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Effects of Phototherapy On Hematological Parameters in Newborns with Indirect Hyperbilirubinemia

İndirekt Hiperbilirubinemili Yenidoğanlarda Fototerapinin Hematolojik Parametreler Üzerine Etkileri

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Abstract

Objective: Indirect hyperbilirubinemia is an important problem in newborns and phototherapy has an important role in treatment. The aim of this study is to evaluate the effects of phototherapy on hematological parameters in newborns with indirect hyperbilirubinemia.

Materials and Methods: January 2021- March 2022, 60 newborns (term = 30 and preterm = 30) diagnosed and treated for indirect hyperbilirubinemia were included in this study.

Results: Twelve of the term babies (40%) had either Rh (n = 3) or ABO incompatibility (n = 9). Ten of the preterms (33%) had either Rh (n = 3) or ABO incompatibility (n = 7). The median values of phototherapy time of term and preterm newborns were 24 (range, 6-40) and 11 hours (range, 4-36), respectively. After phototherapy, WBC and RBC counts, Hb, MCV and RDW values of term newborns decreased significantly. In preterm newborns white blood cell count, red blood cell count, hemoglobin, mean corpuscular volume of red blood cells, red blood distribution width (WBC and lymphocyte, counts and Hb, MCV and RDW) values decreased significantly, while a statistically significant increase in mean corpuscular hemoglobin concentration (MCHC) values and the percentage of monocyte counts were determined.

Conclusion: Our study suggests that phototherapy has various effects on hematological parameters. Some of these effects can be explained by Rh or ABO incompatibility, but prospective studies including more patients are needed to explain the changes in hematological parameters in newborns without Rh or ABO incompatibility.

Keywords: Newborn, Indirect Hyperbilirubinemia, Phototherapy, Hematologic Parameters

&

Öz

Amaç: İndirekt hiperbilirubinemi yenidoğanlarda önemli bir sorundur ve tedavide fototerapi önemli biryere sahiptir. Bu çalışmanın amacı indirekt hiperbilirubinemili yenidoğanlarda fototerapinin hematolojik parametreler üzerindeki etkilerini değerlendirmektir.

Gereç ve Yöntemler: Ocak 2021- Mart 2022 tarihleri arasında indirekt hiperbilirubinemi tanısı alan ve tedavi edilen 60 yenidoğan (term = 30 ve preterm = 30) bu çalışmaya dahil edildi.

Bulgular: Yenidoğan dönemlerinin 12'sinde (%40) Rh uyuşmazlığı (n = 3) veya ABO uyuşmazlığı (n = 9) vardı, erken doğmuş yenidoğanların 10'unda (%33) Rh uyuşmazlığı (n = 3) veya ABO uyuşmazlığı vardı. (n = 7). Zamanında ve erken doğmuş yenidoğanların medyan fototerapi süreleri sırasıyla 24 (dağılım, 6-40) ve 11 saat (aralık, 4-36) idi. Fototerapi sonrası term bebeklerde beyaz küre sayısı, kırmızı küre sayısı, hemoglobin, kırmızı kan hücreleri ortalama korpüsküler hacmi, kırmızı kan dağılım genişliği (WBC ve RBC, Hb, MCV ve RDW) değerlerinin istatistiksel olarak anlamlı oranda azaldığı saptandı. Preterm yenidoğanlarda WBC ve lenfosit, Hb, MCV ve RDW değerlerinde istatistiksel olarak anlamlı oranda azalma olurken, ortalama korpüsküler hemoglobin konsantrasyonu (MCHC) değerleri ve monosit yüzdelerinde artış saptandı.

Sonuç: Çalışmamız fototerapinin bazı hematolojik parametreler üzerine etkisi olduğunu düşündürmektedir. Bu etkilerin bir kısmı Rh veya ABO uyuşmazlığı ile açıklanabilir, ancak Rh veya ABO uyuşmazlığı olmayan yenidoğanlarda hematolojik parametrelerdeki değişiklikleri açıklamak için daha fazla hastada prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Yenidoğan, Direk Hiperbilirubinemi, Fototerapi, Hematolojik Parametreler

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Introduction

Indirect hyperbilirubinemia is one of the most common problems encountered in newborns. It is also one of the most common reason for hospitalizing newborns, especially at first week of the life. Despite that bilirubin-related complications and deaths have been reduced with phototherapy and exchange transfusion, and rhesus immunoglobulin prophylaxis in the presence of rhesus (Rh) incompatibility, hyperbiluribinemia still maintains its importance, especially in underdeveloped and developing countries. The main causes of neonatal hyperbilirubinemia are (i) hemolytic conditions which include ABO incompatibility, Rh incompatibility, subgroup incompatibility, glucose 6 phosphate dehydrogenase deficiency, and/or some others; (ii) dehydration due to lack of proper feeding, (iii) breast milk jaundice, (iv) prematurity, (v) cephalohematoma; (vi) polycythemia; and (vii) other factors (1-3).

Today, the treatment of indirect hyperbilirubinemia is phototherapy and exchange transfusion if necessary. In addition, intravenous immunoglobin administration also may be lifesaving in severe hemolytic diseases of the newborn (4). Apart from these treatments, there are some drugs in use with unproven efficacy such as phenobarbital, ursodeoxycholic acid, metalloporphyrins, and clofibrate.

Phototherapy has some acute and possible long-term side effects. Acute side effects are interference with maternal-infant interaction, imbalance between thermal environment and insensible water loss, electrolyte disturbance, disorder of circadian rhythms, and Bronze baby syndrome. The possible long-term side effects which may be related with photherapy are allergic diseases, melanocytic nevi, melanoma, skin cancer, patency of ductus arteriosus and retinal damage (5). The studies showing the possible unfavorable effects of phototherapy on hematological parameters are quite limited (6-10).

In this study, we aimed to evaluate the effects of phototherapy on hematological parameters in newborns with indirect hyperbilirubinemia.

Materials and Methods

Population and Methods

Date

The charts of newborns who received phototherapy for indirect hyperbilirubinemia in the January 2021-March 2022 were retrospectively analyzed.

Design

This study is a retrospective cross-sectional study.

Hypothesis

Our null hypothesis (H0) is that phototherapy has no effect on hematological parameters. Our alternative hypothesis (H1) is that phototherapy has effect on hematological parameters.

The charts of the newborns who received phototherapy with the diagnosis of indirect hyperbilirubinemia were retrospectively analyzed. Newborns who had complete blood counts one hour before phototherapy starts and one hour after phototherapy ends were included in the study. The newborns included in this study were divided into two groups as preterm and term. The indirect causes of hyperbilirubinemia in the newborns included in the study were as follows; Rh and ABO incompatibility, sepsis, neonatal polycythemia, lactation failure jaundice. The exclusion criteria were as follows; a history of bleeding, preeclampsia or eclampsia, chorioamnionitis, chronic disease, premature rupture of membranes, diabetes, thyroid disease, smoking or receiving drugs such as anticonvulsant, antidepressant, insulin, chemotherapy or cortisone (Except preterm. Because in preterms, cortisone is used for intrauterine use for lung development), exchanged patients, patients who have used IVIG in patients with direct cooms-positive

blood group incompatibility, patients whose complete blood counts was not studied before and after phototherapy. Also, the neonates with birth asphyxia were excluded (6).

If ABO and Rh blood group incompatibility is known in term and preterm babies, bilirubin was measured at 4-hour intervals after birth on the first day, and complete blood count was performed within the first hour after birth. One hour after phototherapy was terminated, complete blood count and bilirubin were checked.

If there was no blood group incompatibility, complete blood count and bilirubin were measured in the preterms within 1 hour and at the 24th hour after birth. Complete blood count and bilirubin were measured at the 24th hour of term.

The bilirubin level at which to provide phototherapy was decided as according to Bhutani nomogram.

In the study, bilateral phototherapy devices were applied to the babies if they were receiving intensive treatment and care in the third stage, and phototherapy was applied with intensive phototherapy devices if their mothers were caring. The duration of application was specific to the patient. The newborns' demographic features including gender, birth weight, gestational age, the age at admission, and duration of phototherapy, newborns' and their mothers' blood groups, complete blood counts before and after phototherapy have been noted.

Ethical Considerations

For this study, the approval was obtained from the local ethics committee of Selcuk University Faculty of Medicine (No: 2021/85). As this study was a retrospective cross-sectional study, informed consent was not obtained from the patients or their guardians. This study was carried out according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines.

Statistical Analysis

In this study, GraphPadPrisim 9 Software was used for statistical analysis (La Jolla, CA, USA). The frequency and percentage values were are given for categorical data. As descriptive statistics, mean ± standard deviation values were used for numeric data with normal distribution, and median (Quartile 1 and Quartile 3) values were used for numeric data without normal distribution. In comparison of hematological parameters before and after phototherapy, paired sample t test was used for comparisons if it satisfied the necessary assumptions for paired t test, and Wilcoxon signed rank test was used if it did not satisfy the necessary assumptions. If alpha (p) value was <.05, it was considered statistically significant.

Results

January 2021- March 2022, 60 newborns diagnosed with indirect hyperbilirubinemia and treated were included in this study. Thirty of the newborns were term and 30 were preterm.

Demographic Features

The demographic features of both term and preterm newborns are shown in Table 1.

The ethnicity of term newborns was Turkish (n = 16, 53%) and Syrian refugees (n = 14, 47%). The term newborns' gestational age ranged from 37 to 41 weeks (median, 39 weeks). There were 16 males (53%) and 14 females (47%) with a median age of five days (1-11 days) at admission. The median birth weight of the term newborns was 3075 grams (range, 2300-4315).

In preterm newborns, 13 were Turkish (43%) and 17 were Syrian refugees (57%). Their gestational age ranged from 26 to 35.5 weeks (median, 33 weeks). The gender distribution of preterm newborns was as six males (20%) and 24 females (80%). Their ages at admission ranged from one to 15 days (median, one day). The median birth weight of the preterm newborns was 2100 grams (range, 870-2940).

Effects of Phototherapy on Hematologic Parameters

Twelve of the neonatal terms (40%) had either Rh incompatibility (n = 3) or ABO incompatibility (n = 9), 10 of the preterm newborns (33%) had either Rh incompatibility (n = 3) or ABO incompatibility (n = 7). The median durations of phototherapy in term and preterm newborns were 24 (range, 6-40) and 11 hours (range, 4-36), respectively.

In Term Newborns

The effect of phototherapy on hematological parameters in term newborns is shown in Table 2 and Figure 1. The mean term newborns'white blood cells counts after the phototherapy were significantly lower than those before phototherapy (p = .0187) (Figure 1a). The decrease in WBC count was significantly more obvious in term babies with either RH or ABO imcompatibility (p = .027). While there was no statistically significant decrease in WBC counts analysed before and after phototherapy in term newborns with neither Rh nor ABO incompatibility (p =.82).

Mean red blood cell counts were significantly lower after phototherapy in all term newborns, term newborns with either Rh or ABO incompatibility, as well as in term neonates with neither Rh nor ABO incompatibility (p values: .0007, .0385, and. 0099, respectively) (Figure 1b).

Similarly, mean Hb levels were significantly lower after phototherapy in all term newborns, term newborns with either Rh or ABO incompatibility, and in term newborns with neither Rh nor ABO incompatibility (p values: .0001, .0225, .0031, respectively) (Figure 1c).

The term newborns' mean MCV level were significantly lower after phototherapy than those before phototherapy (p = .0036) (Figure 1d). The decrease in MCV level was significantly more obvious in term babies with either RH or ABO incompatibility (p = .0036), while there was no statistically significant decrease in term newborns with neither Rh nor ABO incompatibility (p =.3).

The RDW values were lower after phototherapy in all term newborns, term newborns with either Rh or ABO incompatibility, and in term newborns with neither Rh nor ABO incompatibility (p values: <.0001, .0107, .0065, respectively) (Figure 1e).

No statistically significant difference was found in between MCH, MCHC, platelet counts, MPV, PDW, lymphocyte counts and the counts and percentage of leukocyte subgroups in term newborns analysed before and after phototherapy (Table 2).

In Preterm Newborns Preterm Newborns

The preterm newborns' white blood cell counts, Hb levels, red cell distribution width and lymphocyte counts, were statistically lower after phototherapy phototherapy than before phototherapy (p = .002, p=.0131, p = .0036, p = .0081, respectively) (Figure 2a, Figure 2b, Figure 2e, Figure 2f). The decrease in WBC count, Hb level, RDW and lymphocyte count after phototherapy were significantly more obvious in preterm babies with either RH or ABO incompatibility (p = .0058, p = .0112, p = .0529, p = .0119, respectively). While there was no statistically significant decrease in WBC count, Hb level, RDW of preterm newborns without either Rh or ABO incompatibility (p =.08, p=.21, p = .043 and p = .57, respectively).

Mean corpuscular hemoglobin concentrations and the monocyte percentages of all preterm newborns were significantly higher after phototherapy than before phototherapy (p = .0083 and p= <.0001) (Figure 2d and Figure 2g). The monocyte percentages were also significantly higher in preterm newborns with Rh or ABO incompatibility, and in preterm newborns without either Rh or ABO incompatibility after phototherapy (p=.0009 and p=.0067, respectively). The increase in MCV level after phototherapy was significantly more obvious in preterm newborns with either RH or ABO imcompatibility (p = .0277), while there was no statistically significant decrease in preterm newborns without either Rh or ABO incompatibility (p =.07).

No statistically significant difference was observed between RBC, MCH, platelet count, MPV, PDW, neutrophil number and percentage, eosinophil number and percentage, lymphocyte percentage and monocyte count of preterm newborns evaluated before phototerapy and after phototerapy.

Discussion

In this study, changes in various hematological parameters were observed after therapeutic phototherapy for neonatal hyperbilirubinemia.

The studies investigating the effect of phototherapy on hematological parameters are quite limited.6-8 In the study of Timilsina et al. (11) with 120 patients, it was found that phototherapy was associated with a significant decrease in absolute monocyte count and platelet count and increased lymphocyte count and MCH. Hematological parameters that were affected in the results of this study were considered normal in our study. We think that this different result may be related to the causes of indirect hyperbilirubinemia in infants.

Altuntas et al. (6) investigated the effect of phototherapy on WBC parameters and neutrophil volume, volume conductivity scatter (VCS) parameters in 74 newborns with indirect hyperbiluribinemia. While our study reported no changes in eosinophils and basophils, Altuntas et al. (6) found a decrease in leukocyte and neutrophil counts and an increase in eosinophil and basophil counts in newborns who received phototherapy. However, they determined that it had no effect on lymphocyte or monocyte counts. In addition, they observed a significant decrease in neutrophil volume values and a significant increase in neutrophil scatter values after PT.

In another study by Aydin et al. (7), 306 newborns with indirect hyperbilirubinemia were included. In this study, the most common cause of indirect hyperbilirubinemia was ABO incompatibility. The authors indicated a low eosinophil count with a high bilirubin suppression of vascular cell adhesion molecule-1 before phototherapy compared to the control group. They emphasized that the decrease in bilirubin levels after phototherapy and consequently decrease in the pressure on vascular cell adhesion molecule-1 may increase the number of eosinophils. In the study which investigated the effect of phototherapy on lymphocyte subset in newborns with indirect hyperbilirubinemia, the authors determined that the patients' the percentage of CD19+ lymphocyte was lower than control group before phototherapy (8). After phototherapy, it was observed that the low percentage of CD19+ lymphocytes approached the control group. The authors tried to explain this by the inhibitory effect of unconjugated hyperbilirubinemia on CD19 B cells, similar to Khan and Poduval (12). In another study done in Turkey by Karabayir et al. (13), CD4+ lymphocytes were found to increase after eight hours of phototherapy. We cannot discuss such an outcome in our study because we did not compare lymphocyte subgroups.

In our study, a decrease in leukocyte and erythrocyte counts, and Hb, MCV and RDW values were found in term newborns after phototherapy. The decrease in leukocyte count was statistically significant in those with Rh or ABO incompatibility. However, this decrease was not statistically significant in newborns without either Rh or ABO incompatibility. It was observed that the decrease in both erythrocytes count, Hb, and RDW levels was observed in term newborns with Rh or ABO incompatibility and in term newborns without Rh or ABO incompatibility. However, interestingly, a statistically significant decrease in MCV value was observed only in term newborns with Rh or ABO incompatibility.

In preterm newborns, while the leukocyte and lymphocyte count, HB, MCV, and RDW values decreased, MCHC value and monocyte percentage increased. Changes in MCV and monocyte percentage were detected in preterm newborns both with Rh or ABO incompatibility and without Rh or ABO incompatibility. It suggests that Rh or ABO incompatibility plays a more important role in the changes in hematological parameters after phototherapy in all newborns. However, changes in hematological parameters observed in newborns without Rh or ABO incompatibility do not mean to ignore the possible effects of phototherapy.

In our study, platelet count did not change after phototherapy in either term babies or preterm babies. Maj Sanjeev Khera et al. (14) in 2011 found that 35% of the 100 neonates studied had thrombocytopenia after phototherapy. Unlike, Timilsinaet al. (11) and Pishva et al. (15) observed that in their study platelet counts has decreased. It has been emphasized in the literature that this decrease in platelet level may be due to the effect of phototherapythe deterioration of the oxidant-antioxidant balance or the temporary decrease in general DNA, RNA and protein synthesis (10).

There are limitations in our study. Fistly there were not enough newborns with Rh and ABO incompatibility. Another limitation is that we did not include the healthy newborn control group who did not receive phototherapy. If had it been done, we might have some idea of possible normal physiological changes in various blood cell counts. Because of the limited resources, further hematological parameters could not be included in the study.

Conclusion

Our results suggest that although the major causes of hematological changes at newborns treated with phototherapy are related with Rh or ABO incompatibility. Changes in hematological parameters observed in newborns without Rh or ABO incompatibility also may be related to phototherapy.

Ethics Committee Approval: The approval was obtained from the local ethics committee of Selcuk University Faculty of Medicine (No: 2021/85).

Informed Consent: As this study was a retrospective cross-sectional study, informed consent was not obtained from the patients or their guardians.

Conflict of Interest: Authors declared no conflict of interest.

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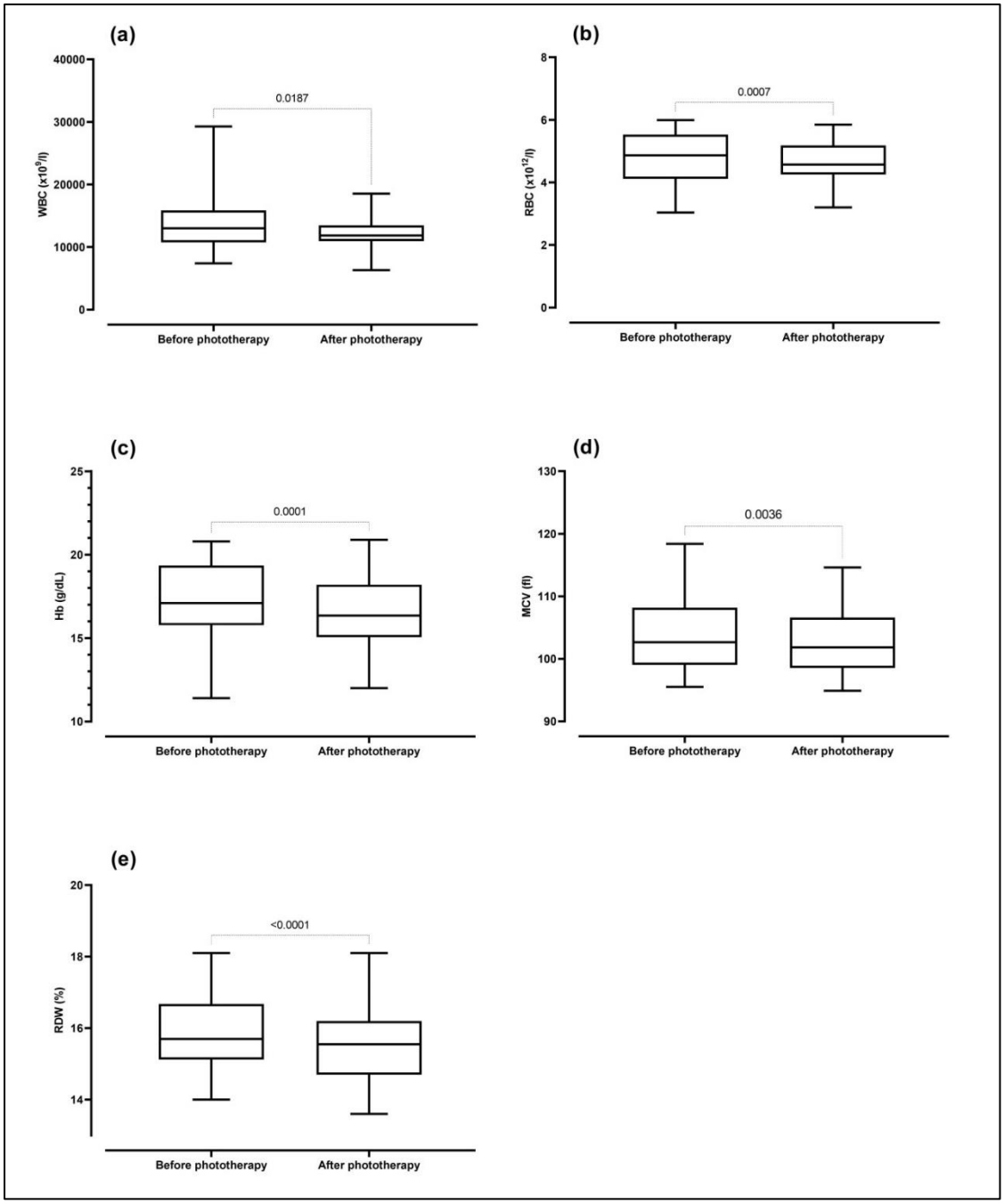
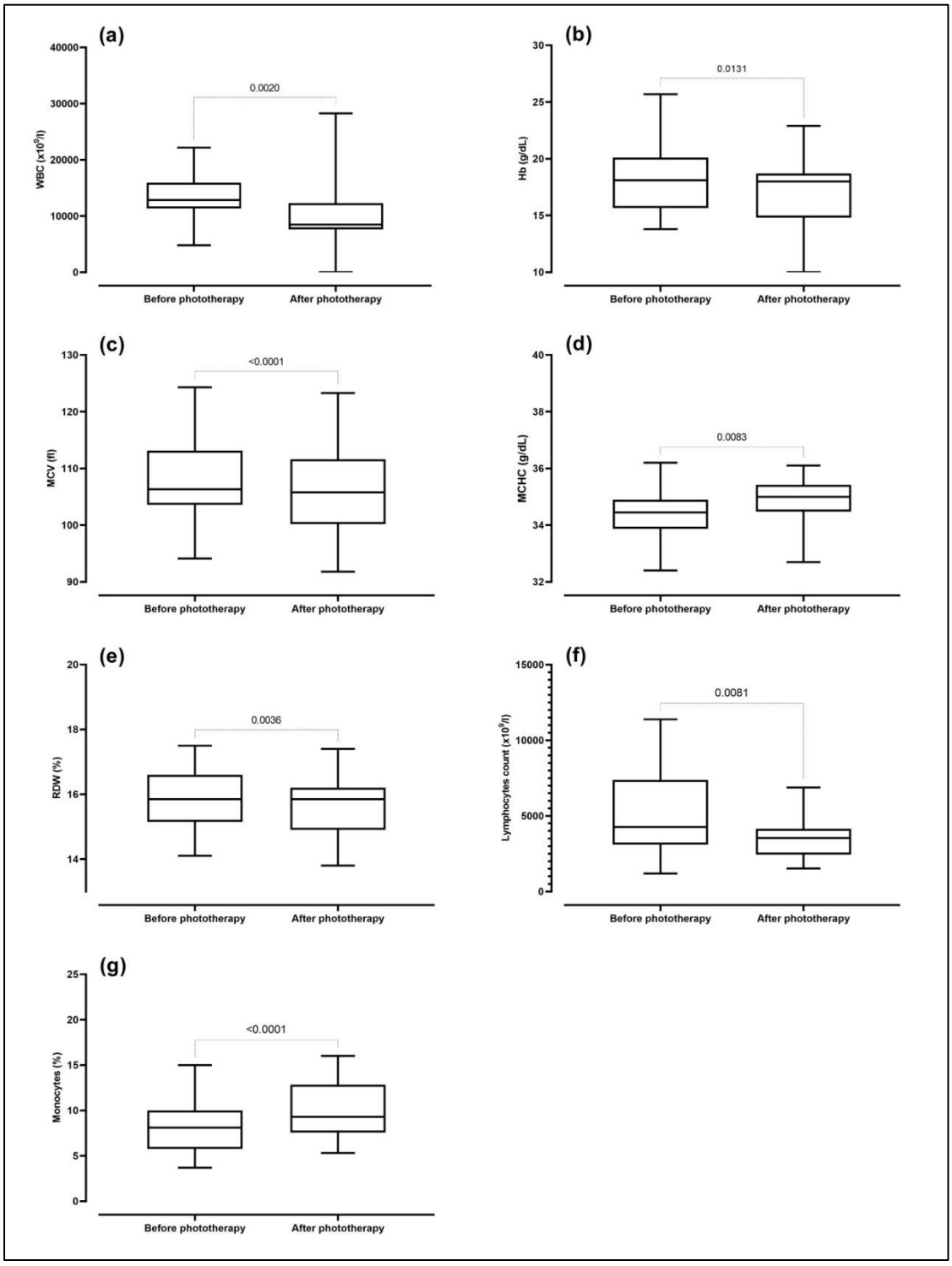


Fgure 1. The changes of (a) white blood cells, (b) red blood cells, (c) Hb, (d) MCV and (e9 RDW values in term newborns



Fgure 2. The changes of (a) white blood cells, (b) Hb, (c) MCV, (d) MCHC, (e) RDW, (f) lymphocyte counts, and (g) the percentage of monocyte in preterm newborns

Table 1.
The demographic features of newborns

	Term newborns	Preterm newborns
Ethnicity		
Turks	16 (53%)	13 (43%)
Refugee (from Syria)	14 (47%)	17 (57%)
Gender		
Male	16 (53%)	6 (20%)
Female	14 (47%)	24 (80%)
Median gestation age (weeks),	39	33
(range)	(37-41)	(26-35.5)
Median birth weight (grams),	3075	2100
(range)	(2300-4315)	(870-2940)
Median age at admission, (days),	5	1
(range)	(1-11)	(1-15)
Median duration of phototherapy, (hours)	24	11
(range)	(6-40)	(4-36)

Table 2.

Effect of Phototherapy on Hematological Parameters in Term Newborns

Parameters	Before Phototherapy (*M* ± *SD*)	After Phototherapy (*M* ± *SD*)	Difference (After – Before) (*M* ± *SD*)	t	df	p
WBC, (x 10^9/l)	14198 ± 5086	12141 ± 2528	-2057 ± 4523	2.491	29	.0187
Incompatibility	15115 ± 5187	11563 ± 2292	-3553 ± 4825	2.551	10	.027
No incompatibility*	12275 (Q1=10530, Q3=15863	11770 (Q1=10945, Q3=13878)	-30	NA	NA	.82
RBC, (x10^{12}/l)	4.843 ± 0.74	4.663 ± 0.65	-0.181 ± 0.26	3.799	29	.0007
Incompatibility	4.531 ± 0.88	4.343 ± 0.73	-0.188 ± 0.28	2.349	10	.0385
No incompatibility	5.052 ± 0.57	4.876 ± 0.51	-0.175 ± 0.26	2.904	17	.0099
Hb, (g/dL)	17.2 ± 2.3	16.5 ± 2.1	-0.72 ± 0.9	4.384	29	.0001
Incompatibility	16.2 ± 2.8	15.4 ± 2.2	-0.783 ± 1.023	2.652	10	.0225
No incompatibility	17.9 ± 1.8	17.2 ± 1.7	-0.678 ± 0.83	3.441	17	.0031
MCV, (fl)	103.8 ± 5.7	102.6 ± 4.7	-1.267 ± 2.187	3.173	29	.0036
Incompatibility	106.0 ± 7.8	103.4 ± 6.1	-2.642 ± 2.48	3.691	10	.0036
No incompatibility	102.4 ± 3.2	102.1 ± 3.6	-0.35 ± 1.398	1.062	17	.3
MCH, (pg)	35.7 ± 1.7	35.5 ± 1.7	-0.18 ± 0.5	1.779	29	.08
MCHC, (g/dL)	34.4 ± 0.9	34.6 ± 0.6	0.24 ± 0.9	1.378	29	.17
RDW, (%)	15.8 ± 1.0	15.6 ± 1.0	-0.2667 ± 0.3	4.662	29	<.0001
Incompatibility	16.2 ± 1.3	15.9 ± 1.1	-0.291 ± 0.3	3.132	10	.0107
No incompatibility	15.5 ± 0.8	15.3 ± 0.8	-0.222 ± 0.3	3.101	17	.0065
Platelets, (x 10^9/l)*	296500 (Q1=249750, Q3=36000)	294500 (Q1=260250, Q3=340250)	-100.0	NA	NA	.99
MPV, (fl)	9.6 (Q1=9.1, Q3=10.3)	9.6 (Q1=9.2, Q3=10.3)	0	NA	NA	.79
PDW, (%)*	16.4 ± 0.3	16.4 ± 0.26	-0.02 ± 0.17	0.712	29	.48
Neutrophil, (x 10^9/l)	4930 (Q1=3200, Q3=8578)	4330 (Q1=3345, Q3=7200)	-165	NA	NA	.17
Neutrophil, (%)	38.6 (Q1=28.6, Q3=56.2)	36.7 (Q1=29.8, Q3=53)	-1.25	NA	NA	.43
Lymphocyte, (x 10^9/l)	5348 ± 1332	4957 ± 1900	-390.7	1.378	29	.17
Lymphocyte, (%)	43.5 (Q1=31.3, Q3=49.6)	45.6 (Q1=27.3, Q3=52.2)	1800	NA	NA	.81
Monocyte, (x 10^9/l)	1348 ± 474.5	1308 ± 487.3	-40.0	0.726	29	.47
Monocyte, (%)	9.9 (Q1=6.6, Q3=12.8)	10.5 (Q1=8.3, Q3=13.8)	0.4	NA	NA	.22
Eosinophil, (x 10^9/l)	579.3 ± 342.5	594.3 ± 312.7	15.0 ± 213.1	0.385	29	.7
Eosinophil, (%)	4.7 ± 3.0	5.0 ± 2.6	0.3	0.945	29	.35

*Wilcoxon t test was used because it could not satisfy the necessary assumption for the paired t test. Therefore, median (Quartile 1 and 3) values were given as descriptive statistics. **NA**: not available, **WBC**: white blood cell, **RBC**: red blood cell, **Hb**: hemoglobin, **MCV**: mean corpuscular volume, **MCH**: mean corpuscular hemoglobin, **MCHC**: mean corpuscular hemoglobin concentration, **RDW**: red cell distribution width, **MPV**: mean platelet volume, **PDW**: platelet distribution width

Table 3.

Effect of Phototherapy on Hematological Parameters in Preterm Newborns

Parameters	Before Phototherapy ($M \pm SD$)	After Phototherapy ($M \pm SD$)	Difference (After – Before)($M \pm SD$)	t	df	p
WBC, (x 10^9/l)	13192 ± 4290	10427 ± 5599	-2765	3.394	29	.002
Incompatibility	13144 ± 3856	8473 ± 2281	-4671	3.590	9	.0058
No incompatibility	13216 ± 4588	11404 ± 6510	-1812 ± 4417	1.835	19	.08
RBC, (x10^{12}/l)*	4.960 (Q1=4.435, Q3=5.323)	4.825 (Q1=4.275, Q3=5.188)	-0.185	NA	NA	.12
Hb, (g/dL)	18.2 ± 2.7	17.2 ± 2.8	-0.99 ± 2.05	2.642	29	.0131
Incompatibility	19.1 ± 2.7	16.7 ± 3.2	-2.370 ± 2.36	3.180	9	.0112
No incompatibility*	17.8 (Q1=15.5, Q3=18.9)	18.0 (Q1=14.8, Q3=18.6)	-0.55	NA	NA	.21
MCV, (fl)*	106.3 (Q1=103.6, Q3=113.1)	105.8 (Q1=100.2, Q3=111.6)	-2.050	NA	NA	<.0001
Incompatibility	111.3 ± 8.4	108.7 ± 8.6	-2.610 ± 2.1	3.945	9	.0034
No incompatibility*	105.6 (Q1=103.5, Q3=111.7)	104.9 (Q1=100.8, Q3=107.9)	-2.050	NA	NA	.0003
MCH, (pg)*	37.4 (Q1=35.2, Q3=38.9)	36.7 (Q1=35.4, Q3=38.8)	-0.1	NA	NA	.17
MCHC, (g/dL)	34.4 ± 0.9	34.9 ± 0.7	0.54 ± 1.0	2.835	29	.0083
Incompatibility	33.9 ± 0.9	34.5 ± 0.8	0.65 ± 0.78	2.623	9	.0277
No incompatibility	36.6 ± 0.8	35.1 ± 0.7	0.48 ± 1.16	1.855	19	.07
RDW, (%)*	15.9 (Q1=15.2, Q3=16.6)	15.9 (Q1=14.9, Q3=16.2)	-0.25	NA	NA	.0036
Incompatibility*	16 (Q1=15.5, Q3=16.6)	15.4 (Q1=14.9, Q3=16.0)	-0.65	NA	NA	.0430
No incompatibility*	15.9 (Q1=15.1, Q3=16.6)	15.9 (Q1=15.1, Q3=16.6)	-0.1	NA	NA	.0529
Platelets, (x 10^9/l)*	214000 (Q1=179000, Q3=287250)	207500 (Q1=181500, Q3=269750)	1000	NA	NA	.81
MPV, (fl)*	9.6 (Q1=8.9, Q3=10.2)	9.5 (Q1=9.0, Q3=10.3)	0.05	NA	NA	.81
PDW, (%)*	16.4 (Q1=16.1, Q3=16.7)	16.5 (Q1=16.2, Q3=16.8)	0.1	NA	NA	.07
Neutrophil, (x 10^9/l)	6552 ± 3978	5991 ± 3996	-561 ± 4350	0.706	29	.48
Neutrophil, (%)	47.6 ± 20.3	52.2 ± 13.5	4.7 ± 26.2	0.974	29	.33
Lymphocyte, (x 10^9/l)	5177 ± 2786	3479 ± 1236	-1698 ± 3274	2.841	29	.0081
Incompatibility	7018 ± 2878	3280 ± 1521	-3738 ± 3763	3.141	9	.0119
No incompatibility*	3755 (Q1=2868, Q3=5468)	3655 (Q1=2443, Q3=4510)	75.0	NA	NA	.57
Lymphocyte, (%)	41.6 ± 21.0	35.0 ± 12.0	-6.5 ± 24.6	1.459	29	.15
Monocyte, (x 10^9/l)	1118 ± 672	1121 ± 752	2333 ± 351	0.036	29	.97
Monocyte, (%)	8.1 ± 3.0	10.0 ± 3.2	1.84 ± 2.1	4.744	29	< .0001
Incompatibility	6.1 ± 2.1	8.3 ± 2.1	2.3 ± 1.5	4.877	9	.0009
No incompatibility	9.2 ± 2.9	10.8 ± 3.3	1.6 ± 2.4	3.045	19	.0067
Eosinophil, (x 10^9/l)	287.3 ± 351.4	273.0 ± 343.6	-14.3 ± 165.9	0.473	29	.63
Eosinophil, (%)*	1.7 (Q1=0.6, Q3=2.3)	1.1 (Q1=0.2, Q3=5.1)	-0.05	NA	NA	.92

*Wilcoxon t test was used because it could not satisfy the necessary assumption for the paired t test. Therefore, median (Quartile 1 and 3) values were given as descriptive statistics. **NA**: not available, **WBC**: white blood cell, **RBC**: red blood cell, **Hb**: hemoglobin, **MCV**: mean corpuscular volume, **MCH**: mean corpuscular hemoglobin, **MCHC**: mean corpuscular hemoglobin concentration, **RDW**: red cell distribution width, **MPV**: mean platelet volume, **PDW**: platelet distribution width

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No Direct Association of Myelin Oligodendrocyte Glycoprotein (MOG) Gene Polymorphism (Val142leu) in Genetic Susceptibility to Migraine

Miyelin Oligodendrosit Glikoprotein (MOG) Gen Polimorfizminin, (Val142Leu) Migrene Genetik Duyarlılık ile Doğrudan Bir İlişkisi Yoktur

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Abstract

Objective: Genes which are involved in immune response portray possible candidate genes in migraine. One of those genes is that myelin oligodendrocyte glycoprotein (MOG) that plays an important role in mediating the complement cascade. The purpose of our study is to show the effect of MOG G511C (Val142Leu; rs2857766) polymorphism in migraine attack frequency.

Materials and Methods: In the cohort of 101 Turkish migraine patients and in a control group of 101 healthy subjects, MOG Val142Leu alleles' distribution was examined. Restriction fragment length polymorphism (RFLP) was carried out to genotype this polymorphism.

Results: Although MOG Leu allele frequency was determined as under-represented in migraine patients, any significant difference between the patient and control groups' genotype, and allele frequencies were not obtained [OR=0.47 (0.21-1.08), p=0.053 for genotypes; OR=0.50 (0.23-1.11), p=0.060 for alleles]. However, a statistically significant relationship between MOG G511C (Val142Leu) polymorphism and the decreased migraine attack frequency was determined [OR=11.71 (1.32-103.77), p=0.013]. Val/Leu genotype frequency increrased in migraine patients with two or fewer attacks per month.

Conclusion: Migraine attack frequency might be related with MOG Val142Leu heterozygote genotype. So we think that MOG gene might be related to genetic susceptibility to migraine in the human leukocyte antigen (HLA) region.

Keywords: Migraine, Myelin oligodendrocyte glycoprotein, Polymorphism, HLA, Val142Leu

&

Öz

Amaç: İmmün yanıtta yer alan genler, migrende olası aday genleri gösterir. Bu genlerden biri, kompleman kaskadına aracılık etmede önemli bir rol oynayan miyelin oligodendrosit glikoprotein genidir (MOG). Çalışmamızın amacı; migren atak sıklığında MOG G511C (Val142Leu; rs2857766) polimorfizminin etkisini göstermektir.

Gereç ve Yöntemler: 101 Türk migren hastası kohortunda ve 101 sağlıklı denekten oluşan kontrol grubunda MOG Val142Leu alellerinin dağılımı incelendi. Bu polimorfizmi genotiplemek için restriksiyon fragman uzunluk polimorfizmi (RFLP) yapıldı.

Bulgular: MOG Leu allel frekansının, migren hastalarında yetersiz temsil edildiğinin belirlenmesine rağmen hasta ve kontrol grubu genotipleri ve allel frekansları arasında anlamlı bir farklılık elde edilemedi [OR=0,47 (0,21-1,08), genotipler için p=0,053; OR=0,50 (0,23-1,11), aleller için p=0,060]. Ancak MOG G511C (Val142Leu) polimorfizmi ile azalmış migren atak sıklığı arasında istatistiksel olarak anlamlı bir ilişki saptandı [OR=11,71 (1,32-103,77), p=0,013]. Ayda iki veya daha az atak geçiren migren hastalarında Val/Leu genotip sıklığı artmıştır.

Sonuç: Migren atak sıklığı, MOG Val142Leu heterozigot genotipi ile ilişkili olabilir. Dolayısıyla MOG geminin, insan lökosit antijeni (HLA) bölgesinde migrene genetik yatkınlıkla ilişkili olabileceğini öngörmekteyiz.

Anahtar Kelimeler: Migren, Miyelin oligodendrosit glikoprotein, Polimorfizm, HLA, Val142Leu

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Introduction

Migraine is a common episodic brain disorder in which environmental and genetic factors involved in its pathophysiology (1). The disease affects 15% of population worldwide. The migraine frequency is three times more common in females (15-18%) than in males (6%). It has been reported that the higher prevalence in women is typically due to hormonal fluctuations or estrogen withdrawal (2). The International Headache Society (IHS) categorized different types of headache [i.e. migraine with aura (MA) and without aura (MO)] based on the aura (1). It is widely believed that cortical spreading depression (CSD) is the pathophysiological mechanism of the aura. Cortical spreading depression is a slowly propagating wave characterized by the loss of all spontaneous or evoked synaptic activity, and activation potentials resulting in near complete depolarization of neuron and glial cells for migraine aura (3).

In heredity studies, the heritability of migraine was approximately determined as 42% (3). Since familial hemiplegic migraine (FHM) is an autosomal dominant inherited disease caused by mutations in three genes encoding ion channels (CACNA1A, ATP1A2 and SCN1A), it is pathophysiologically accepted as a model of hemifegic migraine with aura. With genome-wide association studies, 38 migraine-related ion channel and non-ion channel coding genes were also identified with highly related to common migraine (4). However, etiology and pathophsiology of the migraine is not known well.

In migraine patients, immune system disregulation is supported with the presence of immune activation caused by patients' peripheral blood and cerebrospinal fluids' aberrant amount of proinflammatory cytokines and receptors (4). For normal development of central nervous system (CNS) along with many neuropsychiatric disorder pathogenesis, cytokines are required. The cytokine molecules can change neurotransmitter and neuropeptide systems in addition to affecting nerve cells (5, 6). The human leukocyte antigen (HLA) gene region contains a wide range of highly polymorphic closely linked genes. An increase in shared HLA haplotypes in eight households was showed by investigating the patients with MO. So, this indicates the potential linkage between heredity of migraine and HLA (6). For polymorphisms of three genes [i.e. tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β) and the *16 allele of DRB1] and migraine, an important association was detected (7-9). Myelin oligodendrocyte glycoprotein (MOG) gene is only detected in the mammalian CNS and mapped to 6p21.3-6p22 within 60-kilobase telomeric region of HLA-F locus. The gene polymorphisms have a linkage disequilibrium with other migraine susceptibility genes in HLA region (10). The protein is compromised 245 amino acids, synthesized in the endoplasmic reticulum (ER) in oligodentrocytes and subsequently transfered to the membrane surface of the oligodentrocyte (OL) cell, and outer surface of myelin sheath in the CNS (11). MOG protein is a cellular adhesion molecule, complement sytem activator, regulator of stability of the oligodendrocyte microtubules and mediates associations between myelin, and the immune system. Variants of the MOG gene may affect the advance of autoimmune disorders via mechanisms such as amino acid substitutions and altered gene expression (12). In pathophysiology of migraine with and without aura, responsible genes are still unknown; however, sterile inflammation might play a significant role in cranial vasculature endothelial stage (13). Linkage analysis has localized MOG gene located at 6p21.3 in the major histocompatibility complex (MHC) region which was intensively investigated in migraine. Thus, MOG gene was selected as a candidate (7).

Important polymorphisms identified in the gene include G15A (0.14), CTC repeat in exon 1 (0.04), Val142Leu (0.191), Val145Ile (0.057) in exon 3, 551168A→G (0.131) and 551177C→T (0.02) in intron 4. Although the effect of the identified polymorphisms on the protein structure or function is not known, it was preferred among the known MOG gene polymorphisms in the current study due to the high frequency of polymorphic variants in the population and the presence of a conserved amino acid substitution in the transmembrane domain. There are also various examples of diseases cited as the cause of the migraine including V/L mutations in the transmembrane domains of the proteins encoded by CFTR, ABC7, PSEN1, TSHR, ACHR and KORC1 genes (14, 15). For this step, we used case-control approach to determine the contribution of the MOG variants to migraine.

Materials and Methods

Population of Study

After the approval of Non-Interventional Research Ethics Committee of Firat University (session number: 2021/02-45, dated February 4, 2021) was obtained, the study was carried out by obtaining verbal and written consents of the families. The protocol of this study was approved by ethics committee and informed consent form was signed by all participants. In addition, the principles of the Declaration of Helsinki were taken into account throughout our study. 101 migraineurs and 101 controls were recruited from Elazig province and its surroundings. 90 females and 11 males, and 82 females and 19 males were present in patient and control group, respectively. Diagnosis of the migraine was carried out after six months' follow up according to the International Classification of Headache Disorders (ICHD-II) criteria (16). An experienced neurologist examined all participants. Patients were interviewed to determine their age, gender, family history, intensity and frequency of migraine attacks in the previous 12 months, age of onset, and other clinical features. Details of the clinical symptoms seen in participants were obtained from the daily surveys. For this study, patients with comorbid diseases (e.g. vascular, hormonal and neurological disorders, and genetically transmitted disease); with nonmigrainous headaches, complicated and uncooperative by mental illnesses or other cognitive disfunctions such as in the heart, kidney, liver, or other important organs were excluded. Patients were divided into two groups which were composed of 64 MO patients (ICHD-II code 1.1), and 37 MA patients (ICHD-II code 1.2), respectively. Migraine patients were divided into three subgroups according to the headache attack frequency. First group has at least two, second group has three to five and third group has more than five attacks per month in the past year. Intensity of the pain was scaled as: Low pain=Did not put back daily activities; Tolerable pain=Puts back daily activities; Vigorous pain=Disrupts daily activities. Control subjects were randomly selected from healthy volunteers who had a routine medical examination at the hospital, had no history of migraine or a family history of migraine, and were similar in their age and gender. To exclude the migraine and cluster headaches, a neurologist specializing in headaches screened healthy volunteers.

Genotyping

From peripheral blood, genomic DNA was isolated by using the Wizard Genomic DNA Exraction Kit (Promega, Madison, WI, USA). In the third exon, MOG G511C (Val142Leu) polymorphism was genotyped by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (15). MOG gene spesific primers were: Forward primer 5′-TGCTCCTCCTGCAGATGACT-3′ and reverse primer 5′-GCTCCAAGAAGCCAGCTCAT-3′ (IDT, USA). PCR cycles were carried out at 95 °C for 5 min, 35 cycles at 95 °C 30 sec, 58 °C for 30 sec, 72 °C for 30 sec and 72 °C for 5 min, respectively. Briefly, the PCR reaction mixture was prepared by using 50 ng of genomic DNA (30 µl final volume), 3 µl of 2.5 mM dNTPs (Fermentas, Waltham, MA, USA), 3 µl of MgCl2, 1 µl of NH4 buffer, 0.2 µl of Taq polymerase (Fermentas, Waltham, MA, USA) and 1 µl 10 pmol of each primers per well. To minimize the partial digests, 15 µl aliquot of the product was digested with the 0.5 U Hinf-I (Fermentas, Waltham, MA, USA) restriction enzyme at 37°C overnight. On a 3.5% agarose gel which contained 10 mg/ml ethidium bromide, digested products were run at 120 V for 40 min. Gel screening was performed by using a gel electrophoresis visualization system (Vilber Lourmat, France). After screening, it was determined that Val allele had 121 bp fragments and the Leu allele had 104 bp + 17 bp fragments (Figure 1). Two reviewers scored independently the results of genotyping.

Statistical Analyses

The statistical analyses in this study were performed by using IBM's the Statistical Package for the Social Sciences (SPSS) 22.0 software package (193.255.124.131; SPSS, Chicago, IL, USA). Pearson $\chi 2$ test (Fisher's Exact Test), confidence interval (CI) and Hardy-Weinberg equilibrium (HWE) were used for case-control analyses. The relationship between clinical, demographic features and genotype was examined by using

Pearson's correlation test and Mann Whitney U test. Odds ratios and P values of the variables were calculated at 95% confidence level. In this study, P<0.05 was considered as statistically significant.

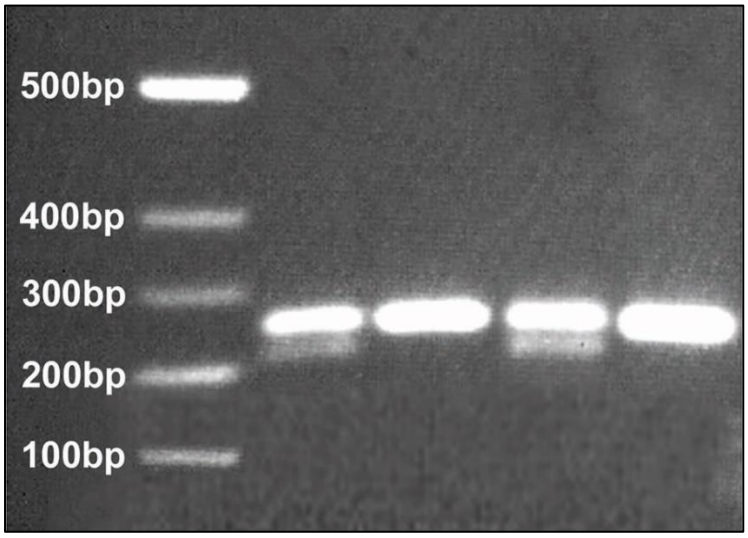

Figure 1. Agarose gel electrophoresis of the PCR products after digestion with Hinf I restriction enzyme showing the presence of Val142Leu polymorphisms of the MOG gene. 3.5% agarose gel electrophoresis, ethidium bromide staining and UV transillumination were performed. M: Molecular weight marker (100 bp each). Lane 1, 2: Bands of 121 bp and 17 bp indicating heterozygote Val/Leu genotype; Lane 3 and 4: Band of 121 bp indicating wild type Val/Val genotype.

Results

Mean ages were calculated as 33.40±9.34 and 34.13±8.4 for patient and control groups, respectively. Thirteen (13.13%) of the patients were receiving paracetamol, seventeen (17.17%) of them were taking non-steroidal anti-inflammatory (NSAII) drugs and seventy one (71.17%) were receiving analgesic-triptan combination therapy during attack. Prophylactically, patients received treatment with amitriptyline or proparapanol. While 83 of the patients had a triggering effect, 18 patients could not identify any trigger. The order in which enviromental agravation factors as the triggers was defined as emotional stress (72/83), physical activity (56/83), and menstruation (22/83). No statistically significant differences between patients and controls were found in terms of the distribution frequency of gender, age, smoking, and drinking status (P>0.05). When MO and MA patients were compared within the patient group, no significant difference was observed in terms of age, gender, age of migraine onset, presence of migraine in relatives, time of pain, frequency of attacks per mounth, intensity of pain, side of pain, aggravation of headache by environmental exposure, therapy, resistance of standard therapy, smoking and drinking parameters (P>0.05). Demographic and clinical characteristics of the patients are shown in Table 1. RFLP method which was used to determine Val142Leu polymorphism is completely appropriate. Agarose gel electrophoresis image are shown in Figure. Genotyping counts determined at polymorphic loci were in Hardy–Weinberg equilibrium for both controls (χ2=1, PHW=0.2) and patients (χ2=0.2, PHW =0.6) (P>0.05). For Val142Leu polymorphism between patients and controls, there was no difference in the genotype and allele distribution [OR=0.47 (0.21-1.08), P=0.053 for genotypes; OR=0.50 (0.23-1.11), P=0.060 for alleles]. The frequency of the Val142Leu genotype was found to be lower than that in controls despite of statistically insignificance in migraine patients. A significant difference was not observed in MA versus MO at the genotypic level (Val/Val) [P=0.53; OR=1.17 (0.30-4.45)] as well as the allelic one [P=0.53; OR=1.16 (0.31-4.25)]. A significant difference was found between the headache attacks frequency and Val142Leu polymorphism (P=0.026) according to Pearson χ2 test. Allelic and genotypic frequencies of polymorphisms are shown in Table 2. The headache attack frequency according to genotypes is presented in Table 3. No significant association was observed among other clinical features such as age, gender, presence of aura, age of migraine onset (years), presence of migraine in relatives, duration of headache attack (hours/attack), pain

index (intensity of pain; visual analog scale), site of pain, agravation of headache by enviromental exposure, therapy, resistance to standard therapy, smoking, drinking when the patients with the Val142Val genotype were compared with the patients with Val142Leu genotype ($p>0.05$). Table 3 presents the characteristics and symptoms of migraine in carriers of Val142Val or Val142Leu. With the post-hoc analyzes made using the G power 3.1 program, 70% power was obtained in the current sampling. The effect size (W) of the study is 0.226.

Table 1
Demographic Characteristics of Study Participants

Parameters	Controls (n=101)	MO (n=64)	MA (n=37)
Age (years)	34.13±8.45	33.2 ± 6.0	32.9 ± 11.2
Familial history	–	54.6	56.7
Sex			
Female /Male	82/19	58/6	30/7
Frequency			
1–3/month	–	18	9
3–5/month	–	16	16
5–10/month	–	30	12
Intensity			
Low	–	2	2
Moderate	–	26	16
Severe	–	36	19
Duration			
12 h	–	9	8
12–24 h	–	21	18
<24 h	–	34	11
Smoking status			
Smoker	18	7	4
Non-smoker	83	57	33
Drinking status			
Drinker	2	1	0
Non-drinker	99	63	37

*MO: Migraine without aura, MA: Migraine with aura, Values are the mean±SD or percentage number of subjects. Pain severity was graded as follows: Low pain=Did not interfere with daily activities; Moderate pain=Interferes with daily activities; Severe pain=Impedes daily activities.

Table 2

MOG genotype and allele distribution in controls and patients of migraine

Genotypes	Patients (n=101)	Controls (n=101)	Odds ratio	P-value
Val/Val	91 (90.09%)	82 (81.18%)		
Val/Leu	10 (0.99%)	19 (18.81%)	0.47 (0.21-1.08)	0.053
Leu/Leu	0 (0%)	0 (0%)		
Allele				
Val	0.9 (95,04%)	0.8 (90,59%)	0,50 (0.23-1.11)	0.060
Leu	0.1 (4.95%)	0.2 (9.40%)		

Table 3

The frequency of attacks according to *MOG* genotypes

The headache attacks frequency per month	Val/Val	Val/Leu	Odds ratio	P-value
Two (a)	21	6	a vs b 2.761 (0.62-12.32) a vs c 11.714 (1.32-103.77)	0.157 **0.013***
Three-Five (b)	29	3	b vs c 4.241 (0.42-42.84)	0.212
More than five (c)	41	1		

*P<0.05; a vs.b, a vs. c and b vs. c odds ratios were calculated as 2.761 (0.619-12.321), 11.714 FF (1.322-103.773) and 4.241 (0.419-42.842), respectively.

Discussion

According to our literature search, this study is the first report determining the genetic association of MOG gene polymorphisms with Turkish migraine subjects. In our study, there was no association between MOG gene rs2857766 polymorphism and migraine or with migraine subtypes. The possible mechanisms responsible for the effect of this polymorphisms on severity or on the risk of developing migraine are unclear. Migraine overlaps many other conditions including bipolar disorder (BD), multiple sclerosis (MS) and epilepsy (8-10). Some authors have suggested that there is an association between MOG gene polymorphism and some diseases such as multiple sclerosis (MS), reading disability and obsessive compulsive disorder, while others published different results (11-13). We showed that Val142Leu polymorphism was underrepresented in migraine patients despite of statistically insignificance. The frequency of the Val142Leu polymorphism in our study was similar to the study of Gomez-Lira et al (14). In our control population, the frequency of the Leu allele was 20%. This value is consistent with 22% (57/248) in Italian population. The gene expression, protein function or activity effect of Val142Leu amino acid substitutions in exon 3 of MOG gene are not exactly known. Changes in splicing, which were reported by Alfonso et al are associated with common genetic variations including Val142Leu in the MOG gene. However, we detected overrepresented Val142Leu genotype in patients with the decreased migraine attack frequency. There may be a number of possible mechanisms underlying the association between Val142Leu genotype and the decreased attack frequency. First, recent studies have implicated that MOG is a complement system classical pathway activator (15). Johns and Bernard proved the binding ability of MOG to C1q. In an unpaired study; C1, C1s and C4 lower levels during early headache are the signs for activation of complement system (12). Val142Leu genotype may decrease the attack frequency by reducing the activation of the compleman system. Second, the pathogenezis of pure menstrual migraine (MM) apperars to be related to estrogen witdrawal, especially MA (17). Oligodendrocytes synthesize nuclear and membrane estrogen receptor alpha (ER-α) and beta (ER-β) receptor protein as well as MOG protein. The classical estrogen signaling activates pathways that lead to differentiation and myelination of OL through activation of ER-α and -β receptors. In support of this datum, it has been shown that the functional remyelination induced by one of the ER-β agonists Indazole-chloride (Ind-Cl) is based on the anti-inflammatory properties of this molecule, as well as the increase in the number of progenitor and mature OL cells. Patients with Val/Leu genotype may decrease the frequency of attacks depending on estrogen effect. However, it is unknown whether MOG expression is changed depending on the estrogen fluctation during menstrual cycle or whether MOG gene is a direct target of ovarian steroid hormones such as estrogen (18). Another datum to support this hypothesis, increased propensity in the female rodents with CSD is consistent with the significant higher prevalence of migraine in women than in men. The exposure to estrogen has been reported to increase CSD susceptibility, whereas exposure to testesterone has the opposite effect (19). Third, the relationships of higher attack frequency, longer disease duration and the increased white matter abnormalities (WMA) in migraine were found (20, 21). Higher rates of hyperintensities of WM and differences in the myelin, and oligodendrocyte-related genes expression are the most reliable findings. Atmaca et al reported that individuals with obsessive-compulsive disorder (OCD) showed a notably higher entire WM volumes in individuals with the Val/Val genotype than Val/Leu and Leu/Leu genotypes (22). We demonstrated a positive correlation between the decreased attack frequency and the Val142Leu genotype. It could be speculated that Val/Leu genotype may be related to reduced risk of the migraine attack frequency and related to decreased WMA. In addition, the intracellular portion of MOG is more immunogenic than the extracellular portion and a predominant MOG epitope containing amino acids located right next to the 142 residues is recognized by CD4+ T cells. In particular, analysis by computer programs showed that the presence of V or L at position 142 altered class I and class II MHC affinity. As a hypothesis, the L residue may elicit a protective effect for migraine by reducing the immunogenicity of this epitope (15).

There are some limitations in our study. First, migraine and subtype case size were not high enough for detection of the small effects from highly low penetrated genes or single nucleotide polymorphisms (SNPs). Obtained data showed the unrelated circumstance of MOG Val142Leu polymorphism and appearance of the migraine. It should be interesting to evaluate the relationship between WMA and MOG polymorphism or MOG antibody positivity rate in patients with migraine. Additional clinical studies on different MOG gene variants in migraine could help give more accurate results. To confirm these results, further genetic, epidemiological and familial aggregation studies in other populations with larger numbers of patients are required.

Conclusion

In our study, no relationship was found between the Val142Leu (rs2857766) polymorphism in the MOG gene and migraine or migraine subtypes in migraine patients for Turkish population. It was figured out that the Val142Leu polymorphism was not adequately represented in migraine patients. In additon, we determined that the Val142Leu genotype was overrepresented in patients with reduced migraine attack frequency i.e. there was a positive correlation between them.

Ethics Committee Approval: The study was approved by the Non-Interventional Research Ethics Committee of Firat University (date: February 4, 2021 and approval number: 2021/02-45).

Informed Consent: Written consent was obtained from the families.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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In Vitro Evaluation of The Protective Effects of Curcumin and Resveratrol Against U87 Cells Induced by Beta-Amyloid

Beta-Amiloid Tarafından İndüklenen U87 Hücrelerine Karşı Kurkumin ve Resveratrol'ün Koruyucu Etkilerinin İn Vitro Değerlendirilmesi

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Abstract

Objective: In this study we aimed to investigate the effects of curcumin and resveratrol antioxidant enzymes in β-amyloid (Aβ)-induced in vitro Alzheimer's Disease (AD) cell model.

Materials and Methods: Three groups were created in this study; The control group consisted of U87 cells, the Aβ group which was the in vitro AD model formed from the β-amyloid-induced U-87 cell lines, and the Aβ+ Curcumin+ Resveratrol group by adding curcumin and resveratrol to the Aβ group. Cell viability in groups was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test. Total Antioxidant Level (TAS), Superoxide dismutase (SOD), Total Oxidant Level (TOS), and Catalase (CAT) enzyme levels, results were analyzed in order to evaluate the antioxidant levels.

Results: When cell viability was evaluated, it was determined that curcumin and resveratrol did not have cytotoxic effects. TAS levels were statistically higher in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$) and TOS levels were found to be significantly low in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$). However, when compared to the control group, CAT and SOD enzyme levels were statistically and significantly low in the Aß group. In contrast, these enzyme levels were found significantly higher in the Aβ+ curcumin and resveratrol group in comparison with the Aß group ($p<0.05$).

Conclusion: In conclusion, it has been shown that curcumin and resveratrol support antioxidant activity.

Keywords: Curcumin, Resveratrol, β-amyloid-induced U-87

&

Öz

Amaç: Çalışmamız, β-amiloid (Aβ) ile indüklenen U87 hücrelerin in vitro Alzheimer Hastalığı (AH) modeli olarak kurkumin ve resveratrol'un antioksidan enzimler üzerindeki etkilerini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntemler: Bu çalışmada; U87 hücrelerden oluşan kontrol grubu, β-amiloid ile indüklenen U-87 hücre dizilerini içeren in vitro AD modelinin oluşturulduğu Aβ grubu ve Aβ grubuna kurkumin ve resveratrol eklenerek Aβ+ Curcumin+ Resveratrol grubu olmak üzere üç grup oluşturuldu. Grupların hücre canlılığı 3-(4,5-dimetiltiazol-2-il)-2, 5-difeniltetrazolyum bromür (MTT) testi ile değerlendirildi. Total Antioksidan Seviyesi (TAS), Süperoksit dismutaz (SOD), Total Oksidan Seviyesi (TOS) ve Katalaz (CAT) enzim seviyeleri, antioksidan durumunu değerlendirmek için sonuçlar analiz edildi.

Bulgular: Hücre canlılığı değerlendirildiğinde kurkumin ve resveratrolün sitotoksik etkilerinin olmadığı belirlendi. Aβ+ kurkumin ve resveratrol grubunda Aβ grubuna göre TAS düzeyleri istatistiksel olarak daha yüksek ($p<0,05$) ve TOS düzeyleri Aβ+ kurkumin ve resveratrol grubunda Aβ grubuna göre istatistiksel olarak anlamlı derecede düşük bulundu($p<0,05$). Ancak kontrol grubu ile karşılaştırıldığında Aß grubunda CAT ve SOD enzim düzeyleri istatistiksel olarak anlamlı düzeyde düşük bulunurken, bu enzim düzeyleri Aβ+ kurkumin ve resveratrol grubunda Aß grubuna göre istatistiksel olarak anlamlı düzeyde yüksek bulundu. ($p<0,05$).

Sonuç: Sonuç olarak, kurkumin ve resveratrolün apoptoza bağlı nörodejenerasyonu azalttığı ve antioksidan aktiviteyi desteklediği gösterilmiştir.

Anahtar Kelimeler: Kurkumin, Resveratrol, β-amiloid kaynaklı U-87

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Introduction

Alzheimer's disease (AD) is most characterized by progressive loss of cognitive capacity as well as severe neurodegeneration leading to dementia (1). It is characterized by memory loss and behavioral and mental dexterity disorders. Amyloid (senile) plaques originating from Amyloid ß (Aß) peptides and neurofibrillary tangles containing tau protein, degeneration, and neuronal loss are seen in Alzheimer's disease (1-4). Although there are many hypotheses (amyloid cascade, glutamatergic, cholinergic, oxidative stress, and tau effects) trying to explain the pathophysiology of AD, nevertheless none of these hypotheses can fully explain the pathophysiology of the disease (5). Since approved drugs available today are insufficient in the treatment of the disease and in relieving symptoms, Scientists have turned to finding new molecules that can be an alternative treatment option. In new therapeutic pursuits today, plants are recognized as one of the main sources of biologically active substances. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a naturally occurring polyphenol. Clinically, resveratrol has exhibited significant anti-inflammatory, antioxidant, anti-cancer, and anti-viral, properties. Experimental studies suggest that resveratrol is active against AD pathogenesis (6-8). Curcumin is a pigment found in the spice castor, also known as turmeric. Curcumin is a compound with powerful antioxidant and anti-inflammatory properties. It has been used in medicine as an anti-inflammatory agent to treat gas, chest pain aches, toothaches, and menstrual difficulties. In a study, the Curcumin-Cu2+ complex was examined in two different segments of the β-amyloid protein to prevent the formation of Curcumin-binding Aβ fibrils. As a result of this study, it was determined that Curcumin binds to Cu2+ and Aβ and acts as a chelator and binding partner to Aβ. Thus, it has been shown that it is possible to use Curcumin in the treatment of AD (9-11).

Oxidative stress also plays an important role in the formation of neurodegenerative diseases such as Alzheimer's by inducing mitochondrial abnormalities. Antioxidants are an important component that plays a protective role in eliminating ROS and protecting from neuronal damage caused by ROS. Antioxidants provide first-line defense against damage from free radicals and are critical in maintaining optimal health and well-being. Enzymatic and low molecular weight antioxidants create the balance between ROS and anti-oxidative mechanisms. While the enzymatic antioxidants are SOD, CAT, and GPx, glutathione (GSH) is one of the low molecular weight antioxidants that are effective in AD (11-13).

We planned this study to investigate the possible effects of curcumin and resveratrol, which we predicted to be used for the treatment of Alzheimer's disease, on the U87 cell line. In accordance with this purpose, we investigated SOD and CAT activities to evaluate antioxidant status, TAS and TOS protein levels, and activities to see their effect on the apoptosis process. In addition, in present study, we examined whether curcumin and resveratrol have a cytotoxic effect on the U87 cells and its effect on cell proliferation by using the MTT test. Before starting experimental animal model studies, determining how the cytotoxic effect depends on the concentration will make a contribution to the drug development stages in the future.

Materials and Methods

U87MG Cell Culture and In vitro Experiments:

The study has been conducted in the laboratory of Cukurova University, Faculty of Medicine, Department of Pharmacology. The materials used in this study were obtained from the laboratory and the chemicals used are listed below. In the study, Aß1-40, Dulbecco's Modification of Eagle's Medium (DMEM), penicillin/streptomycin antibiotic (PSA), fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), U87 MG cell line, 18β-GA from Sigma Aldrich; SOD, KAT, and GPx ELISA kits were obtained from R&D Systems. Literature studies were used to create an in vitro model of AD in U87 MG cells (12).

Culture Medium was created by adding 1% (PSA) and 10% FBS to DMEM broth. Cells were cultured in a 5% humidified and CO2 at 37°C to adhere to the surface in culture flasks. Before proceeding with the assays, the frequency of the cell was at 80%, during which time old media were replaced with new culture media twice a week. The cells, which reached 80% frequency, were washed with serum-free medium after

removing the used medium and kept in serum-free medium for 2 hours. Differentiated U87 MG cells obtained by cell passage were incubated with 5 micromolar (μM) Aß for 24 hours. In vitro groups were formed by performing the corresponding procedures, Control group (U87 MG cells + medium), Aß Group (Aβ-treated U87 MG cells), Aß+18ß GA Group (U87 MG cells treated with 5 μM 18ß-GA 18β-GA + Aβ) with each group having 5x104 cells in each well of the 96-well plate with 6 replicates.

MTT Test:

The MTT assay test was used according to the protocols of manufacturer's instructions to examine cell viability and cytotoxic effects of compounds. Briefly, three groups (Control group, Aβ group, Aß+18ßGA group) were inoculated with a new FBS-free medium containing 10% MTT (5 mg/ml concentration) solution in well plates. The cells were incubated for 24 hours in a dark incubator at 370C and 5% CO2. After incubation, the medium was removed and in order to dissolve the formazan crystals 100 microliters (μl) of DMSO were added to the cells. In order for the formed crystals to dissolve, they were kept in a CO2 (5%) incubator at 37°C for 10 minutes. After all, procedures were completed carefully, absorbance values were recorded by reading each section at 570 nm wavelength.

ELISA Test:

SOD, CAT, and GPx levels, which are antioxidant enzymes, were determined in the control, Aß, and Aß+18ß-GA groups with the ELISA test. The relevant ELISA test procedures are different for each kit and were performed according to the protocols specified in the relevant kits.

Statistical Analysis:

Statistical analyzes of results were performed in the Statistical Package for the Social Sciences program called IBM SPSS 25. As the first step in data analysis, the assumption of normality was checked with the Shapiro Wilk test. ANOVA test for examining the difference between the means of variables with normal distribution and more than two independent groups; Kruskal Wallis test was used to examine the difference between the means of variables that did not have a normal distribution and had more than two independent groups. Bonferroni analysis, one of the post hoc tests, was performed to reveal the group or groups that made the difference. $p<0.05$ was considered statistically significant.

Results

MTT Test:

Change of U87 cell viabilities according to the β-amyloid, Curcumin, and resveratrol applied are shown in Fig.1. It was determined that Curcumin and resveratrol, applied to the human brain cell line (U87), increased the viability and proliferation of the cells and Curcumin applied on cells caused less increase in cell proliferation than resveratrol ($p<0.05$), (Figure 1). IC50 values were calculated as the concentration of the Inula viscosa extract that causes 50% inhibition in cell proliferation.

ELISA Test:

Application of Aβ to the U87 cells reduced the activity of SOD enzymes significantly. Curcumin and resveratrol administration significantly increased this decrease (Figure 2). The application of curcumin and resveratrol significantly increased catalase activity in cells that were treated whit Aβ (Figure 3). Application of Aβ to cells increased the amount of total oxidant (TOS) significantly, and treatment whit Curcumin and resveratrol significantly decreased this increase (Figure 4). Application of Aβ to the cells reduced the amount of TAS, Curcumin, and resveratrol administration significantly increased this decrease (Figure 5).

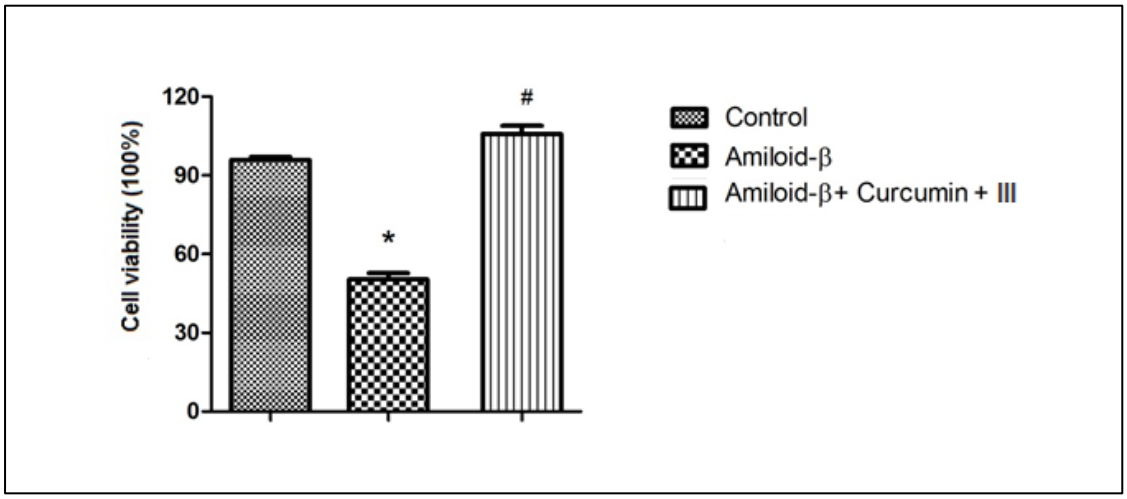

Figure 1. Effect of experimental groups on cell proliferation. p<0.05 relative to control

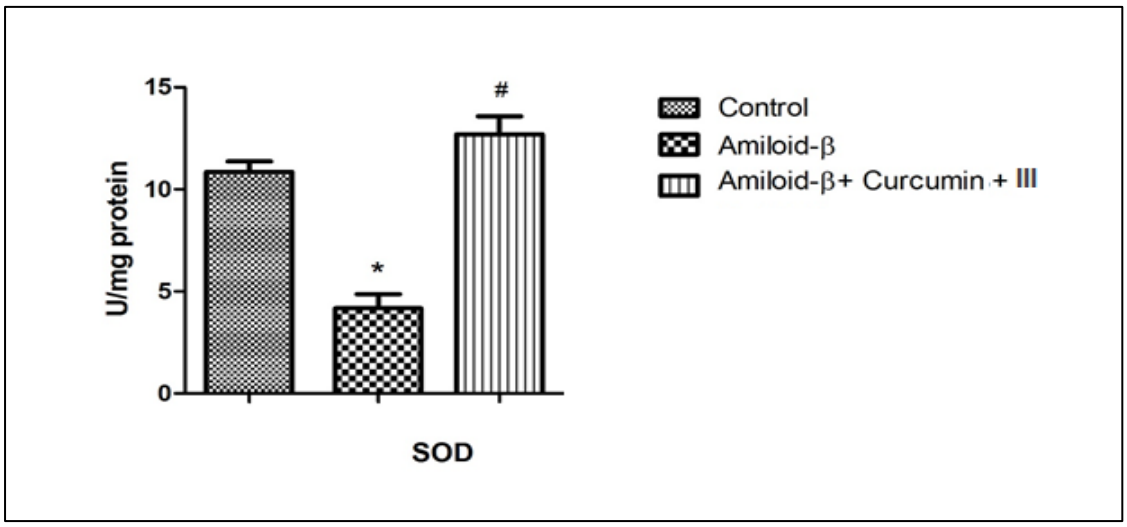

Figure 2. The effect of curcumin and resveratrol administration on SOD activity (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

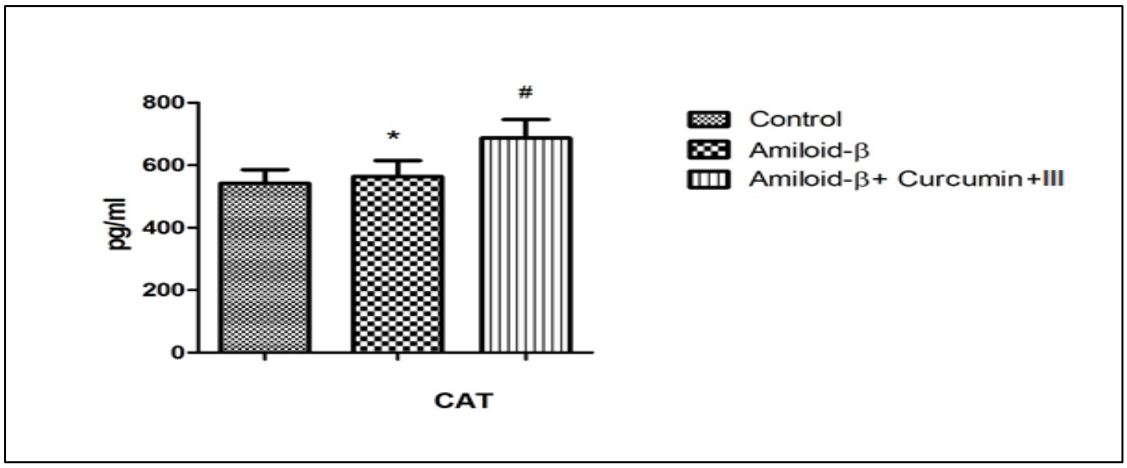

Figure 3. Effect of curcumin and resveratrol application on catalase activity (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

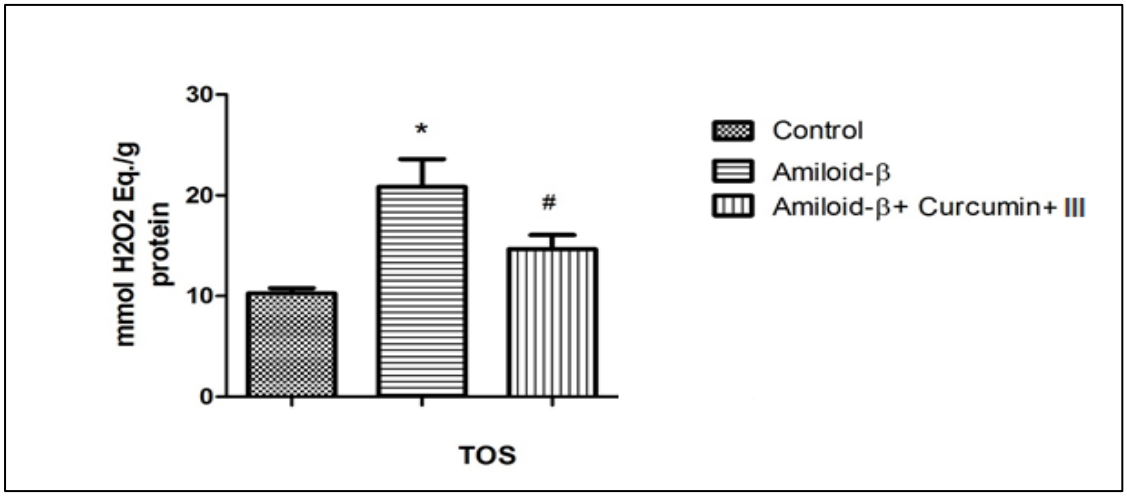

Figure 4. The effect of curcumin and resveratrol application on the amount of oxidant (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

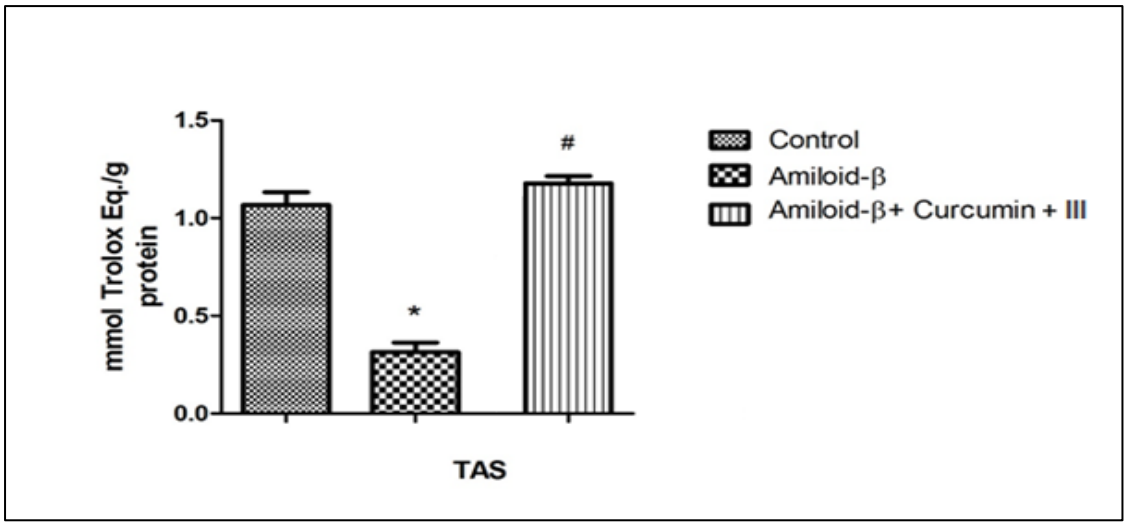

Figure 5. Effect of curcumin and resveratrol application on antioxidant content (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

Discussion

AD is the most common type of dementia in the elderly and is characterized by progressive loss of cognitive capacity as well as severe neurodegeneration. the most important targets in treatment of AD is inhibition of amyloid precursor protein (APP) and Aβ production by blocking Aβ aggregation and inhibiting the inflammatory response and Aβ-induced neurotoxicity (14-16).

Although the relationship between Alzheimer's disease and ROS is not fully understood, it has been reported in the literature that another cause of pathological changes in Alzheimer's disease is the imbalance between ROS and antioxidative mechanisms. While there is indirect evidence showing that oxidative stress mechanisms are increased in AD, ROS has been found to have a potential role in amyloid plaque deposition. It was found that SOD was localized in the brain amyloid plaques of the patients, there was a significant decrease in plasma GPx activity throughout the clinical evolution of the disease, and It has been reported that the role of CAT is in the second defense against ROS in the antioxidant defense system by somehow helping the GPx role (17-19).

In this study, we investigated the effectiveness of curcumin and resveratrol in the Alzheimer's model cell line. the effects of curcumin and resveratrol on cell viability and proliferation was examined by MTT test, and the antioxidant effects of those were evaluated by measuring the levels of SOD, TAS, TOS, and CAT by ELISA.

According to our data, amyloid-B administration on the human brain cell line U87 significantly reduced SOD activity compared to the control. Curcumin and resveratrol administration increased this decrease when compared to the control groups ($p<0.05$). Application of Aβ to the U87 cells increased the amount of total oxidant curcumin and resveratrol application decreased this increase significantly ($p<0.05$ compared to control). The application of Aβ to cells reduced the number of antioxidants significantly. Curcumin and resveratrol administration increased this decrease significantly ($p<0.05$ compared to control). TAS levels were found to be statistically higher in the Aβ + curcumin and resveratrol group compared to the Aβ group ($p<0.05$) and TOS levels were found to be statistically lower in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$). The increase in ROS in the cell and accordingly the defense mechanism activity of the antioxidant may have caused an increase in cell proliferation. Accordingly, we can argue that the application of curcumin and resveratrol increases the activity of SOD enzymes, which constitute the defense mechanism of the cell, together with increased cell proliferation. ROS is effectively cleared by a detoxification defense system such as SOD, and CAT to maintain redox homeostasis in the cellular system. When ROS production is above the cell's detoxification capacity, over-produced ROS causes severe damage to DNA, proteins, and lipids of the cell membrane. It can be suggested here that ROS could act as a second messengers in cell proliferation, possibly through activation of protein kinases and transcriptional factors. Curcumin and resveratrol treatments reduced amyloid-B-induced cell death. Proliferation and antioxidant enzyme activity studies on U87 cell lines, a human brain cell line in vitro, showed significant differences compared to the control groups. Curcumin has been shown to have beneficial effects on Alzheimer's in many studies with its antioxidant, anti-inflammatory, and anti-amyloid effects (20-22).

Conclusion

In our study, we invastigated the effects of Curcumin and resveratrol on the Alzheimer's cell line model. We predict that resveratrol may be more beneficial to Alzheimer's than curcumin. Curcumin and resveratrol can be used as a potential compound that can be an alternative to the few drugs used in the field of AD treatment today and are preferred during treatment.

Ethics Committee Approval: Ethics committee approval was not obtained because it was a cell culture study.

Informed Consent: Not applicable.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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Does St-Elevation Myocardial Infarction Wait for The Vacation to End?

ST-Elevasyonlu Miyokard Enfarktüsü Tatil Dinler mi?

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Abstract

Objective: This is an epidemiological study of patients diagnosed with ST-elevation myocardial infarction (STEMI) in Fethiye, one of Turkey's most popular destinations for domestic and foreign tourists. It aimed to determine a tourist group at risk for STEMI and needs attention for a holiday region that does not have a catheter laboratory and is two hours away from the nearest catheter laboratory.

Materials and Methods: The study was retrospective. Patients diagnosed with STEMI in all hospitals in the district, one state, and two private hospitals during the summer holiday period between June 1, 2021, and October 1, 2021, were examined. The most common STEMI dates and time intervals in tourists admitted to the emergency department were examined. Demographic characteristics and comorbidities of patients, including residents and tourists, were compared.

Results: A total of 331 STEMI patients were observed. 76.7 % (n =254) of the patients were Turkish residents, 1.8 % (n =6) resident foreigners, 19.3 % (n =64) domestic tourists, and 2.1% (n =7) foreign tourists. The average age of the residents was statistically significantly higher than the tourist group (64.15 vs. 57.83, [p=0.01]). A statistically significant difference was found only with hypertension as a comorbidity (P = 0.034).

Conclusion: In STEMI, no situation differs from the local people in the tourist group. Considering that elderly tourists with health problems will be encountered more frequently with the prolongation of human lifespan, efforts should continue to facilitate access to catheter laboratories in holiday regions, mainly for situations that require urgent intervention such as STEMI.

Keywords: Myocardial Infarction, Tourists, Residents, Summer, Vacation

&

Öz

Amaç: Türkiye'nin yerli ve yabancı turist bakımından en gözde beldelerinden olan Fethiye ilçesinde ST elevasyonlu miyokard infarktüsü tanısı alan hastalarının epidemiyolojik inceleme çalışmasıdır. Kateter laboratuarı olmayan, en yakın kateter laboratuarına 2 saat mesafede olan bir tatil yöresi için STEMI açısından riskli olabilecek olan ve dikkat edilmesi gerekecek turist grubunu belirleyebilmek amaçlandı.

Gereç ve Yöntemler: Bu çalışma retrospektif bir çalışmadır. 01.06.2021-01.10.2021 tarihleri arasında yaz tatil döneminde 1'i devlet 2'si özel hastane olmak üzere ilçedeki tüm hastanelerde STEMI tanısı alan hastalar incelendi. Hastaların en sık MI geçirme tarih, gün, saat dilimleri incelendi. Hastalar yerli halk ve turist olmak üzere demografik özellikleri, komorbiditeleri karşılaştırıldı.

Bulgular: Toplam 331 adet STEMI hastası olduğu saptandı. Hastaların %76,7 (n=254)'si yerleşik Türk, %1,8 (n=6) yerleşik yabancı, %19,3 (n=64) Yerli turist ve %2,1 (n=7) yabancı turist idi. Yerleşik halkın yaş ortalaması turist grubuna göre istatistiksel anlamlı olarak daha yüksek izlenmiştir (64,15 vs 57,83, [p=0,01]). Komorbidite olarak sadece hipertansiyon ile istatistiksel olarak anlamlı bir farklılık saptanmıştır (p=0,034).

Sonuç: Turist grubunda ST elevasyonlu MI açısından yerli halktan farklı özellikle dikkat edilmesi gereken spesifik bir hasta grubu yoktur. İnsan ömrünün uzaması ile sağlık problemleri olan yaşlı turistlerle daha sık karşılaşılacağı gözönünde bulundurularak, tatil bölgelerinde özellikle STEMI gibi acil müdahale edilmesi gereken durumlar için katater laboratuarına erişimin daha da kolaylaşması için çabalar devam etmelidir.

Anahtar Kelimeler: Miyokardiyal İnfarktüs, Turist, Yerleşik Halk, Yaz, Tatil

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Introduction

Ischemic heart diseases are the most common cause of mortality according to World Health Organization (WHO) data (1). The European Society of Cardiology reported deaths from ischemic heart diseases as approximately 20% of all deaths in Europe (2). Many factors affect mortality in the ST-elevation myocardial infarction (STEMI) group, the riskiest group among ischemic heart diseases. These are age, Killip score, delay in initiation of treatment, treatment strategy, prior myocardial infarction history, diabetes mellitus, renal failure, and heart failure (2).

Apart from these known factors included in the guidelines, there are many studies on the prognosis and mortality of STEMI. In the last few years, it has been seen that the studies on holidays have increased. Studies on STEMI and holidays are generally the studies of centers with catheter laboratories. Most patients live in the region, and studies are conducted on weekday vs. weekend day comparisons (3,4). As a result of such studies, reasons such as lack of staff, inability to access equipment, and delays in diagnosing patients come to the fore.

Acute coronary syndromes have been reported to be a leading cause of holiday mortality. Still, studies have yet to examine whether patients have STEMI while on vacation away from their region of residence or how many STEMI patients in an area are tourists (5). However, many positive and negative changes occur in the cardiovascular system in the daily life of individuals during the holiday period. If we examine these changes, individuals move away from stress, one of the cardiovascular risk factors, when they go on vacation (6). On the other hand, during the vacation period, the frequency of alcohol use increases, eating habits change, and sleep patterns change (7-9). In addition, according to their destination, there is an altitude difference and a temperature difference, and their bodies show physiological adaptation, and these adaptive changes last for weeks (10-12). These factors may affect the cardiovascular system and ischemic heart diseases (11, 13). Apart from all these, a study examining the risk of myocardial infarction on vacation showed that driving conditions and being less luxurious might also be associated with myocardial infarction (14).

During the holiday season, it can be challenging to diagnose whether it is STEMI, which is a vital emergency in holiday resorts, and to access the catheter laboratory. In this study, we will examine the patients diagnosed with STEMI in Fethiye, one of the most popular destinations in Turkey, in terms of domestic and foreign tourists. The importance of this study is to identify the tourist group that may be at risk in terms of STEMI and needs attention for a holiday resort that does not have a catheter laboratory and is 2 hours away from the nearest catheter laboratory.

Materials and Methods

Study Design and Settings

The study was carried out with the permission of Mugla Sıtkı Kocman University Medicine and Health Sciences Ethics Committee (Date:15.11.2021 – Decision Number: 1). The study was carried out by the principles of the Declaration of Helsinki and the ethical rules. All patients with STEMI in Fethiye between 01.06.2021 and 01.10.2021 were included in the study. Study data were obtained from all hospitals in Fethiye, including one state hospital and two private hospitals. There are cardiology specialists in all three hospitals, and they are on call 24/7. Since there is no catheter laboratory in all three hospitals and the nearest percutaneous intervention center takes more than 120 minutes, thrombolytic treatment is given to STEMI patients, and the patients are referred.

Selection of the Participants

Based on previous studies, it was observed that there should be a minimum of 120 patients in the sample analysis performed by choosing type I error 0.10 and power of 90 % (1- β =0.90). Patients diagnosed with

STEMI in the emergency department were included in the study. Patients with ECG changes conforming to the definitions of ESC 4. Universal myocardial infarction as STEMI was included in the study (15).

Measurements and Outcomes

Patients with STEMI were analyzed for the following parameters; hospital type, gender, age, diabetes mellitus (DM), hypertension (HT), coronary artery disease (CAD), atrial fibrillation (AF), cerebrovascular disease (CVD), and time frame of admission to the emergency department.

Statistical Analysis

SPSS version 25.0 (SPSS Inc., Chicago, Illinois, USA) package program was used for data analysis in the study. Descriptive data on the sociodemographic and clinical information of the patients are given as N and % or mean ± standart deviation (SD) tables.

The study's data were evaluated with Kolmogorov-Smirnov in terms of normality assumptions. An Independent t-test, one of the parametric tests, was applied to determine whether there was a significant difference between the patient's status as residents and tourists. The Chi-Square and Fisher's Exact tests were used to compare categorical variables. $P<0.05$ was considered statistically significant.

Results

A total of 331 STEMI patients were detected. It was observed that 282 (85.2%) of 331 patients were diagnosed in private hospitals, and 49 (14.8%) were diagnosed in state hospitals. 64.4 % of the patient were male (Table 1).

The month with the most STEMI was found to be July (30.8%, [n=102]), and the day was Sunday (17.2%, [n=59]) of all STEMI. The most common STEMI was observed as an 8-16 shift with 37.8% (n =125) (Table 1).

If the patients were divided according to the status of being residents and tourists; 76.7% (n=254) of the Turkish residents, 1.8% (n=6) resident foreigners, 19.3% (n=64) domestic tourists, and 2.1% (n=7) foreign tourists (Table 1).

When we analyzed the patients according to the regions they lived in, 199 (60.1%) lived in Fethiye, 61 (18.4%) lived in districts close to Fethiye but where no cardiologist was available. Patients living in places other than Fethiye were referred here for cardiological evaluation. It has been observed that patients mostly live in the Marmara Region, especially İstanbul, after Fethiye and its surrounding neighborhoods (Table 1). When the patients with STEMI were examined, it was observed that there were no patients in our country who had STEMI from Eastern Anatolia and Southeastern Anatolia regions.

When the countries of foreign tourists were examined, it was observed that patients with STEMI came from Russia, the United Kingdom, the Netherlands, and Ukraine (Table 1).

Considering the comorbidities of the patients who had STEMI, 21.5% had DM, 47.7% had HT, 17.8% had CAD, 3.9 % had AF, and 2.1% had CVD (Table 1).

When the patients were divided into residents and tourists, the average age of the residents was statistically significantly higher than the tourist group (64.15 vs. 57.83, [p=0.01]) (Table 2).

When comparing the residents and tourists, no statistically significant difference was found between the month, the day, and the shift of the STEMI (Table 3). As comorbidity, a statistically significant difference was found with only hypertension (p=0.034).

Table 1.
Sociodemographic and Clinical Data of Patients (n=331)

		N or X_{mean}(Min-Max)	% or Mean ±SD
Age		64.00 (19.00-96.00)	62.80±14.13
Hospital Status	State Hospital	49	14.8
	Private Hospital	282	85.2
Gender	Male	213	64.4
	Female	118	35.6
Month	June	86	26.0
	July	102	30.8
	August	78	23.6
	September	65	19.6
Day	Monday	45	13.6
	Tuesday	52	15.7
	Wednesday	43	13.0
	Thursday	51	15.4
	Friday	39	11.8
	Saturday	42	12.7
	Sunday	59	17.8
Shift	00:00-08:00	86	26.0
	08:00-16:00	125	37.8
	16:00-24:00	120	36.3
Residents' or Tourists' Status	Resident-Turkish	254	76.7
	Resident- Foreign	6	1.8
	Domestic Tourist	64	19.3
	Foreign Tourist	7	2.1
Living Region	Fethiye	199	60.1
	Districts near Fethiye	61	18.4
	Marmara Region	36	10.9
	Aegean Region	11	3.3
	The Mediterranean Region	14	4.2
	The Inner Anatolia Region	3	.9
	Black Sea Region	2	.6
	Russia	2	.6
	The United Kingdom	1	.3
	Netherlands	1	.3
	Ukraine	1	.3
DM	No	260	78.5
	Yes	71	21.5
HT	No	173	52.3
	Yes	158	47.7
CAD	No	272	82.2
	Yes	59	17.8
AF	No	318	96.1

	Yes	13	3.9
CVD	No	324	97.9
	Yes	7	2.1

Table 2.

Comparison of Patients' Ages in Terms of Tourist Status

	Status	**N**	**Mean±SD**	**t**	**p**
Age	Residents	260	64.15±13.31	3.396	**0.001**
	Tourists	71	57.83±15.92		

*Independent samples T-test

Table 3

Comparison of Demographic and Clinical Data Groups By Residents or Tourists

	Residents	**Tourists**	**p**
Gender			
Male	163 (76.5)	50 (23.5)	0.228
Female	97 (82.2)	21 (17.8)	
Month			
June	69 (80.2)	17 (19.8)	0.728
July	80 (78.4)	22 (21.6)	
August	58 (74.4)	20 (25.6)	
September	53 (81.5)	12 (18.5)	
Day			
Monday	33 (73.3)	12 (26.7)	0.811
Tuesday	42 (80.8)	10 (19.2)	
Wednesday	31 (72.1)	12 (27.9)	
Thursday	40 (78.4)	11 (21.6)	
Friday	33 (84.6)	6 (15.4)	
Saturday	34 (81.0)	8 (19.0)	
Sunday	47 (79.7)	12 (20.3)	
Shift			
00:00-08:00	67 (77.9)	19 (22.1)	0.539
08:00-16:00	102 (81.6)	23 (18.4)	
16:00-24:00	91 (75.8)	29 (24.2)	
DM			
No	203 (78.1)	57 (21.9)	0.688
Yes	57 (80.3)	14 (19.7)	
HT			
No	128 (74.0)	45 (26.0)	**0.034**
Yes	132 (83.5)	26 (16.5)	
CAD			
No	209 (76.8)	63 (23.2)	0.103
Yes	51 (86.4)	8 (13.6)	
AF			
No	251 (78.9)	67 (21.1)	0.488*
Yes	9 (69.2)	4 (30.8)	
CVD			

No	255 (78.7)	69 (21.3)	0.645*
Yes	5 (71.4)	2 (28.6)	

Chi-square test, *: Fisher exact test

Discussion

This retrospective study was performed during the summer season in a holiday region on the southwestern coast of Turkey, which has a magnificent view of its mountains and sea; demographic data for tourists admitted to health institutions with the diagnosis of STEMI were analyzed. We aimed to determine the status of STEMI in the summer season for the residents and tourists in the holiday regions far from the catheter laboratory required for STEMI treatment since the holiday regions are relatively denser in the summer season, and the access to the extreme areas is more complicated than the inner parts.

In our study, it was observed that the patients who had STEMI in Fethiye, which is a holiday region, were mostly locals. Among the tourists, there was a higher rate of domestic tourists.

The residents had more STEMI, but the rate was higher than expected. One of the reasons for this was the lack of cardiologists in four districts near Fethiye, and the patients who had STEMI from these districts were directed to Fethiye first. Another reason may be that there are various restrictions on the arrival of foreign tourists due to COVID-19 at the time of the study and the late opening of the crossings.

It is seen that most patients are diagnosed in private hospitals; the reason for this may be that STEMI patients go to the nearest hospital either on their own or by ambulance. Prehospital diagnosis and early initiation of treatment affect prognosis and mortality in STEMI patients (16, 17). For this reason, patients are referred to the hospital, where they can be admitted to the catheter laboratory within 120 minutes. They are referred to the nearest hospital that can give fibrinolytic treatment if they cannot.

According to the literature, there are data on whether it is more, especially in cold weather, cold months, and winter (18-20). The reason why myocardial infarction, associated with respiratory tract infections, is more common in winter may be that respiratory tract infection is more common in winter (21, 22). In our study, the most STEMI was seen in July, not September, considered the coldest month of these four months. The reasons for this may be that we did not carry out a study that would include all months or that the number of domestic tourists was higher because there was a nine-day holiday in July at the time of the study. In addition, upper respiratory tract infections may be less common due to the obligation to wear a mask due to COVID-19.

The average of the residents was older than the tourist group, as we expected. The reason may be that the region is famous for paragliding and water sports, and the average age of the tourists and the general tourist group, especially for these sports, is lower (23).

With the prolongation of the human lifespan, the elderly population is increasing (24). This means encountering older tourists with comorbidities. When tourists go on vacation, they carry comorbid diseases with them. In the study of Eray et al. (25), it was conducted that non-traumatic medical emergency admissions were more common than traumatic injuries. It may be related to the high average and prevalence of residents in our study, which only found a significant difference in hypertension as comorbidity.

There are many limitations to our study. Firstly, our study is a retrospective study. The second of these is that we could not have the opportunity to conduct a study on how many of the incoming tourists have had STEMI. Another is that we needed help to reach information on which day the patients who came as tourists had STEMI. Finally, it is not known by which mode of transportation the tourists arrive.

Conclusion

There is no significant difference in the incidence of STEMI among residents or tourists in holiday regions during the summer months. Considering that elderly vacationers with comorbidities will be encountered more frequently, it is beneficial to act early regarding drugs used and STEMI symptom questioning.

Ethics Committee Approval The study was carried out with the permission of Mugla Sıtkı Kocman University Medicine and Health Sciences Ethics Committee (Date:15.11.2021 – Decision Number: 1).

Informed Consent: As this study was a retrospective study, informed consent was not obtained from the patients.

Conflict of Interest: Authors declared no conflict of interest.

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Oxyresveratrolün, Deneysel Yaşa Bağlı Makula Dejenerasyonunda Oksidatif Strese Karşı Koruyucu Etkisi

Protective Effect of Oxyresveratrol Against Oxidative Stress in Experimental Age-Related Macula Degeneration

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Öz

Amaç: Yaşa bağlı makula dejenerasyonu (YBMD), retina pigment epitel kompleksinin nörodejenerasyonunun neden olduğu görme kaybı ile karakterize kronik bir hastalıktır. Fazla oluşan reaktif oksijen türleri (ROS), makula dejenerasyonu başta olmak üzere retina hastalıklarının gelişmesinde önemli rol oynar. Başlıca ROS'lar ise; peroksinitritler, süperoksit radikaller ve hidrojen peroksitlerdir. Çalışmamızda hücre kültürü ortamında hidrojen peroksit (H_2O_2) ile oluşturulan oksidatif hasar öncesi oksiresveratrolün koruyucu etkisini araştırmayı amaçladık.

Gereç ve Yöntemler: İnsan retina pigment epitel (ARPE-19) hücrelerinde H_2O_2 ile oksidatif stres oluşturuldu. Oksidatif hasar öncesi oksiresveratrol 7 farklı konsantrasyonda uygulandı. Koruyucu etkiler, XTT hücre proliferasyonu testi ile hücre canlılığındaki değişiklik izlenerek araştırıldı. Oksiresveratrolün koruyucu etkisini moleküler düzeyde araştırmak için kaspaz-3 ve hücre ölüm tespit kiti kullanıldı.

Bulgular: Çalışmamızda ARPE-19 hücre hattında H_2O_2 ile oluşturulan oksidatif hasar öncesi oksiresveratrol uygulaması hücre canlılığını arttırarak hücrede oksidatif hasara karşı koruyucu etkinlik göstermiştir. Çalışmamız sonucunda elde ettiğimiz bulgularda; oksiresveratrol ARPE-19 hücrelerinde H_2O_2 ile oluşturulan oksidatif hasar oluşum öncesi uygulandığında 100 µM konsantrasyonda hücre hasarını yaklaşık %15 oranında azaltmıştır, buna ek olarak, hücre ölüm tespiti ve kaspaz-3 sonuçlarına göre, oksiresveratrolün oksidatif hasara karşı apoptotik hücre ölümünü azaltarak koruyucu etkinlik göstermektedir.

Sonuç: Bu in vitro çalışma oksiresveratrolün koruyucu etkisinin geliştirilmesi için ön çalışma niteliğindedir. Oksiresveratrol, deney hayvanları ve klinik çalışmalar sonrasında, başta YBMD olmak üzere retina hastalıklarının önlenmesinde etkin bir terapötik ajan olarak geliştirilebilir.

Anahtar Kelimeler: ARPE-19, Oksiresveratrol, Oksidatif Stres, Yaşa Bağlı Makula Dejenerasyonu

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Abstract

Objective: Age-related-macular-degeneration (AMD) is a chronic disease characterized by vision loss caused by neurodegeneration of the retinal pigment epithelial complex. Excess reactive oxygen species(ROS) play an important role in the development of retinal diseases, especially macular degeneration. The main ROS are; peroxynitrides, superoxide radicals and hydrogen peroxides. In our study, we aimed to investigate the protective effect of before oxidative damage induced by hydrogen peroxide(H_2O_2) in cell culture.

Materials and Methods: Oxidative stress was induced with H_2O_2 in human retinal pigment epithelial(ARPE-19) cells. Oxyresveratrol was applied at 7 different concentrations before oxidative damage. Protective effects were investigated by monitoring the change in cell viability with the XTT cell proliferation assay. Caspase-3 and cell-death-detection kit were used to investigate the protective effect of oxyresveratrol and its molecular mechanism.

Results: In our study, oxyresveratrol application before oxidative damage induced by H_2O_2 in ARPE-19 cell line increased cell viability and showed protective activity against oxidative damage in the cell. In the findings we obtained as a result of our study; When oxyresveratrol was applied to ARPE-19 cells before the oxidative damage induced by H_2O_2, it reduced cell damage by about 15% at 100 µM concentration. In addition, according to cell death detection and caspase-3 results, oxyresveratrol showed protective activity against oxidative damage by reducing apoptotic cell death.

Conclusion: This in vitro study is a preliminary study for the development of the protective effect of oxyresveratrol. Oxyresveratrol can be developed as an effective therapeutic agent in the prevention of retinal diseases, especially AMD, after experimental animals and clinical studies.

Keywords: ARPE-19, Oxyresveratrol, Oksidatif Stres, Yaşa Bağlı Makula Dejenerasyonu

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Giriş

Yaşa bağlı makula dejenerasyonu (YBMD), fotoreseptör RPE kompleksinin nörodejenerasyonunun neden olduğu görme kaybı ile karakterize kronik bir hastalıktır. Dünya çapında yaklaşık 200 milyon insanı etkiler, katarakt ve glokomdan sonra üçüncü sırada yer alan körlük nedenidir. Daha da önemlisi, YBMD prevalansı son yıllarda önemli ölçüde artış göstermektedir. 2040 yılında 288 milyona ulaşacağı öngörülmektedir (1).

Yaş, YBMD gelişimi için ana risk faktörüdür; ancak sigara kullanımı, güneş ışığına maruz kalma, alkol tüketimi, artmış vücut kitle indeksi, hiperlipidemi, hipertansiyon, iris pigmentasyonu, geçirilmiş katarakt cerrahisi, lipid profil bozukluğu genetik faktörler ve oksidatif stres başlıca risk faktörleridir (2).

Retina beyne açılan bir penceredir (3). Merkezi sinir sisteminin bir parçası olan retina, biyoenerjetik (ATP üretimi), ışık algısı ve görsel işleme için büyük miktarda glikoz ve oksijen tüketir (4). Retina ayrıca sıkı kan retina bariyerlerine (KRB) sahiptir ve nöroretini dolaşımdaki bağışıklık hücrelerinden ve plazma bileşenlerinden korur (5). Retina pigment epiteli (RPE), fenestre koriocapillaris'i nöroretinadan ayırır, çubuk ve koni fotoreseptörlerinden oluşan retina dış segmentlerine glikoz, oksijen ve besinleri taşıma işlevini yerine getirir (6, 7). RPE disfonksiyonunun bozulması YBMD ile ilişkiliyken, RPE ve fotoreseptördeki gen mutasyonu retinitis pigmentoza (RP) dahil fotoreseptör dejenerasyonuna ve körlüğe neden olur (8-11).

YBMD gelişimindeki moleküler mekanizmalar tam olarak açıklanamamıştır. Fakat, oksidatif stres Bruch membranının geçirgenliğini etkileyerek, koroidal neovasküler membran (KNV) gelişimine sebep olmaktadır (2). Bunun sonucunda da oksidatif hasar, lipofuksin birikimi, kronik inflamasyon ve apopitozis ile ilişkili mekanizmalardaki mutasyonlar YBMD patogenezinde rol oynayan biyolojik yolaklardır (12).

YBMD, erken, orta ve geç tip YBMD olarak sınıflandırılır. Geç tip YBMD hastalarının % 10'unda ıslak tip, % 90'ında ise kuru tip, YBMD görülür (13).

Kuru (atrofik) tip YBMD genellikle yavaş ve ağrısız seyreder. Düz çizgilerin yamuk görülmesi, görme alanında bulanık bir nokta görme bir veya iki gözde merkezi ve renkli görmede azalma, kuru tip YBMD'nin semptomları arasında yer alır (14, 15).

Islak (neovasküler, eksüdatif) tip YBMD, makula bölgesinin altında gelişen anormal kan damarları ile karakterizedir (16). Kötü şekilde biçimlenmiş bu damarlar doku içinde fazla sıvı sızıntısına neden olarak vasküler bütünlüğü bozar (13, 16). YBMD'nin tedavisi semptomatik tedavilerle sınırlıdır (17). Son yıllarda YBMD'den sorumlu genlerinin tanımlanması, hastalık patogenezinde inflamasyon ve oksidatif stresin rolünün anlaşılması ile koruyucu terapötik yaklaşımların geliştirilmesi için altta yatan mekanizmalar aydınlatılmaya çalışılmaktadır (1). Islak YBMD tedavileri, göz enfeksiyonu, retina dekolmanı, yapısal göz hasarı, daha hızlı katarakt başlangıcı ve şiddetli görme kaybı da dahil olmak üzere komplikasyon riskleri taşımaktadır. Bu nedenle koruyucu tedavi yaklaşımlarına ihtiyaç duyulmaktadır (18).

Oksidatif stres, serbest radikallerin oluşumu ile bunların ortadan kaldırılmasından sorumlu endojen antioksidan savunma mekanizmaları arasındaki dengesizlik sonucunda ortaya çıkar (19). Bu serbest radikaller, DNA, lipidler, proteinler ve nükleik asitlerin, oksijen veya azot türlerinden zarar görmesine neden olur. Yaşlanmanın altında yatan kesin hücresel mekanizmalar hala belirsizdir, ancak oksidatif hasarın, organların, dokuların ve hücrelerin işlev kaybına neden olduğu bilinmektedir (19). Oksidatif stres, glokom, diyabetik retinopati, retinal ven tıkanıklığı ve YBMD gibi retina hastalıklarının gelişmesinde ve hızlanmasında da kritik öneme sahiptir. Başlıca serbest radikaller; süperoksit radikaller, peroksinitritler ve hidrojen peroksitlerdir (20-22).

Stilbenler, bir etanol veya etilen molekülü ile birleştirilmiş iki benzen halkası içerirler, doğal olarak oluşan fitoaleksinlerdir ve fenilpropanoid yolu ile sentezlenirler (23). Stilbenler, trans stereoizomerler olarak bulunan çok çeşitli bitki kaynaklarından elde edilebilirler. Yer fıstığı (Arachis hypogaea), üzüm asması

(Vitis vinifera), çam türleri (Pinustürleri) ve dutlarda bulunurlar (Morus Alba). Antioksidan özellik başta olmak üzere, antiinflamatuar, vazoprotektif, antikanser antimikrobiyal gibi önemli biyolojik özelliklere sahiptir (23).

Oksiresveratrol (2,3',4,5'-tetrahidroksistilben), kimyasal yapıya sahip $C_{14}H_{12}O_4$; moleküler ağırlığı 244,24 g/mol olan doğal bir stilbendir (24). Oksiresveratrol hem serbest hem de glikozidik formlarda meydana gelir ve dört OH grubuna sahiptir (24).

Oksiresveratrol, Morus Alba'nın (beyaz dut) kökünde bulunan bir bileşik olan Mulberroside A'nın hidrolitik aktivasyonu ile sentezlenen bir stilben ve fitoöstrojendir. Antioksidasyon, antienflamatuar, anti-viral, serbest radikal temizleme faaliyetleri tirozinaz inhibitör ve nöroprotektif, aktiviteleri bulunmaktadır (24-28). Bununla birlikte, güncel çalışmalar arasında ARPE-19 hücrelerinde oksiresveratrolün oksidatif stresi önleyici etkisinin araştırıldığı bir çalışma bulunmamaktadır. Yaptığımız bu çalışma ile oksiresveratrolün retina pigment epitel hücrelerinde hidrojen peroksit ile oluşturulan oksidatif hasara karşı koruyucu etkisini tespit etmenin yanı sıra bu etkinin moleküler mekanizmasını da araştırmayı amaçladık.

Gereç ve Yöntemler

Çalışma Hücre Kültürü Uygulamaları

İnsan retina pigment epitel (ARPE-19) hücre hattı, Amerikan Tipi Kültür Koleksiyonundan (ATCC, Manassas, VA, ABD) alındı. Hücre hattı 100 µ/ml penisilin (Sigma-Aldrich, St. Louis, MO, ABD) ve %10 Fetal Bovine Serum (FBS; Gibco/BRL, Gaithersburg, MD, ABD) ile desteklenmiş Dulbecco Modified Eagle Medium/F12 (DMEM/F12) içerisinde 37°C'de %5 CO_2'lik varlığında kültürlendi. Hücreler 2-3 günde bir invert mikroskop ile muayene edildi ve %75-80 yoğunluğa ulaştıklarında seyreltilerek pasajlar yapıldı.

ARPE-19 Hücrelerinde Oksidatif Hasar Oluşum Öncesi Oksiresveratrolün Etkisinin Değerlendirilmesi

Oksidatif hasar oluşumu öncesi oksiresveratrol uygulaması için etkinliğinin belirlenmesi amacıyla 96 kuyucuklu platelere eşit sayıda ekildi %5 CO_2 ve %95 hava içeren etüv içerisinde, 37 °C' de 48 saat boyunca inkübe edildi. Ardından kontrol, çözücü kontrol ve oksiresveratrol (Sigma-Aldrich, Chemie GmbH Eschenstrasse, Taufkırchen) farklı konsantrasyonlarda (100, 10, 1, 0,1, 0,01, 0.001 ve 0.0001 µM) 100 µl uygulanarak, 24 saat inkübasyona bırakıldı. İnkübasyon sonunda üzerine 400 µM konsantrasyonda 100µl H_2O_2 (Merck KGaA, Darmstadt, Germany) uygulandı 20 saat inkübe edildi. Sonrasında XTT solüsyonu hazırlanarak hücre canlılığı değerlendirildi.

Hücre Proliferasyon Canlılık Testi II (XTT)

Bu çalışmada, ARPE-19, Hücre hücre proliferasyonu değerlendirmek amacı ile XTT (2,3-bis [2-metoksi-4-nitro–5-sulfofenil]-2H-tetrazolyum-5- karboksianilid tuzu) yöntemi kullanılmıştır (29). XTT (Cell Proliferation Kit II (XTT) çözeltisi eklenip 4 saat bekletildi sonrasında, absorbans değerleri mikroplate okuyucu ile saptandı.

Yüzde Canlılık = [Örnek ABS ortalama / Kontrol ABS ortalama] x 100

Biyokimyasal Analizler

Hücre Ölüm Tespiti

Çalışmamızda kullandığımız Hücre Ölümü Tespiti Kiti ELISA (Cell Death Detection ELISA Roche 11 544 675 001), apoptoz geçiren hücrelerin sitoplazmasında mevcut olduğu bilinen histonla ilişkili DNA fragmanlarını (mono ve oligonükleozomlar) analiz etmek için kullanıldı. ARPE-19 hücreleri 6 kuyucuklu platelere ekildi, bir gece inkübe edildikten sonra oksiresveratrol ve hidrojen peroksit uygulamaları yapıldı 20 saatlik inkübasyon süresini tamamlamak üzere 37°C inkübatörde bekletildi. Hücre ölümü tespiti kiti protokolü uygulandı. Elisa plaka okuyucu ile birincil dalga uzunluğu olarak 450 nm spektrofotometrede her mikro kuyunun absorbansını okundu.

Kaspaz-3

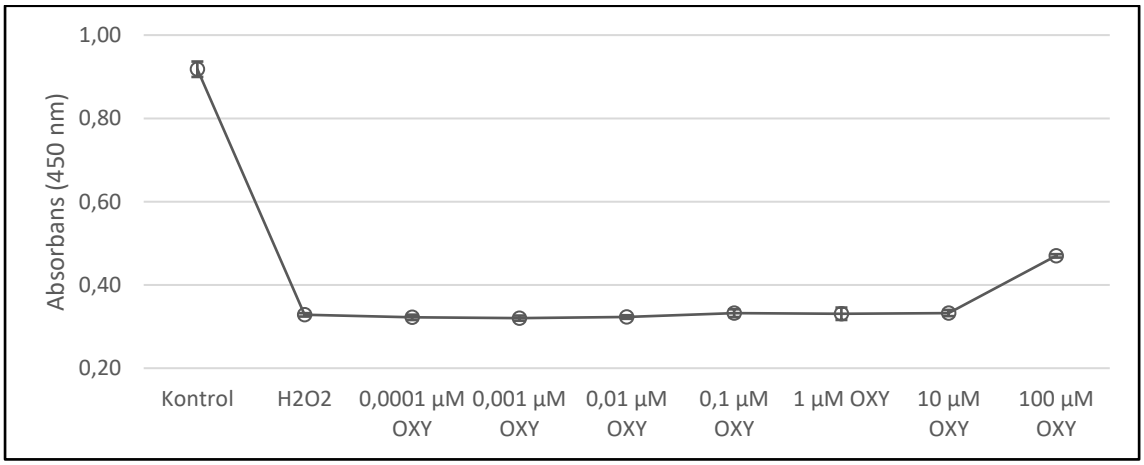
Kaspaz-3'ün kantitatif tespiti için Human Caspase-3 Instant ELISA Kiti kullanılmıştır. ARPE-19 hücreleri 6 kuyucuklu platelere ekildi, bir gece inkübe edildikten sonra oksiresveratrol ve hidrojen peroksit uygulamaları yapıldı 20 saatlik inkübasyon süresini tamamlamak üzere 37°C inkübatörde bekletildi. Human Caspase-3 Instant ELISA Kiti protokolü uygulandı. Elisa plaka okuyucu ile birincil dalga uzunluğu olarak 450 nm spektrofotometrede her mikro kuyunun absorbansını okundu.

İstatistiksel Analiz

Verilerin analizinde normal dağılım önşartı Shapiro-Wilk testiyle analiz edilmiş, basıklık ve çarpıklık katsayıları da incelenmiştir. Grupların karşılaştırılmasında One-Way ANOVA testi kullanılmış, çoklu karşılaştırmalar için Fisher's LSD post hoc test ve uygulamaların kontrol grubu ile kontrolün hidrojen peroksit grubu ile karşılaştırılması için Dunnet post hoc testi kullanılmıştır. Verilerin tanımlayıcı istatistikleri ortalama ve standart sapma ile tablo halinde verilmiştir. İstatistiksel analizler SPSS v.22 paket programı aracılığı ile yapılmış ve anlamlılık düzeyi 0,05 olarak kabul edilmiştir.

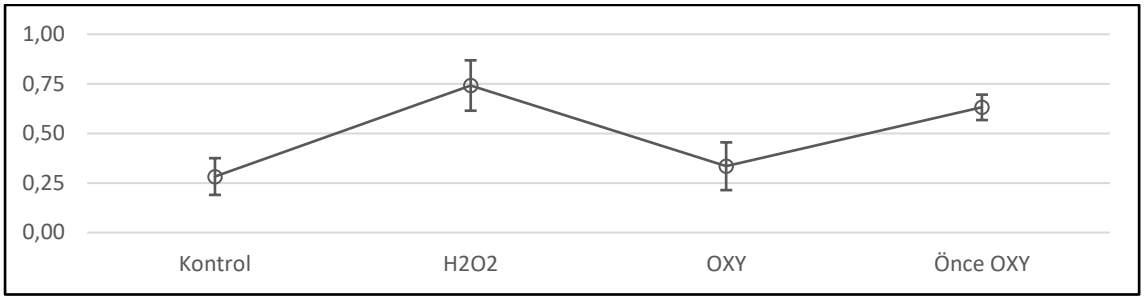
Bulgular

Hücre kültürü ortamında YBMD modelinde, koruyucu ajan olarak kullanılan oksiresveratrol, ARPE-19 hücrelerinde 400 µM H_2O_2 ile oluşturulan oksidatif hasar öncesi farklı konsantrasyonlarda uygulandığında, 100 µM oksiresveratrol konsantrasyonunda hidrojen peroksitin oluşturduğu hücre hasarını yaklaşık %15 oranında azaltarak istatistiksel olarak anlamlı bir etki oluşturmuştur ($p<0,001$; Şekil 1).

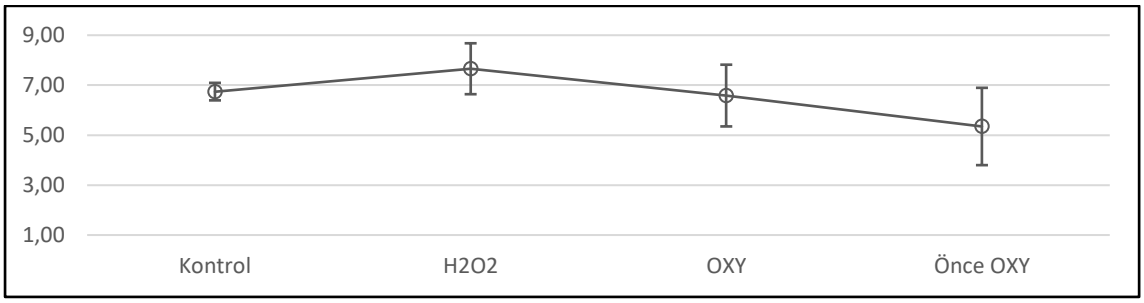


Şekil 1. ARPE-19 hücrelerinde oksidatif hasar oluşum öncesi oksiresveratrolün (OXY) hücre canlılığına etkisi

Nöroprotektif ajan olarak kullanılan oksiresveratrol, ARPE-19 hücrelerinde 400 µM H_2O_2 ile oluşturulan oksidatif hasar öncesinde 100 µM uygulandı, hücre ölümü H_2O_2 grubuna kıyasla tüm çalışma gruplarında azalmıştır. Önce oksiresveratrol grubunda da azalma görülmesine karşın H_2O_2 grubu ile istatistiksel olarak benzerlik göstermektedir. Oksiresveratrolün oksidatif stres altında ARPE-19 hücrelerinde apoptozu baskıladığı görülmektedir ($p<0,001$; Şekil 2). Kaspaz-3 aktivitesi ise, H_2O_2 grubuna kıyasla tüm çalışma gruplarında azalmıştır. Önce oksiresveratrol grubu ile H_2O_2 arasında istatistiksel anlamlı bir şekilde hücre ölümünde azalma görülmektedir ($p<0,001$; Şekil 3). Oksiresveratrolün oksidatif stres altında ARPE-19 hücrelerinde mitokondri aracılı apoptozu baskıladığını göstermektedir ($p<0,001$; Şekil 3).



Şekil 2. ARPE-19 hücrelerinde oksidatif hasar oluşum öncesinde oksiresveratrol (OXY) uygulamasında hücre ölümünün değerlendirilmesi



Şekil 3. ARPE-19 hücrelerinde oksidatif hasar oluşum öncesinde oksiresveratrol (OXY) uygulamasında kaspaz-3 aktivitesinin değerlendirilmesi

Tartışma

Yaşlılık döneminde birçok dejeneratif hastalıkla karşılaşabilmektedir. YBMD de bunlar arasında yer alır. YBMD'nin prevalansı gün geçtikçe artış göstermektedir (1). Mevcut farmakoterapi ise semptomatik tedaviler ile sınırlı olası alternatif tedavi arayışlarını arttırmıştır (30). Son dönemde, sorumlu genlerinin tanımlanması, inflamasyon ve oksidatif hasarın rolünün anlaşılması ile yeni terapötik yaklaşımlar geliştirilmeye çalışılmaktadır (1). Hiperlipidemi, hipertansiyon, iris pigmentasyonu, geçirilmiş katarakt cerrahisi, lipid profil bozukluğu ve genetik olarak risk gurubunda olan bireylerde YBMD gelişmesinin önlenmesi hedeflenmektedir. Bu hasarların önlenmesi ve serbest radikallerin temizlenmesi için çeşitli fitoöstrojenlerin ve antioksidanların etkin olacağı düşünülmektedir (1, 31). Güçlü antioksidan özelliğe sahip olan oksiresveratrolün oksidatif strese karşı doğrudan ROS süpürme ve apoptotik yolaklar üzerinden nöroprotektif etki gösterdiği bilinmektedir (26-28, 32).

Çalışmamızda oksiresveratrolün hidrojen peroksit kaynaklı oksidatif hasarda koruyucu etkilerini hücre kültürü ortamında araştırılmasını hedefledik. Çalışmamız, ARPE-19 hücre hattında oksidatif stresin oluşturduğu hasarda oksiresveratrolün koruyucu rolünün araştırıldığı ilk çalışmadır. Çalışmamız sonucunda elde ettiğimiz bulgularda; H_2O_2 ile ARPE-19 hücrelerinde oluşturulan oksidatif hasar oluşum öncesi oksiresveratrol uygulandığında 100 µM konsantrasyonda hücre hasarını yaklaşık %15 oranında azalttığı, görüldü.

Hu ve Ark., insan mercek epitel hücre (HLEC)'lerinde yaptıkları çalışmada H_2O_2 ile oluşturulan oksidatif hasarda. HLEC hücreleri oksidatif stres öncesi 1 saat boyunca oksiresveratrol (0, 1, 5, 10 veya 20 µM) ile inkübe edilmiştir. Hücre canlılığı tespiti için 3-4,5-dimetil-tiyazolil-2,5-difeniltetrazolyum bromür (MTT) testi kullanılmış ve oksiresveratrolün doza bağlı bir şekilde koruyucu etki gösterdiği sonucuna varılmıştır (33). Lorenz ve Ark., yaptığı çalışmada ise, sıçan astrogliyal hücre hattında H_2O_2 ile oluşturulan oksidatif stresten 30 dakika önce 100 µM oksiresveratrol uygulaması yapılmış ve diklorofloreseine (DCF) ile ölçüm yapılmıştır. Oksiresveratrol uygulaması güçlü bir koruma sağlamıştır (34). Yaptığımız çalışmada da

nöroprotektif etkisini denediğimiz oksiresveratrolün H_2O_2 kaynaklı oksidatif hasara karşı koruyucu etkileri, bulgularımızın literatürlerle uyum içinde olduğunu göstermektedir.

Oksidatif stres sırasında artan aşırı ROS üretimi voltaja duyarlı kalsiyum kanalları ve birçok iyon kanalını uyararak sitozole kalsiyum geçişine bağlı olarak serbest radikallerin üretimini de arttırmaktadır. Bu kısır döngü lipid peroksidasyonunu arttırarak apoptozu indüklemektedir (35). Sıçan kortikal nöronlarında N-metil-D-aspartat (NMDA) ile indüklenen nöronal hücre hasarında oksiresveratrolün hücre içi Ca^{2+} konsantrasyonundaki artışı baskılayarak, sıçan kortikal nöronlarını indüklenen apoptotik hücre ölümüne karşı koruduğu sonucuna varılmıştır (36). Chao ve Ark. oksiresveratrolün parkinson hastalığındaki nöroprotektif etkilerini araştırmak amacı ile yaptıkları çalışmada, nöroblastoma (SH-SY5Y) hücrelerinde 6-hidroksidopamin (6-OHDA) nörotoksisitesi oluşturulmuştur. SH-SY5Y hücreleri 30 dakika boyunca 25 μM oksiresveratrol veya resveratrol ile inkübe edilmiştir, ardından 2 veya 6 saat boyunca 25 μM 6-OHDA inkübe edilmiştir. Nörotoksisite öncesi oksiresveratrol uygulanan gruplarda, oksiresveratrol kaspaz-3 aktivitesini yaklaşık %50 oranında baskılayarak nöroprotektif etki göstermiştir. Resveratrol ile karşılaştırıldığında, oksiresveratrol'ün nöroproteksiyonda daha etkili olduğu bildirilmiştir (37). Çalışmamızda ise; hücre ölümü tespiti ve kaspaz-3 sonuçlarına göre oksiresveratrol, ARPE-19 hücre hattında hidrojen peroksit ile indüklenen hücre hasarına karşı apoptotik hücre ölümünü azaltarak koruyucu etkinlik göstermektedir.

Çalışmamızda antioksidan etkinliğe sahip olduğu bilinen oksiresveratrolün ARPE-19 hücrelerinde H_2O_2 ile oluşturulan oksidatif hasara karşı koruyucu etkileri ortaya konmuş. Bu in vitro çalışma koruyucu etkinin geliştirilmesi için ön çalışma niteliğindedir. Oksidatif stres kaynaklı ilerleyici dejenerasyon, diyabetik retinopati ve YBMD'nin önlenmesinde deney hayvan ve klinik çalışmalar sonrasında koruyucu bir terapötik ajan olarak geliştirilebilir.

Etik Kurul Onamı: Hazır hücre hattı kullanılması nedeniyle etik kurul onayı alınmamıştır.

Bilgilendirilmiş Onam: Çalışmada insan gönüllüler kullanılmamıştır.

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Wilson Hastalığının Nadir Bir Komplikasyonu: Hepatoselüler Karsinom

A Rare Complication of Wilson's Disease: Hepatocellular Carcinoma

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Öz

Bakır metabolizma bozukluğu olan Wilson hastalığı, klinik spektrumu oldukça geniştir. Birikim yaptığı organa spesifik bulgu vermektedir. Bu hastalar gastroenteroloji polikliniğinde asemptomatik transaminaz yüksekliği ile başvurabileceği gibi, ileri komplikasyonlardan olan siroz ve hepatoselüler karsinom şeklinde de prezente olabilmektedir. Olgu sunumunda siroz nedeniyle takipli olan Wilson hastasının rutin takiplerinde AFP (alfa-feto protein) yüksekliği olması nedeniyle ileri araştırmalar sonucunda HCC (hepatoselüler karsinom) tanısı alan hastayı sunmaktayız.

Anahtar Kelimeler: Wilson, Hepatoselüler Karsinom, Siroz

&

Abstract

Wilson's disease, which is a disorder of copper metabolism, its clinical spectrum is quite wide. It gives specific findings to the organ it accumulates. These patients may present with asymptomatic transaminase elevation in the gastroenterology outpatient clinic, as well as cirrhosis and hepatocellular carcinoma, which are advanced complications. In the case report, we present a patient who was diagnosed with HCC (hepatocellular carcinoma) as a result of advanced research due to the high AFP (alpha-feto protein) during routine follow-up of a Wilson patient who was being followed up due to cirrhosis.

Keywords: Wilson, Hepatocelüler Carcinoma, Cirrhosis

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Giriş

Otozomal resesif geçişli olan Wilson hastalığında, bakır taşıyıcı protein ATP7B geninin mutasyona uğraması ile sitozolden bakır atılımında bozulma meydana gelmektedir. Bu proteinde oluşan mutasyon nedeniyle bakırın sitozolden golgi aygıtına taşınmasında problem olmakta, sitozolde biriken ve safraya ekskresyonu yapılamayan bakır kan dolaşımına katılır, daha sonra da vücuttan idrar yoluyla atılımı gerçekleşir (1,2). Wilson hastalığı ile ilgili 800'den fazla mutasyon tanımlanmıştır (3). Dünya Sağlık Örgütü, Wilson hastalığının genel toplum prevalansının 1/10.000 ile 1/30.000 olduğunu düşünmektedir (4).

Esas olarak semptomlar bakırın birikim yaptığı organa spesifik semptomlar şeklindedir. Örneğin korneada Kayser-Fleischer halkaları, sarılık, transaminaz yükseklikleri, dizartri, endokrin bozukluklar, aritmi, titreme ve halsizlik semptom ve bulguları ortaya çıkmaktadır. Karaciğer belirtileri asemptomatik hastalıktan fulminan hepatik yetmezlik spektrumuna kadar geniş aralıkta arasında olabilmektedir. Genel olarak önce hepatik sistem belirtileri daha sonra nörolojik belirtiler olmaktadır. Nörolojik olarak ekstrapiramidal sistem ve bulber tutulum sık olmaktadır. En erken bulgu dizartridir (5). Karakteristik olarak düşük alkalen fosfataz düzeyi, hipoürisemi, coombs negatif hemolitik anemi varlığında Wilson hastalığından şüphelenmemiz gerekmektedir (6).

Wilson hastalığında hepatoselüler karsinom gelişimi bakırın anti-oksidan özellikli olması nedeniyle nadir görülmektedir. Birleşik Krallık ve İsveç'te teşhis edilen 363 Wilson hastasının retrospektif analizinde, 10-29 yıllık takipli hastaların %4,2-5,3'ünün HCC veya kolanjiokarsinom geliştirdiğini ve 39 yıllık takipte de bu oranının %15 olduğu tespit edilmiş olup, yaşla beraber hepatik malignite riski artığından yıllık abdominal ultrason taraması önerilmektedir (7).

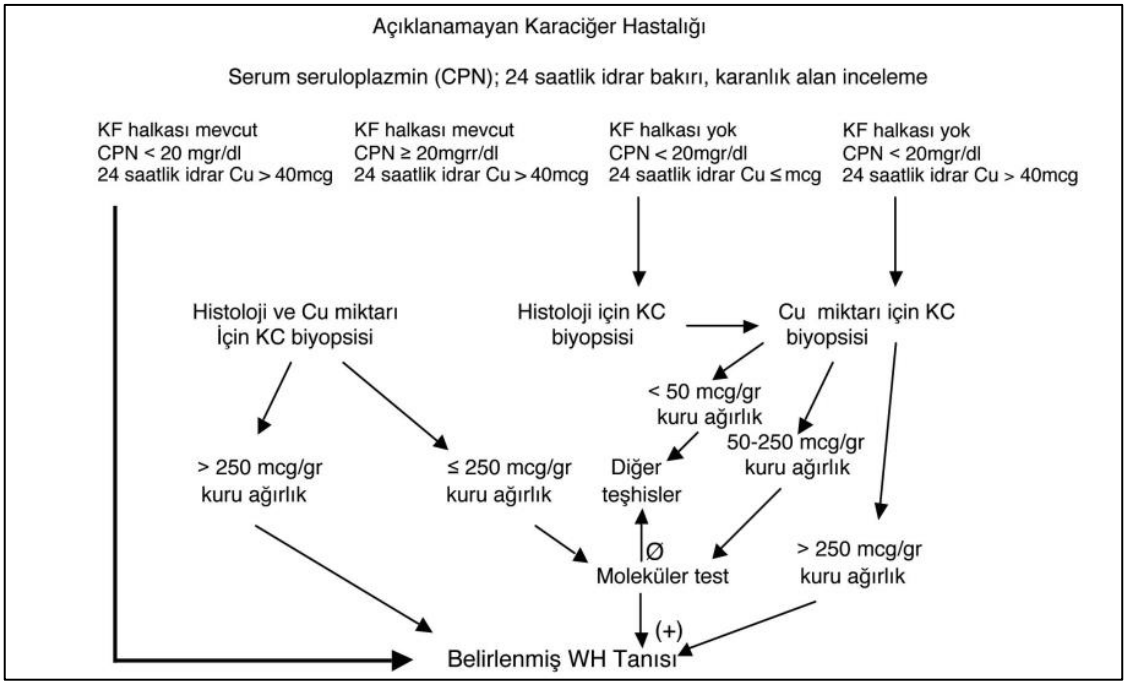
Olgu

43 yaşında erkek hasta 2002 yılda dizartri, kayser-fleischer halkası, transaminaz yüksekliği, nörolojik belirtiler nedeniyle araştırılan hastaya Wilson tanısı konulmuştur. Üniversitemizde 2012 den beri Wilson hastalığına bağlı siroz nedeniyle takip edilmektedir. Hasta o tarihten beri d-penislamin, pirasetam, çinko tedavisi düzenli kullandığını belirtmekteydi. Hastanın dizartrisi devam etmektedir. Özefagusta evre 2 varisleri, trombositopeni, AST ve ALT değerleri normal aralıkta ancak AST>ALT idi, GGT değeri 80 U/L idi. Hastanın 1 yıl önceki polikinik kontrolünde siroz kliniğine ait bulgular dışında patolojik bulgu olmayıp, AFP değerleri normal aralıkta ve abdominal ultrasonda kitle mevcut değildi. Poliklinik başvurusu sırasında AFP değeri 2179 IU/ml (normal aralık 0,5-5,5 IU/ml) çıkması üzerine test tekrarlandı, yeni değeri 2946 IU/ml idi. Yapılan hepatobiliyer ultrasonda karaciğer sağ lob anteriorunda 28 mm boyutunda ekojen görünümlü lezyon olması nedeniyle hepatoselüler karsinom (HCC) açısında dinamik abdomen tomografi çekilmesi planlandı.

Abdominal BT sonucunda karaciğer parankim kaba granüler, konturları irregüler, karaciğer segment 6-7 bileşke düzeyinde aksiyal boyutları 23x20 mm olarak ölçülen arteriyel fazlarda yoğun heterojen kontrastlanan portal ve geç fazlarda wash-out gösteren kitle (HCC?) olarak yorumlandı. Hasta girişimsel radyoloji ile beraber değerlendirildi. Hastanın Child-Pugh skoru sınıf A' idi. Hastaya perkütan tümör ablasyon tedavisi yapıldı. Takiplerde AFP değeri tedrici olarak düştü. Hasta takip ve tedavilerine devam etmektedir.

Tartışma

Wilson hastalığı ATP7B mutasyonu nedeniyle bakırın safraya atılımında bozulma olmasıyla ortaya çıkan bakır birikim hastalığıdır. Karaciğerde aşırı bakır birikimi serbest radikal oluşumu, mitokondriyal hasara neden olarak hepatosit nekrozuna neden olabilmektedir. Bu reaksiyonlar karaciğerde inflamasyon, fibrozise neden olarak siroz oluşumuna neden olmaktadır (8). Şekil 1 de açıklanmayan karaciğer hastalığı olan bir hastaya Wilson açısından tanısal yaklaşımı göstermektedir.



Şekil 1: Wilson Hastalığına tanısal yaklaşım(8)

Wilson hastalığı tanısında Stiernlieb's kriterleri:

1) Kayser-Fleischer halkası,

2) Tipik nörolojik semptomlar,

3) Düşük serüloplazmin düzeyi (250 μgr/gün kuru ekstre).

Kriterlerden 2 veya daha fazlası tanı koydurucudur (9).

Hepatositlerde bakır birikimine bağlı asemptomatik aminotransferaz yüksekliğiyle beraber, akut hepatit, fulminan yetmezlik, hepatoselüler karsinom, intrahepatik kolanjiokarsinom gibi hastalıklara neden olabilmektedir.

Olgumuzda 20'li yaşlarda kompanse karaciğer siroz etyolojisi araştırılırken tanı konulan Wilson tanılı hastada, poliklinik taramaları sırasında AFP yüksekliğinden şüphelenilerek yapılan batın ultrasonografisinde kitlesel lezyon tespitiyle HCC tanısı alan olguyu sunduk.

Wilson hastalığının hepatik malignitelere yol açması diğer kronik karaciğer hastalıkları ile karşılaştırılamayacak kadar düşüktür, Pfeiffenberger ve arkadaşlarının çalışmasına göre bu oran yaş ilerledikçe artmakta olup genel prevalansı %1.2 düzeyindedir (10). Bu genetik hastalığın ileri bir yaşta teşhisi, uzun süredir tedavi edilmemiş Wilson hastalığının hepatoselüler karsinom için bir risk faktörü temsil edebileceğini düşündürmektedir (10). Bu nedenle Wilson hastalarında fibrozis evresinin belirlenmesi ve sirozlu hastaların sürveyans programına alınmasının hepatik malignitelerin daha erken teşhis edilmesine olanak sağlayabilmektedir (10).

Meer ve arkadaşlarının 130 wilson tanılı hasta ile yapılan 15 yıllık takiplerde; HCC'nin yıllık insidansı %0,09 ve sirotik hastalarda %0,14 olarak tespit etmişlerdir (11).

Hastalarda aşırı bakır birikimine bağlı kronik enflamasyonun siroza yol açtığı, sirozlu Wilson hastalarında süreğen rejenerasyon / dejenerasyon sürecinin onkojen potansiyele neden olarak HCC gelişmesine neden olduğu düşünülmektedir (9,10,11). Xu ve arkadaşlarını yaptığı çalışmaya göre genellkle HCC vakaları siroz tanılı Wilson hastaları olduğunu belirtmişlerdir (15).

HCC tanılı Wilson hastalrında özel bir tedavi şeması olmayıp, standart HCC protokollerinde Barselona Kliniği Karaciğer Kanseri (BCLC) evrelemesi yapılıp tedavi kararı verilmiştir.

Wilson hastalığı zemininde HCC gelişimi nadir görülmekle beraber Wilson hastalarının rutin takiplerinde HCC taraması yapılması, HCC açısından dikkatli olunması gerektiğini vurgulamak istedik. Ayrıca özellikle Wilson hastalığına bağlı siroz tanısıyla takip edilen hastada son 1 yıllık poliklinikteki rutin takiplerinde HCC tespit etmiş olup literatüre katkı sunacağımızı düşünerek bu olgu sunumunu gerçekleştiriyoruz.

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Klippel-Trenaunay Sendromunda Alt Gastrointestinal Kanama, Mesane ve Dalakta Hemanjiyomlar: Nadir Görülen Olgu Sunumu

Klippel-Trenaunay Sydrome with Lower Gastrointestinal Bleeding, Bladder and Splenic Hemangiomas: A Rare Case Report

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Öz

Klippel-Trenaunay Sendromu (KTS); genellikle doğumda veya erken bebeklik döneminde ortaya çıkan kütanöz hemanjiyom, doğuştan venöz anomaliler, kemik ve yumuşak dokuda hipertrofi ile karakterizedir. Çoğunlukla periferik bulgularla tanı alan KTS nadirde olsa gastrointestinal sistemi (GİS) içeren vasküler malformasyonlar ile birlikte de karşımıza çıkabilir. Bu yazıda rektal kanama ve anemi şikayeti ile başvuran, herhangi bir periferik bulgusu olmadan distal kolon, rektum, mesane ve dalak tutulumuyla karşımıza çıkan 45 yaşında erkek hasta sunulmuştur. Anemi tedavisi ve transfüzyona yanıt alınamayan hastada küratif tedavi olarak rektosigmoid rezeksiyon (low-anterior rezeksiyon) ve kolorektal anastomoz ameliyatı uygulandı.

Anahtar Kelimeler: Klippel-Trenaunay Sendromu, Gastrointestinal Kanama, Hemanjiyom

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Abstract

Klippel-Trenaunay Syndrome (KTS); It is characterized by cutaneous hemangioma, congenital venous anomalies, bone and soft tissue hypertrophy usually occurring at birth or in early infancy. KTS, which is mostly diagnosed with peripheral findings, can rarely be encountered with vascular malformations involving the gastrointestinal system (GIS). We herein report a 45-year-old male patient who admitted with rectal bleeding and anemia and presented with distal colon, rectum, bladder and spleen involvement without any peripheral findings. Rectosigmoid resection (low-anterior resection) and colorectal anastomosis surgery were performed as curative treatment in the patient who did not respond to anemia treatment and transfusion.

Keywords: Klippel Trenaunay Syndrome, Gastrointestinal Bleeding, Hemangioma

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Giriş

Klippel Trenaunay Sendromu (KTS); variköz venler, porto şarabı lekesi olarak adlandırılan kutanöz kapiller malformasyon ve yumuşak doku yada kemik hipertrofisi triadı (asimetrik ekstremite hipertrofisi, lokalize kapiller hemanjiomlar ve konjenital alt ekstremite varisleri) ile tanımlanır (1). KTS tanılı hastaların %63'ünde bu triad görülürken %37'sinde bu üç bulgudan yalnızca iki tanesi bulunmaktadır. Yani hastalar bu klasik triadın yalnızca iki bulgusu ile de KTS tanısı alabilir (2). Tanısı bahsettiğiniz üzere klinik olarak konmaktadır ve oldukça zordur, nadirde olsa hemanjiomatöz lezyonlar abdominal organlarda görülmektedir ancak literatürde triadın parçaları ile ya da D-dimer yükseliği ve AGGF1 gen mutasyonu ile veriler desteklenmiştir. Hastalar karşımıza bu klasik triad dışında pulmoner emboli, derin ven trombozu ve gastrointestinal kanama gibi hayatı tehdit edici bulgularla da gelebilir (1). Erken tanı hayatı tehdit edici bu komplikasyonları önlemek açısından önemlidir.

Bu yazıda KTS tanısı olmayan rektal kanama şikâyeti ile başvuran 45 yaşında erkek hasta sunulmuştur.

Olgu

45 yaşında erkek hasta yaklaşık 2 yıldır olan makattan kanama, kabızlık ve anemi şikayetiyle genel cerrahi kliniğine başvurdu. Hasta kabızlık ile beraber kanama şikayetinin arttığını belirtti. Hematüri şikayeti olmadı. Hasta kliniğimize başvurusu öncesinde anemi tedavisi almış, gerekli olduğunda kan transfüzyonu yapılmış. Hatta bir dönem ülseratif kolit tanısı ile takip edilmiş. Hastanın bilinen kronik hastalığı bulunmamaktadır. Başvurduğu zamanki vital bulguları stabil olmakla beraber fizik muayenesinde belirgin bir anormallik görülmedi ve rektal tuşede patoloji saptanmadı.

Çekilen abdomen bilgisayarlı tomografisinde rektumda yaklaşık 7cm'lik segmentte 2cm'ye varan, diffüz kitlesel duvar kalınlaşması izlendi. Dalak orta polde yaklaşık 1 cm boyutunda kist ya da hemanjiyom ayrımı net yapılamayan hipodens görünüm izlendi. Hastaya alt GİS kanama nedeniyle kolonoskopi yapıldı. Hastanın yapılan kolonoskopisinde anal mukozadan başlayıp rektum ve sigmoid kolon boyunca dilatetortüyöz venler görüldü.

Hastaya yapılan tanısal laparotomide dalakta, rektosigmoid bölgede ve mesane fundusundan başlayıp mesanenin arka duvarı boyunca uzanan hemanjiyomlar izlendi (Şekil 1-A, 1-B). Hastaya rektosigmoid rezeksiyon (anterior rezeksiyon) ve koloanal anastomoz ameliyatı uygulandı. Kolon rezeksiyonu sonrası intraoperatif mesane arkasındaki hemanjiyomlar kayboldu. Asemptomatik olması nedeniyle splenektomi yapılmadı. Patolojiye gönderilen parça kavernözhemanjiyom şeklinde değerlendirildi.

Taburculuk sonrası hastanın hematokezya şikâyeti tekrarlamadı.

Tartışma

KTS; genellikle neonatal dönemde ortaya çıkan kutanöz hemanjiyom, doğuştan venöz anomaliler, kemik ve yumuşak dokuda hipertrofi ile karakterize olan ve genellikle tek ekstremite tutulumuyla giden hiperplazi sendromlarından biridir (3).

KTS etyolojisi tam olarak bilinmeyen nadir bir konjenital vasküler bir bozukluktur. Çoğunlukla sporadik olarak görülmesine rağmen, otozomal dominant olarak kalıtıldığı ve gen defektinden kaynaklandığı bildirilmiştir. Cinsiyet farkı yoktur (4). Cilt bulguları nedeniyle çoğunlukla çocukluk çağında tanı almakta olan bu hastalık bizim vakamızda 40 yaşında tanı almıştır.

Genelde yaşamı tehdit etmeyen bu sendromda periferik bulguların yanı sıra visseral tutulumun gözlenmesi morbidite ve mortalite artışına neden olabilir. KTS'dekivisseral vasküler malformasyonlar gastrointestinal sistem, karaciğer, dalak, mesane, böbrek, akciğer ve kalp gibi organlarda tanımlanmıştır. GİS tutulumu kendini genellikle rektal kanama ile belli eder. Bu kanama asemptomatik gizli kanamadan masif, hayatı tehdit edici kanamaya kadar değişebilir. GİS'teki en yaygın kanama bölgeleri distal kolon ve

rektumdur (5). Bizim vakamız hematokezya ve anemi şikâyeti ile başvurdu; inen kolon distali, sigmoid kolon, rektum, mesane ve dalakta hemanjiyomları mevcuttu. Daha öncesinde bilinen herhangi bir hastalık tanısı almamıştı.

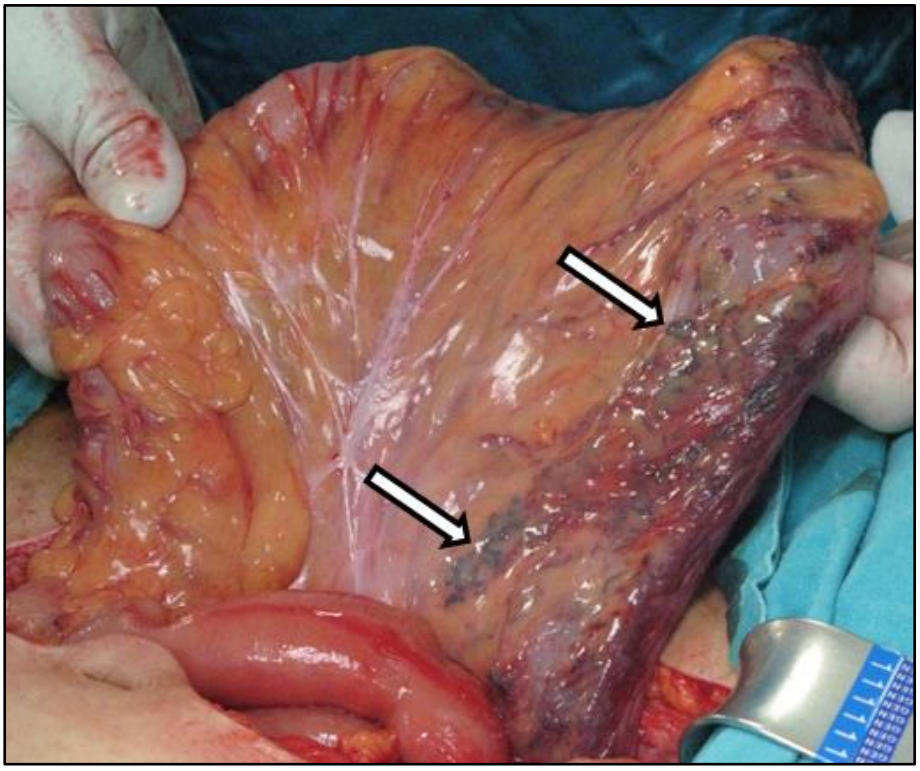
Bilgisayarlı tomografi (BT); visseral vasküler malformasyonları değerlendirmek için basit ve invaziv olmayan bir tanı yöntemidir ancak kesin tanı koydurmada yeterli değildir (5). BT; bizim vakamızda dalaktaki hemanjiyomu göstermiş olup, kalın barsaktaki lezyonların ayrımını net olarak yapamamış, mesanedeki hemanjiyomu gösterememiştir.

GİS kanamasının kesin lokalizasyonu ve yönetimi için gastrointestinal kanalın endoskopik olarak incelenmesi gerekir. Ancak endoskopik inceleme sırasında bu nadir tanının farkında olunmadan vasküler malformasyonlardan biyopsi alınması ölümcül kanama ile sonuçlanabilir. Bu nedenle girişimsel muayene ve tedavi en ideal yöntemdir. Anjiografi vasküler malformasyonlarda altın standart tanı yöntemidir. Ayrıca bazı durumlarda teröpatik müdahale olasılığı vardır; hemostaz, damar içine vazopressin infüzyonu veya anjiografik kateter yardımıyla embolizasyon sağlanabilir. Ek olarak; endoskopik inceleme de olduğu gibi barsak temizliğine ihtiyaç duyulmamaktadır (5). Tüm bu avantajlarının yanında sınırlı sayıda merkezde anjiografinin yapılması dezavantajını oluşturmaktadır.

Hemanjiyom dalağın en yaygın iyi huylu primer tümörüdür. Splenikhemanjiyomlar KTS'nin bir parçası olabilir. Bir hemanjiyomun seyrini tahmin etmek zordur. Büyük hemanjiyomlar (>4cm) daha küçük olanlara göre spontan ya da küçük travmalara bağlı olarak rüptüre olma olasılığı daha yüksektir. Yetişkin hastalarda yapılan son incelemelerde küçük hemanjiyomlu asemptomatik hastaların gözlemle konservatif olarak tedavi edildiği bildirilmiştir (2). Bizim hastamızda dalaktaki hemanjiyomunun 1 cm çapında olması nedeniyle takip edilmiştir, splenektomi yapılmamıştır.

KTS tanılı GİS kanama şikâyeti olan hastaların tedavisinde demir replasmanı, kan transfüzyonu gibi konservatif yöntemler denenebilir. Endoskopik ya da anjiografik olarak müdahaleler yapılabilir. Ancak klinik olarak anlamlı kanaması olan KTS hastalarında ilgili barsak segmentinin rezeksiyonu gerekebilir. Mevcut vakamızda hastaya rektosigmoid rezeksiyon(anterior rezeksiyon) ve koloanal anastomoz ameliyatı uygulandı.

Sonuç olarak; KTS çok nadir görülen bir durumdur. HematokezyaKTS'ningastrointestinal tutulumunun tek ve en ciddi bulgusu olabilir. Görüntülemelerde vasküler malformasyonları olan ve hematokezya şikayeti ile gelen hastalarda KTS tanısı akla gelmelidir. KTS lezyonlarının ilerleyici karakterde olması ve geniş alanlara yayılım göstermesi endoskopik ve anjiografik tedavilerin hastalığın yönetiminde sınırlı rol almasına neden olur. Kontrol altına alınamayan hematokezya şikayeti olan hastalarda tutulum olan barsak segmentinin rezeksiyonu sıklıkla gerekebilir.



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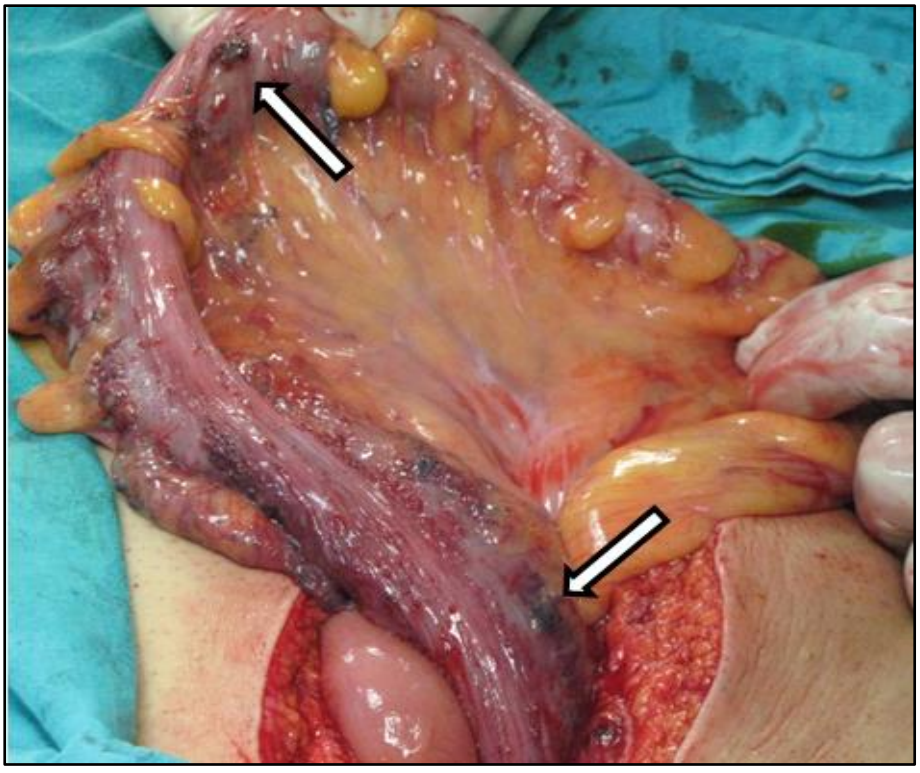

B

Şekil 1. İntraoperatif sigmoid kolon ve rektumu tutan yaygın hemanjiyomlar (oklar) gösterilmektedir.

Bilgilendirilmiş Onam: Bireyden yazılı ve sözlü onam alınmıştır.

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Derleme / Review

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The Benefits of Sacubitril/Valsartan in Low Ejection Fraction Heart Failure

Düşük Ejeksiyon Fraksiyonu ile Kalp Yetmezliğinde Sakubitril-Valsartanın Faydaları

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Abstract

Heart failure (HF) is the cause of impaired exercise capacity due to insufficient peripheral blood flow. The development of natriuretic peptide (NP) through inhibition of the neprilysin enzyme is the therapeutic target in HF. Sacubitril/valsartan reduces mortality and hospitalization and rehospitalization rates for HF compared with enalapril. In HF patients, sacubitril or valsartan may provide significant benefit.

Anahtar Kelimeler: Heart Failure, Natriuretic Peptide, Sacubitril/Valsartan

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Öz

Kalp yetmezliği (KY), perifere yetersiz kan akışı nedeniyle egzersiz kapasitesinin bozulmasının nedenidir. Neprilisin enziminin inhibisyonu yoluyla natriüretik peptit (NP) geliştirmesi, KY' deki terapötik hedeftir. Sakubitril/valsartan, enalapril ile karşılaştırıldığında KY için mortalite ve hastaneye yatış ve yeniden hastaneye yatış oranlarını azaltır. Sakubitril/valsartan KY hastalarında önemli fayda sağlayabilir.

Keywords: Kalp Yetmezliği, Natriüretik Peptit, Sakubitril/Valsartan

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Introduction

The diagnosis of heart failure (HF) is based on symptoms such as dyspnea and/or restricted exercise ability (1). Globally, HF causes significant health and economic costs (2). In the renin-angiotensin-aldosterone system (RAAS) and natriuretic peptide (NP) systems, various neurohormonal mechanisms contribute to the initiation of HF. RAAS activation triggers mechanisms that result in cardiac remodeling. A compensating mechanism inside the brain known as the NP system helps counterbalance the RAAS effects, but not completely (3). Because the enzyme neprilysin destroys NPs, it has been postulated that blocking this enzyme might be a key therapeutic target in HF.

The first dual neprilysin/angiotensin receptor inhibitor (ARNI) is sacubitril/valsartan (4). Both the TRANSITION [reduced ejection fraction (rEF)] and PIONEER-HF [comparison of sacubitril/valsartan medication effect in patients before and after discharge] studies showed treatment effectiveness for ARNI (5). Last but not least, the American College of Cardiology (ACC) and the Canadian Society of Cardiology (CSC) have recently updated their guidelines to recommend sacubitril/valsartan for patients with HF (6,7). Despite PARADIGM-results, the actual processes underlying neprilysin inhibition's therapeutic efficacy remain unknown. Figure 1 depicts the neprilysin substrates.

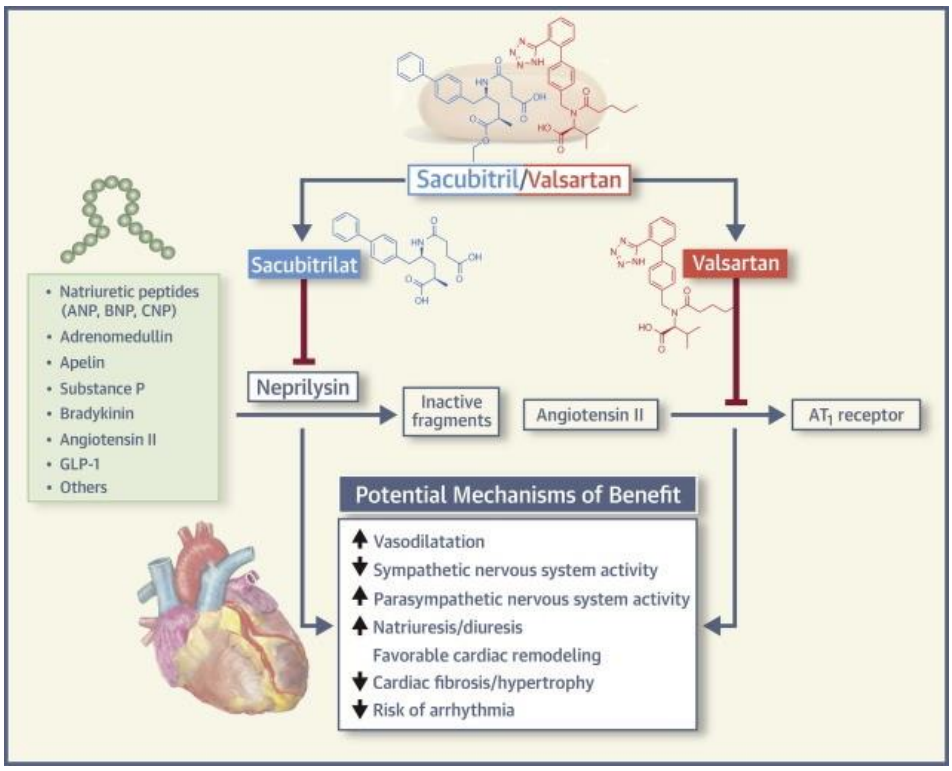

Figure 1. Sacubitril/Valsartan Action Mechanism. Other publication containing the figure in the manuscript include. "Heart Failure 2020, 8(10):800-10."

Sacubitril/valsartan improves quality of life by reducing mortality and disease progression in HF patients. We summarized data on the safety of sacubitril/valsartan in various subpopulations in this review.

Mortality, Sudden Death and Ventricular Arrhythmias

The PARADIGM-HF study showed that sacubitril/valsartan reduced CV mortality by 20 percent compared to enalapril. Aside from improving quality of life, ARNI reduced the risk of mortality by 16% [RR 0.84, 95% (CI) 0.76-0.93, p=0.009] (8).

Arnis And Reduction in Mortality

Sudden death has two basic causes. The first is sustained ventricular arrhythmia, which occurs in HF patients. Bradyarrhythmia or electromechanical dissociation on the ECG are signs of severe left ventricular mechanical failure (9). The positive effects of ARNIs on cardiac remodeling may be more effective than other drugs that decrease the mortality of congestive HF (10). Sacubitril/valsartan improved the clinical situation compared to enalapril in patients with reduced EF (11).

Recurrent Hospitalization

HF is an incurable chronic illness with a poor prognosis. Survival time decreases during hospitalizations. Many registries from other demographics show the same course (12).

Reducing Hospitalizations

In PARADIGM-HF, sacubitril/valsartan reduced hospitalizations for HF by 23%. It reduced recurrent hospitalizations by 33% compared to enalapril (13).

In Acute HF

The PIONEER-HF research was the first to establish that using sacubitril/valsartan therapy in the hospital was safe. After discharge, HF readmissions were lower (8.0%) with sacubitril/valsartan therapy than with enalapril (13.8 percent). Early on after being released from the hospital, the PIONEER-HF trial treatment plan should be favored to prevent readmissions (8)

Cardiac Remodeling

A 10% decrease in the left ventricular end-systolic volume index (LVESVİ) raised the probability of chronic HF mortality by 73%. Reverse cardiac remodeling reduces mortality (8). Increased circulating and myocardial nitric oxide bioavailability leads to increased cyclic guanosine monophosphate (cGMP) and protein kinase G activation. This reduces infarct size and progression. Inhibits pro-inflammatory cytokines and extracellular matrix breakdown, slowing heart remodeling. This avoids LV dysfunction and lowers symptoms of HF (14).

Cardiac Functions

Sacubitril/valsartan improved left ventricular function more significantly than enalapril in the 12-week EVALUATE-HF trial. An early and consistent reduction in NT-proBNP was observed (mainly within 14 days). An increase of 9.4% in LVEF from 28.2% to 37.8% was the most significant result. Overall, all echocardiographic measures showed considerable improvement (15,16). Sacubitril/valsartan, as opposed to angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB), resulted in significant functional improvements in HF patients. Following an acute myocardial infarction (AMI), the PARADISE-MI study showed a 42% reduction in global longitudinal strain compared to ramipril (17,18).

Hemodynamic Effects

The ARNI's hemodynamic effects were initially investigated using candoxatrilat, an ANP-inducing inhibitor. This peptide improves the hemodynamic profile of HF patients with reduced EF by decreasing plasma vasopressin, aldosterone, and renin activity. Systemic vascular resistance remained unchanged. One explanation is that non-selective vasoconstrictor molecules like angiotensin II, endothelin 1, and noradrenaline are degraded and their levels rise, counteracting the vasodilatory effects of NPs (8).

Omapatrilat was the first dual neprilysin and AChE inhibitor (ACE). A randomized, double-blind, placebo-controlled study included 369 HFrEF patients. The first dosage lowered pulmonary capillary pressure and systemic vascular resistance. A drop in blood pressure caused an increase in potentially hazardous hormones such as endothelin-1 and noradrenaline, which recovered to normal with continued usage (19).

The combined inhibition with sacubitril/valsartan has substantial systemic vasodilator effects, resulting in a significant drop in blood pressure. Reduced systolic blood pressure (SBP) is related to HF with reduced

EF. Because they are at great risk for adverse effects, these patients seldom receive disease-modifying medications. Sacubitril/valsartan treatment improves hemodynamics by increasing renal sodium and water excretion, vasodilation, and blood volume reduction. It improves ventricular preload and afterload, which helps cardiac remodeling. It lowers blood pressure, ideally SBP, and has been proven to enhance prognosis in all SBP groups, even those with consistently low SBP (20).

Renal Effect of Neprilysin Inhibition

Mechanical Effect

Inhibition of neprilysin increases NP renal bioavailability. This involves reducing kidney damage and decreasing renal remodeling (21).

Clinical Implications of Neprilysin Inhibition's Renal Action

HF

Despite elevated circulating NP levels, chronic HF is characterized by decreased renal (and extrarenal) NP activity. A meta-analysis of three HFrEF trials found that ARNI improved renal dysfunction and serum creatinine increase (22).

Chronic Kidney Disease (CKD)

In the UK HARP-III study, sacubitril/valsartan was compared to irbesartan on renal function and other outcomes. The results on blood pressure and cardiac indicators were more positive than the renal effects. CV events (particularly those associated with HF) may be reduced in people with chronic renal insufficiency (23).

Metabolic Effects: Type 2 Diabetes (Type 2DM) And Uric Acid

HF and Type 2 DM

HF and Type 2DM have the same risk factors and pathophysiological processes. In clinical trials, all HF medications and devices worked equally well with or without Type 2 DM. Dual RAAS and neprilysin inhibition may improve glycemic control. The PARADIGM-HF study's post-hoc analysis suggests this (24). The Paradigm-HF data also allowed the study of the effects of neprilysin inhibition on the progression of kidney damage in type 2 DM patients. NPs improve adiponectin secretion, adiponectin mobilization, and muscle oxidative capability (17). In diabetics, NP improves the kidneys by boosting urine cGMP content (25).

Two trials found that dapagliflozin lowers the risk of mortality in people with reduced EF and Type 2 DM. These findings imply that the two medicines have distinct but complementary biological effects. Empagliosin substantially lowered the hospitalization rate and CV mortality in the EMPEROR-Reduced study (26,27).

Uric Acid

Uric acid is a pro-oxidant that activates the RAAS. Sacubitril/valsartan lowered uric acid by 0.24 mg/dL and improved clinical outcomes in PARADIGM-HF (28).

Life Satisfaction and Functional Ability

HF sufferers have a poor health-related quality of life. The PARADIGM-HF study discovered that it improved sacubitril/valsartan quality. The Kansas City Cardiomyopathy Questionnaire (KCQ) showed that enalapril increased quality of life 4 months after randomization. This discrepancy lasted over 36 months. The largest gains were shown in domestic and sexual activities. Improving health-related quality of life is becoming a focus of emerging HF therapies (29).

Functional Capacity

Physical intolerance has a negative impact on quality of life. Hospitalizations rose by 8% to 14% for every 50 m lost in nine months. The 6MWT results in clinically meaningful functional capacity increases of 30-50 m. There is enough data to suggest that sacubitril/valsartan improves quality of life and function. It should be a focus in clinical practice to include the patient's viewpoint via objective evaluations of these characteristics (30).

Safety

Renal Failure

Sacuitril/valsartan outperformed enalapril in terms of renal safety. Increased serum creatinine and renal impairment were less common in Paradigm-HF. Patients with an eGFR of 30 mL/min/1.73 m2 have experienced success with the medication (31).

Hyperkalemia

The PARADIGM-HF revealed that those on sacubitril/valsartan had less severe hyperkalemia (6 mEq/L serum potassium) than people taking enalapril. Clinical practice recommends MRAs concurrently to decrease morbidity and death (32).

Arterial Hypertension

There was an increased incidence of symptomatic hypotension in those using sacubitril/valsartan (14 percent vs. 9.2 percent for enalapril), but no increase in medication withdrawal (0.9 percent vs. 0.7 percent, $p = 0.38$). Hypotension necessitates a slower rate of titration (33).

Angioedema

Angioedema was infrequent and did not vary across groups in any investigations (8).

Tolerance

Withdrawal due to adverse effects was uncommon in the PARADIGM-HF study. Acute HF patients on sacubitril/valsartan or enalapril discontinue at equal rates (34).

Recent Studies on Sacubitril/Valsartan

According to Rezq et al. (35), starting sacubitril/valsartan early after ST elevation MI may reduce MACE and HF hospitalizations. However, this additional indication needs to be confirmed on a larger scale with a longer follow-up cohort of patients to assure safety and effectiveness. Murphy et al. (36) found that commencing sacubitril/valsartan quadrupled ANP concentrations in HF patients with poor EF. The extent of future reverse cardiac remodeling was related to early ANP rises.

Using sacubitril/valsartan reduces anemia in patients with cardiorenal syndrome (CRS). These individuals had an increase in cystatin levels. There have been few negative effects. More clinical research is required to verify these findings (37). Sacubitril/valsartan and ivabradine used concurrently reduce adverse effects and improve LV reverse remodeling in patients with hypovolemia. However, sacubitril/valsartan therapy improved EF more than ivabradine treatment did (38).

Zandstra et al. (39) described the first cohort of patients treated with sacubitril/valsartan for systemic right ventricular failure. Treatment improves NT-pro-BNP and echocardiographic function. Sacubitril/valsartan may be an alternative for this patient population. Sacubitril/valsartan is a safe and efficient therapy for HF (40).

It also improves health status and reverses cardiac remodeling in individuals with HFrEF and type 2 diabetes (41). The optimal technique to manage HF patients with electrical devices in their hearts is yet unknown. The clinical utility of sacubitril/valsartan is questioned. It is superior to RAS inhibitors for HF patients (42).

With sacubitril/valsartan treatment, KCQ-23 scores improved rapidly, and this was related to a shift in NT-proBNP (43). Galo et al. (44) discovered that neprilysin is involved in the breakdown of brain beta-amyloid. Theoretically, this might cause plaque build up and eventually Alzheimer's.

Patients in the critical care unit may be safely transitioned to sacubitril/valsartan after a permanent improvement in cardiac index with vasoactive medications. Sacubitril/valsartan improved pulsatility index and preserved left and right ventricular function (45). Adding sacubitril/valsartan medication to symptomatic HF patients on the guideline-recommended medications increased EF, decreased NT-proBNP, and improved quality of life (46).

In conclusion, in patients with HFrEF, sacubitril/valsartan outperforms enalapril in lowering all-cause and cardiovascular death. Its vast range of advantages, including cardiac and extracardiac protection, may be explained by many mechanisms. ARNI may help individuals with HF in both the chronic and acute phases.

Current Studies

Intolerance to modest doses of sacubitril/valsartan is frequent in individuals with advanced chronic HFrEF (47). Sacubitril/valsartan decreased HbA1c and the need for new insulin treatment in HF and diabetic patients with different LVEF, but it may increase the risk of hypoglycemia (48). People with HFrEF saw similar improvements in prognostic biomarkers, health status, and cardiac remodeling at different doses of sacubitril/valsartan (49). Sacubitril/valsartan improves hemodynamic conditions in HFrEF patients (50). Sacubitril/Valsartan may halt renal function decline and reverse myocardial remodeling more efficiently than ACEI/ARB, even at low dosages, while its impact on urine protein is not as favorable (51).

HFrEF patients with varied risk profiles are identified using echocardiographic hemodynamic classification. Sacubitril/valsartan improves outcome across hemodynamic profiles in real-world HFrEF outpatients (52). Treatment with low doses of ARNI might successfully improve cardiac function in hemodialysis (HD) patients with heart failure and hypotension. It was also well tolerated and safe (53). 95% of patients began with low and intermediate sacubitril/valsartan dosages. 30% of patients achieved their target dosage during follow-up. Reverse remodelling was evidenced by a high NT-proBNP level, reduced LV size, and increased LVEF. Park et al. demonstrated the discrepancy between clinical trial and real-world treatment trends (54).

Risks of hypotension, renal failure, hyperkalemia, and angioedema seem minimal and tolerable with expanded sacubitril/valsartan use in randomized clinical trials (RCTs) and worldwide clinical practice (55). The stroke volume index (SVi) is related to full sacubitril/valsartan titration. Low-SVi patients are more likely to have hypotension during titration (56). Sacubitril/valsartan inhibits ventricular remodeling following MI, improves cardiac function, and reduces adverse cardiovascular events, rehospitalization, and death (57).

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Table 1.

Cornerstone Studies On Sacubitril/Valsartan

Reference no.	Authors	Subjects	Number of patients	Main theme
Ref [1]	Bozkurt et al.	HFrEF patients	9 recent clinical trials	HF is a clinical condition characterized by structural and/or functional heart abnormalities, increased natriuretic peptide levels, and pulmonary or systemic congestion.
Ref [2]	Virani et al.	Heart disease and stroke	5000 patients	The Statistical Update is a vital resource for policymakers, media professionals, doctors, healthcare administrators, academics, health activists, and anyone seeking the best available statistics on these causes and disorders.
Ref [5]	Velazquez et al.	HFrEF patients	881 patients	Sacubitril-valsartan suppressed NT-proBNP higher than enalapril in hospitalized HFrEF patients
Ref [8]	Pascual-Figal et al.	HFrEF patients	A review	Sacubitril/valsartan as a key therapy for HFrEF.
Ref [12]	Chun et al.	HFrEF patients	8543 patients	Newly discharged HF patients with ischemic etiology had more cardiovascular events early postdischarge and prefatally.
Ref [17]	Jering et al.	HFrEF patients	5669 patient	PARADISE-MI will investigate whether sacubitril/valsartan is more efficacious than an established ACE inhibitor in reducing HF and cardiovascular mortality after AMI.
Ref [27]	Packer et al.	HFrEF patients	3730 patients	Empagliflozin-treated heart failure patients had a reduced risk of mortality or hospitalization than placebo-treated patients, independent of diabetes.
Ref [34]	Nielsen et al.	HFrEF patients	19086 participants	Sacubitril/valsartan may help more heart failure patients than the guideline recommends.
Ref [35]	Rezq et al.	STEMI patients	200 patients	Sacubitril/valsartan may improve myocardial remodelling in post-STEMI patients.
Ref [39]	Zandstra et al.	HF patients	20 patients	They report the first sacubitril/valsartan-treated systemic RV failure cohort. NT-pro-BNP and echocardiographic function improve with treatment.
Ref [42]	Cheng et al.	HF patients with cardiac implantable electronic devices	A review	Sacubitril/valsartan may improve mortality, SCD, clinical, and echocardiographic results in patients with cardiac implantable electronic devices
Ref [47]	Vader et al.	HFrEF patients	445 subjects	Intolerance to modest doses of sacubitril/valsartan is frequent in individuals with advanced chronic HF with decreased ejection fraction.
Ref [50]	Carluccio et al.	HFrEF patients	727 HFrEF outpatients	Sacubitril/valsartan improves hemodynamic conditions in HFrEF patients.
Ref [55]	Kim et al.	HF patients	15,538 patients	In RCTs and worldwide clinical practice, enhanced sacubitril/valsartan absorption reduces the risk of hypotension, renal dysfunction, hyperkalemia, and angioedema.
Ref [57]	Zhang et al.	AMI patients	A total of 5 articles	Sacubitril/valsartan inhibits ventricular remodeling following AMI, improves cardiac function, and reduces adverse cardiovascular events, rehospitalization, and death.

Abant Tıp Dergisi

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Do We Concern Ourselves with Blood Loss and Blood Transfusions of the Peri-articular Injection of Tranexamic Acid after Unilateral Total Knee Arthroplasty?

Tek Taraflı Total Diz Artroplastisi Sonrası Traneksamik Asit Peri-artiküler Enjeksiyonunun Kan Kaybı ve Kan Transfüzyonları ile İlgili Endişemiz Var mı?

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(Bu makale bir önceki sayımızada hatalı olarak yayınlandığı için bu sayıda tekrar yayınlıyoruz.)

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Abstract

Objective: The means of peri-articular (PA) administration of tranexamic acid (TXA) is not examined sufficiently in unilateral total knee arthroplasty (TKA). The primary purpose of this study was to evaluate postoperative blood loss and transfusions rates after the administration of PA injection of TXA in TKA. In addition, PA TXA may decrease pain owing to reduced hemarthrosis after TKA.

Materials and Methods: In this retrospective study, 113 patients who underwent a primary unilateral TKA with or without a PA injection of TXA were included. A total of 1500 mg/50 ml TXA was injected into the extra-articular soft tissue around the medial, lateral capsules and muscular soft tissue around the quadriceps tendon immediately after cementation the prothesis, but before capsular closure and 15 minutes before the deflation of the tourniquet. A total of 56 patients in the control group did not receive TXA. The surgical procedure was standardized in all of the patients.

Results: There was a statistically significant reduction in hidden blood loss, estimated blood loss, and receiving a postoperative allogeneic blood transfusion in the TXA group compared with the control group (p=0.0001). We found a significant correlation between blood transfusion and the length of hospital stay (p=0.0001). No significant difference was found regarding pain VAS score after postoperative 1st day and postoperative 3rd day (p=0.597 and p=0.183, respectively). 1500 mg/50 ml (30 mg/ml) TXA was a relatively optimal dose to minimize the cytotoxic effects on the soft tissue around the knee compared with 50 mg/ml. No patients encountered any thromboembolic and wound complications.

Conclusion: The PA administration of TXA may offer a significant reduction in postoperative blood loss and transfusions rates as well as the length of hospital stay without increasing the risk of thromboembolic complications and cytotoxic effects on cartilage and peri-articular soft tissue. However, we did not observe a significant reduction in postoperative pain VAS score.

Keywords: Total Knee Arthroplasty, Tranexamic Acid, Peri-Articular Injection, Blood Loss

&

Öz

Amaç: Tek taraflı total diz artroplastisinde (TDA) traneksamik asidin (TXA) peri-artiküler (PA) uygulama şekli yeterince incelenmemektedir. Bu çalışmanın birincil amacı, TDA'da TXA'nın PA enjeksiyonunun uygulanmasından sonra postoperatif kan kaybı ve transfüzyon oranlarını değerlendirmekti. Ek olarak, PA TXA, TDA sonrası hemartrozun azalması nedeniyle ağrıyı azaltabilir.

Gereç ve Yöntemler: Bu retrospektif çalışmaya TXA PA enjeksiyonu olan veya olmayan primer tek taraflı TDA uygulanan 113 hasta dahil edildi. Protezin simante edilmesinden sonra, ancak kapsül kapanmadan ve turnike indirilmeden 15 dakika önce, medial, lateral kapsüller ve kuadriseps tendonu çevresindeki kas yumuşak doku çevresindeki eklem dışı yumuşak dokuya toplam 1500 mg/50 ml TXA enjekte edildi. Kontrol grubundaki toplam 56 hasta TXA almadı. Tüm hastalarda cerrahi prosedür standardize edildi.

Bulgular: Kontrol grubuna kıyasla TXA grubunda gizli kan kaybında, tahmini kan kaybında ve postoperatif allojenik kan transfüzyonu almada istatistiksel olarak anlamlı bir azalma vardı (p=0,0001). Kan transfüzyonu ile hastanede kalış süresi arasında anlamlı bir ilişki bulduk (p=0,0001). Postoperatif 1. gün ve postoperatif 3. günden sonra ağrı VAS skoru açısından anlamlı bir fark bulunmadı (p=0,597 ve p=0,183, sırasıyla). 1500 mg/50 ml (30 mg/ml) TXA, 50 mg/ml. ile karşılaştırıldığında diz çevresindeki yumuşak doku üzerindeki sitotoksik etkileri en aza indirmek için nispeten optimal bir dozdu. Hiçbir hastada herhangi bir tromboembolik ve yara komplikasyonu görülmedi.

Sonuç: TXA'nın PA uygulaması, tromboembolik komplikasyon ve kıkırdak ve periartiküler yumuşak doku üzerinde sitotoksik etki riskini artırmadan, postoperatif kan kaybı ve transfüzyon oranlarında ve hastanede kalış süresinde önemli bir azalma sağlayabilir. Ancak postoperatif ağrı VAS skorunda anlamlı bir azalma gözlemlemedik.

Anahtar Kelimeler: Total Diz Artroplastisi, Traneksamik Asit, Periartiküler Enjeksiyon, Kan Kaybı

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Introduction

One of the most common problems related to total knee arthroplasty (TKA) is intraoperative and postoperative blood loss leading to anemia-related complications, such as the increased risk of infection, cardiovascular complications, hemarthrosis and decreased patient's satisfaction (1,2). To avoid excessive blood loss and transfusion-related complications, various methods have been proposed, such as spinal anaesthesia, careful surgical electrocautery, drain clamping, intraoperative autologous blood transfusion, intraoperative blood saving, hypotensive anesthesia and antifibrinolytic therapy (3,4).

One of the pharmacological agents is tranexamic acid (TXA) which has been well established in a variety of surgical procedures, mostly in TKA (5). Trauma or surgery results in the release of tissue plasminogen activator, which triggers fibrinolysis (6). TXA is an antifibrinolytic agent that acts by competitively blocking a lysine-binding site of plasminogen and thereby inhibiting the formation of plasmin (7,8). The optimal administration means of TXA in TKA is still controversial (8,9). In the literature, most of the studies have suggested that the use of IV TXA is effective in reducing postoperative blood loss and the necessity of an allogenic blood transfusion (9). Many surgeons are, however, concerned that patients who have several comorbidities, such as a history of thrombosis, myocardial infarction, and severe renal dysfunction, may experience thromboembolic problems as the result of major surgery and the use of IV TXA (6,10). Moreover, only a small percentage of the IV TXA diffuses into the target location of soft tissue (8,11). Therefore, some studies focused on the topical application of TXA in the TKA (11,12). The administration of intra-articular (IA) TXA has been documented in many studies in which TXA was found effective and safe for diminishing blood loss after arthroplasty surgery (12,13,14). Nevertheless, Mao et al. thought that the volume of IA TXA solution might be insufficient to reach the anterior soft tissues of the knee joint while the patient is in a supine position until they walk (11). Moreover, IA TXA may have the potential to be cytotic to cartilage (15). Benoni et al. indicated that the positive effects of TXA on blood loss in knee arthroplasty were exerted mainly by inhibition of the fibrinolytic activity locally in the surgical field (16). Hence, some studies suggested the peri-articular (PA) application of TXA in TKA (17,18). However, the means of PA administration of TXA is not examined sufficiently.

We determined that the use of TXA in the way of peri-articular (PA) injection may promise some advantages, including lead to reach sites of soft tissue release and incisal edge, no systemic effects of TXA, reduced thromboembolic events, and decreased cytotoxic effects on cartilage and peri-articular tissue. The primary purpose of this study was to evaluate postoperative blood loss and transfusions rates due to the administration of PA injection of TXA in patients who encountered unilateral TKA. In addition, we hypothesize that PA TXA may decrease pain owing to reduced hemarthrosis after TKA.

Materials and Methods

After approval of the local ethics committee of Medipol University (no, E-10840098-772.02-2616; date, 27/01/2021), a cohort of 113 consecutive patients who performed between November 2017 and January 2021 with unilateral TKA was retrospectively assessed. We commenced applying for PA TXA in the middle of 2019. The patients who met the inclusion criteria in this study were divided into two groups. A total of 57 patients received 1500 mg/50 ml TXA in the TXA group, while 56 patients did not receive TXA in the control group. We excluded the patients who had rheumatoid arthritis, revision TKA, simultaneous bilateral TKA, American Society of Anaesthesiologists (ASA) Level 4, severe allergic history to local anesthetics or TXA, bleeding or clotting disorders, and desperate renal failure. All patients stopped the anticoagulant and non-steroidal anti-inflamatory agents (NSAID) 7 days before the surgery. Written informed constent was obtained from all patients.

All knees were performed according to comorbidity and prefence of the patient under general anesthesia or spinal anesthesia combined with epidural block. All patients were performed through a standard medial

prepatellar approach without patellar eversion to expose the surgical site of the knee joint under tourniquet control. The technique included cemented posterior stabilized NexGen (Zimmer, LPS-Flex Mobile, Warsaw, Indiana and USA) prosthesis without patellar replacement was utilized in all patients. TXA (Transamine, 250 mg/5 ml; Pharmacia, Teva, Turkey) with 50 ml saline solution was prepared in the TXA group. A total of 1500 mg/50 ml TXA was injected into the medial, lateral capsules and muscular soft tissue around the quadriceps tendon immediately after performing knee prosthesis, before capsular closure and 15 minutes before the deflation of the tourniquet (Figure 1). The tourniquet was inflated before the skin incision at 300 mmHg during the procedure. After 15 minutes of injection of the TXA and the cement was completely polymerized, the tourniquet was deflated immediately. When the touniquet was deflated we asked the anesthesiologist to reduce the blood pressure in safe zone. We aimed hypotension during the last period of the surgery until closed the wound. We calculated peroperative blood loss. No patients received any local injection into the knee joint, such as ropivacaine with epinephrine during the procedure. In both groups, the sole drain was placed and clamped for two hours in both groups. We monitored suction drainage blood volume and removed it when the drain 24-h volume of drainage was less than 50 ml after the surgery. All of the patients received the same physical rehabilitation program in which continuous passive machine motion was initiated within 12 h after the surgery and continued on postoperative day 1. After removing the drain, all of the patients began full weight bearing walking with the use of a walker postoperative day 1.

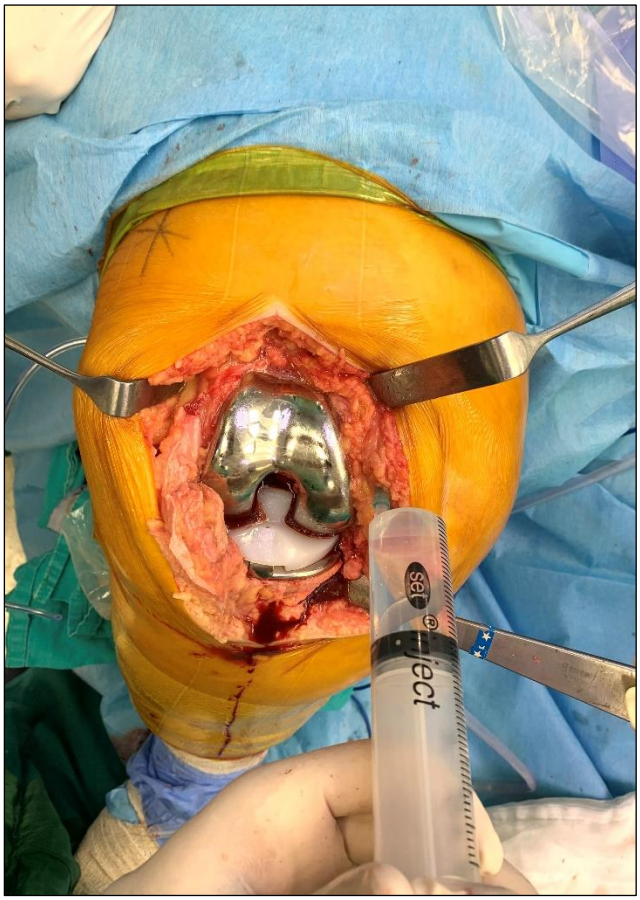

Figure 1. The way of applying PA-TXA

All of the patients were administered a standard course of an antithrombotic agent, which was low molecular-weight heparin (enoxaparin sodium, 40 mg) at eight hours postoperatively and continued three weeks after the surgery. Prophylactic antibiotic (cefazolin sodium, 1000 mg) was administered to all patients 30 minutes before the tourniquet inflated and continued postoperatively over the 24 h.

We recorded the levels of preoperative and postoperative hemoglobin, hematocrit and its drop during the procedure. Laboratory measures were determined from venous blood samples postoperative day 1, 2, and 3. Criteria of allogenic blood transfusion were a postoperative hemoglobin level of ≤ 8 g/dL or a postoperative hemoglobin level between 8 and 10 g/dL with the clinical signs of hemodynamic instability, including light-headedness, presyncope, palpitation or shortness of breath not owing to other causes. Pre-donation of autologous blood was not administered for any patients.

We calculated total blood volume (TBV) using the Nadler method (19). Estimated blood loss (EBL) was monitored using Gross's formula (20), which considers the initial hematocrit before surgery, the minimum postoperative hematocrit level, and the average of the initial and minimum hematocrit levels. Measured blood loss (MBL) was calculated as the sum of the intraoperative blood loss plus the total drain output. Hidden blood loss (HBL) was calculated using Sehat's formula (21), which subtracts the total MBL from the EBL and adds the volume of blood transfused (each packed red blood cell unit contains 200 ml).

We evaluated the two groups regarding blood loss in the volume of intraoperative blood, the volume of drain output, the volume of HBL, the volume of EBL, hemoglobin and hematocrit concentrations, the necessity of allogeneic transfusion, total operation time, pain visual analog score (VAS), the risk of the thromboembolic complications, length of hospital stay, age, sex, body mass index (BMI) and ASA. The surgical procedures and data collection processes were performed by the two authors who participated in this study.

All of the statistical analyses were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Data description was based on the mean ± standard deviation (SD). The Shapiro-Wilk test was used to test for normality. The one–way analysis of variance (ANOVA) test was used to detect differences between patients from each group for normal distributions. Groups were compared using the Student t-test for normally distributed continuous variables. Subgroups were compared with the Newman-Keuls test. The Chi-square test was used to analyse qualitative comparative parameters. A value of $p < 0.05$ was considered to be statistically significant.

Results

In this study, 117 patients were assessed retrospectively from medical records in our institution. No statistically significant differences were found in the demographic variables except for body mass index between the two groups (Table 1).

We found a statistically significant reduction in section drainage blood loss in the TXA group compared with the control group (320.09 ± 89.74 ml and 413.39 ± 118.34 ml, respectively). The hidden blood loss was significantly lower in the TXA group than in the control group (386.78 ± 108.05 ml and 581.87 ± 198.29 ml, respectively). Estimated blood loss was less in the TXA group than in the control group (812.58 ± 134.66 ml and 1025.64 ± 247.08 ml, respectively). Furthermore, hemoglobin reduction was significantly lower in the TXA group compared with the control group at three days postoperatively ($p = 0.0001$). However, there was no significant difference between the TXA and the control group concerning intraoperative blood loss ($p = 0.343$) (Table 2).

In terms of receiving a postoperative allogeneic blood transfusion, there was a highly significant difference between the TXA and the control group ($p = 0.009$) (Table 3). Whereas the TXA group received 6 units, the control group received 20 units. In addition, no patients in the TXA group received more than one unit erythrocyte suspension.

A significant reduction in length of hospital stays was found in the TXA group compared with the control group ($p = 0.01$) (Table 4). No significant difference was observed between the two groups regarding pain VAS score after postoperative 1st day and postoperative 3rd day ($p = 0.597$ and $p = 0.183$, respectively)

(Table 5). However, postoperative pain declined daily in both groups. No patients encountered any thromboembolic and wound complications.

Table 1

Demographic Characteristics Of The Patients.

Variable	TXA group (n = 57)	Control group (n= 56)	P value
Age (years)	67.19 ± 6.86	67.98 ± 7.16	0.551*
Sex (female/male)	47/10	48/8	0.636+
BMI (kg/cm³)	30.11 ± 3.66	32.45 ± 3.38	0.001*
Side (left/right)	30/27	33/23	0.500+
ASA class (I/II/III)	11/36/10	14/30/12	0.583+
Type of anesthesia			
Spinal + epidural	43 (75.44%)	45 (80.36%)	
General + epidural	14 (24.56%)	11 (19.64%)	0.529+
Operation time (min)	73.54 ± 6.33	72.20 ± 6.26	0.258*
Tourniquet time (min)	58.19 ± 5.99	57.86 ± 5.91	0.765*
Hospital stay (day)	3.09 ± 0.29	3.27 ± 0.45	0.01*
Total blood volume (ml)	4405.57 ± 507.89	4411.71 ± 518.53	0.949*

Values presented as mean (standard deviation) and p calculated by using the Student t test (*) and the chi-square test (+).

Table 2

Blood Loss Calculation (ml)

Variable	TXA group (n = 57)	Control group (n= 56)	P value*
Intraoperative blood loss (ml)	103.41 ± 20.69	106.98 ± 19.15	0.343
Drainage blood loss (ml)	320.09 ± 89.74	413.39 ± 118.34	0.0001
Hidden blood loss (ml)	386.78 ± 108.05	581.87 ± 198.29	0.0001
Estimated blood loss (ml)	812.58 ± 134.66	1025.64 ± 247.08	0.0001
Hemoglobin (g/dl)			
Preoperative	12.96 ± 0.97	12.91 ± 0.95	0.794
Postoperative 1st day	11.71 ± 0.86	11.28 ± 0.97	0.014
Postoperative 2nd day	11.03 ± 0.77	10.60 ± 0.95	0.01
Postoperative 3rd day	10.49 ± 0.77	10.14 ± 0.85	0.027
p†	0.0001	0.0001	
Hemoglobin reduction (g/dl)	2.49 ± 0.49	2.85 ± 0.56	0.0001
Hematocrit (%)			
Preoperative	38.92 ± 2.63	38.63 ± 3.05	0.590
Postoperative 1st day	35.69 ± 2.39	34.21 ± 3.01	0.004
Postoperative 2nd day	33.80 ± 2.25	32.10 ± 2.85	0.001
Postoperative 3rd day	32.36 ± 2.19	30.77 ± 2.59	0.001
p†	0.0001	0.0001	
Hematocrit reduction (%)	6.62 ± 1.10	8.02 ± 1.62	0.001

Values presented as mean (standard deviation, range) and p calculated by using the Student t test (*) and the one–way analysis of varience (ANOVA) test (†).

Table 3

The Number of Blood Transfusions

Variable	TXA group (n = 57)	Control group (n= 56)	P value*
Number of patients (%)	6 (10.53%)	17 (30.36%)	0.009
Transfusion (in unit)	6	20	-

Values presented as n (%); *p calculated by using the chi-square test

Table 4

The Correlation Of Blood Transfusion and The Length of Hospital Stay

Variable	TXA group (n = 57)	Control group (n= 56)
Hospital stay in BT (-)	3.08 ± 0.27	3.11 ± 0.31
Hospital stay in BT (+)	3.17 ± 0.41	3.65 ± 0.49
p*	0.0001	0.479

Values presented as mean (standard deviation, range) and p* calculated by using the Student t test. BT= Blood transfusion.

Table 5

Visual Analogue Score (VAS) for pain

Variable	TXA group (n = 57)	Control group (n= 56)	P value*
Preoperative	6.96 ± 0.79	7.09±0.74	0.391
Postoperative 1st day	5.09 ± 0.72	5.02 ± 0.72	0.597
Postoperative 3rd day	3.96 ± 0.71	3.79 ± 0.67	0.183
p†	0.0001	0.0001	

Values presented as mean (standard deviation, range) and p calculated by using the Student t test (*) and the one–way analysis of varience (ANOVA) test (†).

Discussion

The current study suggested that patients who underwent TKA could obtain some advantages, such as smaller reduction of hemoglobin and hematocrit level, less drainage volume, less hidden blood loss, and less transfusion rate without an increase in the risk of thromboembolic disease and cytotoxic effects on cartilage and peri-articular tissue through PA administration of TXA.

Some studies focused on the use of IA TXA (11,12,13). In a recent randomized controlled trial performed by Meshram et al. demonstrated that there was no difference between IA alone and combined IA plus IV regimen of TXA administration (13). In addition, to avoid potential complications associated with systemic administration, they recommended that IA alone is sufficient to reduce blood loss and blood transfusion rates for routine TKA. In contrast, IA TXA may have some disadvantages, such as TXA leakage owing to soft tissue release and cytotoxic impact on chondrocytes (11,22). Therefore, some studies suggested the peri-articular (PA) application of TXA owing to the need to administer a sole dose, its easy employment, and maximum concentration of TXA at the surgical site in TKA (17,18,23).

Few studies have analyzed the effects of PA TXA administration on blood loss in TKA. In the literature, the means of PA administration of TXA is not examined sufficiently. Mao et al. performed a study that provided a significant reduction in the EBL (11). However, they did not calculate intraoperative blood loss and HBL. Pinsornsak et al. compared IV TXA and PA TXA without a control group and calculated only blood loss in the hemovac drain, which is insufficient to evaluate total blood loss (17). Yozawa et al. and Hirose et al. assessed the efficacy of PA TXA after TKA, in which EBL in the PA group was significantly lower than in the control group (18,23). Nevertheless, they did not search for intraoperative blood loss and HBL. Furthermore, there is a lack of consensus on the optimal dose of PA TXA. Mao et al. utilized 2000 mg PA TXA; Pinsornsak et al. used 750 mg; Yozawa et al. and Hirose et al. utilized 1000 mg after TKA (11,17,18,23). We injected 1500 mg PA TXA into the knee after TKA. At the end of the current study, the outcomes revealed that PA injection of 1500 mg TXA obtained a significant reduction in not only the EBL but also the HBL in the TXA group compared with the control group.

According to some reports, intramuscular administration of a single 1 g dose of TXA reaches its maximum plasma concentrations in 30 minutes and then rapidly diffuses into the joint fluid and synovial membranes (24,25). We employed TXA in the way of PA injection technique before 15 minutes tourniquet deflation to gain more soft tissue concentrations of the edge of the bleeding sites before closing the capsule. We found that although the mean calculated postoperative blood loss significantly diminished in the TXA group compared with the control group, intraoperative blood loss was similar in all patients. We concluded that we could employ TXA 30 minutes before tourniquet release.

When we analyzed the data, the PA administration of TXA effectively diminished the need for blood transfusion in the patients who underwent TKA (11,17,18). Yozawa et al. found that only two of 44 (4.5%) cases in the control group received postoperative an allogeneic blood transfusion, whereas no case in the TXA group received a transfusion (18). However, they used an autologous blood transfusion that is not utilized widely in the orthopaedic field and in our institution and did not consider this. In our present study, allogeneic blood transfusions administered to patients in the TXA group were less than those in the control group. This outcome is crucial concerning allogeneic blood transfusion.

We found similar outcomes concerning the length of hospital stay compared with other studies in which the length of hospital stay was higher in patients who did not receive TXA (1,14). The possible reason for this phenomenon is that the patients who administrated blood transfusion owing to intra- and postoperative blood loss needed to stay more days at the hospital. We found a significant correlation between blood transfusion and the length of hospital stay. The control group received more blood transfusion and stayed longer at the hospital compared with the TXA group in the present study.

Huang et al. indicated that the most important findings of their study were significant reduced postoperative knee pain and severity of knee swelling by combing a total dose of 3000 mg IV and topical application of TXA (1). The same conclusion was drawn by Ishida et al., who revealed that 2000 mg IA TXA decreased knee swelling and pain VAS score after TKA (8). Therefore, we hypothesized that PA TXA might decrease pain owing to reduced hemarthrosis after TKA. We found no significant difference with respect to pain between the two groups using a total of 1500 mg TXA, which was low-dose in our study compared with the two previous studies. Likewise, Hirose et al. demonstrated that although a PA injection of 1000 mg TXA was effective for promoting early recovery of knee range of motion (ROM) after TKA, there was no significant difference in pain VAS score (23). However, they injected ropivacaine with epinephrine in both the TXA knee and the control knee. Hence, according to previously reported studies, we assume that the PA TXA dose (1500 mg) used in our study was insufficient to reduce the VAS pain score. In contrast, Wong et al. conducted a randomized and controlled trial that compared the low-dose (1500 mg) and high-dose (3000 mg) topical TXA with a placebo (26). Their report revealed that there was no difference among the three groups in terms of postoperative VAS pain scores. Thus, further study is necessary to optimize dozes and timings to obtain more advantages from the PA technique of TXA.

Reale et al. indicated that TXA did not lead to the risk of thromboembolic complications (27). We observed similar results with no thromboembolic events, such as pulmonary emboli (PE) or deep venous thromboembolism (DVT) in both groups. The means of PA TXA injection around the soft tissue of the knee may suggest minimal TXA resorption into the systemic circulation. Moreover, Astedt et al. showed that TXA tranexamic acid did not suppress the fibrinolytic activity in the vessel walls, which may explain why previous studies and our study have not indicated a higher incidence of thromboembolic complications in patients given TXA (28).

In the literature, effective doses for the topical TXA ranging from 250 mg to 3 g have been applied by most surgeons (22-Goderecci). Although TXA provides a significant reduction of blood loss in total joint arthroplasty, one of the major concerns about the use of topical TXA is that TXA has the potential to be cytotic to cartilage, tendon and synovium, especially in an enclosed joint space (15,22,29,30). Tuttle et al. showed that 50 mg/ml TXA led to cytotoxic effects on cartilage, whereas 25 mg/ml did not result in a cytotoxic impact on chondrocytes (15). We may deal with this problem through PA injection because we do not apply TXA directly into the joint area. Moreover, Mc Lean et al. investigated the interaction between human periarticular tissues and TXA and found that 50 mg/ml or 100 mg/ml of topical TXA triggered significant periarticular tissue toxicity (29). Çıraklı et al. evaluated the effects of 50 mg/ml TXA injected into the soft tissue around Achilles tenotomy surgical sites in rats (30). At the end of the study, they revealed that topical TXA caused a long-term adverse effect on tendon healing. PA injections are partly intramuscular injection, administrating into the muscular soft tissue around the quadriceps tendon. We did not inject TXA directly into the quadriceps tendon, which has poor regenerative capacities. Furthermore, we employed 1500 mg/50 ml (30 mg/ml) TXA to minimize blood loss and the cytotoxic effects on the soft tissue around the knee, which was a relatively optimal dose compared with 50 mg/ml used by Çıraklı et al (27).

The main limitation of this study is its retrospective design. Secondly, IV or IA TXA use should have been chosen as the control group in this study. Another limitation of this study is that we did not evaluate the leg swelling. Postoperative leg swelling is affected by multiple factors, such as cardiovascular or renal dysfunction, soft tissue inflammation, and deep venous thromboembolic complications. Furthermore, we did not monitor TXA in the blood test. Finally, we searched only symptomatic DVT after surgery. We did not search asymptomatic DVT. Thus, we do not know the exact number of DVT in the patients administered TXA.

One of the strengths of our study is that this study has involved a consecutive group of patients who were performed through the same approach and the same implants and standardized perioperative procedure at a single institution. Furthermore, both groups were comparable about demographic characteristics, and preoperative levels of hemoglobin and hematocrit were not significantly different between the two groups.

In conclusion, the PA administration of TXA after unilateral TKA may offer a significant reduction in postoperative blood loss and transfusions rates as well as the length of hospital stay without increasing the risk of thromboembolic complications and cytotoxic effects on cartilage and peri-articular soft tissue. However, we did not observe a significant reduction in postoperative pain VAS score.

Ethics Committee Approval: The study was approved by the Ethics Committee Medipol University (date: 27.01.2021 and approval number: E-10840098-772.02-2616).

Informed Consent: Written consent was obtained from the participants.

Conflict of Interest: Authors declared no conflict of interest.

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