=240)	p value
		Colon cancer (n=124)	Rectal cancer (n=76)		
Sex	**Female**	49 (39.5%)	28 (36.8%)	90 (37.5%)	
	Male	75 (60.5%)	48 (63.2%)	150 (62.5%)	0.457
Smoking	**Yes**	40 (32.2%)	27 (35.5%)	89 (37.1%)	
	No	84 (67.7%)	49 (64.5%)	151 (62.9%)	0.910
Alcohol consumption	**Yes**	21 (16.9%)	17 (22.4%)	36 (15%)	
	No	103 (83.1%)	59 (77.6%)	204 (85%)	0.680
T Staging	**T 1**	6 (4.8%)	3 (3.9%)		
	T 2	24 (19.4%)	10 (13.2%)		
	T 3	60 (48.4%)	37 (48.7%)		
	T 4	34 (27.4%)	26 (34.2%)		
Lymph node involvement	**N 0**	48 (38.7%)	14 (18.4%)		
	N 1	32 (25.85%)	18 (23.7%)		
	N 2	44 (35.5%)	44 (57.9%)		
Distant metastasis	**Yes**	13 (17.1%)	17 (13.7%)		
	Absent	63 (82.9%)	107 (86.3%)		
Tumor differentiation	**Well or moderately**	84 (67.7%)	52 (68.4%)		
	Poorly	40 (32.3%)	24 (31.6%)		

*Values are given as percentage (%) in the table. p-value less than 0.05 was considered as significant. Nodal involvement p:0.08

Table 2. The relationship between genotype/alleles and the colorectal cancer risk

Genotype /alleles	Colorectal cancer patients (n=200)		Control group (n=240)	p value
	Colon cancer (n=124)	Rectal cancer (n=76)		
CC	61	37	95	**0.012***
CT	46	34	91	0.240
TT	17	5	54	0.280
CC + C / T	107	71	186	**0.003***
TT + C / T	73	39	14	0.140

*P-value less than 0.05 was considered as significant. Bold values indicate statistical significance.

Discussion

CRC is one of the most common cancer types and its management is still challenging. Screening programs helps to decrease incidence. Although colonoscopy is generally used for screening it is invasive and expensive.[7] Epigenetic factors such as SNPs in miRNA's that may be useful for diagnosis, prognosis and follow-up. Different types of miRNA's can have spesific correlation with different types of tumor.

miR-196a2 has garnered particular attention due to its potential involvement in oncogenesis, tumor progression, and metastasis in CRC. A growing body of evidence suggests that miR-196a2 modulates the Wnt/β-catenin signaling pathway, a key driver of CRC development. Aberrant activation of this pathway has been shown to contribute to CRC by promoting cellular proliferation and inhibiting apoptosis.[8]

Our study supports that miR-196a2's C / C + C / T genotypes is related with increased risk of CRC development in Turkish population. Indeed, two different meta-analysis reported that miR-196a2 might play a role in pathogenesis of CRC in Iranian and Asian population in concordence of our findings in Turkish population.[9,10] Beside this, miR-196a2 polymorphism was found to be not related with the increasing of CRC development ontrary in Chinese population.[4]

The potential links between several miRNA gene polymorphisms, including miR-27a, miR-146a, miR-196a2, miR-492, and miR-608 and CRC development were investigated in a recent study.[11] Despite prior evidence suggesting that these miRNAs play crucial roles in cancer biology by regulating key oncogenic pathways, the study's findings indicate that genetic variations in these specific miRNAs do not substantially influence CRC susceptibility. These results highlight the complexity of miRNA involvement in cancer and suggest that while miRNAs may contribute to tumor biology, their polymorphisms alone may not be reliable markers for CRC risk.[11]

miRNA's are also promising factors for diagnosis and new treatment strategies.[5] From a clinical perspective, miR-196a2 holds promise as a biomarker for colorectal cancer. Its overexpression in tumor tissues, as well as its detectability in serum, makes it a candidate for non-invasive diagnostic tests.[12]

Also Ge et al., mentioned that high miR-196a2 expression is strongly associated with poor prognosis in colorectal cancer (CRC) patients, consolidating its role as a significant biomarker in predicting clinical outcomes. This supports the hypothesis that miR-196a2 not only contributes to tumor progression through key oncogenic pathways but also exacerbates aggressive tumor behavior, ultimately leading to worse clinical outcomes.[13] miRNAs represent promising therapeutic targets due to their ability to modulate gene networks, making them integral to novel anti-cancer strategies. [14]

MiRNAs are pivotal in modulating key processes such as cell proliferation, invasion, and metastasis, all of which are central to CRC progression. Specific miRNA signatures associated with CRC, such as the downregulation of tumor-suppressive miRNAs and upregulation of oncogenic miRNAs, contribute to the complex molecular mechanisms underlying tumor growth and metastatic potential. For instance, altered expression of miRNAs like miR-21, miR-200, and miR-34a has been linked to enhanced epithelial-mesenchymal transition (EMT), increased stemness and resistance to apoptosis in CRC. These findings suggest that targeting specific miRNA pathways could offer therapeutic benefits in controlling tumor progression and preventing metastasis. [15]

The role of exosomal miRNAs in cancer progression, particularly in chemoresistance and metastasis, has been increasingly recognized in recent years. Kulkarni et al. (2019) highlighted how exosomal miRNAs contribute to these processes by facilitating intercellular communication within the tumor microenvironment. These miRNAs can be transferred between cancer cells and surrounding stromal cells, promoting an environment conducive to tumor survival. In the context of chemoresistance, specific exosomal miRNAs can alter the expression of drug resistance genes, making cancer cells less susceptible to chemotherapy. For example, the upregulation of miRNAs like miR-21 and miR-221 has been associated with resistance to various chemotherapeutic agents. Additionally, exosomal miRNAs play a crucial role in enhancing metastatic potential by regulating genes involved in EMT and cell migration. This dual role of exosomal miRNAs in both drug resistance and metastasis underscores their significance as potential therapeutic targets, where inhibiting their activity could improve treatment outcomes by reducing resistance to chemotherapy and preventing cancer spread.[16]

Therefore, miRNAs can be used as a biomarker for early diagnosis, therapeutic target and also to determine prognosis due to the potential for metastasis.

There are some limitations of our study. First of all, our study size is small for the investigate the association of the risk of CRC development. Secondly, we have investigated only miRNA-196-a2 genotype of miRNA family.Thirdly, we have not investigate the other effects of miRNA-196-a2 genotype as metastasis, prognosis and progression of CRC.The effects of other MiRNA's family on metastasis, prognosis, occurrence of CRC as well as therapeutic option will be important to investigate fort he future studies.

In conclusion, our research highlight the pivotal role of miR-196a2 in the development and of CRC risk. Its involvement in key oncogenic pathways, its impact on chemoresistance, and its potential as both a prognostic biomarker and therapeutic target make miR-196a2 a focal point for future investigations in CRC. As the landscape of precision oncology evolves, targeting miR-196a2 could offer new hope for CRC patients, particularly those with poor prognostic factors. However, it is important to recognize that not all studies have reported a clear link between miRNA polymorphisms and CRC, as evidenced by research that found no significant association between various miRNAs and CRC risk. This discrepancy underscores the complexity of miRNA regulation in cancer and highlights the need for further, more comprehensive investigations. Future studies should focus on larger, more diverse populations and explore additional factors, such as environmental influences and gene-environment interactions, to better understand the precise role of miRNAs in colorectal cancer.

Compliance with Ethical Standards

The study was approved by Biruni University Ethical Committee (2024-BIAEK/03-30).

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

Conception and Design of Study: BCT, MTB, YB, EC. Data Acquisition: BCT, ÜZ, EC. Performing Laboratory Analysis: BCT, ÜZ. Data Analysis: BCT, EC. Drafting Manuscript: BCT. Critical Revision of Manuscript: EC. Final Approval: BCT. Supervision: ÜZ, MTB, YB, EC

Financial Disclosure

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Research Article / Araştırma Makalesi

THYMOQUINONE AFFECTS THE EXPRESSIONS OF HIPPOCAMPAL miR-26b, miR-124, AND miR-29a microRNAs IN HEALTHY RATS

TİMOKİNON, SAĞLIKLI SIÇANLARDA HİPOKAMPAL miR-26b, miR-124 VE miR-29a MİKRORNA'LARININ EKSPRESYONLARINI ETKİLER

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ABSTRACT

Objective: Thymoquinone (TQ), the main bioactive component of *Nigella sativa*, crosses the blood-brain barrier and exerts neuroprotective and neuromodulatory activities. This study aims to investigate the effect of TQ administration on the expressions of microRNAs (miR) 26b, 124, 29a and 29c in the hippocampus of healthy rats

Methods: TQ (20 mg kg−1 d−1) is administered intragastrically to adult rats for 15 days. MicroRNA levels of related genes were analyzed using real-time polymerase chain reaction.

Results: Administration of TQ significantly downregulated the expression profiles of miR-26b and miR-124 and upregulated miR-29a. No significant change was observed in the expression level of miR-29c.

Conclusion: TQ may have a beneficial effect on healthy brain and/or central nervous system (CNS) function by altering the expression of miR-26b, miR-124, and miR-29a, which are highly expressed in the brain.

Keywords: Thymoquinone, Black seed, Black cumin, microRNA, hippocampus

ÖZ

Amaç: *Nigella sativa*'nın ana biyoaktif bileşeni olan Thymoquinone (TQ), kan beyin bariyerini geçerek nöroprotektif ve nöromodülatör aktiviteler gösterir. Bu çalışmanın amacı, TQ uygulamasının sağlıklı sıçanların hipokampüsünde 26b, 124, 29a ve 29c mikroRNA'larının (miR) ekspresyonları üzerindeki etkisini araştırmaktır.

Yöntem: TQ (20 mg kg-1 d-1) yetişkin sıçanlara 15 gün boyunca intragastrik olarak uygulanmıştır. İlgili genlerin mikroRNA seviyeleri gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak analiz edilmiştir.

Bulgular: TQ uygulaması miR-26b ve miR-124'ün ifade profillerini önemli ölçüde aşağı düzenlerken miR-29a'nın ifadesini yukarı düzenlemiştir. miR-29c'nin ifade düzeyinde anlamlı bir etki gözlenmemiştir.

Sonuç: TQ, beyinde yüksek oranda ifade edilen miR-26b, miR-124 ve miR-29a'nın ifadelerini değiştirerek sağlıklı beyin ve/veya merkezi sinir sistemi fonksiyonlarında faydalı bir role sahip olabilir.

Anahtar Kelimeler: Timokinon, kara tohum, kara kimyon, mikroRNA, hipokampüs.

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Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally, thereby providing fine-tuned regulation of hundreds of targets.[1] Accordingly, every biological process is subject to miRNA-dependent regulation. Therefore, deregulation and/or alterations of specific miRNAs could disrupt the maintenance of health and contribute to diseases. It is predicted that approximately 70% of all miRNAs are expressed in the brain, and they play a pivotal role in regulating brain development and function,[2,3] modulating neurodevelopment[4] and neurodegeneration.[5] Abnormal miRNA expression profiles in the hippocampus have been identified as a risk factor in neuropathologies characterized by oxidative stress and apoptosis. [5]

Medicinal herbs are used as an alternative to chemical agents to alleviate health disorders. *Nigella sativa* (NS) has been utilized as a natural source of remedies and health-care in traditional medicine in various cultures for centuries. The seeds of this medicinal plant are often known as “black seed”, “black cumin”, or “black caraway”[6] and are extensively cultivated in the Mediterranean countries, Middle East, Eastern Europe and Western Asia.[7] Black seed and its extracts have been shown in numerous studies to be effective therapeutic agents with hepatoprotective, neuroprotective, cardioprotective, gastroprotective, antioxidant, antihistamine, antidiabetic, anticancer, antihypertensive, anti-inflammatory, antimicrobial, immunemodulatory, analgesic, and spasmolytic properties.[8-10] The broad spectrum of biological activities originated from its bioactive constituents, including thymoquinone (TQ), thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethole, sesquiterpene, α-pinene, and thymol.[8] Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) is the major bioactive component (30 – 48%) of the seed's essential oils and is attributed as responsible for the therapeutic properties of *Nigella sativa*.[8,11] TQ has the ability to cross the blood-brain barrier due to its small size and lipophilicity and perform neuromodulatory activities. This makes it an attractive potential substance for targeting the brain in the treatment of neurological disorders.[9]

TQ has been shown to provide neuroprotective effects in several degenerative diseases of the central nervous system, including cerebral ischemia,[11] epilepsy,[12] Parkinson's disease[13] and Alzheimer's disease.[14] It has been reported that TQ enhances antioxidant capacity and inhibits neuroinflammation.[13] Besides, it improves the memory and cognitive function,[15] sleep quality and ameliorates stress, anxiety[16] and depression.[17] The hippocampus is the region in which learning, memory, emotional regulation and pain perception occurs.[18] Therefore, in the present study, we aimed to investigate the effects of TQ on the expression of brain abundant microRNAs, including miR-26b, miR-29a, miR-29c and miR-124 related to brain development and function, neurodevelopment and neurodegeneration,[3,4] in the hippocampus of a healthy rat brain.

Methods

Animals

In the present study, 24-week-old female Spraque Dawley rats obtained from Bezmialem Vakif University were used. Animals were housed under standard laboratory conditions (12h light/dark cycles, 22 °C, and 60% humidity) with ad libitum food and water. All experimental procedures were performed according to the ethical approval obtained from the Committee for Animal Research Ethics at Bezmialem Vakif University (2015/229).

Thymoquinone (TQ) administration

Rats were randomly divided into two groups; control (C) (n = 5) and thymoquinone (TQ) (n = 5). TQ (Sigma–Aldrich, Darmstadt, Germany) was dissolved in corn oil as a final concentration of 20 mg mL−1 (w/v). All animals were treated with either TQ (20 mg kg−1 d−1) or corn oil according to their weights by intragastric gavage for 15 days.

Quantitative real-time PCR analysis

The homogenization for total RNA extraction was performed to the right hippocampal tissues of rats. Using the miRNA isolation kit (Thermo Fisher Scientific, Inc.), miRNAs were isolated from total RNA, which is obtained by TRIzol and the PureLink RNA mini kit. Firstly, at room temperature, supernatants of tissue homogenates were incubated with 200 µL of chloroform for 3 min. After centrifugation at 12 000 × g for 15 min at 4°C, 70% ethanol was added to the transparent part of the supernatant in a 1:1 ratio. After washing steps with special columns, isolated RNA was collected to eppendorf tubes on ice. After determination of the amount of isolated RNA by Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham), cDNA reverse-transcribed by miRNA specific cDNA synthesis kit. A reaction mixture including SybrGreen (Bioline, Luckenwalde, Germany), DNA polymerase, dinucleotide, and buffer solution was mixed with the template and reactions were performed in an appropriate thermal cycle with CFX96 Touch Real-Time PCR (Bio-Rad Laboratories, California). Primer against miR-26b, miR-29a, miR-29c, and miR-124 with housekeeping U6 genes (Table 1)[19] were purchased from Sentromer Technology (Istanbul, Turkey). Gene cards were analyzed using the threshold cycle (CT) relative quantification method. CT values were normalized for endogenous reference (ΔCT = CT [U6] − CT [miRNA gene]) and compared with control using the ΔΔCT formula (ΔΔCT = ΔCT [TQ group] − ΔCT [control]). Data were analyzed using logarithmic transformation of fold induction ratios according to the relative quantification (RF) formula (2−ΔΔCt).

Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). Unpaired t-test with Welch’s correction were performed to calculate the statistical significance between groups using GraphPad Prism 8.01 (GraphPad Software Inc., La Jolla, CA, USA). The statistically signi-

ficant difference was considered as *p < 0.05, ** p < 0.01, *** p < 0.001.

Table 1. Information about the primer sets

Gene	Forward sequence	Reverse sequence
miR-26b	TTCAAGTAATTCAGGATAGGT	-
miR-29a	TAGCACCATCTGAAATCGGTTA	-
miR-29c	TAGCACCATTTGAAATCGGTTA	-
miR-124	TAAGGCACGCGCTGAATGCC	-
U6	CGCAAAUUCGUGAAGCGUUC	-

Results

As shown in Figure 1 and Table 2, RT-PCR analysis revealed that TQ administration led to alterations in the expression of miR-26b, miR-124 and miR-29a in the healthy rat brain. No significant change was determined in the expression of miR-29c. The expression of miR-26b ($p > 0.01$) and miR-124 ($p > 0.05$) were significantly down-regulated (0.38-fold and 0.52-fold, respectively) in the TQ-treated rat group compared to the control group. However, the expression of miR-29a ($p > 0.05$) was markedly up-regulated (1.36-fold) in the TQ-treated rat group relative to the control group.

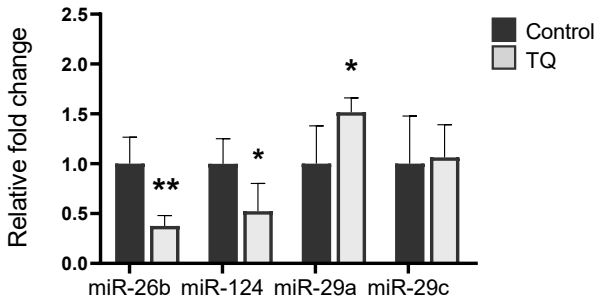

Figure 1. MicroRNA expression with Real-time RT-PCR. The microRNA expression levels of miR-26b, miR-124, miR-29a and miR-29c in the TQ administrated healthy rat hippocampus relative to control group (n = 5 in each group). Error bars represent SEM (standard error of mean). Statistically significance difference; *p < 0.05 and **p < 0.01.

Discussion

The present study investigated the effect of TQ supplementation on the expression of miR26a, miR-124, miR-29a and miR-29c in the healthy rat brain by RT-PCR. The results of the present study showed that TQ treatment reduced the mRNA expression of miR-26b and miR-124 while increasing the expression of miR-29a in comparison to the control group (Table 1, and Fig. 1). The expressions of miR-29c did not exhibit a notable difference.

It is well known that mRNAs are highly expressed in the brain and play a pivotal role in regulating brain development and function.[2,3] miR-26 has been identified as a functional miRNA that plays a role in a number of bio-

Table 2. Expression level of MicroRNAs (miRNAs) analysed by qRT-PCR in control and hippocampus.

miRNA	Control	TQ-treated Hippocampus
miR26b	0,04247 ± 0,01129	0,01598 ± 0,00442** ↓
MiR124	0,05880 ± 0,01478	0,03084 ± 0,01633* ↓
MiR29a	0,26712 ± 0,10160	0,36314 ± 0,09917* ↑
miR29c	0,17419 ± 0,08326	0,18525 ± 0,05720 ↑

The results are shown as mean ± SEM. The degree of significance is denoted as *p < 0.05 and **p < 0.01.

logical processes, including neural cell specification,[4] cell proliferation, apoptosis and the development of normal tissues and tumors.[20] It has been reported that loss of miR-26b results in protective effects on oxidative stress damage by regulating neuronal apoptosis.[21] Dill et al.[22] showed that it regulates neuronal differentiation. Caputo et al.,[23] found that miR-26b negatively regulates brain-derived neurotrophic factor (BDNF) expression at the post-transcriptional level that is involved in neuronal development and plasticity. Reduced BDNF expression is thought to be a risk factor for major depressive disorder (MDD). A previous study showed that miR-26b expression increased in MDD patients.[23] Additionally, decreased levels of BDNF, in the hippocampus are strongly associated with cognitive impairments in animals with Alzheimer disease.[24] In addition, miR-26b and miR-207 were found to be consistently dysregulated in obstructive sleep apnea patients following intermittent hypoxia, a characteristic pathophysiological change of obstructive sleep apnea, which can alter the expressions of apoptosis/anti-apoptosis proteins in the hippocampus coexisting with mnemasthenia.[25] It has also been identified as related to Alzheimer's disease since increased expression of miR-26b activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons.[22] It has recently been demonstrated that healing effect on AD patients through down-regulated miR-29c and miR-26b expressions in the Aβ1–42-induced rat hippocampus by increasing cell viability and decreasing apoptosis rate.[19] Considering all these studies, it can be concluded that decreased miR-26b expression may provide neuroprotective effects and reduce the risk of CNS diseases in healthy individuals.

miR-124, a brain-enriched mRNA, regulates neuroinflammation,[26] neural development and differentiation.[27] It is extensively expressed in neurons[4], suggesting its key function in the CNS. The expression level of miR-124 is undetectable or extremely low in neural progenitors, while its expression increases gradually in differentiating and mature neurons.[4,28] Highly expressed miR-124 can induce neuron-specific differentiation and govern the dendritic plasticity of neural stem cells.[29,30] miR-124 has been reported to be up-regulated in chronic stress and acts as a regulator of structural plasticity and behavioral responses to chronic stress.[31] Bahi et al.[32] also found that stress in the rat hippocampus causes increased expression of miR16 and miR-124, linked to the development of depressive-like symptoms. Pan-Vazquez, et al.[33] demons-

trated that exercise has a positive effect on stress resilience via increased expression of the glucocorticoid receptor (Nr3c1) and decreased expression of miR-124 in the hippocampus. Moreover, it was shown that expression levels of miR-124 up-regulated in the peripheral blood of MDD patients affect alterations in brain miRNA expression directly. Its expression levels were also found to be significantly correlated with the connectivity of both intra- and inter-networks in the brain.[34] Additionally, miR-124 has been shown to function as a regulator to alleviate cell death correlated with the expression of BACE1/β-secretase in Alzheimer's disease (AD).[35] Another study reported repressed tyrosine-protein phosphatase non-receptor type 1 (PTPN1) by up-regulation of miR-124 in the temporal cortex and hippocampus in AD patients, which indicates region-specific abnormalities linked to synaptic, plasticity and memory dysfunction.[36] Furthermore, miR-124 regulates translation of GluA2 primarily in the somatic cytoplasm of neurons rather than in dendrite, which could cause functional alterations in synaptic strength and connectivity.[37] Khan et al.[38] suggest that TQ may be directly linked with the miR-124 expression levels, which are involved in neuronal development and differentiation and this fact decreases ERK phosphorylation and ameliorates cognitive impairments in the rat hippocampus. Therefore, we may suggest that TQ-induced down-regulated miR-124 expression may improve memory, synaptic strength and plasticity. As mentioned before, the expression level of miR-124 has been found to be increased in chronic stress, AD and major depressive disorder; thereby, reduced miR-124 expression can also protect individuals from the development of neurodegenerative diseases.

The miR-29 family, consisting of miR-29a, miR-29b and miR-29c, has been implicated in neuronal proliferation, differentiation, plasticity, and survival.[39] miR-29a is abundant in the brain, and particularly in hippocampal neurons[40] and regulates dendritic spine morphology,[41] neural development and morphology.[40] In addition, it is important in fine-tuning motor function.[42] miR-29a, has been shown to be up-regulated during normal aging in the CNS.[43] Dysregulation of the miR-29 family is associated with many neurodegenerative disorders including AD, Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis and Parkinson's disease.[39] Ma et al.[44] found that overexpression of miR-29a regulates neurite outgrowth and development of neuronal stem cells by targeting extracellular matrix-related genes. Thus, increaseed miR-29a expression levels due to TQ treatment may affect neural development and morphology.

TQ, a major bioactive compound of *Nigella sativa* seed's oil, has been extensively studied for its biological activities and therapeutic potential and shown to possess neuroprotective, antioxidant and anti-inflammatory properties. It has been shown that TQ exerted a strong neuroprotective effect on Aß-induced neurotoxicity and aggregation by increasing cell viability and decreasing apoptosis rate via inhibition of ROS formation, and mitochondrial membrane depolarization in the hippocampal and cortical neurons.[45] The diminished TQ effect on Aß-induced inhibition of synaptic vesicle recycling was also reported in the same study. Bin Sayeed et al.[15] demonstrated that *Nigella sativa* (500 mg capsule twice daily for 9 weeks) improved age-related cognitive decline, memory and attention in healthy humans. The authors also reported that daily consumption of one NS capsule (500 mg for 4 weeks) as a nutritional supplement stabilized mood, decreased anxiety, and improved memory in healthy adolescent males.[46]

Overall, these studies and our results showed that TQ administration alters the expression of miR-26b, miR-124 and miR-29a, which play a role in maintaining healthy brain function. All these three miRNAs are abundant in the brain, indicating the importance of their activity in the CNS and their activities affect neuronal development, differentiation, plasticity, survival and synaptic function. Therefore, TQ may be suggested to be used as a natural supplement for the maintenance and improvement of brain health.

Conclusion

The results of the present study indicate that the TQ administration can affect brain-enriched microRNA expressions, which have functions in neural development and differentiation, neuroinflammation, memory, synaptic strength and plasticity. Regular expressions of these brain enriched miRNAs are critical for the maintenance of healthy CNS. Administration of TQ may help to improve memory, cognitive abilities and prevent the development and progression of neurodegenerative diseases. However, further research is required to identify the full potential of these natural agents for CNS in health and diseases.

Compliance with Ethical Standards

The experimental protocol of this study was approved by the Committee for Animal Research Ethics at Bezmialem Vakif University (2015/229).

Conflict of interest

The authors declare no conflict of interest.

Authorship Contributions

B.E. designed research and performed data analysis; T.D. performed research together with M.B., who also aided data analysis; and A.D contributed to data analysis and wrote the paper. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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Olgu Sunumu | Case Report

BARİATRİK CERRAHİ SONRASI ORTAYA ÇIKAN MİDE ADENOKARSİNOMU: OBEZİTE VE MİDE KANSERİ ARASINDAKİ GENETİK İLİŞKİNİN İNCELENMESİ

GASTRIC ADENOCARCINOMA AFTER BARIATRIC SURGERY: INVESTIGATION OF THE GENETIC RELATIONSHIP BETWEEN OBESITY AND GASTRIC CANCER

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ÖZ

Dünya genelinde ciddi bir sağlık sorunu olan obezitenin en etkin tedavisi olarak cerrahi işlemler uygulanmaktadır. Sleeve gastrektomi en sık uygulanan obezite cerrahisi tekniğidir. Obez bireylerde kanser riskinin arttığına dair görüşler mevcuttur. Ancak obezite cerrahisi sonrası da mide kanseri gelişen bazı olgular bildirilmiştir. Morbid obezite tanısı ile sleeve gastrektomi uygulanan bir hastanın ameliyatından 8 ay sonra dispeptik şikayetleri gelişmeye başlamıştır. Tetkikler neticesinde striktür tanısı konulan hastaya gastrik bypass revizyon cerrahisi uygulanmıştır. Hastanın bu operasyonundan sonra da şikayetlerinin devam etmesi üzerine yapılan detaylı incelemede hastaya mide adenokarsinomu tanısı konulmuştur ve hastaya total gastrektomi ameliyatı yapılmıştır. Obezite ciddi bir sağlık problemi olmasının yanında beraberinde birçok hastalık içinde risk faktörü olarak değerlendirilmektedir. Obez bireylerde kanser riskinin arttığı yönünde literatürde ciddi çalışmalar mevcuttur. Ancak obezite cerrahisi sonrasında kanser riskinin arttığına yönelik bazı çalışmalar mevcut olsa da henüz genel bir kanı oluşmamıştır. Ancak kanser riski artmış olan obez bireylerde cerrahi tedavi öncesinde kanser taraması amacıyla kontrol endoskopi yapılması ciddi şekilde önerilmektedir.

Anahtar Kelimeler: Sleeve gastrektomi, mide adenokarsinomu, morbid obezite

ABSTRACT

Surgical procedures are applied as the most effective treatment of obesity, which is a serious health problem worldwide. Sleeve gastrectomy is the most common bariatric surgery technique. There are opinions that the risk of cancer increases in obese individuals. However, some cases of gastric cancer after bariatric surgery have been reported. A patient who underwent sleeve gastrectomy for morbid obesity developed dyspeptic complaints 8 months after surgery. As a result of the examinations, the patient was diagnosed with stricture and underwent gastric bypass revision surgery. When the patient's complaints continued after this operation, the patient was diagnosed with gastric adenocarcinoma and total gastrectomy was performed. Obesity is not only a serious health problem but also a risk factor for many diseases. There are serious studies in the literature indicating an increased risk of cancer in obese individuals. However, although there are some studies showing an increased risk of cancer after bariatric surgery, there is no general opinion yet. However, in obese individuals with an increased risk of cancer, control endoscopy is strongly recommended for cancer screening before surgical treatment.

Keywords: Sleeve gastrectomy, gastric adenocarcinoma, morbid obesity

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Giriş

Obezite Dünya Sağlık Örgütü tarafından kronik bir hastalık olarak kabul edilmektedir ve giderek yaygınlığı artmaktadır. Obezitenin neden olduğu sorunların yanı sıra obeziteye eşlik eden çok sayıda patolojik durum sağlık sistemleri üzerinde ciddi bir yük oluşturmaktadır. Birçok ülkede obezitenin kalıcı ve etkin çözümü için cerrahi uygulamalar yaygınlık kazanmıştır. Bu nedenle başta mide olmak üzere sindirim sistemine yönelik farklı cerrahi girişimler içeren yöntemler geliştirilmiştir. Laparoskopik sleeve gastrektomi son yıllarda tanımlanmış ve günümüzde en yaygın olarak yapılan bir ameliyat tekniği haline gelmiştir.[1]

Mide kanseri gastrointestinal sistem kanserleri içinde kolon kanserinden sonra 2. sırada, genel kanser sıklığı sıralamasında ise ülkelere göre değişiklik göstermekle birlikte 5. sırada yer almaktadır.[2] Bariatrik cerrahi girişimlerden sonra özefagus kanseri insidansında artış olduğu literatürde belirtilmiştir.[3] Ancak mide kanserlerinde artış olduğuna dair henüz bir veri mevcut değildir. Literatürde obezite cerrahisinden 1 sene sonra ve 10 seneye kadar süre içerisinde mide kanseri tanısı alan hastalar olduğu bildirilmiştir.[4]

Bu yazımızda sleeve gastrektomi yapılan bir hastada ortaya çıkmış olan mide kanseri olgusu sunulacak ve obezite ile mide kanseri arasındaki genetik ilişki incelenecektir.

Olgu Sunumu

45 yaşında erkek hasta, dispeptik şikayetler ile polikliniğimize başvurdu. Yutma güçlüğü ve kusma şikayetleri olan hastanın 16 ay önce geçirdiği bariatrik cerrahi işlemler sonrasında beklenenden fazla ve hızlı kilo verdiği belirlendi. Hastaya 16 ay önce morbid obezite tanısı ile (VKİ: 44,9) laparoskopik sleeve gastrektomi ameliyatı yapılmıştır. Ameliyattan 8 ay sonra ortaya çıkan yutma güçlüğü, kusma ve aşırı kilo kaybı sebebiyle hastada striktür tanısı konularak revizyon cerrahisi planlanmış ve striktürün üstünden mide devamlılığı bozulmadan laparoskopik side-to-side Roux N-Y Gastrik Bypass ameliyatı yapılmıştır. İkinci ameliyatından 8 ay sonra tekrar yutma güçlüğü ve kusma şikayeti ile kliniğimize başvuran hastanın yapılan tetkiklerinde gastrik kitle tespit edilmiş ve endoskopik olarak alınan biyopside mide adenokarsinomu tanısı konulmuştur. Kliniğimizde total gastrektomi ve D2 lenf nodu diseksiyonu yapılmıştır.

Tartışma

Mide kanseri sağlıklı bireylerde Dünyada en sık karşılaşılan 5. kanser olarak kabul edilmektedir.[2] Kanser riskini artıran veya kanser gelişimine sebep olan birçok faktör sorgulanmaktadır. Obezite birçok metabolik soruna sebep olan bir hastalık olması sebebi ile kanser etyolojisi için risk faktörlerinden birisi olarak kabul edilmektedir.[5] Artmış kanserojen içerikli beslenme, adipoz dokunun artmasına bağlı hormonal düzenin bozulması gibi sebeplerle genetik mutasyonların artabileceği düşünülmektedir.

Herediter diffüz mide kanseri, cadherin 1 geni (CDH1) değişikliklerinin neden olduğu en tanınmış ailesel mide kanseridir. Herediter diffüz mide kanseri otozomal dominant geçiş gösteren bir kanser sendromudur. Aile öyküsü olan hastalarda gastrik karsinom riski, böyle bir öyküsü olmayan bireylere göre yaklaşık üç kat daha yüksektir.[6] İnsan obezitesinde, vücut ağırlığı durumundaki değişkenliğin %40 ila %50'sini oluşturan, ancak normal kilolu bireyler arasında daha düşük (yaklaşık %30) ve obezite ve şiddetli obezite alt popülasyonunda önemli ölçüde daha yüksek (yaklaşık %60-80) olan genetik bir bileşen vardır.[7] En az 15 gendeki kusurlar, çoğunlukla leptin-melanokortin sinyal yolundaki eksikliklerden kaynaklanan monojenik obezite vakalarının nedenidir.[7] Bu sebeplerden dolayı obezite cerrahisi planlanacak olan hastalara işlem öncesinde mutlaka endoskopik kontrol önerilmektedir. Sunulan olgunun ilk ameliyatından önce gastroskopik inceleme yapılıp yapılmadığı bilgisine ulaşılamamıştır. Eğer hastalarda ameliyat öncesinde mide içerisinde premalign veya malign bir lezyon varlığı mevcut ise bariatrik girişim öncesi bunun saptanması önemlidir. Aksi taktirde yapılacak olan cerrahi işleme bağlı olarak bu lezyonun progresyonunu olumsuz yönde etkileyebileceği gibi tanı konulmasının gecikmesi de söz konusu olabilmektedir.

Çoğu mide kanseri sporadiktir ancak %5-10 hastada ailesel olarak izlenebilmektedir.[8] Hastamızda cerrahi öncesi endoskopi bilgisi olmadığı için hastalığının etyolojisi ve gelişim süreci araştırılmak istenmiştir. Aile öyküsünde birinci dereceden akrabasında kolon kanseri ve ikinci dereceden akrabalarında yine kolon kanseri ve meme kanseri öyküleri mevcuttur. Cerrahi sonrası bu kadar kısa süre içerisinde kanser gelişmesinin altında genetik bir hastalık varlığı araştırılmak istenmiştir. Bu sebeple hastamızdan PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), KRAS (Kirsten rat sarcoma viral oncogene homolog) ve HER-2 (human epidermal growth factor receptor 2) genetik testleri yapılmıştır. Ayrıca rutin kanser paneli de çalışılmıştır. Testler sonucunda kanser etiyolojisinde genetik bir mutasyon saptanmamıştır.

Sonuç olarak obez bireylerde mide kanseri riskinin arttığı bilinmektedir. Dünyada kanserler arasında 5. sıklıkta izlenen mide kanserinin tanısının konulması için endoskopi altın standart olarak görülmektedir. Özellikle obezite cerrahisi geçirecek olan hastaların erken evre veya ileri evre mide kanseri varlığı durumunda ameliyat öncesi bu durumun açığa çıkarılmaması durumunda mide kanserinin tanı ve tedavisinin gecikebileceği açıktır. Özellikle ailesinde kanser öyküsü olan hastalarda genetik mutasyonların da eşlik edebileceği ve mide kanseri riskinin artacağı unutulmamalıdır. Bu gibi durumların yaşanmaması için cerrahi işlem öncesinde kontrol endoskopinin yapılmasının gerekliliği ve önemi açıktır.

Mide kanseri ile obezite arasında genetik bir ilişki olup olmadığı konusunda literatürde henüz bir veri mevcut değildir. Ancak özellikle bariyatrik girişimden 1-2 yıl sonra mide kanseri gelişen hastaların genetik açıdan

incelenmesi bu konuda değerli bilgiler verecektir. Obezite ile mide kanseri arasında ortaya konulacak bir genetik korelasyon halinde bariyatrik cerrahiye aday hastaların ameliyat öncesi endoskopik incelemelerinin yapılmasının yanı sıra yapılacak genetik analizlerde yol gösterici olacaktır.

Bu olgu vesilesiyle obezite ve mide kanseri arasında ilişki olup olmadığı konusuna dikkat çekilmesi istenmiştir. Ancak bu konuda çok merkezli çalışmalara ihtiyaç duyulmaktadır.

Etik Standartlara Uygunluk

Hastanın gizliliği korunarak etik unsurlara dikkat edilmiştir.

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Derleme | Review

BAŞ BOYUN KANSERLERİNDE ONKOGENLER VE TÜMÖR BASKILAYICI GENLER

ONCOGENES AND TUMOR SUPPRESSOR GENES IN HEAD AND NECK CANCERS

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ÖZ

Baş ve boyun kanserleri (BBK), ağız boşluğu, farenks, gırtlak, burun boşluğu ve tükürük bezleri gibi baş ve boyun çevresindeki anatomik bölgelerden kaynaklanan çeşitli malignite gruplarını kapsar. Bu kanserlerin gelişimi ve ilerleyişi, özellikle tümör baskılayıcı genler ve onkogenleri içeren genetik ve/veya epigenetik değişikliklerle karmaşık bir şekilde bağlantılıdır. Tümör baskılayıcı genler, hücre döngüsü regülasyonu, genomik stabilitenin korunması ve tümör oluşumunun önlenmesi gibi mekanizmalarda kritik rol oynarlar. Bu genlerin inaktivasyonu kontrolsüz hücre çoğalması ve kanser gelişimi ile sonuçlanabilmektedir. Öte yandan, onkogenler ise hücre büyümesini ve bölünmesini destekleyen ve proto-onkogen olarak adlandırılan normal genlerin mutasyona uğramış veya aşırı eksprese edilmiş versiyonlarıdır. BBK'nde onkogenlerin aktivasyonu, malign dönüşümü ve tümör büyümesini uyarmaktadır. Bu genlerin rollerinin anlaşılması, BBK de dahil olmak üzere tüm kanser türlerinde altta yatan moleküler mekanizmaların aydınlatılması ve kişiye özel tedavi stratejilerinin geliştirilmesinde bu mekanizmaların hedeflenerek daha spesifik bir tedavi uygulanması açısından önem taşımaktadır.

Anahtar Kelimeler: Baş boyun kanserleri, tümör baskılayıcı genler, onkogenler

ABSTRACT

Head and neck cancers (HNC) include various groups of malignancies originating from anatomical regions around the head and neck, such as the oral cavity, pharynx, larynx, nasal cavity and salivary glands. The development and progression of these cancers are intricately linked to genetic and/or epigenetic changes, especially those involving tumor suppressor genes and oncogenes. Tumor suppressor genes play critical roles in mechanisms such as cell cycle regulation, maintenance of genomic stability and prevention of tumorigenesis. Inactivation of these genes can result in uncontrolled cell proliferation and cancer development. Oncogenes, on the other hand, are mutated or overexpressed versions of normal genes called proto-oncogenes that support cell growth and division. Activation of oncogenes in HNC stimulates malignant transformation and tumor growth. Understanding the roles of these genes is important for elucidating the underlying molecular mechanisms in all types of cancer, including HNC, and also for the development of personalized targeting-based treatment strategies.

Keywords: Head and neck cancer, tumor suppressor genes, oncogenes

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Giriş

Baş boyun kanserleri, en yaygın yedinci kanser türü olup küreselleşme süreciyle birlikte risk faktörlerine daha fazla maruz kalınması hastalığın insidansını giderek arttırmaktadır.[1] Amerikan Kanser Derneğinin verilerine göre 2023 yılı için 54.540 oral kavite ve farengeal kanser vakası ön görülmektedir.[2] Kulak, burun, paranazal sinüsler, nazofarenks, majör ve minör tükürük bezleri, oral kavite, orofarenks, larenks, hipofarenks, tiroid ve paratiroid bezi gibi baş ve boyun çevresindeki farklı anatomik bölgelerdeki malign neoplazmlar baş boyun kanserlerinin alt türlerini oluşturmaktadır.

Gelişiminde biyolojik ve biyolojik olmayan faktörlerin rol oynadığı bilinen baş boyun kanserleri için bireyin yaşam tarzı ve alışkanlıkları doğrultusunda karsinojenlere maruz kalması en temel risk etmenleri arasında yer almaktadır.[3,4] Özellikle çeşitli formlarda tütün ürünlerinin tüketimi bu kanser türü için başlıca risk etmeni olarak bildirilmiş ve dünya çapında tüm oral kanserlerin %20-30'u tütün ürünleri kullanımıyla ilişkilendirilmiştir.[5] Bunun yanı sıra fazla miktarda ve sürekli olarak alkol tüketimi, sağlıksız ve dengesiz beslenme, radyasyon veya bazı meslek grupları için formaldehit gibi zararlı kimyasallara sürekli maruz kalınması, genetik yatkınlık, çeşitli viral enfeksiyonlar gibi risk faktörleri ve yaşanılan coğrafi bölgenin kültürüne bağlı olarak değişen yaşam koşulları oral kanser oluşumuna zemin hazırlayan faktörlerdir.[6] Literatür incelendiğinde, özellikle insan papilloma virüsü (HPV) ve herpes simpleks virüs (HSV) enfeksiyonlarının oral kavite, nazofarenks ve larenks kanserlerinin gelişiminde mutajen olarak önemli rol oynadıkları görülmektedir.[7] Son yıllarda yapılan çalışmalar, bu enfeksiyonların yalnızca kanser gelişiminde değil baş boyun kanserlerinin prognozunda ve uygulanan tedavinin etkinliğinde de önemli fark oluşturduğunu ortaya koymaktadır.[8,9] Moleküler mekanizması henüz tam olarak aydınlatılamamasına rağmen, HPV-pozitif baş boyun kanseri hastalarında tümör mikroçevresinin T hücre aktivasyonu, immun infiltrasyon ve immunregülasyon açısından daha aktif olduğu gösterilmiş olup, HPV-negatif baş boyun kanseri hastalarına göre daha iyi prognoz ve hayatta kalım oranları sergiledikleri vurgulanmıştır.[10]

Kanser hücrelerinin benzersiz doğası ve hastalığın seyrinden tedavi yanıtına kadar tüm sürecin her bireyde farklı ilerleyebilme potansiyeli bu alana yönelik araştırmaların devamlılığını gerektirmektedir. Kanser gelişimi ve ilerleyişinde genetik ve epigenetik birçok değişiklikle birlikte, literatürde baş boyun kanserleri ile ilişkili onkogen ve tümör baskılayıcı genlerin bütüncül bir perspektifte değerlendirildiği bir çalışma bulunmaması bu alanda bir boşluk oluşturmaktadır. Bu noktadan yola çıkılarak, bu yazı kapsamında baş boyun kanserleri ile ilişkili olduğu bilinen onkogenler ve tümör baskılayıcı genlerin kanser gelişimindeki rollerine odaklanılması amaçlanmıştır.

Baş Boyun Kanserlerinde Karsinogenez

Karsinogenez normal hücrelerin maligniteye doğru ilerlediği çok basamaklı bir süreci kapsamaktadır. Bu süreçte meydana gelen hücresel ve genetik değişimlerin hem her kanser türünde hem de her bireyde farklı bir işleyişte gelişmesi bu sürecin karmaşık doğasına hizmet etmektedir. Genetik ve epigenetik anormallikler ve bunların birbirleriyle etkileşimi tüm kanser türlerinde önem taşımaktadır. Karsinogenez sürecinde rol oynayan mekanizmalar tam olarak bilinmemesine rağmen hem onkogen aktivasyonunun hem de tümör baskılayıcı genlerin inaktivasyonunun tümör gelişiminde kritik rol oynadığı bilinmektedir.

Onkogenler gen amplifikasyonu, translokasyon, nokta mutasyonları gibi genetik değişikliklerin veya metilasyon yoluyla susturulmuş haldeki genlerin metilasyon profillerinin değişmesi gibi birtakım epigenetik farklılaşmaların bir sonucu olarak anormal hücre çoğalmasını uyarmakta ve bu şekilde karsinogenez sürecini desteklemektedirler.[11] Diğer yandan tümör baskılayıcı genler ise mutasyon, allel kaybı, delesyon gibi genetik veya metilasyon, asetilasyon gibi epigenetik değişiklikler sonucunda hücre döngüsü ve çoğalması üzerindeki işlevlerini kaybederek karsinogenez sürecine katkıda bulunmaktadırlar.[12] Onkogenler ve tümör baskılayıcı genlerdeki bu değişiklikler bilimsel literatürde, sırasıyla, fonksiyon kazanımı (gain of function) ve fonksiyon kaybı (loss of function) olarak tanımlanmaktadır.

Onkogenler ve baş boyun kanserleri ile ilişkileri

Proto-onkogenler, hücre büyümesi ve farklılaşması gibi normal hücresel olayları düzenleyen proteinleri kodlayan genlerdir. Onkogenler, proto-onkogen adı verilen normal hücresel genlerin mutasyona uğramış ve bunun sonucu olarak etkinleşmiş formlarıdır. Onkogenler, genellikle fonksiyon kazandıran mutasyon özelliğine sahip olup hücre farklılaşmasını uyarma, anjiogenezi teşvik etme ve apoptozu engelleme gibi mekanizmalarla malign forma dönüşümü tetiklemektedirler.[11] Onkogenler, hücresel seviyede baskın bir etkiye sahip olup etkin hale geldiklerinde veya ifadeleri arttığında, tek bir mutant allelleri nedeniyle bir hücreyi benign fenotipten malign fenotipe dönüştürebilmektedir. Onkogenler tarafından kodlanan proteinler, fonksiyon kazandıran mutasyonlar ya da genin bir allelinin artmış veya aşırı ifadesi ile kanser oluşumuna neden olmaktadır. Baş-boyun kanser gelişimi ile ilişkili ve en sık çalışılmış olan onkogenler *siklin D1*, *Ras*, *EGFR*, *STAT3* genleri ve bunlarla ilişkili moleküler yolaklardır.[13] Onkogenler, ürünü oldukları proto-onkogenlerin fonksiyonel ve biyokimyasal özellikleri temel alınarak ilişkili oldukları hücresel yolaklara göre 5 ana sınıfta kategorize edilmektedir (Tablo 1).[11,14]

Büyüme faktörleri, hücre dışından salgılanarak, belirli bir büyüme faktörüne yanıt vermek üzere özelleşmiş reseptöre sahip olan hedef hücrelerin çoğalmasını uyaran polipeptitlerdir.[11] Platelet-kaynaklı büyüme faktörü (platelet-derived growth factor, PDGF), epidermal büyüme faktörü (epidermal growth factor, EGF) ve fibroblast büyüme faktörü (fibroblast growth factor, FGF) farklı hücrelerin uyarılmasında etki gösteren ve moleküler aktiviteleri iyi karakterize edilmiş başlıca büyüme faktörleridir. Bu faktörler ve retroviral onkogenler arasındaki ilişkiye dair ilk çalışmalar bir maymun fibrosarkomundan izole edilen ve bir retrovirüs olan

simian sarkom virüsünün 'sis' onkogeninin incelenmesiyle ortaya koyulmuştur.[15,16]

Tablo 1. Onkogenlerin dahil oldukları hücresel yolaklara göre sınıflandırılmaları.[14]

Sinyal Yolağı	Örnekler
Büyüme faktörleri	*PDGF, EGF, Wnt ailesi, sis, int-2*
Büyüme faktörü reseptörleri	*EGFR, VEGFR, erbB-2*
Sinyal iletimi	*H-ras, K-ras, N-ras, siklin D1*
Transkripsiyon faktörleri	*erbA, c-myc, N-myc, myb*
Programlanmış hücre ölümü regülasyonu	*Bcl-2, siklin D1*

Bu çalışma kapsamında dizi analizi ile sis onkogeninin PDGF'nin beta zincirini kodladığı gösterilmiştir. Bu bulgular aynı zamanda büyüme faktörlerindeki ifade artışının kendi reseptörleri üzerinde otokrin aktivasyon sağlayarak kontrolsüz çoğalmayı teşvik edebileceklerinin de ilk göstergesi olmuştur.[16] Baş boyun kanseri hastalarında ekspresyonel ve fonksiyonel değişiklikleri sıklıkla gözlenen büyüme faktörü reseptörlerinden biri EGFR'dır.[17] Kromozom 7p12'de lokalize olan EGFR, tirozin kinaz proteinlerinin ErbB alt ailesine ait bir reseptör tirozin kinaz (RTK)'dır. RTK'lar, üç ana bölgeden oluşan karakteristik bir protein yapısına sahiptir: (1) hücre dışı ligand bağlama bölge, (2) transmembran bölge ve (3) hücre içi tirozin kinaz aktivitesi gösteren katalitik alan şeklindedir.[11] RTK'ların katalitik aktiviteleri; genellikle dışarıdan gelen bir sinyal neticesinde uyarılmanın ardından hücre içi kompartımanda, mitojenle aktifleştirilen protein kinaz (MAPK)'lar, PI3K/AKT veya STAT gibi çeşitli proteinlerden oluşan hücre içi yolakların aktivasyonu sürdürülür. Bu hücresel yolaklar ise spesifik RTK'ya bağlı olarak farklı şekillerde etkinleştirilir ve genellikle hücrenin hem apoptotik uyaranlardan kaçmasına hem de kontrolsüz çoğalmasına aracılık ederler.[17] Yapılan bazı çalışmalar *EGFR* geninin baş boyun kanserleri de dahil olmak üzere epitel kökenli kanserlerin birçoğunda sağlıklı hücrelere oranla daha fazla ifade edildiğini göstermektedir.[18] *EGFR* genindeki aşırı anlatımın, baş boyun kanseri türleri içinde en çok larenks kanserinde olduğu bildirilmiştir. Aynı zamanda, gen ifadesindeki bu artışın radyoterapiye dirençte rol oynayabileceği, lenf nodu metastazı ve tümörün tekrarlama riski, kötü prognoz ve sağ kalımda azalma ile ilişkili olabileceğinin de altı çizilmiştir.[19,20] EGF/EGFR aktivasyonu neticesinde uyarılan STAT tirozin kinaz sistemi de bu alanda en çok araştırılan konularındandır. Etkinleşmiş EGFR, STAT proteinlerini karışık bir mekanizma ile aktive etmekte ve sürekli aktive olan STAT proteinleri hücre farklılaşmasını tetiklemektedir.[21] Baş boyun kanseri hastalarında STAT3'ün aşırı ifade edildiği gösterilmiş ve buna bağlı olarak DNA'ya bağlanmasının da belirgin ölçüde arttığı not edilmiştir.[21]

Hücre döngüsü kontrolünde aktif çalışan siklin proteinleri de karsinogenez sürecinin başlatılmasında ve ilerletilmesinde kritik etkiye sahip olan proteinlerdir. *CCND1* geni insanda kromozom 11q13'te lokalize olup siklin D1 proteinini kodlamaktadır. Siklin D1, Rb'yi fosforilleyerek hücre döngüsünün ilerletilmesini sağlamaktadır. Yapılan araştırmalarda, baş boyun kanseri hastalarında, *siklin D1* gen ifadesinin aşırı arttığı bildirilmiştir.[13] Larenks kanserinde hücre döngüsü düzenlenmesindeki sık rastlanan anormalliklerden biri de Rb yolağının en önemli üyesi olan *CCND1*'in gen ifadesindeki olağandışı artıştır.[22] Bazı baş boyun kanserlerinde *CCND1* ifadesindeki aşırı artışın bu kromozomal bölgedeki amplifikasyon sonucunda oluştuğu görülmüştür.[11]

Bunların dışında, kanserlerin çoğunda başlatıcı etken olarak sıklıkla rastlanan bir diğer grup *Ras* gen ailesi tarafından kodlanan *H-ras*, *K-ras* ve *N-ras* onkogenleridir. Bu gen ailesinin, nokta mutasyonları sonucu proto-onkogenlerin etkinleşmesi ile kanser oluşumunda rol oynadığı bilinmektedir. Bu proto-onkogenlerin kodladığı proteinler, büyüme faktörlerine sinyal ileterek hücre büyümesinin uyarılmasını sağlar. Yapılan çalışmalar, *Ras* gen ailesindeki mutasyonların baş boyun kanserlerinin gelişiminde etkili olduğunu ve bu etkinin de çoğunlukla *K-ras* ve *H-ras* ile ilişkili olduğunu, *N-ras*'ın etkisinin daha az olduğunu göstermiştir.[23,24]

Tümör baskılayıcı genler ve baş boyun kanserleri ile ilişkileri

Tümör baskılayıcı genler, hücre döngüsünün ve kontrollü hücre çoğalmasının negatif regülatörleri olarak bilinmekte olup hücre proliferasyonunun baskılanması ve tümör gelişiminin inhibisyonu üzerinden aktivite gösterirler.[12] Çoğu tümörde bu genler baskılanarak veya etkisiz hale getirilerek tümör hücrelerinin anormal çoğalmasına katkıda bulunan bir süreç uyarılmaktadır. Bu genler tarafından kodlanan proteinlerde herhangi bir işlev kaybı kontrolsüz hücre çoğalmasına ve programlı hücre ölümünün (apoptoz) hatalı şekilde sürdürülmesine neden olmaktadır. Bir tümör baskılayıcı genin her iki allelinin de işlevini kaybetmesine neden olan bir mutasyon meydana gelmesi durumunda ise ilgili protein etkinliğini tamamen kaybederek karsinogenez sürecinin başlamasına yol açabilmektedir. Baş boyun kanserleri ile ilişkilendirilen ve en sık çalışılmış olan tümör baskılayıcı genler; *TP53*, *retinoblastoma (Rb)*, *p16*, *p21*, *p27*, *PTEN* ve *APC* genleridir (Tablo 2).[12]

Baş boyun kanserlerinde, p53 ve Rb proteinlerinin kontrol ettikleri yolaktaki fonksiyon bozuklukları en fazla görülen genetik faktörlerdendir. p53 yolağının görevi, hücre siklusunun düzenlenmesi, hücresel strese apoptoz yoluyla yanıt verilmesi ve hücre büyümesinin kontrollü bir şekilde gerçekleşmesini sağlamaktır.

TP53 geni, 53kDa ağırlığında, 17. kromozomun (17p13.1) kısa kolunda yer almakta ve hücre döngüsünün durdurulması, hücresel yaşlanma, DNA onarımı ve apoptoz süreçlerinde rol oynayan proteinlerin ekspresyonlarını ve bu yolaklardaki aktivitelerini kontrol eden ve bir transkripsiyon faktörü olan p53 proteinini kodlamaktadır.[25] Radyasyon veya kimyasal maddeler gibi mutajenik ajanların etkisiyle DNA'da bir hasar meydana gelmesi durumunda, p53 proteini stabil hale gelerek hücre çekirdeğinde birikmeye başlar. Biriken normal yapıdaki p53, DNA'da ilgili bölgelere bağlanarak hücre döngüsünün G1 fazında duraklamasına neden olur ve bu sayede hasarlı

DNA'nın onarılması için hücreye zaman tanınır. Bu süre içerisinde DNA onarımı yapılamazsa p53, DNA hasarı içeren bu hücrelerin çoğalmasını engellemek üzere hücre ölüm sürecini uyararak genetik hasarın sonraki hücrelere aktarılmasını engeller.[25]

Tablo 2. Tümör baskılayıcı genlerin dahil oldukları hücresel yolaklara göre sınıflandırılmaları.[12]

Sinyal Yolağı	Örnekler
Hücre döngüsü regülasyonu	*pRB, p16, INK4*
Programlanmış hücre ölümü (apoptoz) indüksiyonu	*TP53, PTEN*
Büyüme ve gelişmeyi uyaran sinyallerin inhibisyonu	*TGF-β, APC*
DNA tamir mekanizmaları	*TP53, MSH2*
DNA hasarı veya kromozomal anormaliler ile ilişkili	*BRCA1, p16, p14*

p53'ün transkripsiyon faktörü rolüyle ekspresyonunu düzenlediği diğer proteinler hücre döngüsünün kontrolü, apoptoz uyarımı, gelişim ve farklılaşma gibi birçok hücresel süreçte rol almakta olup bu proteinlerin hücresel seviyelerindeki kontrolsüz değişiklikler organizmada malign sürecin ilerlemesine neden olabilmektedir.[12,25] Nitekim yapılan çalışmalar, kanser olgularının çoğunda *TP53*'ün homozigot delesyon (HD), heterozigosite kaybı (loss of heterozygosity, LOH), nokta mutasyonları ve/veya metilasyon yoluyla etkisizleştirildiğini ve erken başlangıçlı malignensi gelişimine katkı sağladığını vurgulamaktadır.[12,26] *TP53* geninin kodladığı proteinin tümör baskılayıcı aktivitelerini bozan somatik değişiklikler tüm kanserlerin %50'sinde gözlenmektedir. Normal şartlarda yarı ömrü oldukça kısa olan p53 proteini birtakım değişiklikler ve/veya mutasyonlar sonucunda doğal konformasyonunu kaybederek DNA'ya bağlanamaz ve buna bağlı olarak hücre döngüsü ve ölümü üzerindeki kontrol edici etkinliğini gerçekleştiremez.[25] *TP53*'te gözlenen en baskın değişiklik olarak ön plana çıkan yanlış anlamlı mutasyonların yaklaşık %90'ı, p53 proteininin DNA'ya bağlanma bölgesini kodlayan dizide meydana gelmektedir.[27,28] Genetik veya epigenetik etkenlerin dışında, hücresel p53 seviyelerinin düzenlenmesinde ubikuitinasyon aracılı proteozomal degradasyon mekanizması da sıklıkla rol oynamaktadır.[12] Bu mekanizma üzerinden *MDM2* (mouse double minute 2 homolog), *MDM4*, herpesvirüs ilişkili ubikuitin spesifik proteaz, gibi genler ile insan papilloma virüsü E6 proteinin de hücresel p53 seviyeleri üzerinde etkili olduğu çeşitli çalışmalarda gösterilmiştir.[12] Literatürdeki bu bulgular, ubikuitin proteazom yolağının, p53 fonksiyonunun kaybıyla ilişkili olarak tümör oluşumu üzerinde teşvik edici bir etkiye sahip olduğunu desteklemektedir.

Tümör baskılayıcı aktiviteleri birçok kanser türünde sıklıkla çalışılmış olan ve hücre döngüsünün belirli fazlarına giriş ve çıkışın kontrolünde görev alan diğer tümör baskılayıcı genler *Retinoblastoma 1* (*Rb1*) ve *INK4* genleridir.[29]

İlk olarak ailesel retinoblastoma hastalığında mutasyona uğradığı gösterilen *Rb1* geninin ürünü pRb1'in daha sonra hücre döngüsü düzenleyicisi olarak kritik rol oynadığı keşfedilmiştir. pRb, hücre döngüsünün G1 fazında durdurulmasını sağlayarak, döngünün ilerlemesinde rol oynayan genlerin transkripsiyonunu kontrol etmektedir. pRb'nin fonksiyonel aktivitesi, normal durumda fosforilasyonla düzenlenmektedir. Sağlıklı hücrelerde, hücre döngüsü boyunca siklin bağımlı kinaz 2 (cyclin-dependent kinase 2, *cdk2*) ve siklin D (cyclin D, CCND1) kompleksleri tarafından pRb fosforillenerek inaktive edilir ve bu sayede pRb'nin hedef genleri üzerindeki baskılayıcı etkisi ortadan kaldırılır.[25] *INK4* ise bir CDK-inhibitörü olan p16 proteinini kodlamakta olup bu protein de pRb'ye benzer şekilde hücre döngüsünün G1-S fazı geçişinde kritik rol oynamaktadır.[30] Buna ek olarak *INK4*'ün inaktivasyonu sonucunda pRb'nin kontrolsüz olarak fosforile edildiği gösterilmiştir.[25] Çoğu kanserde Rb proteini tarafından yapılan bu kontrol sürecinin sıklıkla bozulduğu bilinmektedir. Bu durum da hücre döngüsünün sürekli olarak devam ettirilmesine ve kontrolsüz hücre çoğalmasına neden olmaktadır.[31] Larenks kanserinde sıklıkla görülen genetik değişikliklerden biri kromozom 9p21'de yerleşik olan *siklin-bağımlı kinaz inhibitörü 2A* (*cyclin dependent kinase inhibitor 2A*, *CDKN2A*) genindeki etkinlik kaybıdır. *CDKN2A* geninin etkinliğini kaybetmesine bağlı olarak p16 proteinin üretimi de azalmaktadır.[30] Yapılan çalışmalarda baş boyun ve diğer kanserlerde bu genin HD ya da LOH durumlarına ek olarak DNA metilasyonu veya nokta mutasyonu gibi genetik ve epigenetik değişikliklerin de *CDKN2A* ifadesi üzerinde etkili olduğu gösterilmiştir.[32]

Öte yandan, normalde hücre büyümesi kontrol elemanlarından biri olmasına rağmen her kanser türündeki etkileri değişiklik gösteren ve buna bağlı olarak da tedavi süreci ve stratejilerinin değişebildiği bazı genler veya gen aileleri de mevcuttur. Bunlardan biri olarak, *TGF-β* gen ailesi hücre büyümesi, hayatta kalım, farklılaşma, göç ve apoptoz gibi pek çok kritik hücresel yolakta görev alan ve insanlarda TGF-β1, β2 ve β3 şeklinde 3 farklı izoformu bulunan multifonksiyonel proteinleri kodlamaktadır.[33] TGF-β1, normal insan oral keratinositleri de dahil olmak üzere birçok hücre tipi için güçlü bir büyüme inhibitörüdür.[34] Bu proteinlerin etkili oldukları sinyal yolaklarındaki değişiklikler karsinogenez süreci de dahil olmak üzere birçok hastalıkla ilişkilendirilmiştir. TGF-β sinyal yolağındaki deregülasyon çoğu kanser türünde gözlenmekle beraber özellikle karsinogenezin başlatılmasında, ilerleyişinde ve metastatik sürecin tetiklenmesinde bu sinyal yolağının kritik roller üstlendiği bilinmektedir.[33] Ancak yolağın kanser hücrelerindeki tümör baskılayıcı veya tümör oluşumunu destekleyici rolü günümüzde halen daha tartışma konusudur. Karsinogenezin erken ve geç aşamalarındaki farklı etkinliklerinin yanı sıra TGF-β izoformları ve reseptörlerinin regülasyonu her kanser türüne göre de farklılık göstermektedir.[34] Örnek olarak, meme, kolon, pankreas kanserlerinde TGF-β1 aşırı ekspresyonun hastalığın ilerleyişi ile korele olduğu gözlenirken bazı kanserlerde TGF-β izoformlarının otokrin üretiminde önemli ölçüde azalma rapor edilmiştir.[34] Yapılan bir çalışmada oral karsinom hücre hatlarında, tümör hücrelerinin otokrin TGF-β1 üretimini baskılayarak *TGF-β*'nin büyüme kontrolü üzerindeki negatif etkisinden kaçtığı gösterilmiş.[34]

Moleküler çalışmalar, tümör baskılayıcı genlerdeki inaktivasyonun genellikle, tümör baskılayıcı lokusların içinde veya yakınında konumlanan polimorfik belirteçlerdeki heterozigozite kaybına yol açan ve sitogenetik olarak saptanamayan mikrodelesyonlarla ilişkili olduğunu göstermektedir.

Sonuç olarak; bu derleme kapsamında baş boyun kanserleri özelinde kanser gelişimi, prognozu, kanser hücresinin metastatik karakter kazanması, tedavi stratejileri ve/veya tedavi sonrası kanserin nüksü gibi karsinogenez sürecinde onkogen ve tümör baskılayıcı genlerin rollerinden bahsedilmiştir. Onkogenler ve tümör baskılayıcı genlerdeki genetik ve epigenetik değişiklikler hastalığın erken tanısı, prognozu, risk sınıflandırılması ve terapötik direnç gibi noktalarda baş boyun kanseri hastaları için hayati öneme sahip biyobelirteçler olarak hizmet edebilirler. Örneğin, *TP53*'teki mutasyonlar veya *EGFR*'nin aşırı ekspresyonu, geleneksel tedavilere verilen yanıtı etkileyebileceğinden bireysel temelde bu durumun bilinmesi geleneksel uygulama yerine kombine tedavilerinin kullanımına rehberlik edebilir. Bunun yanı sıra özellikle gelişen teknolojik ve bilimsel ilerlemeler sayesinde kişiye özel tedavi seçenekleri de gün geçtikçe artmaktadır. Buna yönelik olarak, spesifik onkogenleri (örn; EGFR inhibitörleri, RTK inhibitörleri gibi) veya tümör baskılayıcı kaybıyla bozulan sinyal yolaklarını (örn. PI3K/AKT yolu inhibitörleri gibi) hedef alan tedavi stratejileri de son yıllarda dikkat çekmektedir. Bu sayede yalnızca hastalığı değil bireyi de temel alan kişiselleştirilmiş ve daha yüksek etki kapasitesine sahip tedaviler uygulanabilmektedir. Ancak yine de karsinogenez sürecinin sadece genetik açıdan değerlendirilmesi hastalığın ve tedavi sürecinin yönetiminde eksik kalınmasına yol açabilmektedir. Genetik olarak bireyin sahip olduğu kalıtsal miras her ne kadar karsinogenez sürecinde çok büyük bir faktör olarak rol oynasa da elbette ki bu sürecin gelişimi ve ilerleyişinde çevresel etmenler de kritik önem taşımaktadır. Bu nedenle tanı ve tedavi süreçlerinde genetik ve çevresel faktörlerin karmaşık etkileşimi her zaman göz önünde bulundurulmalıdır.

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