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The Relationship Between Liver Fibrosis and Golgi Protein 73 in Patients with Chronic Hepatitis B

Kronik Hepatit B'li Hastalarda Karaciğer Fibrozisi ve Golgi Protein 73 Arasındaki İlişki

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ABSTRACT

Aim: This study aimed to determine the relationship between the degree of liver fibrosis and serum Golgi protein 73 (sGP73) concentration in patients with chronic hepatitis B (HBV) infection.

Materials and Methods: A total of 124 (78 HBV-positive, 40 healthy) individuals were included in the study. The participants were classified as negative healthy individuals (Group 1); HBV surface antigen-positive, HBV DNA <2000 IU/mL, chronic HBV-infected patients without liver biopsy (Group 2); and individuals with HBV DNA ≥2000 IU/mL and liver biopsy (Group 3). Group 3 was divided into subgroups as those with fibrosis lower than stage 2 (F1) and those with fibrosis of stage 2 or higher (F2). When regrouped according to the hepatic activity index (HAI), Group 1 and 2 remained the same, while Group 3 was divided into patients with an HAI below 6 (A1) and those with HAI of 6 or higher (A2).

Results: sGP73 concentrations were 11.40±7.05 ng/mL in Group 1, 16.78±6.01 ng/mL in Group 2, 43.23±10.99 ng/mL in subgroup F1, and 48.75±10.93 ng/mL in subgroup F2. These values were significantly higher in F1 and F2 compared to Groups 1 and 2 ($p<0.05$), with no statistical difference between F1 and F2. When the mean sGP73 concentrations of Group 1, Group 2, subgroup A1 (43.42±11.15 ng/mL), and subgroup A2 (46.74±11.11 ng/mL) were compared, there was no significant difference between A2 and A1 ($p>0.05$), while the differences between the other groups were statistically significant ($p<0.05$).

Conclusion: A relationship was observed between sGP73 and liver damage in patients with HBV. Although sGP73 concentration was associated with the presence of fibrosis, the relationship between sGP73 and degree of fibrosis was weakly positive and non-significant.

Keywords: GP73, hepatitis B virus, fibrosis

ÖZ

Amaç: Çalışmamızda kronik hepatit B (HBV) tanılı bireylerde karaciğer fibrozisi derecesiyle serum Golgi protein 73 (sGP73) seviyeleri arasındaki ilişkinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya 124 (78 HBV pozitif, 40 sağlıklı) birey dahil edildi. Hastalar karaciğer biyopsisi varlığı ve biyopside fibrozis derecesine göre gruplara ayrıldı. Sağlıklı bireyler (Grup 1), HBV yüzey antijen pozitif, HBV-DNA <2000 ıu/mL, biyopsi yapılmayan kronik HBV enfeksiyonlu bireyler (Grup 2), HBV-DNA ≥2000 IU/mL olup biyopsi yapılan bireyler (Grup 3) olarak sınıflandırıldı. Grup 3 kendi içinde fibrozis evresi 2'nin altında olan bireyler (F1), fibrozis evresi 2 ve üzeri olan bireyler (F2) olarak ayrıldı. Bu gruplama hepatik aktivite indeksi (HAİ) skoruna göre tekrar yapıldığında Grup 1 ve 2 değişmezken Grup 3 HAİ derecesi 6'nın altında olan bireyler G1, HAİ derecesi 6 ve üzeri olan bireyler G2 olarak tanımlandı.

Bulgular: Grup 1 (11,40±7,05 ng/mL), Grup 2 (16,78±6,01 ng/mL), F1 (43,23±10,99 ng/mL) ve F2 (48,75±10,93 ng/mL) sGP73 düzeyleri açısından karşılaştırıldığında F2 ile F1 arasındaki fark istatistiksel açıdan anlamsız bulunurken ($p>0,05$) diğer gruplar arasındaki farklılık istatistiksel olarak

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anlamlı bulundu (p<0,05). Grup 1, Grup 2, G1 (43,42±11,15 ng/mL) ve G2 (46,74±11,11 ng/mL)'nin ortalama sGP73 düzeyleri karşılaştırıldığında G2 ile G1 arasındaki fark istatistiksel açıdan anlamsız bulunurken (p>0,05) diğer gruplar arasındaki farklılık istatistiksel olarak anlamlı bulundu (p<0,05).

Sonuç: sGP73 ile HBV pozitif hastaların karaciğer hasarı arasında ilişki vardır. Fibrozis varlığı ile sGP73 düzeyi ilişkili olmakla birlikte fibrozis derecesindeki artış ile sGP73 düzeyi arasındaki ilişki pozitif yönlü, zayıf ve istatistiksel açıdan anlamsız bir ilişkidir.

Anahtar Kelimeler: GP73, hepatit B virüsü, fibrozis

INTRODUCTION

Hepatitis is characterized by inflammation and necrosis of the liver. Hepatitis caused by hepatitis B virus (HBV) infection is a global public health issue[1]. It is generally accepted that early diagnosis of cirrhosis and elimination of its cause can halt liver damage, increase the chances of successful transplantation, and reduce mortality. Liver biopsy is the gold standard in the diagnosis of cirrhosis[2-4]. Golgi protein 73 (GP73), which was discovered by Kladney et al.[5] in 2000, is a transmembrane glycoprotein expressed by biliary epithelial cells of the liver and normally found in the cis-Golgi complex[6]. Previous studies have shown significant increases in serum GP73 (sGP73) levels with both viral and non-viral liver diseases[7]. There are also studies showing that sGP73 levels are associated with disease stage in alcoholic liver disease and chronic hepatitis[8]. We conducted the present study to determine the relationship between the degree of fibrosis and sGP73 levels in patients positive for HBV surface antigen (HBsAg).

MATERIALS AND METHOD

Our study was conducted in accordance with the principles of the Declaration of Helsinki at Sivas Cumhuriyet University Faculty of Medicine between April and October 2018. Written informed consent was obtained from all participants. Ethical approval was obtained from the Ethics Committee of Sivas Cumhuriyet University Faculty of Medicine (decision no: 2018-01/04, date: 09.01.2018). The study was supported by Cumhuriyet University Scientific Research Projects Unit.

Patient Selection

A total of 124 individuals (78 HBV-positive, 46 HBV-negative) who were over the age of 18 years and had not received any antiviral treatment were included in the study. Subjects with any viral infection other than HBV [e.g., Human Immunodeficiency virus (HIV) or hepatitis D virus were excluded.

The participants were divided into groups according to the Ishak scoring system as HBsAg-negative healthy individuals (Group 1); HBsAg-positive, HBV DNA <2000 IU/mL, chronic HBV-infected individuals without biopsy (Group 2); and HBV DNA ≥2000 IU/mL, biopsied individuals (Group 3). Group 3 was divided into subgroups as those with fibrosis lower than stage 2 (F1) and stage 2 or higher (F2). When the classification was reassessed according to the hepatic activity index (HAI), Groups 1 and 2 remained the same, while Group 3 patients were divided into those with HAI below 6 (A1) and HAI 6 or higher (A2). Figure 1A, Figure 1B shows the algorithm for the classification of the study subjects.

Variables

Age, sex, HAI, fibrosis score, alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, direct bilirubin, albumin, platelet (PLT) count, international normalized ratio, serum HBV DNA levels, AST to PLT ratio index (APRI), fibrosis index based on 4 factors (FIB-4), and sGP73 concentrations were compared among the groups.

$$APRI = [AST \times (\text{upper limit of normal}) / PLT\ (10^9/L)] \times 100$$

$$FIB\text{-}4 = [\text{Age (years)} \times AST\ (IU/L)] / [PLT\ (10^9/L) \times \sqrt{ALT\ (IU/L)}]$$

Measurement of sGP73

Routine biochemical tests, HBV DNA, and sGP73 were analyzed from venous blood samples taken after at least 12 hours of fasting for all subjects and before the initiation of medical treatment in patients with HBV. sGP73 was measured using a SunRed ELISA kit as per the manufacturer's instructions. Briefly, a standard solution series (concentrations of 48, 24, 12, 6, and 3 ng/mL) was prepared, and 50 µL of each standard and 40 µL of each study sample were placed into the wells of ELISA plates. Each well was added 10 µL of anti-GP73 antibody, followed by 50 µL of streptavidin-horseradish peroxidase. The plate was incubated at 37 °C for 60 minutes, then washed 5 times with a washing solution. Next, 50 µL of chromogen solution A and 50 µL of chromogen solution B were added and the plates were incubated at 37 °C for 10 minutes. After adding 50 µL of stop solution, absorbance values were read at 450 nm.

Statistical Analysis

The IBM SPSS Statistics version 22.0 (IBM Corp.) program was used to analyze the data. The data were evaluated for normal distribution with the Kolmogorov-Smirnov test. Comparisons of more than two independent groups were made using ANOVA with Tukey's post-hoc test for variables showing normal distribution and using the Kruskal-Wallis test with post-hoc Mann-Whitney U test for variables not showing a normal distribution. ROC curve analysis was performed to

determine the discriminative power of sGP73. Qualitative data were tested using chi-square tests. Differences with p<0.05 were considered statistically significant.

RESULTS

There were no significant differences among the groups in terms of demographic characteristics (Table 1). ALT values were significantly higher in F2 than in all other groups and in F1 compared to Group 1 (p<0.05). The F2 subgroup also had significantly higher AST and APRI values and lower PLT count than the other groups (p<0.05). There was also a significant difference in the comparison of FIB-4, with higher values in F2 than in all other groups (p<0.05). No other significant differences were detected among the groups (Table 2).

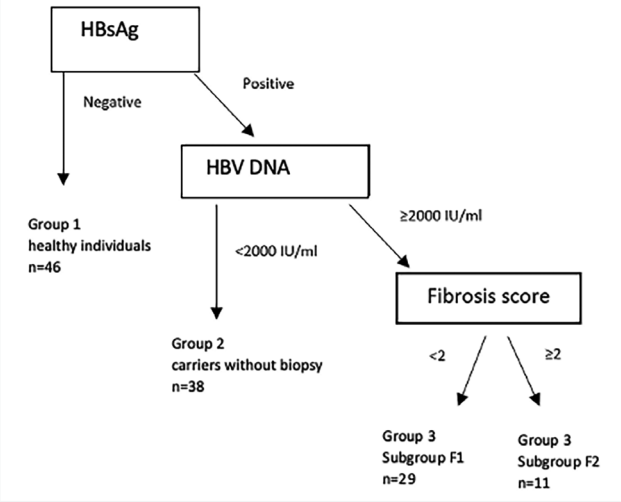

Figure 1A. The algoritm of groups according to fibrosis score
HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B, HAI: Hepatic activity index

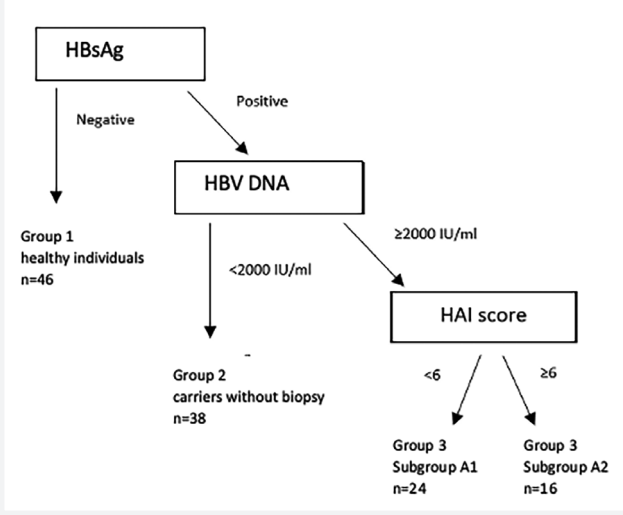

Figure 1B. The algoritm of groups according to HAI scoring
HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B, HAI: Hepatic activity index

Table 1. Subject demographics

	Patients (n)	Gender (F/M)	Age (years) (mean ± SD)
	HBV DNA level		
Group 1	46	28/18	47.65±10.75
Group 2	40	18/22	46.30±13.11
Group 3	38	20/18	45.63±13.81
p-value		0.338	0.751
	Fibrosis stage		
Group 1	46	28/18	47.65±10.75
Group 2	38	17/21	45.29±12.65
F1	29	17/12	42.62±12.87
F2	11	4/7	57.18±12.23
p-value		0.287	**0.007**
	HAI grade		
Group 1	46	28/18	47.65±10.75
Group 2	38	17/21	45.29±12.65
G1	24	13/11	42.58±12.78
G2	16	8/8	52.69±14.34
p-value		0.525	0.068

F1 group: Fibrosis stage <2, F2 group: Fibrosis stage ≥2, G1 group: HAI score <6, G2 group: HAI score ≥6, SD: Standard deviation, HAI: Hepatic activity index, F: Female, M: Male, HBV: Hepatitis B

The mean sGP73 values were 11.40±7.05 ng/mL in Group 1, 16.92±5.93 ng/mL in Group 2, and 46.08±9.66 ng/mL in Group 3 (p<0.05). sGP73 concentration increased significantly between Group 1 and Group 2 and between Group 2 and Group 3 (Table 3).

When further examined by subgroup, the mean sGP73 values were 43.23±10.99 ng/mL in F1 and 48.75±10.93 ng/mL in F2. Although there was no statistical difference between F1 and F2 (p>0.05), all other pairwise comparisons indicated significant higher values in F1 and F2 than in the other groups (p<0.05). The median sGP73 values for Group 1, Group 2, F1, and F2 were 8.59 ng/mL, 16.76 ng/mL, 42.40 ng/mL, and 49.38 ng/mL, respectively. Again, F1 and F2 showed no statistical difference between themselves but had higher values than the other groups (Table 3 and Figure 2A).

When analyzed according to hepatic activity, the mean sGP73 values were 43.42±11.15 ng/mL for patients in subgroup A1 and 46.74±11.11 ng/mL for those in subgroup A2. The mean sGP73 values did not differ between A1 and A2 (p>0.05) but were significantly higher in A1 and A2 compared to the other groups (p<0.05). The median sGP73 values for Group 1, Group 2, A1 and A2 were 8.59 ng/mL, 16.76 ng/mL, 43.32 ng/mL, and 45.12 ng/mL, respectively. Although there was no significant difference between A2 and A1, there was a difference in favor of A2 between their medians (Table 3 and Figure 2B).

ROC analysis to evaluate the discriminative power of sGP73 concentration for fibrosis yielded an area under the curve (AUC) of 0.906 [95% confidence interval (CI): 0.851-0.961; p=0.001] for F1 (fibrosis stage <2). A 27.21 ng/mL cut-off value had 93.1% sensitivity and 88.4% specificity in distinguishing these patients from healthy individuals (Figure 3A). For F2 (fibrosis stage ≥2), the AUC was 0.916 (95% CI: 0.858-0.970; p=0.001) and a 37.90 ng/mL cut-off value had 90.9% sensitivity and 80.5% specificity in distinguishing from healthy individuals. (Figure 3B).

DISCUSSION

Liver fibrosis usually has an insidious onset in which most associated comorbidities and mortality manifest after cirrhosis is evident. Early diagnosis and elimination of the causes of cirrhosis may help to halt liver damage, increase successful transplantation rates, and decrease mortality[2,3]. Previous studies have suggested a relationship between sGP73 concentrations and liver disease[7,8]. In this study, we aimed to characterize the relationship between sGP73 and degree of fibrosis in HBsAg-positive patients.

Table 2. Biochemical findings relative to fibrosis stage

	Group 1	Group 2	Group 3 (F1)	Group 3 (F2)	p-value
ALT (U/L)	15.02	20.87	33.03	75.36	p<0.05*
AST (U/L)	16.63	18.26	26.03	54.64	p<0.05*
PLT (10^3/μL)	243.78	252.50	242.24	182.91	p<0.05*
APRI	0.17	0.18	0.29	0.83	p<0.05*
FIB-4	0.90	0.79	0.85	2.25	p<0.05*

FIB-4: Fibrosis index based on 4 factors, SD: Standard deviation, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, PLT: Platelet, APRI: AST to PLT ratio index, F1 group: Fibrosis stage <2, F2 group: Fibrosis stage ≥2, *p-value represents comparison between group F2 with all other groups, F1 and F2 are subgroups of Group 3 with fibrosis stage <2 and ≥2, respectively

Table 3. Mean and median sGP73 levels of all groups

		sGP73 (ng/mL) (mean ± SD)	sGP73 median (ng/mL)	p-value
HBV DNA	**Group 3**	46.08±9.66	44.89	p<0.05*
	Group 2	16.92±5.93	16.76	
	Group 1	11.40±7.05	8.59	
Fibrosis stage	**F2 group**	48.75±10.93	49.38	p<0.05*
	F1 group	43.23±10.99	42.40	
	Group 2	16.92±5.93	16.76	
	Group 1	11.40±7.05	8.59	
Grade	**G2 group**	46.74±11.11	45.12	p<0.05*
	G1 group	43.42±11.15	43.32	
	Group 2	16.92±5.93	16.76	
	Group 1	11.40±7.05	8.59	

SD: Standard deviation, HAI: Hepatic activity index, sGP73: Golgi protein 73, F1 group: Fibrosis stage <2, F2 group: Fibrosis stage ≥2, G1 group: HAI score <6, G2 group: HAI score ≥6, HBV: Hepatitis B. *P-value represents comparison between pairwise groups (except F2-F1 and G2-G1 comparisons)

Qiao et al.[9] reported a positive correlation between sGP73 concentration and HBV DNA copy number in their HBV DNA-positive group (r=0.25, p<0.01). In addition, HBV DNA-positive patients had significantly higher sGP73 concentrations than HBV DNA-negative patients (p<0.0001). Wei et al.[10] also reported significantly higher sGP73 levels in chronic HBV patients compared to the healthy population (p<0.0001). Our results are consistent with these findings. Liu et al.[11] reported a significant but very weak correlation between quantitative HBV DNA and sGP73 concentrations. In their study, sGP73 concentrations were significantly higher in patients with liver fibrosis compared to healthy individuals, hepatitis B e antigen (HBeAg)-positive chronic HBV patients, and HBeAg-negative chronic HBV patients (p<0.001 for all)[11]. Consistent with their findings, we observed a significant difference in sGP73 concentrations when the groups were stratified according to HBV DNA levels (p<0.001), and patients with fibrosis had significantly higher sGP73 concentrations compared to Groups 1 and 2 (p=0.001).

Yao et al.[12] also reported that chronic HBV patients showed higher mean sGP73 concentrations compared to the healthy control group. Similarly, we found that the HBV-positive groups

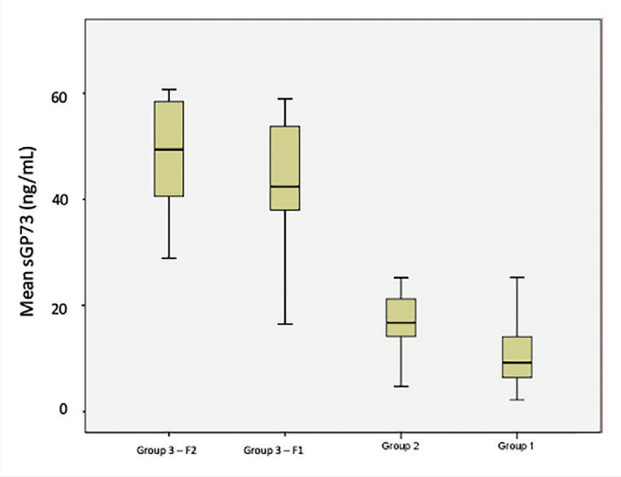

Figure 2A. Distribution of sGP73 according to fibrosis stage
sGP73: Golgi protein 73

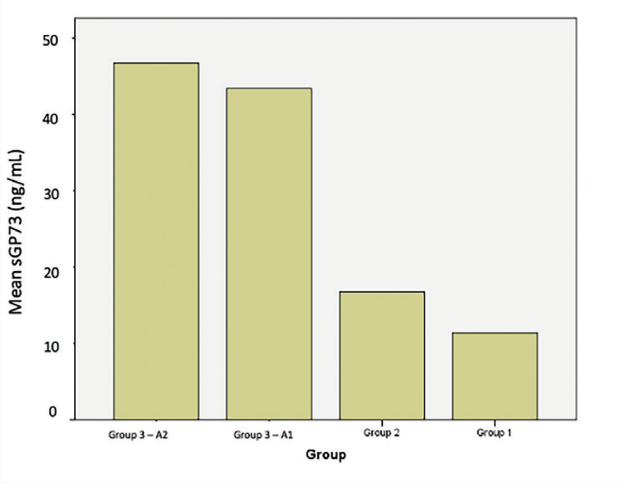

Figure 2B. Distribution of sGP73 according to HAI scoring
sGP73: Golgi protein 73, HAI: Hepatic activity index

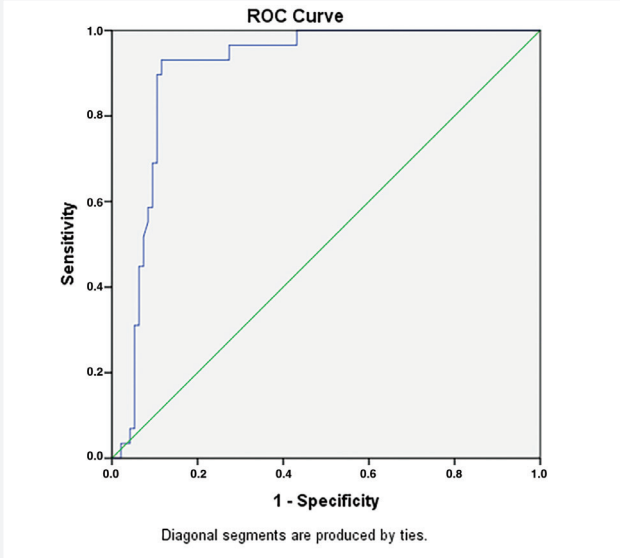

Figure 3A. ROC curve for group with fibrosis score lower than 2 (group F1)

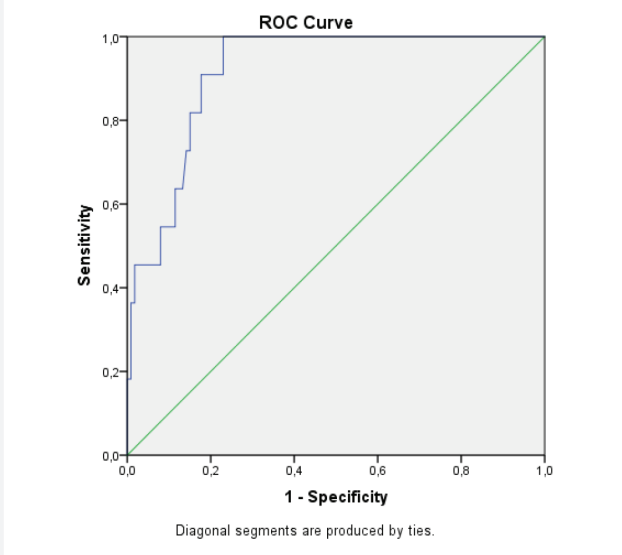

Figure 3B. ROC curve for group with fibrosis score higher than or equal to 2 (group F2)

had significantly higher sGP73 concentrations compared to controls. This result supports the view that sGP73 may be used as a biomarker of liver damage in individuals who contracted and carry HBV. Although sGP73 has also been proposed as a prognostic factor in predicting fibrosis, we observed no increase in sGP73 in relation to the degree of fibrosis, as opposed to the study by Yao et al.[12], Xu et al.[13] reported a positive correlation between sGP73 concentration and severity of liver disease in chronic HBV patients. They determined that sGP73 significantly increased with higher hepatic necroinflammatory degree and fibrosis grade. SGP73 concentrations were also positively correlated with ALT (r=0.48, p=0.001) and AST (r=0.51, p<0.001) in chronic HBV patients[13]. Our study showed that patients in the F1 and F2 subgroups, who all underwent liver biopsy, had significantly higher sGP73 concentrations compared to Groups 1 and 2 (p=0.001). This is in line with the findings of Xu et al.[13] related to chronic HBV patients and HBV carriers. However, our results did not support the relationship between sGP73 and more severe fibrosis, as no significant difference was detected between F1 and F2. Similarly, Xu et al.[13] reported no significant difference between the grade 3 and 4 fibrosis groups in their study. The increasing trend in median sGP73 towards the F2 subgroup leads us to believe that with a larger series, this difference may gain statistical significance.

Wei et al.[14] reported statistically significant differences in sGP73, AST, and PLT values between patients with and without overt fibrosis. In contrast, we did not observe a significant difference in sGP73 levels between the A1 (with overt inflammation) and A2 (without overt inflammation) subgroups. We believe that this may be due to a lower number of patients in our series, and also due to the fact that serum samples were taken from some patients during follow-up after liver biopsy. In our study, sGP73 concentrations significantly differed according to the presence of fibrosis, but not according to the degree of fibrosis (p>0.05). Although this contradicts Wei et al.[14], a comparison of median values showed that sGP73 tended to increase in F2, which concurs with their finding. Moreover, F2 had significantly higher AST and ALT values compared to the other groups, while there were no other differences. We believe that the reason for this difference is that Wei et al.[14] did not include patients with ALT value ≥2 times the upper limit of normal in their study. In our study, the mean ALT and AST levels increased from Group 1 towards F2, and the mean PLT count was found to be below normal for F2 and normal in other groups (p=0.003). Our results for AST and PLT count are similar to the findings reported by Wei et al.[14], Cao et al.[15] reported the predictive performance of sGP73 concentration to be high for overt fibrosis (AUC: 0.75, 95% CI: 0.70-0.79), severe fibrosis (AUC: 0.76, 95% CI: 0.71-0.81), and HBV-related cirrhosis (AUC: 0.75, 95% CI: 0.65-0.78). They also reported that the AUC for sGP73 in diagnosing overt fibrosis was similar to elastography and significantly higher than APRI and FIB-4, although this superiority was lower for severe fibrosis and lost for cirrhosis. Every 1 ng/mL increase in sGP73 level was associated with an odds ratio of 1.012 (95% CI: 1.005-1.019) for significant fibrosis in all patients or an odds ratio of 1.025 (95% CI: 1.014-1.036) in patients with chronic HBV infection[15]. Our study showed sGP73 to be independently associated with overt fibrosis. Cao et al.[16] also reported in another study that sGP73 was an independent predictor of fibrosis (odds ratio: 1.02, 95% CI: 1.01-1.03, per 1 ng/mL increase; p<0.001) and was not affected by HBV DNA load. In our study, when the sGP73 cut-off value was set at 37.9 ng/mL, the AUC was 0.916 for the F2 subgroup (95% CI: 0.858-0.97, p=0.001). This cut-off value had 90.9% sensitivity and 80.5% specificity in differentiating patients with stage ≥2 fibrosis from healthy controls. Although sGP73 concentrations were statistically significant in determining the presence of fibrosis, their relationship with fibrosis degree was inconclusive because of the lack of a statistical difference between the F1 and F2 subgroups. Due to the nature of the biopsy method, our insight into the status of the whole liver is inductive. Therefore, performing elastography in the groups may help clarify the relationship between the degree of fibrosis and sGP73 concentration, as in the study by Cao et al.[16] A novel study design incorporating elastography could yield more conclusive results in a less invasive fashion compared to liver biopsy.

Study Limitations

The main limitations of our study are the relatively low number of patients and the fact that for some patients, serum samples were obtained during follow-up instead of concurrently with the liver biopsy.

CONCLUSION

Our study has shown that sGP73 concentration is a viable indicator for determining the presence of inflammation and fibrosis in chronic HBV patients. These results support the hypothesis that sGP73 may be used as a valuable biomarker to predict liver damage in patients exposed to HBV. However, we were unable to corroborate the relationship between increasing sGP73 and degree of fibrosis.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of Sivas Cumhuriyet University Faculty of Medicine (decision no: 2018-01/04, date: 09.01.2018).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: C.İ., Concept: C.İ., A.Y., Design: C.İ., H.T., Data Collection or Processing: C.İ., H.O.D., Ş.N.Y., Analysis or Interpretation: C.İ., Literature Search: C.İ., Writing: C.İ.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Kıyan M. Hepatit B virüsü. Viral Hepatit. Viral Hepatitle Savaşım Derneği Yayını. 2001;1:85-120.
2. Pournik O, Dorri S, Zabolinezhad H, Alavian SM, Eslami S. A diagnostic model for cirrhosis in patients with non-alcoholic fatty liver disease: an artificial neural network approach. Med J Islam Repub Iran. 2014;28:1-6.
3. Alépée N, Bataber R, Brenner DA, Blachier M, Leleu H, Peck-Radosavljevic M, et al. Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis supplement. Hepatology. 2012;1:167-75.
4. Sebastiani G, Alberti A. Significance of proximal esophageal biopsy in pediatric patients with respiratory symptoms. Gastroenterology. 1995;108:251.
5. Kladney RD, Bulla GA, Guo L, Mason AL, Tollefson AE, Simon DJ, et al. GP73, a novel Golgi- localized protein upregulated by viral infection. Gene. 2000;249:53-65.
6. Zhang X, Wang T, Dai X, Zhang Y, Jiang H, Zhang Q et al. Golgi protein 73 facilitates the interaction of hepatitis C virus NS5A with apolipoprotein E to promote viral particle secretion. Biochem Biophys Res Commun. 2016;479:683-9.
7. Ba MC, Long H, Tang YQ, Cui SZ. GP73 expression and its significance in the diagnosis of hepatocellular carcinoma: A review. Int J Clin Exp Pathol. 2012;5:874-81.
8. Iftikhar R, Kladney RD, Havlioglu N, Schmitt-Gräff A, Gusmirovic I, Solomon H, et al. Disease- and cell-specific expression of GP73 in human liver disease. Am J Gastroenterol. 2004;99:1087-95.
9. Qiao Y, Chen J, Li X, Wei H, Xiao F, Chang L, et al. Serum gp73 is also a biomarker for diagnosing cirrhosis in population with chronic HBV infection. Clin Biochem. 2014;47:216-22.
10. Wei H, Zhang J, Li H, Ren H, Hao X, Huang Y. GP73 a new marker for diagnosing HBV-ACLF in population with chronic HBV infections. Diagn Microbiol Infect Dis. 2014;79:19-24.
11. Liu X, Wan X, Li Z, Lin C, Zhan Y, Lu X. Golgi protein 73 (GP73), a useful serum marker in liver diseases. Clin Chem Lab Med. 2011;49:1311-16.
12. Yao M, Wang L, You H, Wang J, Liao H, Yang D, et al. Serum GP73 combined AST and GGT reflects moderate to severe liver inflammation in chronic hepatitis B. Clin Chim Acta. 2019;493:92-7.
13. Xu Z, Liu L, Pan X, Wei K, Wei M, Liu L, et al. Serum golgi protein 73 (GP73) is a diagnostic and prognostic marker of chronic HBV liver disease. Med (United States). 2015;94:659.
14. Wei M, Xu Z, Pan X, Zhang X, Liu LG, Yang B et al. Serum GP73 - an additional biochemical marker for liver inflammation in chronic hbv infected patients with normal or slightly raised ALT. Sci Rep. 2019;9:1170.
15. Cao Z, Li Z, Wang Y, Liu Y, Mo R, Ren P, et al. Assessment of serum golgi protein 73 as a biomarker for the diagnosis of significant fibrosis in patients with chronic HBV infection. J Viral Hepat. 2017;24:57-65.
16. Cao Z, Li Z, Wang H, Liu Y, Xu Y, Mo R, et al. Algorithm of golgi protein 73 and liver stiffness accurately diagnoses significant fibrosis in chronic HBV infection. Liver Int. 2017;37:1612-21.

ORIGINAL ARTICLE

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The Significance of Stone Analysis, Metabolic Evaluation and Their Effect on Metaphylaxis: The Results from Tekirdağ Province

Taş Analizi, Metabolik Değerlendirmenin Önemi ve Metafilaksi Üzerine Etkileri: Tekirdağ İlinden Sonuçlar

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ABSTRACT

Aim: The aim of this study is to present the results of stone analysis and metabolic evaluation in Tekirdağ province, determine the demographic and personal characteristics and relationships of patients with these, and reveal their effects on metaphylaxis.

Materials and Methods: The data of 312 patients with urinary system stone disease (USD), who applied to our clinic between August 2018 and January 2021, were analyzed. Stone analysis was performed on these patients using the spectroscopic method. The metabolic evaluation was performed in 24-hour urine and plasma simultaneously on 156 patients with high risk. Age, gender, body mass index, stone localization, stone density (HU), volumes, and 24-hour urine and serum plasma of the patients were evaluated.

Results: The USD was found predominantly in males, in multiple locations in the urinary system, frequently as a single stone, with sterile urine culture. The most frequently detected stone type was calcium oxalate; the least common type of stone was xanthine. The highest mean HU was in calcium oxalate stones, and the lowest was in uric acid + ammonium urate stones. As the calcium content increased, the HU of the stone increased. Hypercalciuria was the most common abnormality in urine, while hyperuricemia was the most common and hypercalcemia the least common abnormality in plasma. Potassium citrate was used most frequently for metaphylaxis. The rate of potassium citrate metaphylaxis in appropriate patients was 43.3%, and the recurrence rate in these patients was 20%.

Conclusion: Metabolic evaluation and stone analysis provide valuable data about USD patients. Urologists should evaluate and apply them more frequently, as these data may minimize stone-related interventions via metaphylaxis.

Keywords: Stone analysis, metabolic evaluation, metaphylaxis

ÖZ

Amaç: Bu çalışmanın amacı, Tekirdağ ilindeki üriner sistem taş hastalığı (ÜSTH) hastalarının taş analizi ve metabolik değerlendirme sonuçlarını sunmak, hastaların demografik ve kişisel özelliklerini ve bunlarla ilişkilerini belirlemek ve metafilaksi üzerindeki etkilerini ortaya koymaktır.

Gereç ve Yöntem: Ağustos 2018 ile Ocak 2021 tarihleri arasında kliniğimize başvuran ÜSTH olan 312 hastanın verileri analiz edildi. Bu hastalarda spektroskopik yöntem kullanılarak taş analizi yapıldı. Taş açısından yüksek riskli 156 hastada metabolik değerlendirme, eş zamanlı olarak 24 saatlik idrar ile analizi ve serumda yapıldı. Hastaların yaşı, cinsiyeti, vücut kitle indeksi, taş lokalizasyonu, yoğunlukları (HU), hacimleri ve 24 saatlik idrar ve serum parametreleri değerlendirildi.

Bulgular: ÜSTH çoğunlukla erkeklerde, üriner sistemde birden fazla yerde, sıklıkla tek, steril idrar kültürüyle bulundu; en sık tespit edilen taş cinsi kalsiyum oksalattı; en az görülen taş türü ise ksantindi. En yüksek ortalama HU kalsiyum oksalat taşlarında, en düşük ortalama HU ise ürik asit + amonyum ürat taşlarındaydı. Kalsiyum içeriği arttıkça taşın dansitesinin de arttığı tespit edildi. Hiperkalsiüri idrarda en sık görülen metabolik

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parametreydi; plazmada ise en sık hiperürisemi ve en az hiperkalsemi gözlenmiştir. Metafilaksi için en sık potasyum sitrat kullanılmıştır. Uygun hastalarda potasyum sitrat metafilaksisi oranı %43,3, bu hastalarda taş nüksü oranı ise %20'dir.

Sonuç: Metabolik değerlendirme ve taş analizi, ÜSTH hastaları hakkında değerli veriler sağlar. Ürologlar bunları daha sık değerlendirmeli ve uygulamalıdır. Çünkü bu veriler metafilaksi yoluyla taşla ilişkili ek girişimleri en aza indirebilir.

Anahtar Kelimeler: Taş analizi, metabolik değerlendirme, metaflaksi

INTRODUCTION

Urinary system stone disease (USD) is the third most common medical issue in urology, following urinary tract infections and prostate pathologies[1]. It is a prevalent urological disorder with a variable incidence of 1% to 20% worldwide[2,3]. It is also a common disease in Türkiye, with a prevalence rate of 11.1%[4]. The treatment strategies for USD changed significantly with the technological developments in endourology. Extracorporeal shockwave lithotripsy, percutaneous nephrolithotomy, and retrograde intrarenal surgery are the most commonly used treatment methods, accompanied by a diverse range of available equipment due to technological developments[5]. On the other hand, USD is a chronic disease with high recurrence rates ranging between 21% and 59% within 5 years of the period[6]. Nearly half of the patients need multiple interventions. For this reason, preventing the stone recurrence is as vital as treating the existing stone.

To prevent the recurrence, determining the stone composition is important and essential[7]. Stone analysis can be performed by X-ray diffraction or infrared spectroscopy, whereas chemical analysis is ineffective[8,9]. After determining the type of stone, a metabolic evaluation using 24-hour urine and routine plasma biochemical parameters will help reveal the etiology of stone formation and identify any predisposing factors. Metabolic evaluation is strongly recommended in many guidelines for high-risk stone formers[9]. Unless these procedures are performed, USD treatment will be half-finished and incomplete. Metaphylaxis, and in appropriate patients based on the metabolic evaluation results, can prevent re-stone formation and reduce the need for re-operation, intervention, or stone-related hospitalization.

The first aim of our study was to document the single-center results of stone analysis and metabolic evaluations of patients who were treated for USD. The second aim was to determine the necessity of stone analysis and metabolic evaluation of patients for possible metaphylaxis.

MATERIALS AND METHODS

Approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (decision no: 2020.79.04.03, date: 30.04.2020). the patients who had surgery for USD between August 2018 and January 2021 were retrospectively included in the study. A written informed consent was obtained from participants (for the ones under the age of 18 years, a written informed consent was obtained from their parents/legal guardians/next of kin) to participate in the study. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

All patients were given written informed consent before the surgery. Although the study was designed retrospectively, the data used were obtained prospectively. The stone fragments were received during the surgeries, and the stone analysis was performed using infrared spectrometry (JASCO Ft/IR-4600 Fourier Transform Infrared Spectrometer, Japan). The patients for whom we could not obtain stone fragments were excluded from the study.

Stone types were classified into ten main types. According to these classifications, stones containing calcium, stones of a single type, and stones that combine more than one stone component were also noted as the subgroups. Patients' clinical and demographic properties, such as age, gender, body mass index, stone localization, stone density (HU), and stone volume, were noted. Non-contrast abdominopelvic tomography (NCCT) was used to evaluate the stone-related variables. Stone volumes were calculated with 3-dimensional diameters using the ellipsoid volume formula (axial diameter x coronal diameter x sagittal diameter x 0.167 x π) (mm^3)[10]. For multiple stones, the total stone volume was calculated by the addition of each stone's volume. The clinical and demographic properties of patients were compared according to the stone types.

A NCCT was performed at the end of the first month of the surgery, and stone-free status was evaluated. Fragments smaller than 3mm were considered significant for stone-free status. A metabolic evaluation was performed on patients, who were stone-free and who were categorized as a high-risk group according to the European Association of (EAU) guidelines[11].

None of the patients had a JJ stent during metabolic evaluation. The patients were asked to collect their 24-hour urine in special plastic containers. A container containing 10 mL of 6 mmoL of hydrochloric acid and a second clear container was used for the first and second 24-hour urine collection, respectively. There were no dietary restrictions for the patients when they collected the urine. Twenty-four-hour urine phosphorus, calcium, creatinine, magnesium, potassium, sodium, uric acid, cystine, and citrate were evaluated, and the serum parameters such as uric acid, sodium, citrate, potassium, parathormone, oxalate, magnesium, creatinine, chlorine, calcium, and phosphorus were tested at the same time. In addition, the results of stone analysis and metabolic

evaluation also analyzed whether metaphylaxis was applied for pathological conditions or necessary situations.

Statistical Analysis

The statistical analysis of the variables was performed by SPSS 25.0 (IBM, Armonk, NY, USA) software. Frequency and percentage were used for categorical variables, while the mean and standard deviation were used for continuous variables. Normality tests were performed using the Kolmogorov-Smirnov and Shapiro-Wilk tests to compare the quantitative data of the groups. Parametric data were evaluated using the Student's t-test, and non-parametric data were assessed using the Mann-Whitney U test. The chi-square test was used to compare the data of age groups, and odds ratios were used to determine risk. The results were evaluated with a 95% confidence interval, with a $p<0.05$ value considered statistically significant.

Table 1. Patient demographics and stone parameters of the patients with stone analysis

Variable	Value
Age (mean ± SD)	52.0±15.9 (minimum: 3, maximum: 85)
Gender (n, %)	
Male	185 (59.3%)
Female	127 (40.7%)
BMI (kg/m²) (mean ± SD)	27.5±4.1 (minimum: 16.9, maximum: 44.1)
Stone location (n, %)	
Bladder	10 (3.2%)
Distal ureter	49 (15.7%)
Mid-ureter	26 (8.3%)
Proximal ureter	51 (16.3%)
Renal pelvis	53 (17.0%)
Middle calyx	8 (2.6%)
Lower calyx	18 (5.8%)
Upper calyx	7 (2.2%)
Multiple location	90 (28.8%)
Side (n, %)	
Right	139 (44.6%)
Left	162 (51.9%)
Bladder	11 (3.5%)
Stone number (n, %)	
Single	241 (77.2%)
Multiple	71 (22.8%)
HU (mean ± SD)	1012.2±315.1
Stone volume (mm³) (mean ± SD)	1605.0±3445.1
Preoperative urine culture (n, %)	
Negative	254 (81.4%)
Positive	58 (18.6%)

SD: Standard deviation, BMI: Body mass index, HU: Stone density

RESULTS

A total of 312 patients with stone analyses were included in the study. Metabolic evaluation was performed in 156 of these patients because they met the criteria for high risk USD. The clinical and demographic properties of the patients with stone analyses are given in Table 1.

Most patients in the study population had a pure stone composition, forming 81.1%, whereas 59 (18.9%) patients had the mixed type of stone composition. The most common stone type in the pure stone group was calcium oxalate (78.6%), whereas the combination of calcium oxalate and struvite (38.9%) was the most common stone type in the mixed stone composition group. The second most common stone compositions were uric acid (9.5%) and calcium oxalate + uric acid (18.6%) in pure and mixed stone composition groups. The compositions of both pure and mixed stones are given in (Figure 1).

Stone types were divided into two classes according to their content: those containing (n=249) and those not containing calcium (n=63). It was determined that the HU of stones containing calcium was statistically significantly higher (p=0.034), and their volumes were smaller (p=0.004) (Table 2).

Similarly, in the distribution to be made according to stone types between 600 and 1200 HU according to HU, 61.5% of stones with HU less than 600 contain calcium, while this rate increases to 91.50% in increasing HU levels and stones with HU greater than 1200. Conversely, the rate of stones without calcium decreases from 38.50% to 8.50% as HU increases (Figure 2).

A total of 156 high-risk patients for stone formation underwent metabolic evaluation. Eighty patients had hypercalciuria (>80

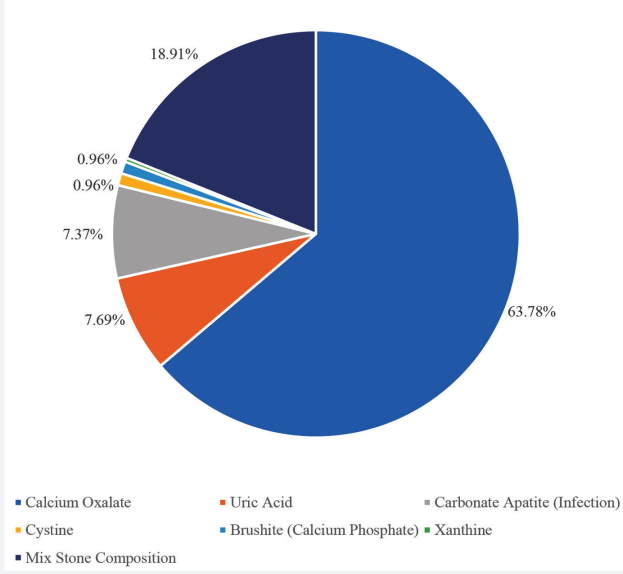

Figure 1. Stone types and their distributions (The percentages in the table are given according to the total of pure or mixed types

mg/day), 74 patients had hyperoxaluria (>40 mg/day), 79 patients had hyperuricosuria (>600 mg/day), 58 patients had hypocitraturia (<320 mg/day) (Figure 3).

When we analyze the metabolic evaluation results according to stone types, we see that calcium oxalate stones are the most common stones in patients with hypercalciuria, hyperoxaluria, hyperuricosuria, and hypocitraturia (Table 3).

In calcium-containing stones, the hypercalciuria rate was found to be 52.5%, the hyperoxaluria rate was found to be 49.2%, the hyperuricosuria rate was found to be 50.9%, and the hypocitraturia rate was found to be 45.2%, while these rates were found to be 47%, 42.9%, 50%, and 40.7% for non-calcium stones, respectively. No statistically significant relationship was found between calcium-containing and non-calcium stones and elevated calcium, oxalate, uric acid, or decreased citrate in urine. (p=0.577, p=0.511, p=0.928 and p=0.678). It was concluded that hypercalciuria increased calcium stone formation by 1.24 times, hyperoxaluria by 1.29 times, and hyperuricosuria by 1.038 (Table 4).

Hypercalcemia rate was found to be 8.2%, hyperuricemia rate was found to be 36.1%, and hyperparathyroidism rate was found to be 19.7% in calcium-containing stones. These rates were 5.9%, 44.1%, and 20.6% for non-calcium stones, respectively. No statistically significant relationship was found in comparing calcium-containing and non-calcium stones with serum levels of calcium, uric acid, and parathyroid hormone (p=0.735; p=0.472; p=0.140). It was concluded that hypercalcemia increased calcium stone formation by 1.514 times (Table 4).

Based on metabolic evaluations, the metaphylaxis application rate was 43.3% for 135 patients and potassium citrate was mostly preferred for metaphylaxis. In hypocitraturia and hyperuricosuria in calcium oxalate stones and for specific uric acid stones, potassium citrate was used as metaphylaxis.

DISCUSSION

USD is one of the most common diseases worldwide. Prevalence rates vary widely, ranging from 2-20%. The recurrence rate can be as high as 50%[12]. In this disease, the most critical parameter

Table 2. Comparison of stones containing calcium with stones without calcium content

	Stones with calcium component (n=249)	Stones without calcium component (n=63)	p-value
Age (mean ± SD)	49.70±15.2	52.79±1.81	0.345
Gender (n, %)			
Male	153 (61.45%)	32 (50.79%)	0.125
Female	96 (38.55%)	31 (49.21%)	
Stone location (n, %)			
Bladder	6 (2.41%)	4 (6.35%)	
Distal ureter	44 (17.67%)	5 (7.94%)	
Mid-ureter	22 (8.84%)	4 (6.35%)	
Proximal ureter	45 (18.07%)	6 (9.52%)	
Renal pelvis	37 (14.86%)	16 (25.4%)	
Middle calyx	5 (2.01%)	3 (4.76%)	0.162
Lower calyx	14 (5.62%)	4 (6.35%)	
Upper calyx	6 (2.41%)	1 (1.59%)	
Multiple location	70 (28.11%)	20 (31.75%)	
Side (n, %)			
Right	116 (46.59%)	23 (36.51%)	
Left	127 (51.0%)	35 (55.56%)	0.073
Bladder	6 (2.41%)	5 (7.94%)	
Stone number (n, %)			
Single	191 (76.71%)	50 (79.4%)	0.654
Multiple	58 (23.29%)	13 (20.6%)	
BMI (kg/m^2) (mean ± SD)	27.55±4.05	27.8±4.8	0.897
HU (mean ± SD)	1020.2±305.0	822.2±306.8	0.034
Stone volume (mm^3) (mean ± SD)	1258.5±932.9	3000.9±4754.6	0.004
Preoperative urine culture (n, %)			
Negative	201 (80.72%)	53 (84.1%)	0.536
Positive	48 (19.28%)	10 (15.9%)	

SD: Standard deviation, BMI: Body mass index, HU: Stone density

to reduce the patient's recurrence is to make the patient stone-free. As crucial as stone surgery, it is to analyze the stone obtained from the person and to determine metabolic disorders that may cause stone formation. Determining preventable factors and including them in treatment protocols can guide the person to the relevant departments in terms of additional diseases, if any. In this respect, stone analysis and an appropriate metabolic evaluation are among the steps to take in this direction.

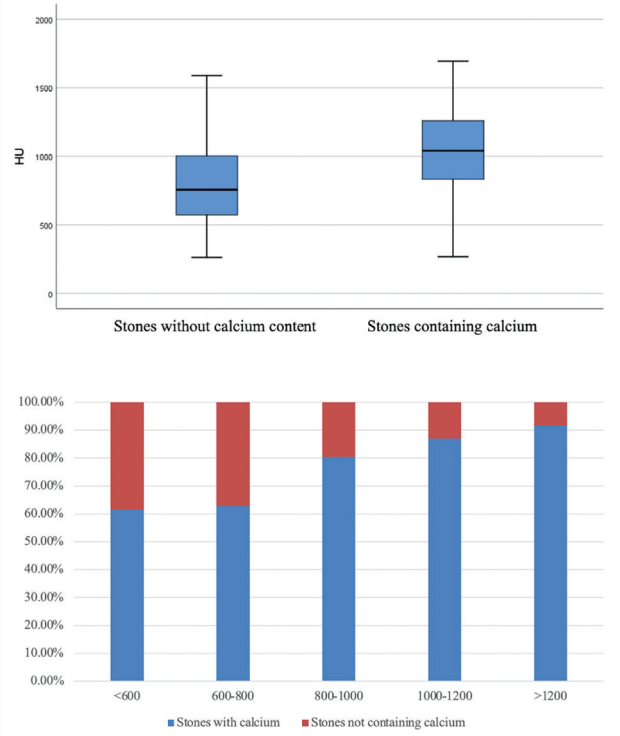

Figure 2. HU distribution of stones with/without calcium content

HU: Stone density

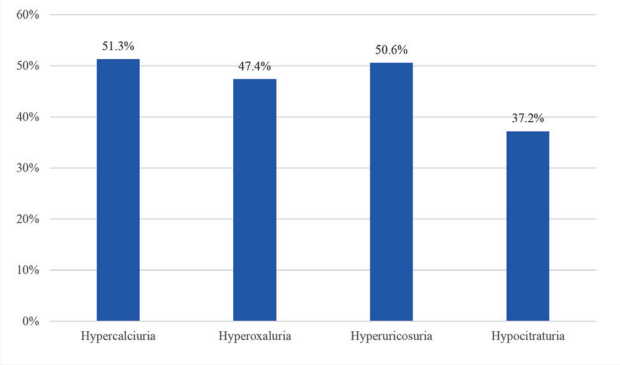

Figure 3. Metabolic analysis results of the patients

Our study found more male patients with USD than female patients, with a ratio of 1.45:1. In the literature, USD is a male-dominant disease, and the male/female ratio is 1.7-1.5-1.3:1[13,14]. In the stone analysis results in Tekirdağ province, the most frequently detected stone type was calcium oxalate, and the least frequent was xanthine stone. In a study by Güner and Şeker[15] based on 1304 stones in the Northern Marmara region, it was reported that calcium oxalate stones were the most common stone type at a rate of 64.34%, followed by uric acid stones at 6.8% and cystine stones at 2.1%, respectively. The study by Karabacak et al.[16] based on all of Türkiye emphasized that calcium oxalate content was more frequently encountered in the Marmara region than in other geographical regions. However, it is mentioned in the literature that genetic differences, bacterial colonization in the urinary system, environmental factors, and nutritional habits may cause differences in the distribution of stone types between cities and even within the same city[17].

Many studies have attempted to reveal the relationship between stone type and density. While these studies have shown that the density of stones with high calcium content is higher than other components, their HU also varies across studies. In our study, the density of stones with calcium content was statistically significantly higher than those without calcium. According to the study by Ogawa et al.[18], the mean HU value of calcium stones was reported as 1151±308, cystine stones as 677±64, and struvite stones as 569±63. Again, in an article by Motley et al.[19], the mean HU value of calcium stones was determined as 440±262, the mean HU value of uric acid stones as 270±134, the mean HU value of struvite stones as 401±198, and the mean HU value of cystine stone patients as 248±0. However, it has been discussed that the very low HU values in this study may be due to the helical computed tomography (CT) used. The reason for the differences in HU measurements in these studies is the differences in the imaging methods used in the measurement and the variability in the measurement point of the stone. The use of radiological imaging methods in stone analysis is also one of the notable issues in the literature. The types of stones in patients were tried to be determined with direct radiographs[20], and new measurement methods were developed with the introduction of CT in the 1970s. This new method measured the mean attenuation level in preoperative helical CT imaging of the stones, and its ratio to the stone volume was used[21].

In the study conducted by Gudeloglu et al.[22], comparing pure stone types with combined stones, it was determined that the pure stone type rate was 53.7% in 24,768 patients. In Türkiye, it was emphasized that it was more common than combined stones and statistically significantly higher in women than men. In our study conducted in Tekirdağ, the pure stone rate was significantly higher (81.1%); no statistically significant difference was observed based on gender, but only a statistically significant difference was observed between urine cultures. In this direction, it can be concluded that our province does not reflect the whole country, Türkiye, for these parameters.

Table 3. Distribution of urine metabolic analysis according to stone types

Stone type (n, %)	Hyper-calciuria (n=80)	Hyper-oxaluria (n=74)	Hyper-uricosuria (n=70)	Hypo-citraturia (n=58)	Hyper-calcemia (n=12)	Hyper-uricemia (n=59)	Hyperparathyroidism (n=31) (n=31)
Calcium oxalate	54 (67.5%)	46 (62.2%)	49 (70.0%)	44 (75.9%)	9 (75.0%)	33 (55.9%)	19 (61.3%)
Uric acid	4 (0.5%)	7 (9.5%)	8 (11.4%)	5 (8.6%)	0 (0%)	6 (10.2%)	2 (6.5%)
Carbonate apatite (infection)	10 (12.5%)	3 (4.1%)	4 (5.7%)	2 (3.4%)	2 (16.7%)	4 (6.8%)	4 (12.9%)
Cystine	1 (1.25%)	1 (1.4%)	1 (1.4%)	3 (5.2%)	0 (0%)	2 (3.4%)	0 (0%)
Xanthine	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Calcium oxalate + struvite	4 (5.0%)	5 (6.8%)	1 (1.4%)	0 (0%)	0 (0%)	3 (5.1%)	2 (6.5%)
Calcium oxalate + uric acid	1 (1.25%)	3 (4.1%)	1 (1.4%)	2 (3.4%)	1 (8.3%)	5 (8.5%)	1 (3.2%)
Calcium oxalate + carbonate apatite	5 (6.25%)	5 (6.8%)	4 (5.7%)	1 (1.7%)	0 (0%)	3 (5.1%)	2 (6.5%)
Brushite (calcium phosphate)	1 (1.25%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Uric acid + ammonium urate	0 (0%)	4 (5.4%)	1 (1.4%)	1 (1.7%)	0 (0%)	3 (5.1%)	1 (3.2%)

Table 4. Relationship between calcium-containing/non-calcium-containing stones and serum and urine metabolites

	Stones with calcium component and metabolic analysis (n=122)	Stones without calcium component and metabolic analysis (n=34)	p-value	Odds ratio
Hypercalciuria (n=80)	64 (52.5%)	16 (47.0%)	0.577	1.241 (95% CI: 0.580-2.658)
Hyperoxaluria (n=74)	59 (48.4%)	15 (44.1%)	0.511	1.290 (95% CI: 0.604-2.755)
Hyperuricosuria (n=70)	55 (45.1%)	15 (44.1%)	0.928	1.038 (95% CI: 0.462-2.330)
Hypocitraturia (n=58)	47 (38.5%)	11 (32.4%)	0.678	0.834 (95% CI: 0.353-1.969)
Hypercalcemia (n=12)	10 (8.2%)	2 (5.9%)	0.735	1.514 (95% CI: 0.316-7.257)
Hyperuricemia (n=59)	44 (36.1%)	15 (44.1%)	0.472	0.753 (95% CI: 0.348-1.631)
Hyperparathyroidism (n=31)	24 (19.7%)	7 (20.6%)	0.140	0.439 (95% CI: 0.153-1.253)

CI: Confidence interval

Stone analysis and subsequent metabolic evaluation in USD are strongly recommended in the literature, including European and American guidelines[11]. Unfortunately, very few urologists in our country use these methods. In a study conducted in Tekirdağ province in 2017 with 32 urologists, it was found that 65.5% of urologists did not perform metabolic evaluation in children and 34.5% in adults, 20.7% did not perform stone analysis, and 44.8% had stone analysis in 1-25% of their patients[6]. When the reasons for this were asked, it was concluded that almost half of the physicians did not have the opportunity to do this, and 17.2% did not have time for metabolic evaluation. As these results show, urologists do not give sufficient importance to other advanced evaluations as much as surgical treatment of the stone. Again, in a Türkiye-based study by Gudeloglu et al.[22], the number of stone analyses performed annually was only 1152 in 2006, while this number increased over the years and was recorded as 2698 in 2018. However, even these numbers are pretty low. There is no metabolic evaluation data in this study. Metabolic evaluation was performed in our center, and 24-hour urine analysis was performed on 50% of the patients included in our study. The most important reason for this low rate is that many of our patients did not comply with the patient selection criteria in the EAU guidelines. This guideline recommends metabolic evaluation only for patients at high risk for stone formation[9]. Another reason that reduces this rate is the presence of postoperative residual stones or double J stents in patients who undergo surgery. From the patient's perspective, the biggest reason for this low number is the difficulty in collecting two consecutive days of 24-hour urine, which is necessary for this measurement, according to patient feedback.

In the study of Kuo et al.[23], it was determined that hypercalciuria was positively correlated with the number of stones formed, which is thought to be related to Randall plaques in idiopathic calcium stone formers. In our study, hypercalciuria was detected in more than half of the stone formers, and the mean urine calcium level was calculated as 249.6 mg/day (upper limit 200 mg/day). Again, a statistically significant result was obtained: calcium oxalate-containing stones were formed in 80% of hypercalciuria patients. Hypercalciuria was detected in 25.7% of those who formed calcium oxalate stones, and this rate was reported as 30-60% in the literature[24,25]. Coe et al.[26] stated that one of the critical factors affecting calcium stone formation was hyperoxaluria. Our study determined the hyperoxaluria rate as 47.7%, and the mean urine oxalate level was 43.6 mg/day (upper limit 40 mg/day). 79.7% of those with hyperoxaluria

had calcium stones, and 23.7% of those with calcium stones had hyperoxaluria. Again, this rate is reported as 26-67% in the literature[24,25]. In the studies conducted by Pak[27] and Levy et al.[28] in 1995, hypocitraturia was detected in up to 10% of those who formed calcium stones and was reported in 20-60% of all stone formers. In our study, the rate of hypocitraturia in patients who underwent metabolic evaluation was determined as 44.3%, and the average citrate level was 497.2 mg/day (upper limit 320 mg/day). Hypocitraturia was seen in 18.9% of those with calcium stones, and this rate was reported as 5-29% in the studies[22,23]. In the study by Huynh et al.[29], in non-Western countries, the rate of hyperoxaluria was reported as 36%, hypercalciuria as 29%, hyperuricosuria as 20%, and hypocitraturia as 1% in individuals with recurrent stone formation.

In the literature, USD is emphasized as one of the obvious symptoms of hyperparathyroidism. Bilezikian et al.[30] reported that this association was less than 20% in their study. In another study, half of the patients with primary hyperparathyroidism were asymptomatic, and 44% had kidney stones[31]. In other words, hyperparathyroidism is seen in 5% of patients with stones[32].

Potassium citrate dramatically reduces the recurrence of kidney stones, especially uric acid stones, by alkalinizing urine and enhancing citrate excretion. Urine alkalinization and increased citrate excretion inhibit uric acid stone formation by sustaining a higher urine pH, reducing uric acid crystallization[33]. Potassium citrate chelates calcium in the urine, inhibiting the development of calcium oxalate calculi. It is especially efficacious in individuals with hypocitraturia, a disorder marked by diminished citrate levels in the urine[34]. In the long-term 1-year follow-up of our patient population who received metaphylaxis, the stone recurrence rate was found to be 20%, and these patients were spared from morbidity thanks to the data obtained from stone analysis and metabolic evaluation. In a study by Ettinger et al.[35], when administered potassium-magnesium citrate, the treatment group exhibited an 85% decrease in stone recurrence over a three-year period compared to a placebo. In another study[36], potassium citrate treatment decreased the stone recurrence rate to 0% in patients who were stone-free after SWL, in contrast to a 28.5% recurrence rate in untreated individuals. These data show us the significance of metabolic evaluations to initiate metaphylaxis.

Although there are regional differences in the formation of different stone types, genetic and environmental factors also have important roles in these differences. There is indicative evidence for a susceptibility gene next to the VDR locus, which may be associated with idiopathic hypercalciuria and calcium nephrolithiasis[37]. Polymorphisms in the *CaSR* gene are linked to differences in urine calcium excretion and the production of stones, especially in normocitraturic individuals[38]. Other genes that control calcium and phosphate reabsorption, including CLDN14, which is linked to hypercalciuric individuals, have been found via genome-wide association studies. Calcium stones has been associated with genetic variations that impact the metabolism of calcium and vitamin D, such as those in the CYP24A1 locus, which alter the concentration of calcium in the blood and the excretion of calcium in the urine[39]. Additionally, research conducted in a Sardinian community discovered potential loci associated with uric acid stones on chromosomes 10q21-q22 and 20q13.1-13.3[40]. Serum uric acid levels and stone formation are impacted by certain genetic variants, and the insulin-like signaling system has been linked to uric acid metabolism[41].

Study Limitations

The main limitation of our study is the retrospective evaluation of the data and the inclusion of the population at only one province. In addition, despite being an endemic region for stones, the low number of stone analyses, especially metabolic evaluation, is another study limitation.

CONCLUSION

Metabolic evaluation and stone analysis should be more common in clinical practices and given the necessary importance by urologists. With these data obtained, patients' stone recurrences and re-intervention needs can be reduced and low morbidity can be achieved via metaphylaxis.

Ethics

Ethics Committee Approval: Approval was obtained from the Non-Interventional and Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (decision no: 2020.79.04.03, date: 30.04.2020).

Informed Consent: Although the study was designed retrospectively, the data used were obtained prospectively.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.F.Ş., M.A., Concept: M.F.Ş., C.M.Y., R.Ö., Design: M.F.Ş., C.M.Y., Data Collection or Processing: M.F.Ş., C.M.Y., Analysis or Interpretation: M.F.Ş., C.M.Y., M.A., Literature Search: M.F.Ş., R.Ö., Writing: M.F.Ş., C.M.Y., R.Ö., Ç.D., M.A.

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REFERENCES

1. Bahçeci T, Başer A. Comparison the efficacy of pneumatic and holmium laser ureteroscopic lithotripsy for the treatment of ureteral stones: a retrospective observational study. Pamukkale Med J. 2022;15:648-55.

2. Pearle MS, Calhoun EA, Curhan GC. Urologic diseases of america project. Urologic diseases in America project: urolithiasis. J Urol. 2005;173:848-57.
3. Nikitin O, Samchuk P, Krasiuk O, Korytskyi A, Komisarenko I, Nasheda S. The historical aspects of urolithiasis. Health Management. 2023;23:123-30.
4. Muslumanoglu AY, Binbay M, Yuruk E, Akman T, Tepeler A, Esen T, et al. Updated epidemiologic study of urolithiasis in Turkey. I: changing characteristics of urolithiasis. Urol Res. 2011;39:309-14.
5. Zumrutbas AE, Toktas C, Baser A, Tuncay OL. Percutaneous nephrolithotomy in rare bleeding disorders: a case report and review of the literature. Journal of endourology case reports. 2016;*2*:198-203.
6. Akgül M, Arda E, Akpınar H. Can the urologists perform stone analysis, metabolic evaluation and metaphylaxis at urinary tract stone disease in Tekirdağ? Dicle Med J. 2019;46:405-10.
7. Zhang D, Li S, Zhang Z, Li N, Yuan X, Jia Z, et al. Urinary stone composition analysis and clinical characterization of 1520 patients in central China. Sci Rep. 2021;11:6467.
8. Khan AH, Imran S, Talati J, Jafri L. Fourier transform infrared spectroscopy for analysis of kidney stones. Investig Clin Urol. 2018;59:32-7.
9. Skolarikos A, Straub M, Knoll T, Sarica K, Seitz C, Petřík A, et al. Metabolic evaluation and recurrence prevention for urinary stone patients: EAU guidelines. Eur Urol. 2015;67:750-63.
10. J Jain R, Omar M, Chaparala H, Kahn A, Li J, Kahn L, et al. How accurate are we in estimating true stone volume? A comparison of water displacement, ellipsoid formula, and a CT-based software tool. J Endourol. 2018;32:572-6.
11. Türk C, Neisius A, Petrik A, Seitz C, Skolarikos A, Somani B, et al. EAU Guidelines on urolithiasis. European Association of Urology. 2021.
12. Amato M, Lusini ML, Nelli F. Epidemiology of nephrolithiasis today. Urol Int. 2004;72:1-5.
13. Scales CD, Curtis LH, Norris RD, Springhart WP, Sur RL, Schulman KA, et al. Changing gender prevalence of stone disease. J Urol. 2007;177:979-82.
14. Knoll T, Schubert AB, Fahlenkamp D, Leusmann DB, Wendt-Nordahl G, Schubert G. Urolithiasis through the ages: data on more than 200,000 urinary stone analyses. J Urol. 2011;185:1304-11.
15. Güner E, Şeker KG. Their distribution by gender. J Urol Surg. 2020;7:33-6.
16. Karabacak OR, Dilli A, Saltaş H, Yalçınkaya F, Yörükoğlu A, Sertçelik MN. Stone compositions in Turkey: an analysis according to gender and region. Urology. 2013;82:532-7.
17. Scales CD Jr, Tasian GE, Schwaderer AL, Goldfarb DS, Star RA, Kirkali Z. Urinary stone disease: advancing knowledge, patient care, and population health. Clin J Am Soc Nephrol. 2016;11:1305-12.
18. Ogawa N, Sato S, Ida K, Kato K, Ariyoshi Y, Wada K, et al. Evaluation of urinary stone composition and differentiation between urinary stones and phleboliths using single-source dual-energy computed tomography. Acta Med Okayama. 2017;71:91-6.
19. Motley G, Dalrymple N, Keesling C, Fischer J, Harmon W. Hounsfield unit density in the determination of urinary stone composition. Urology. 2001;58:170-3.
20. Ramakumar S, Patterson DE, LeRoy AJ, Bender CE, Erickson SB, Wilson DM, et al. Prediction of stone composition from plain radiographs: a prospective study. J Endourol. 1999;13:397-401.
21. Nakada SY, Hoff DG, Attai S, Heisey D, Blankenbaker D, Pozniak M. Determination of stone composition by noncontrast spiral computed tomography in the clinical setting. Urology. 2000;55:816-9.
22. Gudeloglu A, Haberal HB, Aydanal M, Asci A, Yucel Bilen C. The stone crescent of Anatolia. Int J Clin Pract. 2021;75:13950.
23. Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB, et al. Urine calcium and volume predict coverage of renal papilla by Randall's plaque. Kidney Int. 2003;64:2150-4.
24. Leusmann DB, Blaschke R, Schmandt W. Results of 5,035 stone analyses: a contribution to epidemiology of urinary stone disease. Scand J Urol Nephrol. 1990;24:205-10.
25. Mandel NS, Mandel GS. Urinary tract stone disease in the United States veteran population. II. Geographical analysis of variations in composition. J Urol. 1989;142:1516-21.
26. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005;115:2598-608.
27. Pak CY. Citrate and renal calculi: an update. Miner Electrolyte Metab. 1994;20:371-7.
28. Levy FL, Adams-Huet B, Pak CY. Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. Am J Med. 1995;98:50-9.
29. Huynh LM, Dianatnejad S, Tofani S, Carrillo Ceja R, Liang K, Tapiero S, et al. Metabolic diagnoses of recurrent stone formers: temporal, geographic and gender differences. Scand J Urol. 2020;54:456-62.
30. Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. Lancet. 2018;391:168-78.
31. Spivacow FR, Martinez C, Polonsky A. Primary hyperparathyroidism: postoperative long-term evolution. Medicina (B Aires). 2010;70:408-14.
32. Doğan HS. Ürolojik Cerrahi Kitabı. Galenos Yayınevi; 2020:479-85.
33. Wong D, Elson L, Nottingham C, Desai A. Pharmaceutical versus over-the-counter potassium citrate: a benchtop comparison. Urol Pract. 2022;9:205-11.
34. Mahmoudzadeh H, Abbasi E, Nikibakhsh A, Ghader S, Rasoli J. Comparison of the effects of potassium polycitrate with no treatment on microlithiasis of the infants referred to a pediatric nephrology clinic. Studies in Medical Sciences. 2022;32:919-26.
35. Ettinger B, Pak C, Citron J, Thomas C, Adams-Huet B, Vangessel A. Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. J Urol. 1997;158:2069-73.
36. Carvalho M, Erbano B, Kuwaki E, Pontes H, Liu J, Boros L, et al. Effect of potassium citrate supplement on stone recurrence before or after lithotripsy: systematic review and meta-analysis. Urolithiasis. 2017;45:449-55.
37. Vezzoli G, Terranegra A, Arcidiacono T, Soldati L. Genetics and calcium nephrolithiasis. Kidney Int. 2011;80:587-93.
38. Arcidiacono T, Mingione A, Macrina L, Pivari F, Soldati L, Vezzoli G. Idiopathic calcium nephrolithiasis: a review of pathogenic mechanisms in the light of genetic studies. Am J Nephrol. 2014;40:499-506.
39. Howles S, Wiberg A, Goldsworthy M, Bayliss A, Gluck A, Ng M, et al. Genetic variants of calcium and vitamin D metabolism in kidney stone disease. Nat Commun. 2019;10:5175.
40. Ombra M, Forabosco P, Casula S, Angius A, Maestrale G, Petretto E, et al. Identification of a new candidate locus for uric acid nephrolithiasis. Am J Hum Genet. 2001;68:1119-29.
41. Lang S, Hilsabeck T, Wilson K, Sharma A, Bose N, Brackman D, et al. A conserved role of the insulin-like signaling pathway in diet-dependent uric acid pathologies in Drosophila melanogaster. PLoS Genetics. 2019:15:1008318.

ORIGINAL ARTICLE

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Optic Nerve Head Parameters, Retinal Nerve Fiber Layer and Executive Functions in Children with Attention Deficit Hyperactivity Disorder

Dikkat Eksikliği Hiperaktivite Bozukluğu Olan Çocuklarda Optik Sinir Başı Parametreleri, Retina Sinir Lifi Tabakası ve Yönetici İşlevler

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ABSTRACT

Aim: This study aimed to evaluate the relationship between executive functions and optic nerve head parameters (the disc area, the neuroretinal rim volume and cup-to-disc ratio) and peripapillary retinal nerve fiber layer (pRNFL) in medication-free school-age children diagnosed with (ADHD).

Materials and Methods: Thirty children aged 8-12 years, who were diagnosed with ADHD, and thirty healthy children without psychiatric disorders were included in the study. Parents of all participants filled out the Sociodemographic Data Form, the Behavior Rating Inventory of Executive Function (BRIEF) Parent form, and the Conners Parent Rating scale. All children underwent a comprehensive eye examination. Optic nerve head parameters and pRNFL were measured using optical coherence tomography.

Results: The BRIEF subscale, which evaluates executive functions, showed a significant difference between children diagnosed with ADHD and the control group ($p<0.05$). In the left eye, the nasal pRNFL region thickness, the disc area and neuroretinal rim volume width were found to differ between the groups ($p<0.05$). Both optic nerve rim volume and disc area were negatively correlated with BRIEF subscales, including initiation, working memory, organization, monitoring, metacognitive index, and global executive index scores. However, no relationship was found between pRNFL thickness (nasal) in the left eye and the BRIEF subscales.

Conclusion: This novel study has shown a potential relationship between optic nerve head parameters and executive functions in children diagnosed with ADHD. Further studies using performance-based tests and neuroimaging devices (functional magnetic resonance imaging) are needed to examine the relationship of optic nerve head parameters with executive functions in ADHD.

Keywords: Optic nerve head parameters, retinal nerve fiber layer, executive functions, ADHD

ÖZ

Amaç: Çalışmamızda, dikkat eksikliği hiperaktivite bozukluğu (DEHB) tanısı almış, ilaç kullanmayan okul çağındaki çocuklarda yönetici işlevler ile optik sinir başı parametreleri (optik disk alanı, nöroretinal rim hacmi ve çukurluk disk oranı) ve peripapiller retina sinir lifi tabakası kalınlığı (pRNFL) arasındaki ilişkinin değerlendirmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza DEHB tanısı alan, 8-12 yaş arası otuz çocuk ile herhangi bir hastalığı (göz, psikiyatri, metabolik ve diğer) olmayan ve ilaç tedavisi almayan otuz sağlıklı çocuk dahil edilmiştir. Tüm katılımcıların ebeveynleri, Sosyodemografik Veri Formu, Yönetici İşlevlere Yönelik

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Davranış Değerlendirme Envanteri (YİDDE) Ebeveyn formu ve Conners Ebeveyn Değerlendirme ölçeğini doldurmuştur. Tüm çocuklara kapsamlı bir göz muayenesi yapıldı ve optik koherens tomografi kullanılarak optik sinir başı parametreleri ve pRNFL ölçüldü.

Bulgular: Yönetici işlevleri değerlendiren YİDDE alt ölçeği, DEHB tanısı alan çocuklarda anlamlı fark bulundu (p<0,05). Her iki grup arasında sol gözde, nazal pRNFL bölge kalınlığı, optik disk alanı ve nöroretinal rim hacmi istatistiksel farklılık gösterdi (p<0,05). Ayrıca, optik sinir nöroretinal rim hacmi ve optik disk alanı ile başlatma, çalışma belleği, organizasyon, izleme, metakognitif indeks ve genel yönetici indeks gibi YİDDE alt ölçekleri arasında negatif yönlü bir ilişki saptandı.

Sonuç: Bu çalışma, DEHB tanısı almış çocuklarda optik sinir başı parametreleri ile yönetici işlevler arasında potansiyel bir ilişki olabileceğini göstermiştir. Optik sinir başı parametrelerinin DEHB'deki yönetici işlevlerle ilişkisini incelemek için performansa dayalı testler ve nörogörüntüleme cihazları (fonksiyonel manyetik rezonans görüntüleme) ile yapılan daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Optik sinir başı parametreleri, retina sinir lif tabakası, yönetici işlevler, DEHB

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by the symptoms of inattention, hyperactivity, and impulsivity that are not developmentally appropriate and negatively affect psychosocial functioning[1]. As the most common psychiatric disorder of childhood, the prevalence of ADHD worldwide varies between 1.7% and 17.8%, with recent epidemiological studies reporting a median prevalence of 4% in children and adolescents[2]. In a study conducted in Türkiye by Ercan et al.[1], the prevalence of ADHD was found to be 12.7%. Executive functions (EFs) encompass a range of cognitive skills necessary for the purposeful planning and execution of thoughts and behaviors, including directing attention, inhibiting inappropriate stimuli (inhibition), maintaining active information, and ensuring the transition among information[3]. Working memory, inhibition, and cognitive flexibility are core components of EFs[3]. In the existing literature, numerous studies have utilized different neuropsychological tests to asses EFs in children with ADHD[4]. Meta-analyses have consistently found weaker EF skills in children diagnosed with ADHD, including deficits in inhibition, sustained attention, working memory, and cognitive flexibility[4].

The retina originates from the cerebral cortex and embryological prosencephalon, containing unmyelinated axons and glial cells[5]. Due to these factors, it is believed that changes in the structure and function of the brain may be reflected in the retina[5]. Optical coherence tomography (OCT) is a non-invasive and non-contact imaging technique that provides cross-sectional images of retinal tissue[6]. OCT enables the measurement of peripapillary retinal nerve fiber layer (pRNFL), ganglion cell layer (GCL), and macular thickness, which can be used to monitor and assess treatment response in ophthalmic diseases such as glaucoma and macular disorders[7]. In recent years, studies have examined pRNFL, GCL, and macular thickness in certain psychiatric disorders such as bipolar disorder, ADHD[8-11], and autism spectrum disorder (ASD)[12], and have revealed differences. Additionally, OCT has been reported to detect chronic axonal degeneration and structural abnormalities in the central nervous system[13].

In neuroimaging studies on the etiology of ADHD, it has been observed that cases diagnosed with ADHD have atypical brain structure. In these cases, a decrease in total brain volume has been reported in the prefrontal cortex (PFC), basal ganglia, cerebellum, and parietotemporal areas[14]. However, in a meta-analysis study evaluating studies examining the retinal features (pRNFL and GCL) of cases diagnosed with ADHD, it was found that there was a decrease in global peripapillary RNFL thickness and thinning in the GCL layer[10]. Additionally, literature has shown a negative correlation between frontal and parietal cortical thickness and EFs in childhood and adolescence (healthy individuals)[15,16]. Although the relationship between cortical thickness and EFs is not clear, it has been suggested that this difference between childhood and adulthood may be related to central nervous system maturation[17]. When the literature is examined, it is seen that many studies, in which EFs such as response inhibition, working memory and visuospatial perception were evaluated with functional brain imaging techniques, have reported increased activation in the dorsolateral PFC and parietal cortex[18]. In a study conducted with 1485 healthy volunteers, evaluating the relationship between RNFL thickness and cognitive functions, it was shown that RNFL thickness was significantly higher in people with better neuropsychological test scores[19].

This study aimed to evaluate the relationship between EFs and optic nerve head (ONH) parameters (the disc area, the neuroretinal rim volume and cup-to-disc ratio) and pRNFL in medication-free school-age children diagnosed with ADHD.

MATERIALS AND METHOD

Sample

Our study included 30 children aged 8-12 years, diagnosed with ADHD according to DSM-V-TR, who sought treatment at the child and adolescent psychiatry outpatient clinic. Additionally, 30 healthy children without any psychiatric disorders were recruited. Additionally, individuals with any acute or chronic diseases related to neurology, genetics, endocrinology, infection, or the cardiovascular system were excluded. Additionally, those with known eye diseases

(especially glaucoma and ocular hypertension) and those using medication, except for ±2 spherical refractive error, were also excluded. Participants whose parents did not consent to participate were also not included in the study. The study received approval from Erzurum Regional Training and Research Hospital Ethics Committee (decision no: 2022/13-140, date: 05.09.2022). Prior to the study, written and verbal informed consent was obtained from the parents of all participants.

Procedure

Parents of all participants were asked to fill out a Sociodemographic Data form, the Behavior Rating Inventory of Executive Function Parent (BRIEF) form, and the Conners' Parent Rating Scale (CPRS) Short Form. A detailed eye examination was conducted on all 60 children participating in the study to exclude ophthalmological pathologies. Each participant underwent measurements of refractive errors using an autorefractometer, determination of best-corrected visual acuity with a Snellen chart, intraocular pressure measurement using Goldmann applanation tonometry, and a detailed ophthalmic examination including slit-lamp biomicroscopy and dilated fundus examination. Subsequently, non-invasive OCT measurements of both eyes were performed using the RTvue device (Optovue, CA, USA). Based on the study by Pitkänen et al.[20], only measurements of pRNFL thickness (superior, inferior, nasal, and temporal region thickness) and ONH parameters (the disc area, the neuroretinal rim volume, and cup-to-disc ratio) of the left eye were included in the evaluation for each participant.

Data Collection Tools

Sociodemographic Data Form: This form, prepared by the researchers, was designed and implemented to collect information about participants and family members (age, gender, delivery time, delivery type, developmental milestones (walking, talking, toilet training), family structure, and settlement).

Hollingshead-Redlich Scale: The Hollingshead-Redlich scale evaluates socioeconomic status (SES) by considering parental education and occupation. It categorizes SES into five levels, with the highest-achieving parent determining the score. Levels 1 and 2 denote high SES, Level 3 corresponds to middle SES, and Levels 4 and 5 indicate low SES[21].

Conners' Parent Rating Scale Short Form: The Conners' rating scale is the most widely used behavior rating scale to assess the characteristics of ADHD in children and adolescents with sub-scales for inattention, hyperactivity, and oppositional behavior. The scale has been adapted into Turkish by Kaner et al.[22] Parents were asked to rate their children's behaviors on a four-point Likert scale for each item. The scale is scored as never (0), rarely (1), often (2), and always (3)[22]. In our study, the ADHD index calculated from the scale utilized the Inattention and Hyperactivity subscales. The obtained high scores indicate the severity of ADHD symptoms.

Behavior Rating Inventory of Executive Function Parent Form: BRIEF was developed to assess EFs, problem-solving skills, and adaptive behaviors in children. This scale is filled out by an individual familiar with the child (parent, teacher) based on the child's behavior and attitudes over the last 6 months. The scale consists of a total of 86 items, and 8 subscale and 3 index scores related to a person's EFs can be calculated. The obtained high scores indicate weak executive skills. The Turkish standardization of the scale was conducted by Batan et al.[23]. The description of BRIEF Subscales is as follows[23]:

Inhibition: The capacity to regulate impulses and halt one's behavior at appropriate moments.

Shifting: The ability to transition flexibly between different situations, tasks, perspectives, or aspects of a problem.

Emotional Control: The skill of managing and regulating emotional responses.

Initiate: The ability to independently begin tasks or activities and generate ideas.

Working Memory: The capacity to hold and manipulate information in mind to complete tasks or respond appropriately.

Planning/Organizing: The ability to anticipate future events, set goals, create structured steps in advance, execute tasks systematically, and effectively communicate key ideas.

Organization of Materials: The ability to manage task-related demands while considering both present and future situational requirements.

Monitoring: The capacity to oversee work, assess performance, and track both one's own and others' efforts.

Behavioral Regulation Index (BRI): A composite score derived from the inhibition, shifting, and emotional control subscales.

Metacognition Index (MCI): A total score encompassing the initiate, working memory, planning/organizing, organization of materials, and monitoring subscales.

Global Executive Composite Index: Calculated as the sum of the BRI and MCI scores.

OCT Measurement and Device Specifications: Measurements were made using the RTvue device (Optovue, CA, USA) employing a non-invasive technique that provides tomographic sections of tissues at the micron level by measuring the reflection, delay time, and intensity of infrared light at approximately 840 nm wavelength sent to

tissues and reflected from different tissues. A detailed eye examination was conducted for each participant to exclude ophthalmological pathologies. During measurement, low-quality scans were rejected. All image qualities were controlled using the signal strength index (SSI). Only scans with SSI >50 were included in the study. For RNFL thickness measurements, a three-dimensional (3D) disc and a 4 mm diameter ONH map were used. pRNFL thickness measurements were obtained along 13 circular B-scans manually positioned over the optic disc to create a peripapillary RNFL thickness map. The pRNFL thickness measurement was calculated as the difference in distance between the inner limiting membrane and the outer edge of the inner plexiform layer within a circle with a diameter of 3.45 mm. The ONH scan provides a 3D view and is created using 12 radial B-scans with a fixed length of 3.7 mm for ONH shape analysis. In each scan series, average pRNFL thickness, quadrant pRNFL thickness (superior, inferior, temporal, and nasal), and optic disk volume and cup-disk areas with the cup-to-disk ratio were analyzed. Data were recorded with information about race, age, and gender. The device's recorded normative database outputs show color-coded normal distribution percentages among individuals of the same age. The lower 1% of normal measurements is shown in red, the lower 5% in yellow, the upper 5% in white, and the remaining 90% in green. Results were analyzed based on this information[6].

Statistical Analysis

The statistical analysis was performed using SPSS 23.0 statistical software package. Descriptive statistics of the evaluation results were presented as numbers and percentages for categorical variables and as mean, standard deviation, minimum, and maximum for numerical variables. The Kolmogorov-Smirnov test was used to assess the normal distribution conditions of the data. When the normal distribution condition was met for comparing numerical variables among three or more independent groups, the ANOVA test was used; otherwise, the Kruskal-Wallis test was used; otherwise, the Kruskal-Wallis test was used; otherwise, the Kruskal-Wallis Variance analysis was used. The Bonferroni test from post-hoc test statistics was employed to determine the source of significant differences among more than two groups. Spearman's rank correlation coefficient was used to analyze the relationship between numerical variables in two groups. The chi-square test was employed to analyze the differences in the proportions of categorical variables between independent groups. A significance level of $p<0.05$ was accepted for statistical significance.

RESULTS

A total of 60 children aged between 8 and 12 years participated in the study. The groups were comparable in terms of age, gender, years of education, and SES ($p>0.05$) (Table 1). Significant differences were observed in the BRIEF subscale,

Table 1. Sociodemographic characteristics of the participants, RNFL thicknesses, and ONH parameters

	Children with ADHD (mean ± SD)	Controls (mean ± SD)	p-value
Age (years)	9.63±1.93	9.23 ±1.35	0.68*
Gender (boy/girl)	22/8	16/14	0.108
Education (year)	4.37±1.75	4.85± 2.71	0.887*
Hollingshead-Redich scale (mean ± SD)	3.47±1.22	3.76 ± 1.1	0.197*
CPRS			
Inattention	9.8±2.7	1.4±1.3	**<0.001***
Hyperactivity	10.3±7.22	1.16.±1.2	**<0.001***
ADHD index	14.7±3.13	3.06±1.8	**<0.001***
pRNFL			
pRNFL superior (µm) L	121.32±9.90	125.41±17.29	0.285*
pRNFL inferior (µm) L	122.93±11.52	125.67±13.41	0.424**
pRNFL temporal (µm) L	75.25±8.92	77.9±11.03	0.427**
pRNFL nasal (µm) L	71.82±6.41	75.59±7.07	**0.043***
Optic nerve head parameters			
Optic disc area (mm^2) L	2.04±0.50	2.27±0.36	**0.048***
Optic neuroretinal rim volume (mm^3) L	1.51±0.31	1.84±0.39	**0.001****
Optic cup-to-disc ratio L	0.24±0.16	0.19±0.11	0.245**

ADHD: Attention deficit hyperactivity disorder, p: Probability of significance, $p<0.05$, µm: Micrometers (data in the table presented in units of), pRNFL: Peripapillary retinal nerve fiber layer, L: Left eye, SD: Standard deviation, CPRS: Conners' parent rating scale, *Kruskal-Wallis, **ANOVA, ONH: Optic nerve head

which assesses EFs, between children diagnosed with ADHD and the control group (p<0.05) (Table 2). When examining pRNFL and ONH parameters between the groups, differences were observed in the pRNFL nasal region thickness in the left eye (p=0.043), the disc area belonging to the ONH (p=0.048), and the neuroretinal rim volume (p=0.01) (Table 1). Both optic neuroretinal rim volume and disc area showed a negative correlation with the sub-scores of the BRIEF scale, including initiation, working memory, plan/organize, organization of materials, monitoring, metacognitive index, and global executive composite index scores (Table 3). However, there was no relationship found between the pRNFL thickness (nasal) in the left eye and BRIEF subscales. Additionally, the ADHD index score calculated from the CPRS scale exhibited a negative correlation with both optic neuroretinal rim volume and optic disc area (Table 3).

DISCUSSION

In recent years, studies investigating RNFL and optic nerve parameters using OCT in psychiatric disorders have started to increase. In the study, children diagnosed with ADHD exhibited significant differences in pRNFL nasal region thickness, optic disc area, and neuroretinal rim volume compared to the control group. Additionally, optic disc area and neuroretinal rim volume were found to be associated with BRIEF subscales in the ADHD group.

In the study, it was observed that both the optic disc area and the neuroretinal rim volume were thinner in the ADHD-diagnosed group (p<0.05). Furthermore, these parameters of the left eye's ONH were found to be associated with both EFs, including initiation, working memory, plan/organize, organization of materials, monitoring, metacognition index and global executive composite scores, and also the ADHD index score. In other words, it was found that children diagnosed with ADHD who had a larger optic disc area and neuroretinal rim volume exhibited stronger EFs skills and less severe ADHD symptoms. Studies in the literature that examine the relationship between ocular findings and cognitive impairment have reported controversial results. In a cohort study, Pitkänen et al.[20] found that individuals with a larger optic disc area had a higher level of education, completed the Humphrey 24-2 perimetric test (HFA) in a less time, but performed worse on the grade point average (GPA) test. In the same study, greater neuroretinal volume was detected to be associated with higher GPA, shorter HFA completion time, and fewer errors in the paired associates learning test. Similarly, in the Beijing Eye Study, the average age of the population was 56.2±10.6 years, and it was emphasized that optic disc head size was associated with education level and perimetric test duration time. The author noted that education level was significantly associated with increasing disc size and shorter perimetric test duration[24]. Another study found that a larger optic cup-to-disc ratio in females was independently associated with poorer cognitive abilities[25]. The data obtained in our study are consistent with the findings of Pitkänen et al.[20] and Jonas et al.[24]. This interpretation suggests that an increase in both optic disc size and neuroretinal rim volume may be associated with stronger EFs skills and fewer symptoms in children diagnosed with ADHD. Since existing studies in the literature have primarily been conducted on adult populations, there is a need for more OCT studies with larger sample sizes in children with ADHD, assessing EF skills using diverse methodologies.

ADHD has been linked to dysfunctions in somatomotor and visual networks, alongside impairments in higher-level

Table 2. Comparison of behavior rating inventory of executive function, of children with ADHD and controls

	Children with ADHD (mean ± SD)	Controls (mean ± SD)	p-value
BRIEF			
Inhibition	24.40±14.15	16.43±7.6	**0.009***
Shifting	15.6±4.17	13.6±1.74	**0.005***
Emotional control	21.52±5.08	16.35±4.23	**<0.001***
Initiate	15.83±3.04	11.85±3.30	**<0.001***
Working memory	24.35±3.40	17.08±4.68	**<0.001***
Plan/organize	33.09±7.53	21.69±6.57	**<0.001***
Organization of materials	16.39±4.35	11.73±3.36	**<0.001***
Monitoring	18.35±3.71	12.23±3.69	**<0.001***
Behavioral Regulation index	73.26±14.76	52.12±11.38	**<0.001***
Metacognition index	91.61±15.30	62.85±16.90	**<0.001***
Global executive composite	164.87±27.19	114.96±26.91	**<0.001***

p: Probability of significance, p<0.05, ADHD: Attention deficit hyperactivity disorder, BRIEF: Behavior Rating Inventory of Executive Function, SD: Standard deviation, *Independent sample t test

Table 3. Correlation between properties of ONH, pRNFL (nasal) and the BRIEF scale

	L optic neuroretinal rim volume	L optic disc area	L optic pRNFL nasal (µm) L
BRIEF			
Inhibition	p=0.588 r=-0.075	p=0.010 r=-0.344*	p=0.041 r=-0.276
Shifting	p=0.072 r=-0.271	p=0.028 r=-0.328*	p=0.827 r=-0.034
Emotional control	p=0.242 r=-0.178	p=0.133 r=-0.228	p= 0.371 r=-0.137
Initiation	**p=0.003 r=-0.436****	**p<0.001 r=-0.543****	p= 0.327 r=-0.149
Working memory	**p=0.005 r=-0.415****	**p=0.005 r=-0.408****	p=0.129 r=-0.230
Plan/organize	**p=0.01 r=-0.378***	**p=0.013 r=-0.366***	p=0.264 r=-0.170
Organization of materials	**p<0.001 r=-0.478****	**p<0.001 r=-0.500****	p=0.125 r=-0.232
Monitoring	**p=0.02 r=-0.442****	**p=0.003 r=-0.443****	p=0.094 r=-0.253
Behavioral Regulation index	p=0.24 r=-0.336	p=0.016 r=-0.358*	p=0.122 r=-0.234
Metacognition index	**p=0.003 r=-0.435****	**p=0.002 r=-0.445****	p=0.165 r=-0.211
Global executive composite	**p=0.005 r=-0.409****	**p=0.004 r=-0.425****	p=0.128 r=-0.230
CPRS			
Inattention	**p=0.003 r=-0.428****	p=0.016 r=-0.350*	p=0.297 r=-0.157
Hyperactivity	**p=0.011 r=-0.370***	p=0.102 r=-0.241	p=0.576 r=-0.085
ADHD index	**p=0.001 r=-0.459****	**p=0.009 r=-0.376****	p=0.220 r=-0.184

ADHD: Attention deficit hyperactivity disorder, CPRS: Conners' Parent Rating scale, pRNFL: Peripapillary retinal nerve fiber layer, L: Left eye, SD: Standard deviation, ONH: Optic nerve head, p: Probability of significance, p<0.05, BRIEF: Behavior Rating Inventory of Executive Function Parent

cognitive and behavioral functions. Neurotransmitters such as dopamine, glutamate, and GABA play a crucial role not only in retinal function but also in post-ocular structures like the thalamus and visual cortex[26]. Research has shown that ADHD is associated with decreased GABA levels and increased glutamate levels in the PFC and striatum, alongside disruptions in dopaminergic signaling[27]. Dopamine is known to support retinal function in multiple ways, whereas glutamate, when present in excess, acts as a neurotoxin that damages retinal GCL. Notably, studies have found a significant reduction in RNFL thickness in parkinson's disease, where dopamine levels are diminished[28]. Similarly, retinal layer thinning has been observed in adults with restless legs syndrome, a condition frequently comorbid with ADHD and sharing overlapping pathophysiological mechanisms with parkinson's disease[26]. Some studies indicate that pRNFL thickness does not show a significant difference in children diagnosed with ADHD compared to the control group[8,29,30], while others have observed thinning in pRNFL nasal quadrant thickness in individuals with ADHD[9,31]. In a study on ASD, the author interpreted the differences in pRNFL thinning in children diagnosed with ASD as an indicator of atypical brain development[12]. In our study, consistent with the findings of Hergüner et al.[9] and Kaymak et al.[31], a significant reduction in the nasal quadrant thickness of pRNFL was observed in children diagnosed with ADHD (p<0.05). However, no significant association was observed between this difference and the ADHD index score. When evaluating pRNFL and ONH findings together, this difference in ONH structure may be attributed to variations in axonal volume, deficiencies in dopaminergic function, or structural abnormalities in glial cells among children with ADHD. However, to better elucidate the underlying mechanisms of these alterations, further studies with larger sample sizes incorporating neuroimaging techniques and biochemical markers related to dopamine function are warranted.

In recent years, studies conducted on adult samples have found a relationship between pRNFL thickness and cognitive function[19,32]. Additionally, one of these studies has emphasized that macular GCL thickness is more strongly linked to cognitive functions than pRNFL thickness[32]. This relationship observed in the healthy population has also been evaluated in neurodegenerative diseases such as parkinson's disease and multiple sclerosis[33,34]. Thinning in pRNFL inferior quadrant thickness has been specifically associated with cognitive impairment in parkinson's patients[33]. In multiple sclerosis patients, changes in both pRNFL and GCL inner plexiform layer may be associated with cognitive impairment, suggesting a connection to neurodegeneration[34]. Another study investigated the relationship between EF skills, also known as advanced cognitive skills, and pRNFL in adults diagnosed with ADHD, like our study, and found no relationship between pRNFL and EFs[31]. The varied results in studies investigating the relationship between pRNFL and cognitive function in the literature may arise from differences in the scales used in the studies, the variability in age groups and the differences in the etiology of neurodegenerative diseases and neurodevelopmental disorders. To investigate the relationship between pRNFL and cognitive function, studies with larger samples in a similar age group within neurodevelopmental disorders are needed.

Study Limitations

The present study has some limitations. One is its small sample size. The other is that the scale used to assess EFs in our study relies on parental feedback. Further studies with large samples are needed using performance-based tests and neuroimaging devices such as functional magnetic resonance imaging.

CONCLUSION

In conclusion, our study is important as the first investigation of EFs and OCT parameters in school-age children diagnosed with ADHD. In school-age children with ADHD in our study, ONH parameters, specifically were found to be smaller and associated with poorer EF skills. On the other hand, pRNFL was only found to be thinner in the nasal quadrant in children with ADHD, and no relationship was found with EF skills. In the etiopathogenesis of ADHD, which is a neurodevelopmental disorder, changes in optic nerve parameters such as optic disc area and neuroretinal rim volume rather than pRNFL may suggest the indicators of atypical brain development. Therefore, we hope that our study will provide clinicians with a different perspective and serve as a guide in this regard.

Ethics

Ethics Committee Approval: The study received approval from Erzurum Regional Training and Research Hospital Ethics Committee (decision no: 2022/13-140, date: 05.09.2022).

Informed Consent: Prior to the study, written and verbal informed consent was obtained from the parents of all participants.

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Footnotes

Authorship Contributions

Concept: G.Y.T., B.S.Ö., G.Ö., B.U., B.Ş., Design: G.Y.T., B.S.Ö., G.Ö., B.Ş., Data Collection or Processing: G.Y.T., B.U., B.Ş., Analysis or Interpretation: G.Y.T., B.S.Ö., G.Ö., A.Ç., B.U., B.Ş. Literature Search: G.Y.T., B.S.Ö., G.Ö., AB.U., B.Ş., Writing: G.Y.T., B.S.Ö., B.Ş.

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REFERENCES

1. Ercan ES, Bilaç Ö, Uysal Özaslan T, Rohde LA. Is the prevalence of ADHD in Turkish elementary school children really high? Soc Psychiatry Psychiatr Epidemiol. 2015;50:1145-52.
2. Merikangas KR, Nakamura EF, Kessler RC. Epidemiology of mental disorders in children and adolescents. Dialogues Clin Neurosci. 2009;11:7-20.
3. Diamond A. Executive functions. Annu Rev Psychol. 2013;64:135-68.
4. Willcutt EG, Doyle AE, Nigg JT, Faraone SV, Pennington BF. Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. Biol Psychiatry. 2005;57:1336-46.
5. London A, Benhar I, Schwartz M. The retina as a window to the brain—from eye research to CNS disorders. Nat Rev Neurol. 2013;9:44-53.
6. Iverson SM, Sehi M. The comparison of manual vs automated disc margin delineation using spectral-domain optical coherence tomography. Eye. 2013;27:1180-7.
7. Jindahra P, Hedges TR, Mendoza-Santiesteban CE, Plant GT. Optical coherence tomography of the retina: applications in neurology. Curr Opin Neurol. 2010;23:16-23.
8. Işik Ü, Kaygisiz M. Assessment of intraocular pressure, macular thickness, retinal nerve fiber layer, and ganglion cell layer thicknesses: ocular parameters and optical coherence tomography findings in attention-deficit/hyperactivity disorder. Braz J Psychiatry. 2020;42:309-13.
9. Hergüner A, Alpfidan İ, Yar A, Erdoğan E, Metin Ö, Sakarya Y, et al. Retinal nerve fiber layer thickness in children with ADHD. J Atten Disord. 2018;22:619-26.
10. Li SL, Kam KW, Chee ASH, Zhang XJ, Chen LJ, Yip WWK, et al. The association between attention-deficit/hyperactivity disorder and retinal nerve fiber/ganglion cell layer thickness measured by optical coherence tomography: a systematic review and meta-analysis. Int Ophthalmol. 2021;41:3211-21.
11. Tonkaz GY, Özyurt G, Çakir A, Turan B, Utlu B, Özbay AD. Evaluation of retinal nerve fiber layer, ganglion cell thickness, and macular thickness in children with comorbid specific learning disorder and attention-deficit hyperactivity disorder. J Pediatr Ophthalmol Strabismus. 2024;61:128-36.
12. Bozkurt A, Say GN, Şahin B, Usta MB, Kalyoncu M, Koçak N, et al. Evaluation of retinal nerve fiber layer thickness in children with autism spectrum disorders. Research in Autism Spectrum Disorders. 2022;98:102050.
13. Galetta KM, Calabresi PA, Frohman EM, Balcer LJ. Optical coherence tomography (OCT): imaging the visual pathway as a model for neurodegeneration. Neurotherapeutics. 2011;8:117-32.
14. Valera EM, Faraone SV, Murray KE, Seidman LJ. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biological psychiatry. 2007;61:1361-9.
15. Tamnes CK, Østby Y, Walhovd KB, Westlye LT, Due-Tønnessen P, Fjell AM. Neuroanatomical correlates of executive functions in children and adolescents: a magnetic resonance imaging study of cortical thickness. Neuropsychologia. 2010;48:2496-508.
16. Burzynska AZ, Nagel IE, Preuschhof C, Gluth S, Bäckman L, Li SC, et al. Cortical thickness is linked to executive functioning in adulthood and aging. Hum Brain Mapp. 2012;33:1607-20.
17. Vaidya CJ. Neurodevelopmental abnormalities in ADHD. Curr Top Behav Neurosci. 2012;9:49-66.
18. Mostofsky SH, Simmonds DJ. Response inhibition and response selection: two sides of the same coin. J Cogn Neurosci. 2008;20:751-61.
19. van Koolwijk LM, Despriet DD, Van Duijn CM, Oostra BA, van Swieten JC, de Koning I, et al. Association of cognitive functioning with retinal nerve fiber layer thickness. Invest Ophthalmol Vis Sci. 2009;50:4576-80.
20. Pitkänen J, Veijola J, Barnett J, Liinamaa J, Saarela V. Optic Nerve Parameters and Cognitive Function in the Northern Finland Birth Cohort Eye Study. Ophthalmic Epidemiol. 2022;29:189-97.
21. Hollingshead, AB. Four factor index of social status. Yale University, Department of Sociology. 1975.
22. Kaner S, Büyüköztürk S, İşeri E. Conners' Parent rating scale-revised short: turkish standardization study. Noro-Psikyatri Arsivi. 2013;50:100-9.
23. Batan SN, Öktem-Tanör Ö, Kalem E. Reliability and validity studies of Behavioral Rating Inventory Of Executive Function (BRIEF) in a Turkish normative sample. Elementary Education Online. 2011;10:728-40.

24. Jonas JB, Wang YX, Li JJ, Xu L. Optic nerve head size and cognitive function. Acta Ophthalmol. 2013;91:83-4.

25. Kravets S, Rupnow RA, Sethi A, Espeland MA, Pasquale LR, Rapp SR, et al. Association between cognitive function and large optic nerve cupping, accounting for cup-disc-ratio genetic risk score. Plos one. 2022;17:258564.

26. Tünel M, Keşkek NŞ. Retinal scan with optical coherence tomography in adult attention deficit hyperactivity disorder. Turk Psikiyatri Derg. 2021;32:176-82

27. Edden RA, Crocetti D, Zhu H, Gilbert DL, Mostofsky SH. Reduced GABA concentration in attention-deficit/hyperactivity disorder. Arch Gen Psychiatry. 2012;69:750-3.

28. Inzelberg R, Ramirez JA, Nisipeanu P, Ophir A. Retinal nerve fiber layer thinning in Parkinson disease. Vision Res. 2004;44:2793-7.

29. Bodur Ş, Kara H, Açıkel B, Yaşar E. Evaluation of the ganglion cell layer thickness in children with attention deficit hyperactivity disorder and comorbid oppositional defiant disorder. Turkish J Clinical Psychiatry. 2018;21:222-30.

30. Ayyildiz T, Ayyildiz D. Retinal nerve fiber layer, macular thickness and anterior segment measurements in attention deficit and hyperactivity disorder. Psychiatry and Clinical Psychopharmacology. 2019;29:760-4.

31. Kaymak D, Gündoğmuş İ, Dalkıran M, Küçükevcilioğlu M, Uzun Ö. Retinal nerve fiber layer thickness and its relationship with executive functions in adult attention deficit hyperactivity disorder patients. Psychiatry Investig. 2021;18:1171-9.

32. Ward DD, Mauschitz MM, Bönniger MM, Merten N, Finger RP, Breteler MMB. Association of retinal layer measurements and adult cognitive function: a population-based study. Neurology. 2020;95:1144-52.

33. Chang Z, Xie F, Li H, Yuan F, Zeng L, Shi L, et al. Retinal nerve fiber layer thickness and associations with cognitive impairment in Parkinson's disease. Front Aging Neurosci. 2022;14:832768.

34. Dreyer-Alster S, Gal A, Achiron A. Achiron, Optical coherence tomography is associated with cognitive impairment in multiple sclerosis. J Neuroophthalmol. 2022;42:14-21.

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Survival Impact of Adjuvant Chemoradiotherapy in Resected Pancreatic Cancer

Opere Pankreas Kanserinde Adjuvan Kemoradyoterapinin Sağkalım Etkisi

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ABSTRACT

Aim: Pancreatic cancer (PC) is one of the most aggressive cancers, with a 5-year survival rate of less than 50% in resectable stages. The aim of this study was to determine the survival effect of adjuvant chemoradiotherapy (CRT) in resected PC.

Materials and Methods: We retrospectively analyzed 156 patients with resected PC, who received adjuvant chemotherapy with/without CRT. The Cox regression and Kaplan-Meier analyses were used to determine the factors related to survival rate. Subgroup analyses were performed according to clinical characteristics.

Results: The number of patients with lymph node metastases was statistically significantly higher in patients receiving CRT (p=0.007). No effect of CRT on both disease-free survival (DFS) and overall survival was detected (p>0.05). Subgroup analysis of DFS showed that adjuvant CRT was associated with poor prognosis in patients with in patients with in patients with low Eastern Collaborative Oncology Group performance scores (p=0.043), low T-stage (p=0.024), or low De-Ritis ratio (p=0.030). Subgroup analysis indicated that the overall survival benefit of adjuvant CRT was more significant in patients without diabetes mellitus (p=0.040), low serum carbohydrate antigen 19-9 levels (p=0.047), or low hemoglobin values (p=0.046).

Conclusion: Our study has shown that the survival benefits of adding CRT to adjuvant chemotherapy in operated PC patients are limited. Patients who will receive CRT must be carefully selected according to their clinicopathological characteristics.

Keywords: Pancreatic cancer, adjuvant therapy, chemoradiotherapy, radiotherapy, survival

ÖZ

Amaç: Pankreas kanseri (PK), rezeke edilebilir evrelerde 5 yıllık sağkalım oranı %50'den az olan en agresif kanserlerden biridir. Bu çalışmanın amacı rezeke edilmiş PK'de adjuvan kemoradyoterapinin (CRT) sağkalım etkisini belirlemektir.

Gereç ve Yöntem: Opere edilmiş PK'li ve adjuvan kemoterapi alan 156 hastayı retrospektif olarak analiz ettik. Sağkalım oranıyla ilişkili faktörleri belirlemek için Cox regresyon ve Kaplan-Meier analizleri kullanıldı. Alt grup analizleri klinik özelliklere göre yapıldı.

Bulgular: Lenf nodu metastazı olan hasta sayısı, CRT alan hastalarda istatistiksel olarak anlamlı derecede daha yüksekti (p=0,007). CRT'nin hem hastalıksız sağkalım (DFS) hem de genel sağkalım üzerinde bir etkisi saptanmadı (p>0,05). DFS'nin alt grup analizi, adjuvan CRT'nin düşük Doğu İşbirliği Onkoloji Grubu performans skorları (p=0,043) veya düşük T-evresi (p=0,024) veya düşük De-ritis oranı (p=0,030) olan hastalarda kötü prognozla ilişkili olduğunu gösterdi. Alt grup analizi, adjuvan CRT'nin genel sağkalım faydasının non-diabetes mellitus (p=0,040) veya düşük serum

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karbohidrat antijeni 19-9 seviyeleri (p=0,047) veya düşük hemoglobin değerleri (p=0,046) olan hastalarda daha anlamlı olduğunu gösterdi.

Sonuç: Çalışmamız, ameliyat edilen PK hastalarında adjuvan kemoterapiye CRT eklemenin sağkalım faydalarının sınırlı olduğunu gösterdi. CRT alacak hastalar klinikopatolojik özelliklerine göre dikkatlice seçilmelidir.

Anahtar Kelimeler: Pankreas kanseri, adjuvan tedavi, kemoradyoterapi, radyoterapi, sağkalım

INTRODUCTION

Pancreatic cancer (PC) is the twelfth most common cancer in the world and one of the leading causes of cancer deaths. PC exhibits a lethal disease course and is expected to become the second most common cause of cancer-related deaths in the next decades despite improved treatment strategies[1,2]. While its overall survival (OS) is 2.6%, it is responsible for 4.7% of cancer-related deaths, and the expected 5-year OS in the advanced stage is 3%[3]. In the operable stages, that is to have been potentially curable, the 5-year survival is less than 50%, even if they have received all planned treatments[2].

Surgery, chemotherapy, and radiotherapy (RT) (with/without chemotherapy) are substantial treatment options in the early or locally advanced stages of PC. The benefit of adjuvant chemotherapy after surgery in localized patients is clear and there is no controversy in this setting on guidelines[4-6]. Contrary to strong recommendations for chemotherapy, the importance of RT is unclear. In the light of studies and guidelines in the literature, RT is either included as a poor recommendation next to chemotherapy or is not recommended[4,7,8]. In addition, it is unclear whether RT provides a survival benefit or is a poor prognostic factor for survival.

In this study, we investigated the effect of adjuvant chemoradiotherapy (CRT) on survival by performing a comprehensive prognostic factor analysis in operated PC patients receiving adjuvant chemotherapy. In this way, we aimed to identify the patient populations that could benefit from adjuvant RT and to determine the most appropriate prognostic factors to guide cancer treatment professionals in clinical practice.

MATERIALS AND METHODS

Study Population

This study was planned as a multicenter-retrospective study and conducted in accordance with the Declaration of Helsinki. Approval from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (TNKU) Faculty of Medicine (decision no: 2022.159.09.06, date: 27.09.2022). Data were collected from participating medical oncology clinics. Patients aged >18 years who underwent surgery for PC and received adjuvant chemotherapy between March 2016 and June 2022 were included. Patients with metastatic disease, concomitant or previous malignancy, positive margins, having received neoadjuvant therapy, or patients who did not complete the planned adjuvant chemotherapy were excluded from the study.

All patients who received RT were given treatment as CRT (with capecitabine/5-fluorouracil). As a RT protocol in the included centers, patients generally receive RT (3-dimensional conformal RT or intensity-modulated RT) for 5 days a week (28 fractions) for approximately 5.5 weeks within 7-21 days after the completion of chemotherapy. During RT, patients receive either capecitabine twice a day for 5 days a week or 5-fluorouracil continuously for 5.5 weeks or until RT is completed.

Data Collection

Three centers with oncology expertise were included in the study. Patients' demographics, clinicopathologic data, and serum laboratory parameters measured prior to initial chemotherapy were documented. Prognostic nutritional index (PNI), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and De Ritis (aspartate transaminase-to-alanine transaminase ratio) were measured and recorded from laboratory data. The calculation formula for the globulin was total protein-serum albumin, and that for PNI was 10 × serum albumin (g/dL) + 0.005 × lymphocyte count (per mm^3).

Statistical Analysis

Statistical analyses were performed using SPSS Statistic software 24 (SPSS Inc., Chicago, III). The ROC curve and area under the curve were used as optimal cut-off values for laboratory parameters and indices, but the optimal cut-off could not be determined. Therefore, median values were accepted as cut-off values. For the analysis of categorical variables, the chi-square test or Fisher's exact test were utilized. These cut-offs were used to differentiate the two groups as "low" and "high." Firstly, univariate and multivariate analyses of factors affecting OS and Progression-free Survival were performed with Cox Proportional Hazards Model. Secondly, two separate groups were formed with and without CRT, and potential prognostic factors were investigated by subgroup analysis using univariate Cox analysis. Hazard ratio (HR) was reported with the corresponding 95% confidence intervals (CI)

(95% CI). Thirdly, the factors that were found to be statistically significant in the subgroup analysis were compared with the Kaplan Meier and Log-Rank tests. The OS was calculated as the time from randomization to all-cause death or the last follow-up date used for censoring. Disease-free survival (DFS) was considered as time to relapse or all-cause death, whichever came first. Statistical significance was accepted as $p<0.05$.

RESULTS

Patient Characteristics

A total of 1128 patients were examined. Eight hundred forty-nine patients were found to be in the metastatic stage, and 123 patients had no laboratory data before the first treatment. These patients were excluded, and the study was completed with 156 patients. The median age was 62 years (range: 35-82 years). One hundred (64.1%) of the patients were male. Of all study patients, 114 (73.1%) relapsed and 104 (66.7%) died of cancer-related causes. The median follow-up time among all studies was 73.0 months (range: 63.7-82.3). The median DFS was 20.6 months (95% CI: 16.6-24.6) in all patients, 17 months (95% CI: 13.2-20.9) in those receiving adjuvant CRT and 23.3 months (95% CI: 18.9-27.7) in those not receiving CRT. The median OS was 80 months (95% CI: 70.5-89.5) in all patients, 101.3 months (95% CI: 56.4-146.2) in those receiving CRT and 77.5 months (95% CI: 65.3-89.7) in those not receiving CRT.

Demographic and clinicopathologic characteristics of patients who received CRT and those who did not receive RT were similar. The number of patients with lymph node metastases was statistically significantly higher in patients who received only CRT (p= 0.007). The general characteristics and laboratory data of the patients, compared according to the CRT groups, are shown in Table 1.

Survival Analysis

For survival analysis, the median values of 2.92 for carcinoembryonic antigen, 39.94 for carbohydrate antigen 19-9 (CA 19-9) 12.1 for hemoglobin, 0.75 for total bilirubin, 2.63 for NLR, 155.12 for PLR, 1.09 for De-Ritis, and 48.9 for PNI were accepted as cut-off. In the Cox regression analysis, low body mass index (HR=1.50, 95% CI: 1.0-2.23, p=0.047), higher T-stage (HR=1.55, 95% CI: 1.06-2.27, p=0. 026), lymph node metastasis (HR=1.67, 95% CI: 1.13-2.47, p=0.010) and higher De-ritis (HR=1.50, 95% CI: 1.03-2.17, p=0.032) were associated with lower DFS. Diabetes mellitus (DM) (HR=2.24, 95% CI: 1.22-4.12, p=0.009), tumor localization outside the pancreatic head (HR=1.91, 95% CI: 1.08-3.37, p=0.026) and high CA 19-9 level (HR=1.99, 95% CI: 1.09-3.64, p=0.025) were found to be poorly prognostic for OS. Adjuvant CRT had No survival effect for both DFS and OS (p=0.490, p=0.090, respectively) (Table 2). In established multivariate models, lymph node status (HR=1.67, 95% CI: 1.13-2.47, p=0.010) for DFS and DM (HR=3.06, 95% CI: 1.61-5.81, p=0.001) and tumor localization (HR=2.37, 95% CI: 1.31-4.30, p=0.004) for OS were found to be independent prognostic factors.

Association of Adjuvant CRT with Survival, Subgroup Analysis

Subgroup analyses were performed to identify groups that could benefit or suffer from CRT. No subgroup that adjuvant CRT contributed positively to DFS was found. Adjuvant CRT was associated with worse survival in good ECOG performance status (HR=1.65, 95% CI: 1.02-2.69, p=0.043), low pathologic T (pT) (HR=2.05, 95% CI: 1.10-3.82, p=0.024) and low De-ritis ratio (HR=1.85, 95% CI: 1.06-3.24, p=0.030) (Table 3). Survival curves were made by the Kaplan-Meier analysis. The corresponding mDFS values according to ECOG performance scores and pT stage were 17.0 (95% CI: 12.3-21.8) versus 29.2 months (95% CI: 18.0-40.5) (log rank p=0.040), 17.0 months (95% CI: 7.7-26.3) versus 52.8 months (95% CI: 23.4-82.2) (log rank p=0.021), and 16.8 (95% CI: 12.4-21.2) versus 23.5 months (95% CI: 16.2-30.8) (log rank p=0.046), respectively, with significant difference (Figure 1).

The Cox regression analysis found that adjuvant CRT also did not provide survival for OS. In subgroup analysis, CRT provided longer OS in those with non-DM (HR=0.46, 95% CI: 0.22-0.97, p=0.040), low CA 19-9 levels before treatment (HR=0.42, 95% CI: 0.18-0.99, p=0.047) and low hemoglobin values (HR=0.35, 95% CI: 0.13-0.98, p=0.046) (Table 3). Survival curves were made by the Kaplan-Meier analysis. The corresponding mOS values according to without-DM, low CA 19-9, and low hemoglobin values were 139 (95% CI: 48.4-229.7) versus 80 months (95% CI: 75.6-84.5) (log rank p=0.045), 139 months (95% CI: 97.9-180.2) versus 80.1 months (95% CI: 66.4-94.8) (log rank p=0.042), and 101.3 (95% CI: 58.3-144.3) versus 69.9 months (95% CI: 54.8-85) (log rank p=0.037), respectively, with significant difference (Figure 1).

DISCUSSION

In this study, we addressed the relationship between adjuvant CRT and survival in PC patients who underwent surgery and received adjuvant chemotherapy. Our study found that adjuvant CRT had no impact on both DFS and OS. In subgroup analyses, CRT was associated with worse survival times with good ECOG performance, low pT stage and low De-ritis rate. CRT was associated with longer OS times in without-DM, low CA 19-9 values and low hemoglobin values. Significant survival benefits have been achieved in operable PC with adjuvant chemotherapy[9,10]. However, chemotherapy alone does

not provide the desired contribution to survival, indicating the need for new treatment methods. Among the studies on adjuvant CRT, although the Gastrointestinal Tumor Study Group study conducted in 1974 was superior to RT alone, the EORTC-3 study with a similar design revealed that CRT was not superior to the observation arm[11,12]. A large patient analysis conducted by 2013 reported that chemotherapy followed by CRT was not favorable in prolonging survival.

Table 1. Demographic and clinicopathological features of patients regarding chemoradiotherapy and chemotherapy alone

	Total	Non-CRT	CRT	
	n (%)	n (%)	n (%)	p-value
Clinicopathological characteristics	156 (100)	101 (64.7)	55 (35.3)	
Age (year)				
<65	96 (61.5)	60 (62.5)	36 (37.5)	0.458
≥65	60 (38.5)	41 (68.3)	19 (31.7)	
ECOG-PS				
<2	95 (60.9)	56 (58.9)	39 (41.1)	0.059
≥2	61 (29.1)	45 (73.8)	16 (26.2)	
BMI				
<25	110 (70.5)	69 (62.7)	41 (37.3)	0.415
≥25	46 (29.5)	32 (69.6)	14 (30.4)	
Sex				
Male	101 (64.7)	69 (38.3)	32 (31.7)	0.206
Female	55 (35.3)	32 (58.2)	23 (41.8)	
Smoking history				
Yes	97 (62.2)	58 (59.8)	39 (40.2)	0.097
No	59 (37.8)	43 (72.9)	16 (27.1)	
Alcohol history				
Yes	30 (19.2)	23 (76.7)	7 (23.3)	0.128
No	126 (80.8)	78 (61.9)	48 (38.1)	
DM history				
Yes	53 (34)	36 (67.9)	17 (32.1)	0.551
No	103 (66)	65 (63.1)	38 (36.9)	
Primary site				
Head	102 (65.4)	63 (61.8)	39 (38.2)	0.284
Other	54 (34.5)	38 (70.4)	16 (29.6)	
pT				
<3	64 (41)	43 (67.2)	21 (32.8)	0.594
≥3	92 (59)	58 (63)	34 (37)	
Lymph node status				
Negative	59 (37.8)	46 (78)	13 (22)	**0.007**
Positive	97 (62.2)	55 (56.7)	42 (43.3)	
Histologic type				
Ductal adeno	143 (91.7)	91 (63.6)	52 (36.4)	0.545
Others	13 (8.3)	10 (76.9)	3 (23.1)	
Histologic grade				
Grade 1-2	133 (85.3)	85 (63.6)	48 (36.4)	0.600
Grade 3	23 (14.7)	16 (69.6)	7 (30.4)	
Chemotherapy regimes				
Gemcitabine based	97 (62.2)	60 (61.9)	37 (38.1)	0.333
İrinotekanlı/irinotekansız oksaliplatin	59 (37.8)	41 (69.5)	18 (30.5)	

CRT: Chemoradiotherapy, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, DM: Diabetes mellitus, pT: Pathologic T, BMI: Body mass index

Table 2. Univariate analyses of factors for disease-free survival and overall survival

		DFS		OS	
Clinicopathological Characteristics	**Category**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p-value**
Age (year)	<65/≥65	1.14 (0.78-1.68)	0.497	1.15 (0.65-2.03)	0.635
Gender	Female/male	0.97 (0.66-1.41)	0.845	0.94 (0.53-1.68)	0.839
BMI	<25/≥25	1.50 (1.0-2.23)	0.047	1.71 (0.89-3.31)	0.110
ECOG	0-1/≥2	1.38 (0.95-2.01)	0.090	1.30 (0.72-2.35)	0.382
Smoking history	No/Yes	0.99 (0.68-1.45)	0.977	1.03 (0.58-1.84)	0.910
Alcohol history	No/Yes	1.07 (0.66-1.74)	0.778	1.36 (0.67-2.75)	0.393
DM history	No/Yes	1.07 (0.73-1.59)	0.724	2.24 (1.22-4.12)	**0.009**
Primary site	Head/Others	0.72 (0.48-1.07)	0.107	1.91 (1.08-3.37)	**0.026**
pT stage	<3/≥3	1.55 (1.06-2.27)	**0.026**	0.78 (0.45-1.36)	0.387
Lymph node status	Negative/Positive	1.67 (1.13-2.47)	**0.010**	1.69 (0.93-3.07)	0.085
Histologic type	Ductal Adeno/Others	0.78 (0.40-1.55)	0.479	1.11 (0.47-2.65)	0.810
Histologic grade	1/2-3	1.33 (0.83-2.14)	0.236	1.08 (0.55-2.14)	0.822
Chemotherapy regimes	Gemcitabine/Oxaliplatin	1.03 (0.69-1.55)	0.880	0.66 (0.36-1.21)	0.179
CRT	No/Yes	1.15 (0.78-1.68)	0.490	0.60 (0.33-1.08)	0.090
Laboratory parameters					
NLR	<2.63/≥2.63	0.90 (0.63-1.31)	0.592	0.76 (0.44-1.33)	0.343
PLR	<155.12/≥155.12	1.01 (0.70-1.46)	0.959	0.99 (0.57-1.72)	0.960
De-Ritis	<1.09/≥1.09	1.50 (1.03-2.17)	0.032	0.86 (0.48-1.55)	0.625
PNI	<48.9/≥48.9	1.36 (0.94-1.96)	0.106	1.04 (0.59-1.81)	0.901
CEA (ng/mL)	<2.92/≥2.92	1.26 (0.87-1.82)	0.223	1.00 (0.58-1.74)	0.991
CA 19-9 (U/mL)	<39.94/≥39.94	1.45 (0.94-2.25)	0.096	1.99 (1.09-3.64)	0.025
Hemoglobin (g/dL)	<12.10/≥12.10	1.29 (0.89-1.86)	0.183	0.77 (0.44-1.35)	0.360

DFS: Disease-free survival, OS: Overall survival, HR: Hazard ratio, CI: Confidence interval, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, DM: Diabetes mellitus, pT: Pathologic T, CRT: Chemoradiotherapy, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, PNI: Prognostic Nutritional Index, CEA: Carcinoembryonic antigen, CA 19-9: Carbohydrate antigen 19-9, BMI: Body mass index

Moreover, it was observed that the combination arm was more toxic than chemotherapy alone[13]. Consistently, in our study, CRT did not provide a survival benefit for either DFS or OS. In addition to studies with no survival benefit, there are also studies in the literature reporting the negative effect of RT on survival times[14,15]. The conflicting results in the literature regarding the impact of adjuvant CRT on survival have prevented the adoption of adjuvant CRT in the guidelines.

Although CRT has a modest impact on survival, it is important to identify high-risk patients for whom CRT may be effective and patient groups for whom it has an adverse effect. The study by Shi et al.[16] and the study by Opfermann et al.[17] reported a survival benefit of CRT in lymph node positive patients. In our study, lymph node positivity was significantly higher in patients who received CRT compared to those who did not. In line with the literature, this suggests that lymph node status is considered an important factor in the selection of CRT candidate patients in our institution. However, in our study, lymph node status was not associated with survival times for CRT. In subgroup analysis, lymph node involvement was not associated with CRT for survival. This may be related to the fact that, unlike previous studies, all patients in our study received chemotherapy. This difference may be due to the fact that the studies showing a positive effect of CRT were mostly conducted against observation. Consistent with our study, CRT did not show a positive effect in the study of Van Laethem et al.[18], which included patients receiving chemotherapy. In addition, the significant numerical difference between the CRT and non-CRT groups may have contributed to this result.

Given the doubts about the survival effects of CRT and the complications of RT, it is important to choose the right treatment candidates for CRT[19,20]. In our study, no survival benefit was demonstrated for CRT in most of the subgroups. Moreover, the results were detrimental for DFS in groups including performance status, stage and De-Ritis rate. To the best of our knowledge, our study is the first to report

Table 3. Survival effect of chemoradiotherapy among subgroups

	DFS		OS	
Clinicopathological characteristics	**HR (95% CI)**	**p**	**HR (95% CI)**	**p-value**
Age (year)				
<65	1.18 (0.73-1.90)	0.496	0.62 (0.29-1.31)	0.208
≥65	1.017 (0.52-1.97)	0.960	0.46 (0.15-1.43)	0.181
ECOG-PS				
<2	1.65 (1.02-2.69)	**0.043**	0.56 (0.27-1.17)	0.121
≥2	0.66 (0.33-1.32)	0.237	0.67 (0.22-1.98)	0.465
BMI				
<25	1.02 (0.64-1.63)	0.924	0.67 (0.35-1.31)	0.242
≥25	1.63 (0.79-3.34)	0.186	0.44 (0.10-2.01)	0.294
Sex				
Male	1.09 (0.66-1.78)	0.746	0.64 (0.30-1.35)	0.242
Female	1.35 (0.73-2.51)	0.343	0.43 (0.14-1.40)	0.161
Smoking history				
Yes	1.07 (0.66-1.73)	0.796	0.55 (0.18-1.68)	0.298
No	1.35 (0.70-2.58)	0.368	0.63 (0.30-1.34)	0.231
Alcohol history				
Yes	0.51 (0.15-1.77)	0.292	1.00 (0.25-4.02)	0.995
No	1.33 (0.88-2.01)	0.182	0.61 (0.31-1.19)	0.147
DM history				
Yes	1.12 (0.56-2.24)	0.751	1.14 (0.41-3.17)	0.804
No	1.15 (0.72-1.82)	0.566	0.46 (0.22-0.97)	**0.040**
Primary site				
Head	1.05 (0.67-1.66)	0.825	0.55 (0.24-1.26)	0.155
Other	1.57 (0.82-3.00)	0.175	0.84 (0.34-2.07)	0.709
pT				
<3	2.05 (1.10-3.82)	**0.024**	0.53 (0.20-1.43)	0.211
≥3	0.78 (0.48-1.28)	0.325	0.58 (0.25-1.35)	0.208
Lymph node status				
Negative	0.90 (0.42-1.91)	0.784	0.39 (0.11-1.40)	0.153
Positive	1.09 (0.69-1.73)	0.708	0.56 (0.26-1.20)	0.137
Histologic type				
Ductal adeno	1.30 (0.87-1.92)	0.200	0.71 (0.38-1.34)	0.294
Others	0.14 (0.02-1.18)	0.071	0.02 (0.00-100.30)	0.371
Histologic grade				
Grade 1	1.21 (0.48-3.01)	0.685	0.16 (0.02-1.31)	0.087
Grade 2-3	1.12 (0.74-1.72)	0.590	0.72 (0.37-1.38)	0.717
Chemotherapy regimes				
Gemcitabine based	1.07 (0.69-1.66)	0.753	0.50 (0.25-1.00)	0.051
Oxaliplatin with/without irinotecan	1.78 (0.71-4.93)	0.205	2.82 (0.71-11.16)	0.141
NLR				
<2.63	0.81 (0.46-1.42)	0.454	0.88 (0.40-1.90)	0.737
≥2.63	1.57 (0.92-2.67)	0.100	0.40 (0.15-1.09)	0.072
PLR				
<155.12	1.04 (0.60-1.80)	0.895	0.84 (0.39-1.82)	0.656
≥155.12	1.21 (0.71-2.09)	0.486	0.43 (0.15-1.24)	0.119
PNI				
<48.9	1.18 (0.67-2.08)	0.564	0.51 (0.23-1.13)	0.098
≥48.9	1.08 (0.64-1.83)	0.783	0.90 (0.32-2.56)	0.845

Table 3. Continued

	DFS		GS	
Clinicopathological characteristics	**HR (95% CI)**	**p**	**HR (95% CI)**	**p-value**
De-Ritis				
<1.091	1.85 (1.06-3.24)	0.030	0.61 (0.27-1.38)	0.238
≥1.091	0.71 (0.41-1.23)	0.222	0.67 (0.25-1.78)	0.422
CEA (ng/mL)				
<2.92	1.15 (0.64-2.07)	0.645	0.40 (0.16-1.04)	0.059
≥2.92	1.08 (0.64-1.81)	0.776	0.89 (0.39-2.06)	0.791
CA 19-9 (U/mL)				
<39.94	1.01 (0.62-1.65)	0.975	0.42 (0.18-0.99)	0.047
≥39.94	1.47 (0.79-2.73)	0.224	0.90 (0.38-2.13)	0.817
Hemoglobin (g/dL)				
<12.10	1.40 (0.80-2.47)	0.241	0.35 (0.13-0.98)	0.046
≥12.10	0.99 (0.58-1.67)	0.954	0.87 (0.39-1.93)	0.727

DFS: Disease-free survival, OS: Overall survival, HR: Hazard ratio, CI: Confidence interval, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, BMI: Body mass ındex, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, PNI: Prognostic Nutritional index, CEA: Carcinoembryonic antigen, CA 19-9: Carbohydrate antigen 19-9, HR: Hazard ratio, pT: Pathologic T

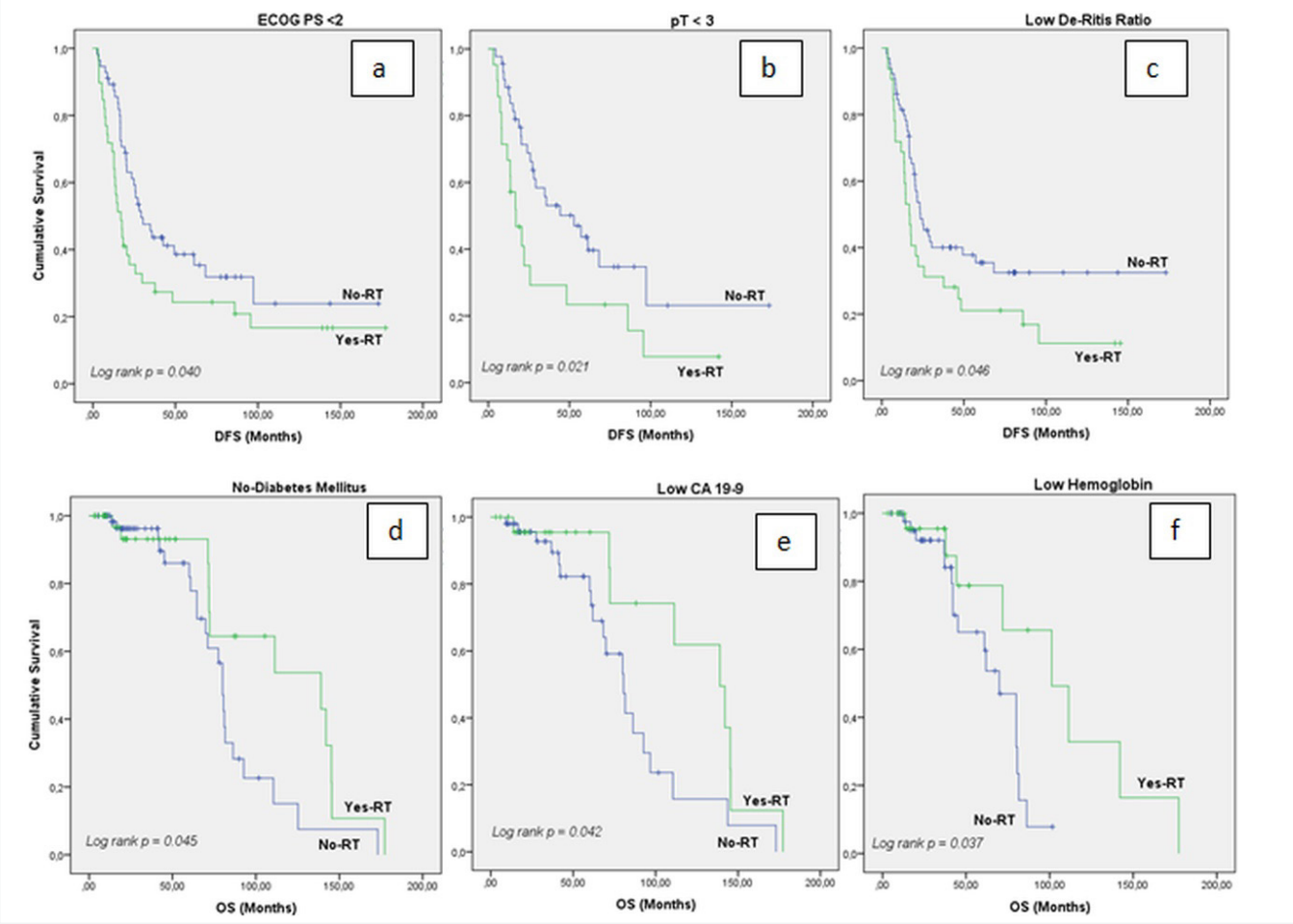

Figure 1. Comparison of survival times for patients treated with chemotherapy alone and patients treated with chemoradiotherapy in Kaplan-Meier analyses

DFS: Disease-free survival, OS: Overall survival, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, pT: Pathologic T

these results. Our findings suggest that risk-based decision making and patient selection should be applied more comprehensively and carefully.

Study Limitations

Our study has some limitations. Firstly, it has a retrospective design. Secondly, although the patient selection criteria were carefully chosen, laboratory markers could still be influenced by various circumstances. Thirdly, lymph node status was significantly different for those who received CRT and those who did not receive CRT; although accurate regression analyses were performed to minimize error, this difference may have affected generalizability. Investigation of the prognostic values of some indices for CRT for the first time, including real-life data, and performing subgroup analysis are the strengths of our study. In addition, the fact that it has a multicenter design and consists of patients who all underwent optimal surgery is important for the generalizability of the results.

CONCLUSION

In conclusion, our study has shown that there are limited survival benefits of adding CRT to adjuvant chemotherapy in operated PC, and CRT candidates should be chosen carefully considering the risk of detrimental effects on some patient groups. More reasonable and optimized prospective randomized clinical trials are needed to further evaluate the benefits of RT in resectable PC.

Ethics

Ethics Committee Approval: Approval from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (TNKU) Faculty of Medicine (decision no: 2022.159.09.06, date: 27.09.2022).

Informed Consent: This study was planned as a multicenter-retrospective study and conducted in accordance with the Declaration of Helsinki.

Footnotes

Authorship Contributions

Concept: E.Ç., K.K., A.S., O.A., Design: E.Ç., K.K., Y.İ., A.S., Y.B., O.A., Data Collection or Processing: E.Ç., K.K., Y.İ., A.S., Y.B., O.A., Analysis or Interpretation: E.Ç., K.K., Y.İ., A.S., Y.B., O.A., Literature Search: E.Ç., K.K., Writing: E.Ç., K.K., O.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Chang JS, Chen LT, Shan YS, Chu PY, Tsai CR. The incidence and survival of pancreatic cancer by histology, including rare subtypes: a nation-wide cancer registry-based study from Taiwan. Cancer Med. 2018;7:5775-88.
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics. CA Cancer J Clin. 2024;74:12-49.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, I et al. Global cancer statistics. 2020: GLOBOCAN estimates of ıncidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-49.
4. Tempero MA, Malafa MP, Al-Hawary M, Behrman SW, Benson AB, Cardin DB, et al. Pancreatic adenocarcinoma, version 2.2021, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2021;19:439-57.
5. Ducreux M, Cuhna AS, Caramella C, Hollebecque A, Burtin P, Goéré D, et al. Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26:56-68.
6. Khorana AA, Mangu PB, Berlin J, Engebretson A, Hong TS. Potentially curable pancreatic cancer: american society of clinical oncology clinical practice guideline update. J Clin Oncol. 2017;35:2324-8.
7. Boyle J, Czito B, Willett C, Palta M. Adjuvant radiation therapy for pancreatic cancer: a review of the old and the new. J Gastrointest Oncol. 2015;6:436-44.
8. Pasqualetti F, Sainato A, Morganti R, Laliscia C, Vasile E, Gonnelli A, et al. Adjuvant radiotherapy in patients with pancreatic adenocarcinoma. is it still appealing in clinical trials? a meta-analysis and review of the literature. Anticancer Res. 2021;41:4697-704.
9. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet. 2017;389:1011-24.
10. Oettle H, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. JAMA. 2013;310:1473-81.
11. Klinkenbijl JH, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg. 1999;230:774-6.
12. Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg. 1985;120:899-903.
13. Liao WC, Chien KL, Lin YL, Wu MS, Lin JT, Wang HP, et al. Adjuvant treatments for resected pancreatic adenocarcinoma: a systematic review and network meta-analysis. Lancet Oncol. 2013;14:1095-103.
14. Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med. 2004;350:1200-10.
15. Merchant NB, Rymer J, Koehler EA, Ayers GD, Castellanos J, Kooby DA, et al. Adjuvant chemoradiation therapy for pancreatic adenocarcinoma: who really benefits? J Am Coll Surg. 2009;208:829-41.
16. Shi X, Peng J, Jiang H, Gao Y, Wang W, Zhou F. Impact of Adjuvant chemoradiotherapy on survival of resected pancreatic adenocarcinoma cancer: a Surveillance, Epidemiology and End Results (SEER) Analysis. Front Oncol. 2021;11:651671.
17. Opfermann KJ, Wahlquist AE, Garrett-Mayer E, Shridhar R, Cannick L, Marshall DT. et al. Adjuvant radiotherapy and lymph node status for pancreatic cancer: results of a study from the Surveillance, Epidemiology, and End Results (SEER) Registry Data. J Clin Oncol. 2014;37:112-6.
18. Van Laethem JL, Hammel P, Mornex F, Azria D, Van Tienhoven G, Vergauwe P, et al. Adjuvant gemcitabine alone versus gemcitabine-based chemoradiotherapy after curative resection for pancreatic cancer: a

randomized EORTC-40013-22012/FFCD-9203/GERCOR phase II study. J Clin Oncol. 2010;28:4450-6.

19. Vento P, Mustonen H, Joensuu T, Kärkkäinen P, Kivilaakso E, Kiviluoto T. Impact of preoperative chemoradiotherapy on survival in patients with resectable pancreatic cancer. World J Gastroenterol. 2007;13:2945-51.

20. Versteijne E, Suker M, Groothuis K, Akkermans-Vogelaar JM, Besselink MG, et al. Preoperative chemoradiotherapy versus immediate surgery for resectable and borderline resectable pancreatic cancer: results of the dutch randomized phase III PREOPANC trial. J Clin Oncol. 2020;38:1763-73.

ORIGINAL ARTICLE

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The Effect of Uric Acid/Albumin Ratio on Prognosis of Patients Followed Up with COVID-19 Diagnosis

Ürik Asit/Albümin Oranının COVID-19 Tanısı ile Takip Edilen Hastaların Prognozuna Etkisi

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ABSTRACT

Aim: The aim of this study was to investigate the effect of the uric acid to albumin ratio (UAR) on prognosis in Coronavirus Disease 2019 (COVID-19) patients followed up in the intensive care unit (ICU) and wards.

Materials and Methods: A single center, retrospective study was organized to observe the UAR values of 204 COVID-19 patients (>18 years of age) hospitalized at the Sultan 2. Abdulhamid Han Training and Research Hospital between May 1, 2020 and April 1, 2022. Patients were divided into two groups according to UAR, as low and high UAR groups, using Receiver Operating Characteristic curve analysis to determine the optimal cut-off value for UAR. The cut-off value was determined as 1.63. Demographic clinical characteristics and laboratory parameters of the participants during their hospitalization were retrospectively obtained from the hospital's electronic medical records.

Results: Patients with high UAR (≥1.63) required longer ICU hospitalization (14.5% vs. 51%, p<0.001) and showed higher in-hospital mortality (0.05% vs. 43.5%, p<0.001).

Conclusion: In this study, we have concluded that UAR is a useful tool independent of other parameters in predicting in-hospital mortality in patients with COVID-19 followed in ICU and wards.

Keywords: UAR, COVID-19, prognosis, mortality

ÖZ

Amaç: Bu çalışmada, yoğun bakım ünitesinde (YBÜ) ve servislerde takip edilen Koronavirüs Hastalığı 2019 (COVID-19) hastalarında asit albümin oranının (UAR) prognoz üzerine etkisinin araştırılması amaçlandı.

Gereç ve Yöntem: 2. Abdülhamid Han Eğitim ve Araştırma Hastanesi'nde 1 Mayıs 2020 1 Nisan 2022 tarihleri arasında yatan 204 COVID-19 hastasının (>18 yaş) UAR değerlerini gözlemlemek için tek merkezli, retrospektif bir çalışma düzenlendi. Hastalar, UAR için optimal kesme değerini belirlemek üzere Alıcı İşletim Özelliği eğrisi analizi kullanılarak UAR'ye göre düşük ve yüksek UAR grupları olmak üzere iki gruba ayrılmıştır. Kesme değeri 1,63 olarak belirlendi. Katılımcıların hastanede yattıkları süre boyunca demografik klinik özellikleri ve laboratuvar parametreleri hastanenin elektronik tıbbi kayıtlarından retrospektif olarak elde edilmiştir.

Bulgular: Yüksek UAR (≥1,63) olan hastalar daha uzun süreli YBÜ yatış gerektirmiş (%14,5'e karşı %51, p<0,001) ve daha yüksek hastane içi mortalite (%0,05'e karşı %43,5, p<0,001) göstermiştir.

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Sonuç: Bu çalışmada, YBÜ ve servislerde takip edilen COVID-19 tanılı hastalarda, diğer parametrelerden bağımsız olarak, UAR'nin hastane içi mortaliteyi öngörmek için yararlı bir araç olduğu sonucuna varılmıştır.

Anahtar Kelimeler: UAR, COVID-19, prognoz, mortalite

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) emerged in China in December 2019 and was accepted by the World Health Organization as a pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) on March 11, 2020, and has caused the death of millions of people since its emergence[1,2]. While most of the COVID-19 patients recover without any complications, a significant number of patients face serious complications and death. Patients with COVID-19 present with a variety of symptoms as well as different prognoses, including recovery, intensive care unit (ICU) admission, and death. While only fever and cough can be seen in mild cases, critical cases can be seen in various presentation such as Acute Respiratory Distress Syndrome (ARDS), sepsis or septic shock. Early detection of high-risk patients likely to develop critical illness is essential to allocate limited resources to treat the disease[3].

It is known that immunosuppression, malignancy, diabetes, and hypertension (HT) adversely affect the prognosis in COVID-19 patients and cause high mortality[4]. A severe and uncontrolled immune inflammatory response is an effective predictor of disease severity and poor prognosis. Many different serological markers have been identified as the indicators of inflammatory response. In previous studies, C-reactive protein (CRP), erythrocyte sedimentation rate (erythrocyte sedimentation rate), ferritin, proinflammatory cytokines in addition to lactate dehydrogenase (LDH), D-dimer, and high-sensitivity cardiac troponin I were determined to be predictors of serious morbidity and mortality[5-7]. Albumin is an important molecule in the body's defense mechanisms, which plays a role in antiinflammation, antiapoptosis and protection of the body from oxidative stress[8,9]. Low albumin level was also found to be significant in predicting disease severity and mortality in previous studies[10-13].

Uric acid (UA) is a catabolic product of purine from RNA and DNA as a metabolic index, it is less affected by other factors than drugs and high purine diet. Previous studies have shown that UA is closely related to the activation of the immune system and scavenging of free oxygen radicals[14-16]. High UA levels can be detected in chronic diseases such as HT, diabetes, chronic kidney disease, obesity, and gout. In addition, hyperuricemia was found to be associated with increased mortality in these diseases[17,18]. It has been reported that kidney and gastrointestinal involvement rates are high in COVID-19 patients. Since the kidneys and intestines are both targets of SARS-CoV2 and primary sites of UA excretion, serum UA concentrations have been shown to be lower in patients with COVID-19 disease. Hypouricemia has also been found to be strongly associated with poor prognosis and mortality[19-23]. However, it is known that hyperuricemia is associated with hypoxia and systemic inflammation in respiratory tract diseases, and some studies have found that both hyperuricemia and hypouricemia are associated with increased mortality[3,24].

In recent studies, systemic immune inflammatory indices such as neutrophil lymphocyte ratio (NLR), derived neutrophil to lymphocyte ratio, platelet lymphocyte ratio (PLR), monocyte lymphocyte ratio and systemic inflammatory index (SII) have been shown to be useful in predicting disease severity and mortality in COVID-19[25-27]. Uric acid to albumin ratio (UAR) is an index that has been shown to predict prognosis in many diseases such as acute renal failure, pneumonia, and acute coronary diseases, and unlike these diseases, there is no study conducted in COVID-19 patients. In this study, we planned to investigate the effect of UAR on prognosis in COVID-19 patients followed in ICU and other wards[28-30].

MATERIALS AND METHODS

Patient Selection

COVID-19 patients (>18 years old), hospitalized at the Sultan 2. Abdulhamid Han Training and Research Hospital in the internal medicine clinic between May 1, 2020 and April 1, 2022 were the main focus of this single-center, retrospective study. COVID-19 diagnoses were made by a positive result from a real-time reverse transcriptase (RT) polymerase chain reaction (PCR) assay of nasal and pharyngeal swab specimens. Patients who had negative RT-PCR results or patients using drugs to decrease serum UA level and patients under 18 years old were excluded from the study. Ethics committee approval for the study was obtained from the University of Health Sciences Türkiye, Hamidiye Clinical Research Ethics Committee (decision no: E-46418926-050.99-133138, date: 31.05.2022).

Statistical Analysis

The power analysis of the study was performed with G*Power 3.1.9.4. When the effect size d: 0.5, α err prob: 0.04, Power (1-β error prob): 0.964, the total sample size was found to be 204. For the study, 364 patients who were followed up with a diagnosis of COVID-19 were screened and patients with undesirable UA levels during hospitalization were excluded from the study. When the number of 204 patients identified

after power analysis was reached, data collection was terminated through the system. The patients were divided into two groups according to UAR as the low and high UAR groups.

The demographic, clinical characteristics and laboratory parameters of the participants during their hospitalization were retrospectively obtained from the hospital's electronic medical records. The duration of follow up in the ICU, the treatments they received, and the duration and doses of these treatments were recorded.

Laboratory Analysis

Blood samples were obtained on the first day of the Sultan 2. Abdulhamid Han Education and Research Hospital admission. Immediately after sampling, complete blood count parameters were determined by a hematology analyzer (ABX Pentra DX 120). Serum UA and albumin levels were measured using a Roche Diagnostics Cobas 8000 c502 analyzer (Roche Holding AG, Basel, Switzerland). Serum albumin level was determined using the bromocresol green method.

ICU Evaluation Criteria

The patients were admitted to the ICU according to the following criteria: 1) Meeting criteria for ARDS or needs O^2 >6 liters per minute to maintain SpO^2 >92% (or rapid escalation of oxygen requirement), 2) Respiratory rate>30 per minute, 3) Systolic blood pressure <90 mmHg, mean arterial pressure <65 mmHg, tachycardia and other signs of shock, 4) Arterial blood gas with pH <7.3 or partial CO^2 pressure >50 mmHg or above patient's baseline, lactate >2 mmol/liter, 5) Concerning clinical appearance (cyanosis fast breathing, grunting, chest indrawing, inability to drink, lethargy, convulsions, mottled or cool skin). The decision of ICU admission was based on the criteria by Brigham and Women's Hospital COVID-19 guidelines which were updated on December 20, 2020[31].

RESULTS

The primary outcome was defined as all cause in-hospital mortality in this study. The secondary outcome was defined as ICU admission.

Continuous variables were presented as mean (standard deviation) or median (interquartile range), or median interquartile range (25^{th} to 75^{th}) according to data distribution, and as absolute number (and percentage) for categorical variables; the distribution was tested with the Shapiro-Wilk test. ROC curve was used to determine the optimal cut-off value (by Youden index) for UAR. Differences between patient outcomes were studied with t-test for independent groups or with the Mann-Whitney U test if non-parametric analysis was required.The evaluation of differences between groups of categorical data was carried out with the chi-square statistics. In order to calculate the probability of mortality for patients admitted to the ICU, binary logistic regression analysis was used. Stepwise binary logistic regression was employed, where variables with p<0.05 were included in the multivariable model. The effect on mortality (survival) over time was investigated with the Kaplan-Meiere (Log-Rank-Mante Cox) and Cox Regression in the Statistical Analysis field. All confidence intervals were established at 95%; two-tailed significance level was established to be <0.05. Statistical data analysis was performed using the R v4.01(Vienna; Austria) with "rms" "hmisc" "epi" "survival" "ggfortify" packages.

Two hundred and four patients with COVID-19 (PCR positive) who were followed in hospital were included in the study. ROC curve analysis was made to determine optimal cut-off value for UAR. According to the ROC analysis, optimal cut-off with highest Youden index was found as 1.63 (Figure 1). Sensitivity of this cut-off value for in-hospital mortality was 86%, while specificity was 71%. Patients were divided into two groups as low UAR and high UAR regarding this cut-off value. Comparison of baseline characteristics, laboratory parameters and duration of hospitalization for these two groups were given in Table 1. The first group (UAR <1.63) included 119 patients, while the higher UAR group (≥1.63) included 85 patients. Although the mean age for the general population was 63±16.3 years, the mean age was 57.4±15.9 years in the low UAR group, and 70.9±13.2 in the high UAR group (p<0.001). Variables including the presence of HT, diabetes mellitus (DM),

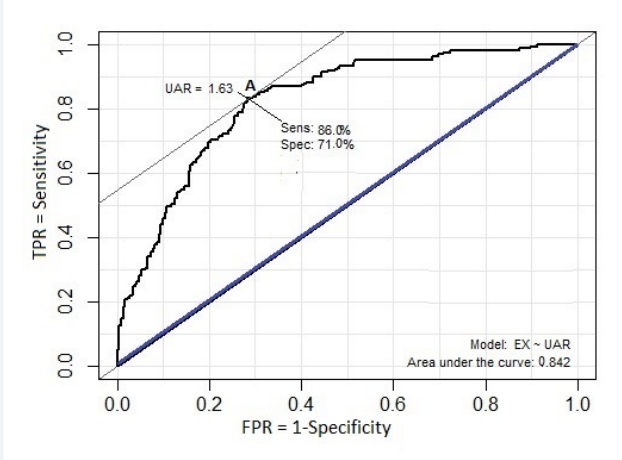

Figure 1. ROC curve of UAR for predicting in-hospital mortality

Navy blue line: a diagnostic test with the lowest discriminatory ability, which is no better than chance, area under the curve (AUC) =0.5, black line: a test with good discriminatory ability (AUC =0.842 x-axis: coordinate points with 1 - specificity y-axis: sensitivity as the all cut-off values measured from the test results, A: Youden Index cut-off point (reference) value, UAR: Uric acid albumin ratio, FPR: False positive rate, TPR: True positive rate

chronic heart failure, coronary artery disease, and male gender were comparable between the low and high UAR groups (p=0.71, p=0.64, p=0.73, p=0.22, and p=0.08, respectively), whereas chronic obstructive pulmonary disease and chronic renal disease frequencies were found to be higher in the high UAR group (p=0.02 and p<0.001, respectively), while glomerular filtration rate (GFR) was lower in the high UAR group (80.60±21 vs. 58.5±24, p<0.001). Inflammatory laboratory markers including CRP, ferritin, D-dimer, white blood cell count (WBC), UA, creatinine, aspartate aminotransferase (AST) and LDH levels were found to be higher in the high UAR group (p<0.001, p=0.002, p=0.002, p=0.002, p<0.001, p<0.001, p=0.003, and p<0.001, respectively). Albumin, GFR, lymphocyte count, and hemoglobin level demonstrated lower levels in the high UAR group (p<0.001, p<0.001, p=0.007, and p<0.001 respectively). Patients with high UAR required more ICU admission (14.5% vs. 51%, p<0.001) and demonstrated higher in-hospital mortality (0.05% vs. 43.5%, p<0.001). There was a significant difference in mortality between the two groups. We predicted that the difference in age and renal failure rate between the two groups significantly affected mortality.

Univariable logistic regression analysis to predict in-hospital mortality is present in Table 2. The parameters included in univariable logistic regression analysis were as follows: UA level, albumin level, UAR, age, creatinine level, CRP, AST, LDH, ferritin, hemoglobin, D-dimer levels, and leucocyte count. Among these variables, UA, albumin, CRP, AST, LDH, D-Dimer, ferritin, hemoglobin levels, UAR, age and leucocyte count were related to in-hospital mortality in univariable logistic regression analysis (p<0.001 for all).

Table 1. Comparison of basal characteristics, laboratory parameters and hospitalization duration between the high UAR and low UAR groups

Variable	All patient	Low UAR group (n=119)	High UAR group (n=85)	p-value
Age (mean ± SD)	63±16.3	57.4±15.9	70.9±13.2	p<0.001*
Gender (male) (n, %)	125 (61.2%)	67 (56.3%)	58 (68.2%)	0.08**
Hypertension (n, %)	29 (14.2%)	16 (13.4%)	13 (15.3%)	0.71**
DM (n, %)	14 (6.9%)	9 (7.6%)	5 (5.9%)	0.64**
COPD (n, %)	7 (3.4%)	1 (0.8%)	6 (7.1%)	0.02**
CRF (n, %)	35 (17.2%)	6 (5%)	29 (34.1%)	p<0.001**
CAD (n, %)	14 (6.9%)	6 (5%)	8 (9.4%)	0.22**
CHF (n, %)	4 (2%)	2 (1.7%)	2 (2.4%)	0.73**
Uric acid (mg/dL) (mean ± SD)	5.9±3.3	4.5±0.9	7.8±4.3	p<0.001*
Albumin (g/L) (mean ± SD)	3.3±0.7	3.6±0.6	2.8±0.6	p<0.001*
Hospitalization (mean ± SD)	10±6.9	10.1±6.4	11.7±7.6	0.10*
Creatinine (mg/dL) (mean ± SD)	1.2±0.9	1±0.3	1.5±1.3	p<0.001*
GFR (mL/dL/1.73 m^2) (mean ± SD)	71.5±25	80.6±21	58.5±24	p<0.001*
AST (U/L) (mean ± SD)	79.1±182	54±73	114±268	0.03*
LDH (U/L) (mean ± SD)	763±755	591±353	944±1017	p<0.001*
CRP (mg/dL) (mean ± SD)	109±89	81±80	149±88	p<0.001*
Ferritin (ng/mL) (mean ± SD)	1546±4400	714±2020	2739±6280	0.002*
D-dimer (µg/L) (mean ± SD)	2827±5190	2100±4600	3870±5745	0.002*
WBC (mm^3) (mean ± SD)	6380±4470	5470±3190	7700±6180	0.002*
Lymphocyte (mm^3) (mean ± SD)	1040±660	1150±590	880±740	0.007*
Hemoglobin (g/dL) (mean ± SD)	11.1±2.1	11.7±2.1	10.3±1.9	p<0.00**
Platelet ($10^3/mm^3$) (mean ± SD)	193±101	200±101	183±101	0.25*
ICU admission (n, %)	61 (30.3%)	17 (14.5%)	44 (51%)	p<0.001**
In-hospital mortality (n, %)	43 (21%)	6 (0.05%)	37 (43.5%)	p<0.001**

UAR: Uric acid to albumin ratio, DM: Diabetes mellitus, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, CAD: Coronary artery disease, CHF: Chronic heart failure, GFR: Glomerular filtration rate, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, WBC: White blood cell, ICU: Intensive care unit, *Student's t-test, **Chi-square test

Table 2. Univariable and multivariable logistic regression analysis (modeling to binary) to predict in-hospital mortality

	Univariable			Multivariable		
Variable	**Odds ratio**	**Confidence interval**	**p-value***	**Odds ratio**	**Confidence interval**	**p-value***
UAR	4.650	2.660-8.150	<0.001	3.300	1.570-6.981	0.002
Age	1.700	1.050.1.100	<0.001	1.120	1.040-1.200	0.003
Creatinine (mg/dL)	1.030	0.700.1.500	0.88	-	-	-
CRP (mg/dL)	1.009	1.004-1.010	<0.001	1.001	0.990-1.011	0.81
AST (U/L)	1.008	1.003-1.01	<0.001	1.003	0.99-1.011	0.61
LDH (U/L)	1.003	1.002-1.004	<0.001	1.004	1.002-1.006	0.81
WBC (mm^3)	1.002	1.001-1.003	<0.001	1.002	1.001-1.002	0.03
D-dimer (µg/L)	1.002	1.001-1.003	<0.001	1.001	0.999-1.002	0.07
Ferritin (ng/mL)	1.002	1.001-1.003	<0.001	1.000	0.999-1.001	0.27
Hemoglobin (g/dL)	0.590	0.480-0.720	<0.001	0.686	0.471-1.001	0.06

UAR: Uric acid albumin ratio, CRP: C-reactive protein, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, WBC: White blood cell, *Univariable and Multivariable logistic regression analysis

In Table 2, multivariate logistic regression analysis to predict in-hospital mortality was given. The variables UAR, age, AST, LDH, CRP, ferritin, D-dimer, WBC, ferritin, and hemoglobin were included in the analysis. Among these variables, UAR, older age and WBC count independently predicted the in-hospital mortality (p=0.002, p=0.003, and p=0.03, respectively). The distribution of UAR levels for survivor (n=161) and non- survivor (n=43) was given in Box/Violin Plot (Figure 2). Wider distribution of UAR with a higher mean level was observed with this plot. The effect of UAR (reference: <1.63) on mortality over time (survival) was statistically significant (Kaplan-Meiere; 96.086±8.273 versus 17.065±1.623; Log-Rank (Mantel-Cox) p<0.0001; Figure 3). The mortality rate of patients with high UAR values over time was 3.164 times higher than the other patients [Cox Regression; Exp (b)= 3.164 (1.883-5.317); p<0.0001].

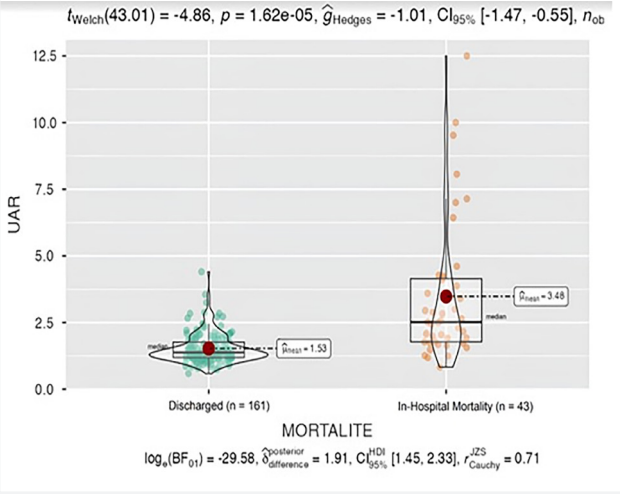

Figure 2. Box and Violin plot of UAR for discharged and in-hospital mortality. The median and mean values of patients who were discharged are close to each other, with the median and quartiles also being relatively close. The majority of the data are distributed between the median and the mean. In contrast, in patients with in-hospital mortality, the UAR value increases, the distribution is more elongated with extreme values emerge. It is seen that there is a statistical difference between the UAR levels of the discharged and in-hospital mortality

UAR: Uric acid albumin ratio, CI: Confidence interval

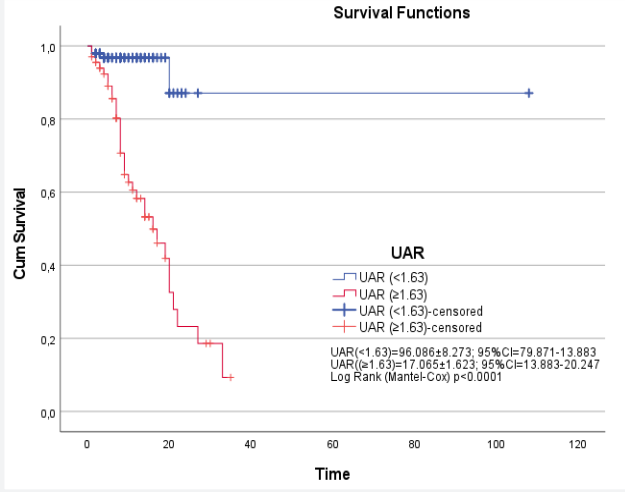

Figure 3. Kaplan-Meier curves to assess the association between UAR and mortality over time

UAR: Uric acid albumin ratio

DISCUSSION

Since its emergence, COVID-19 has emerged with a pandemic that has arisen with a wide spectrum ranging from mild symptoms such as cough and fever to pneumonia, severe respiratory distress, need for care, and it still continues[1-3]. The course of COVID-19 in this wide range varies according to the basal characteristics of the patient (gender, age, HT, DM etc.). Moreover, COVID-19 affects the respiratory, urinary and gastrointestinal systems at different levels in patients, and it affects the blood values of the patients, especially the inflammation markers, at different levels. This shows the course of the disease and is a predictor for the course of the disease[4-6].

One of these parameters is albumin, and some studies have shown that low albumin levels are associated with high mortality[10-13]. It has been shown in previous studies that albumin is decreased in malnutrition and as a negative acute phase reactant secondary to inflammation, and hypoalbuminemia has been shown to be associated with mortality even in the general population[32,33]. Hypoalbuminemia may be due to the presence of a systemic inflammatory condition and malnutrition in COVID-19. It is known that due to increased capillary permeability due to inflammation, serum albumin may be extravasated into the interstitial space and the volume distribution of albumin increases[34,35].

In a study conducted by Shoji et al.[36] in 2024, it was shown that the risk of serious disease was higher in diabetic patients infected with COVID-19 due to hypoalbuminemia, as assessed by the blood albumin level at the time of diagnosis.

Another parameter affected by COVID-19 is UA. In previous studies, hypouricemia has been shown as a poor prognostic factor in patients with intra-abdominal sepsis[37], radiation pneumonitis[38] and COVID-19[19-23]. It remains unclear whether the poor outcomes, particularly in COVID-19 patients, are due in part to a lack of antioxidants. Hyperuricemia has also been found to be associated with a variety of diseases, including coronary heart disease [39], HT[40], kidney failure[41], and exacerbations of chronic obstructive pulmonary disease[42]. This may be due to the direct pathophysiological effects of high UA concentrations, such as increased oxidative stress, inflammation, endothelial dysfunction, activation of the renin angiotensin aldosterone system, and insulin resistance[43]. In addition, there are studies showing that both hypouricemia and hyperuricemia increase mortality in COVID-19 patients[3,24]. Although hypouricemia and hyperuricemia are thought to be related to the increase in mortality in COVID-19 patients due to the reasons mentioned above, these reasons are open to discussion.

UAR has been shown to predict mortality in various diseases, alongside indices such as NLR, PLR, C-reactive protein albumin ratio (CAR), and SII[25-27]. In studies, it has been shown that it is a better predictor of mortality than CAR in some patient groups such as non-ST-elevation myocardial infarction[30].

In a study conducted by Ertan et al.[44] in 2024, it was shown that the UAR associated with 28-day mortality in patients with acute kidney injury developing in the ICU was important in showing mortality with 39.3% sensitivity and 84.1% specificity.

This study evaluated UAR as a predictor of mortality in COVID-19 patients. Patients were divided into the low and high UAR groups based on a ROC analysis, determining the cut-off value. Results showed that patients with high UAR were associated with more ICU admissions. UAR independently predicted in-hospital mortality. These findings suggest that UAR may be a valuable, accessible parameter for risk stratification in ICU and ward patients with COVID-19. Future studies with larger datasets are needed to validate these findings.

Study Limitations

Firstly, the retrospective nature of this single center study is one of its limitations. Secondly, this study had a relatively small number of patients. Thirdly, we only analyzed serum concentrations of UA and albumin on admission and did not have access to follow up measurements over time. Another limitation is that, unlike other biomarkers, there is no consensus on the standard cut-off values of UAR. Therefore, future studies including larger data sets are needed to test the results of our study.

CONCLUSION

UAR is a useful ratio that can be easily calculated with routine blood tests. In this study, we showed that UAR could independently predict hospital mortality in patients diagnosed with COVID-19, followed in the ICU and onwards.

Ethics

Ethics Committee Approval: Ethics committee approval for the study was obtained from the University of Health Sciences Türkiye, Hamidiye Clinical Research Ethics Committee (decision no.: E-46418926-050.99-133138, date: 31.05.2022).

Informed Consent: The main focus of this single-center, retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.Ç.G., N.T., Z.S., Y.Ö.T., A.E.K., M.K., Concept: B.Ç.G., N.T., Z.S., Y.Ö.T., A.E.K., M.K., Design: B.Ç.G., İ.K., M.K., Data Collection or Processing: B.Ç.G., N.T., Z.S., Y.Ö.T., A.E.K., M.K., Analysis or Interpretation: İ.K., Literature Search: B.Ç.G., İ.K., B.G., N.K., Z.S., Writing: B.Ç.G., İ.K., B.G.

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REFERENCES

1. Izcovich A, Ragusa MA, Tortosa F, Lavena Marzio MA, Agnoletti C, Bengolea A, et al. Correction: prognostic factors for severity and mortality in patients infected with COVID-19: a systematic review. PLoS One 2020;15:0241955.
2. World Health Organization. (2020). Novel Coronavirus (2019-nCoV): situation report, 18. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200207-sitrep-18-ncov.pdf
3. Chen B, Lu C, Gu HQ, Li Y, Zhang G, Lio J, et al. Serum uric acid concentrations and risk of adverse outcomes in patients with COVID-19. Front Endocrinol (Lausanne). 2021;12:633767.
4. Li J, Huang DQ, Zou B, Yang H, Hui WZ, Rui F, et al. Epidemiology of COVID-19: a systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. J Med Virol. 2021;93:1449-58.
5. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020;84:106504.
6. Nalbant A, Kaya T, Varim C, Yaylaci S, Tamer A, Cinemre H. Can the neutrophil/lymphocyte ratio (NLR) have a role in the diagnosis of coronavirus 2019 disease (COVID-19). Rev Assoc Med Bras (1992). 2020;66:746-51.
7. Gallo Marin B, Aghagoli G, Lavine K, Yang L, Siff EJ, Chiang SS, et al. Predictors of COVID-19 severity: a literature review. Rev Med Virol. 2021;31:1-10.
8. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Mol Aspects Med. 2012;33:209-90.
9. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. FEBS Lett. 2008;582:1783-87.
10. Wang Y, Shi L, Wang Y, Duan G, Yang H. Albumin and total bilirubin for severity and mortality in Coronavirus Disease 2019 patients. J Clin Lab Anal. 2020;34:23412.
11. Parohan M, Yaghoubi S, Seraji A. Liver injury is associated with severe coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of retrospective studies. Hepatol Res. 2020;50:924-35.
12. Paliogiannis P, Mangoni AA, Cangemi M, Fois AG, Carru C, Zinellu A. Serum albumin concentrations are associated with disease severity and outcomes in coronavirus 19 disease (COVID-19): a systematic review and meta-analysis. Clin Exp Med. 2021;21:343-54.
13. Acharya R, Poudel D, Bowers R, Patel A, Schultz E, Bourgeois M, et al. Low serum albumin predicts severe outcomes in COVID-19 infection: a single-center retrospective case-control study. J Clin Med Res. 2021;13:258-67.
14. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature. 2003;425:516-21.
15. Kool M, Soullié T, van Nimwegen M, Willart MA, Muskens F, Jung S, et al. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. J Exp Med. 2008;205:869-82.
16. Sautin YY, Johnson RJ. Uric acid: the oxidant-antioxidant paradox. Nucleosides Nucleotides Nucleic Acids. 2008;27:608-19.
17. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. Arthritis Rheum. 2011;63:3136-41.
18. Abdelmalek MF, Diehl AM. Nonalcoholic fatty liver disease as a complication of insulin resistance. Med Clin North Am. 2007;91:1125-49.
19. Gabarre P, Dumas G, Dupont T, Darmon M, Azoulay E, Zafrani L. Acute kidney injury in critically ill patients with COVID-19. Intensive Care Med. 2020;46:1339-48.
20. Jin X, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, et al. Epidemiological, clinical and virological characteristics of 74 cases of Coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. Gut. 2020;69:1002-9.
21. Werion A, Belkhir L, Perrot M, Schmit G, Aydin S, Chen Z, et al. SARS-CoV-2 causes a specific dysfunction of the kidney proximal tubule. Kidney Int. 2020;98:1296-07.
22. Dufour I, Werion A, Belkhir L, Wisniewska A, Perrot M, De Greef J, et al. Serum uric acid, disease severity and outcomes in COVID-19. Crit Care. 2021;25:212.
23. Li G, Wu X, Zhou CL, Wang YM, Song B, Cheng XB, et al. Uric acid as a prognostic factor and critical marker of COVID-19. Sci Rep. 2021;11:17791.
24. Bartziokas K, Papaioannou AI, Loukides S, Papadopoulos A, Haniotou A, Papiris S, et al. Serum uric acid as a predictor of mortality and future exacerbations of COPD. Eur Respir J. 2014;43:43-3.
25. Paliogiannis P, Zinellu A, Scano V, Mulas G, De Riu G, Pascale RM, et al. Laboratory test alterations in patients with COVID-19 and non COVID-19 interstitial pneumonia: a preliminary report. J Infect Dev Ctries. 2020;14:685-90.
26. Labenz C, Toenges G, Wörns MA, Sprinzl MF, Galle PR, Schattenberg JM. Liver injury in patients with severe acute respiratory syndrome Coronavirus-2 infection: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2021;33:1194-00.
27. Fois AG, Paliogiannis P, Scano V, Cau S, Babudieri S, Perra R, et al. The systemic inflammation index on admission predicts in-hospital mortality in COVID-19 patients. Molecules. 2020;25:5725.
28. Tian Y, Li Y, Jiang Z, Chen J. Urea-to-albumin ratio and in-hospital mortality in severe pneumonia patients. Can J Infect Dis Med Microbiol. 2021;2021:5105870.
29. Özgür Y, Akın S, Yılmaz NG, Gücün M, Keskin Ö. Uric acid albumin ratio as a predictive marker of short-term mortality in patients with acute kidney injury. Clin Exp Emerg Med. 2021;8:82-8.
30. Çakmak EÖ, Bayam E, Çelik M, Kahyaoğlu M, Eren K, Imanov E, et al. Uric acid-to-albumin ratio: a novel marker for the extent of coronary artery disease in patients with non-ST-elevated myocardial ınfarction. Pulse (Basel). 2021;8:99-107.
31. Brigham and women's hospital COVID protocols. December 20, 2020. Available from: https://Covidprotocols.org/en/chapters/patient-assessment/#disease-severity-and-disposition-t4tualt31n26
32. Tseng CH. Correlation of uric acid and urinary albumin excretion rate in patients with type 2 diabetes mellitus in Taiwan. Kidney Int. 2005;68:796-01.
33. Chronic Kidney Disease Prognosis Consortium; Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet. 2010;375:2073-81.
34. Huang J, Cheng A, Kumar R, Fang Y, Chen G, Zhu Y, et al. Hypoalbuminemia predicts the outcome of COVID-19 independent of age and co-morbidity. J Med Virol. 2020;92:2152-58.
35. Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: Pathogenesis and Clinical Significance. JPEN J Parenter Enteral Nutr. 2019;43:181-93.

36. Shoji Mayumi, Teramoto N, Ishikawa T, ett al. Hypoalbuminemia on admission in diabetic patients correlates with severity of illness in COVID-19: A retrospective clinical study and literature review. Endocrine and Metabolic Science (2024): 100196.

37. Abou-Mourad NN, Chamberlain BE, Ackerman NB. Poor prognosis of patients with intra-abdominal sepsis and hypouricemia. Surg Gynecol Obstet. 1979;148:358-60.

38. Wang JY, Chen KY, Wang JT, Chen JH, Lin JW, Wang HC, et al. Outcome and prognostic factors for patients with non-small-cell lung cancer and severe radiation pneumonitis. Int J Radiat Oncol Biol Phys. 2002;54:735-41.

39. White J, Sofat R, Hemani G, Shah T, Engmann J, Dale C, et al. Plasma urate concentration and risk of coronary heart disease: a Mendelian randomisation analysis. Lancet Diabetes Endocrinol. 2016;4:327-36.

40. Kuwabara M, Hisatome I, Niwa K, Hara S, Roncal-Jimenez CA, Bjornstad P, et al. Uric acid is a strong risk marker for developing hypertension from prehypertension: a 5-year Japanase cohort study. Hypertension. 2018;71:78-6.

41. Srivastava A, Kaze AD, McMullan CJ, Isakova T, Waikar SS. Uric acid and the risks of kidney failure and death in individuals with CKD. Am J Kidney Dis. 2018;71:362-70.

42. Kahnert K, Alter P, Welte T, Huber RM, Behr J, Biertz F, et al. Uric acid, lung function, physical capacity and exacerbation frequency in patients with COPD: a multi-dimensional approach. Respir Res. 2018;19:110.

43. Joosten LAB, Crişan TO, Bjornstad P, Johnson RJ. Asymptomatic hyperuricaemia: a silent activator of the innate immune system. Nat Rev Rheumatol. 2020;16:75-6.

44. Ertan OES, Gökçe O, Bal C, Kocaturk E, Ertan O, Mutluay R. ınvestigation of the relationship between serum uric acid-to-albumin ratio and 28-day mortality in patients with and without acute kidney injury. J Acute Med. 2024;14:152-9.

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Impact of Breastfeeding Duration on Infant Health Outcomes in Türkiye: A Cross-Sectional Analysis

Emzirme Süresinin Bebeklerin Sağlık Sonuçları Üzerine Etkisi: Türkiye'den Kesitsel Bir Çalışma

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ABSTRACT

Aim: The present study aims to examine the relationship between breastfeeding duration and health outcomes among children aged 12-24 months in Türkiye to provide evidence-based recommendations for improving public health strategies and better breastfeeding practices.

Materials and Methods: This study relies on data from the Turkish Statistical Institute, Türkiye Health Survey 2022 dataset on children aged 0-14 years. The dataset contains information about breastfeeding duration and the health outcomes in children. Health outcomes, including general health status, chronic diseases, restrictions in daily life, and infectious diseases were analyzed using the chi-square and Mann-Whitney U tests. A p-value of <0.05 was considered statistically significant.

Results: Among the 343 infants aged between 12 and 24 months, 83% were breastfed for at least six months and 65% for at least 12 months. Infants who were breastfed for six months or longer were found to have better health status (p=0.021), lower rates of chronic diseases (p=0.042), and fewer restrictions in daily life (p=0.014). Breastfeeding for at least six months was associated with reduced history of communicable diseases (p=0.026) and lower respiratory tract infections (p=0.010). No significant differences were observed for acute gastroenteritis or urinary tract infections. Breastfeeding for a period longer than 12 months was associated with lower communicable disease history (p=0.047).

Conclusion: Our study highlights the significant benefits of breastfeeding. Particularly breastfeeding at least for 6 months was associated with improved infant health outcomes and reduced history of acute and chronic diseases in early childhood.

Keywords: Breastfeeding, infant health, communicable disease, chronic disease

ÖZ

Amaç: Bu çalışmanın amacı, Türkiye'de 12-24 aylık çocuklar arasında emzirme süresi ile sağlık sonuçları arasındaki ilişkiyi inceleyerek halk sağlığı stratejilerinin geliştirilmesi ve daha iyi emzirme uygulamaları için kanıta dayalı öneriler sunmaktır.

Gereç ve Yöntem: Bu çalışma, Türkiye İstatistik Kurumu, Türkiye Sağlık Araştırması 2022 veri setinde yer alan 0-14 yaş arası çocuklara ilişkin verilere dayanmaktadır. Veri seti, emzirme süresi ve çocuklarda sağlık sonuçları hakkında bilgi içermektedir. Genel sağlık durumu, kronik hastalıklar, günlük yaşamdaki kısıtlamalar ve bulaşıcı hastalıklar dahil olmak üzere sağlık sonuçları ki-kare ve Mann-Whitney U testleri kullanılarak analiz edilmiştir. <0,05 p-değeri istatistiksel olarak anlamlı kabul edilmiştir.

Bulgular: Yaşları 12 ila 24 ay arasında değişen 343 bebeğin %83'ü en az altı ay, %65'i ise en az 12 ay anne sütüyle beslenmiştir. Altı ay veya daha uzun süre anne sütüyle beslenen bebeklerin sağlık durumlarının daha iyi olduğu (p=0,021), kronik hastalık oranlarının daha düşük olduğu (p=0,042) ve günlük yaşamda daha az kısıtlama yaşadıkları (p=0,014) saptandı. En az altı ay emzirilenlerde, daha az bulaşıcı hastalık (p=0,026) ve alt solunum

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yolu enfeksiyonu (p=0,010) öyküsü ile ilişkili bulundu. Akut gastroenterit veya idrar yolu enfeksiyonları için anlamlı bir farklılık gözlenmedi. Emzirme süresinin 12 aydan uzun olması daha düşük bulaşıcı hastalık öyküsü ile ilişkiliydi (p=0,047).

Sonuç: Çalışmamız anne sütü ile beslenmenin önemli faydalarını vurgulamaktadır; özellikle en az 6 aylık anne sütü ile beslenme, bebek sağlığı sonuçlarının iyileşmesi ve erken çocukluk döneminde akut ve kronik hastalık öyküsünün azalması ile ilişkili bulunmuştur.

Anahtar Kelimeler: Emzirme, bebek sağlığı, bulaşıcı hastalık, kronik hastalık

INTRODUCTION

Breastfeeding is widely regarded as the most ideal infant feeding method and offers significant health benefits for infants and mothers alike. The World Health Organization highlights the importance of exclusive breastfeeding in the first six months of life and continued breastfeeding with appropriate complementary foods up to two years of age or beyond. This approach is considered critical in achieving the optimum growth, development, and health outcomes in children[1]. The literature indicates that breastfeeding has an added advantage to the health of infants; it decreases the likelihood of infectious diseases such as respiratory tract infections, diarrhea, and otitis media[2]. In addition, the longer the child is breastfed, the lower the risks of chronic diseases such as obesity and diabetes are. Also, a positive correlation is suggested in the literature between breastfeeding duration and the child's cognitive development in later childhood[3].

The national health policy in Türkiye also emphasizes the importance of breastfeeding. According to the Türkiye Health Survey (THS) of the Turkish Statistical Institute (TurkStat), breastfeeding practices among children aged 0-14 years old provide critical insights into the present state of child health in the country[4]. However, the prevalence of exclusive breastfeeding is still below the global targets[5,6]. Socio-economic, cultural, and health system-related factors influence the rates of breastfeeding[7]. An understanding of the effect that breastfeeding duration has on child health outcomes in the context of Türkiye can contribute to developing targeted interventions that improve these rates and promote child health.

This study aimed to examine the association between breastfeeding duration and health outcomes among children aged 1-2 years using data from THS in order to provide evidence-based recommendations that may improve the effectiveness of public health programs and contribute to increasing breastfeeding rates in Türkiye.

MATERIALS AND METHODS

Source of Data

This study relies on the TurkStat, THS-2022 data, which was collected in cross-sectional survey design research. TurkStat conducts survey in every 3 years. Approval for the use of anonymized secondary data from the Turkish Statistical Institute Presidency, Department of Information Distribution and Communication dataset was obtained (decision no: 27964695-622.03-E.9930, date: 20.04.2018). This study was conducted under the ethical standards outlined in the Declaration of Helsinki. THS-2022 used the sampling frame obtained from updated version of the National Address Database in August 2022. The sample size was calculated by applying a stratified two-stage cluster sampling method with the external stratification criterion being the urban-rural distinction. In the first stage, clusters (blocks) consisting of approximately 100 addresses were selected randomly using a probability proportional to size approach. In the second stage, systematic random sampling was employed to select household addresses from each chosen block. A total of 1.117 clusters have been identified, from which 10 household addresses were selected per cluster, resulting in 11.170 household addresses across Türkiye[4].

Variables

In the TurkStat questionnaires, the health conditions of the participants in the previous six months were investigated. In this study, the duration of breastfeeding was determined by the number of months the participants had breastfed. To evaluate the outcomes of breastfeeding for a minimum of six months and a minimum of one year during the infantile period, we used the data of infants between 12 and 23 months of age in the THS survey for "0-14 age group". Independent variable of the study was the duration of breastfeeding in months. Breastfeeding duration was categorized into two different variables: Breastfeeding at least 6 months and 12 months. Dependent variables consist of gender, perceived general health status, and oral and dental health status along with the past 6 months' occurrence of chronic conditions, restriction in daily life, communicable diseases, upper respiratory tract infections (URTI), lower respiratory tract infections (LRTI), acute gastroenteritis (AGE) and urinary tract infections (UTI). "general health status" and "oral and dental health status" were 5-item Likert scale questions and "restriction in daily life" was a 3-item question. The variable definitions can be found in the Supplementary Table.

Statistical Analysis

Data were analyzed by using IBM SPSS Statistics version 29.0. Descriptive statistics were used to summarize the baseline characteristics (gender, general health status, oral and dental health status), breastfeeding duration, and health outcomes of the children. Frequencies and percentages were calculated for categorical variables, while means and standard deviations were calculated for continuous variables. The Shapiro-Wilk test and graphical analyses were used to evaluate the conformity of continuous variables to normal distribution. The Mann-Whitney U test was used to compare continuous variables that did not show normal distribution between two groups, and the t-test was used for comparisons of continuous variables that conformed to normal distribution. The Fisher-Freeman-Halton test and Pearson chi-square test were employed to examine the associations between breastfeeding duration categories and acute (e.g., respiratory infections, diarrhoea) and chronic illnesses. General health status and oral and dental health status variables were dichotomized in chi-square analysis, in which a p-value of less than 0.05 was considered statistically significant.

RESULTS

The study included data from 343 infants aged between 12 and 23 months, sourced from THS-2022 data. Among the infants, 55.1% were female. The mean breastfeeding duration was 12.39 (±6.3) months [median: 13, minimum: 0, maximum: 24, interquartile range (IQR): 8-17 months], and the rate of infants aged between 12-24 months and breastfed for at least six months was 83%, and it was 65% for those breastfed for at least 12 months. The general health status of 93.6% and oral health status of 96.5% of the infants was good. The rate of disease lasting longer than 6 months (chronic disease) was 8.5%, and 8.7% of the infants had experienced restrictions in daily life due to an illness in the last 6 months. According to the questions about acute infectious diseases in the last 6 months, 3.2% of the infants had communicable disease

Table 1. Descriptive characteristics of breastfeeding and health outcome data by gender

		Female		Male		Total	
		n	%	n	%	n	%
Breastfeeding ≥6 months	Yes	152	80.4	132	85.7	284	82.8
	No	37	19.6	22	14.3	59	17.2
Breastfeeding ≥12 months	Yes	120	63.5	103	66.9	223	65.0
	No	69	36.5	51	33.1	122	35.0
General health status	Good	176	93.1	145	94.2	321	93.6
	Fair	10	5.3	5	3.2	15	4.4
	Bad	3	1.6	4	2.6	7	2.0
Oral and Dental health status	Good	182	96.3	149	96.6	331	96.5
	Fair	6	3.2	4	2.6	10	2.9
	Bad	1	0.5	1	0.6	2	0.6
Chronic disease	Yes	14	7.4	15	9.7	29	8.5
	No	175	92.6	139	90.3	314	91.5
Restriction in daily life	Yes	15	7.9	15	9.7	30	8.7
	No	174	92.1	139	90.3	313	90.3
Communicable disease history*	Yes	5	2.6	6	3.9	11	3.2
	No	184	97.4	148	96.1	332	96.8
URTI	Yes	22	11.6	25	16.2	47	13.2
	No	167	88.4	129	83.8	296	86.3
LRTI	Yes	12	6.3	13	8.4	25	7.3
	No	177	93.7	141	91.6	318	92.7
AGE	Yes	68	36.0	62	40.3	130	37.9
	No	121	64.0	92	59.7	213	62.1
UTI	Yes	5	2.6	4	2.6	9	2.6
	No	184	97.4	150	97.4	334	97.4

URTI: Upper respiratory tract infections, LRTI: Lower respiratory tract infections, AGE: Acute gastroenteritis, UTI: Urinary tract infections, THS-2022: Türkiye Health Survey-2022, *In the THS-2022 questionnaire, the communicable disease history question was designed to determine the history of infectious diseases, such as chicken pox, mumps, etc

(such as varicella or mumps), 13.7% had URTI, 7.3% had LRTI, 37.9% had AGE, and 2.6% had UTI. No gender differences were found in the assessment of health outcomes. Descriptive characteristics of breastfeeding and health outcome data by gender are shown in Table 1.

In the assessments of health outcomes regarding breastfeeding durations, infants general health status was "good" for 86.4% of infants who were breastfed for less than 6 months, whereas this rate was 95.1% for infants who were breastfed for at least 6 months, and the difference was statistically significant (p=0.021). The rates of chronic disease (disease lasting longer than 6 months) and restriction in daily life were higher in infants breastfed for less than 6 months (p=0.042 and p=0.014, respectively). There was no significant difference in the oral-dental health status of children aged 12-24 months in relation to the duration of breastfeeding. The rates of communicable diseases (such as rubella, mumps, etc.) and LRTI history were higher in infants who were breastfed for less than six months (p=0.026 and p=0.010, respectively). No statistically significant difference was observed in URTI, AGE and UTI history rates (p=0.652, p=0.851, p=0.624, respectively). The comparison of health outcomes according to breastfeeding for at least 6 months are presented in Table 2.

Higher rates of good general health and oral-dental health status were found in infants who were breastfed for at least 12 months, but the difference was not significant. There was no difference between breastfeeding for at least 12 months and chronic disease and restriction in daily life (Table 3). No statistically significant difference was observed in the breastfeeding duration of children regarding general health status, oral-dental health status and chronic disease history (p=0.190, p=0.144 and p=0.323, respectively), but the median breastfeeding duration in infants with a history of restrictions in daily life was longer (11.5 vs. 13 months, p=0.019). The mean differences in breastfeeding duration according to the health status of the children were presented in Table 4.

In the second year of life, the median (IQR) duration of breastfeeding in children with a history of communicable disease in the previous six months was 6 (10) months, while the median duration in infants without a history of infectious disease was 13 (10) months (p=0.005). No statistically significant difference was observed in the breastfeeding duration of children with a history of URTI, LRTI, AGE and UTI (p=0.951, p=0.198, p=0.836, p=0.816, respectively). Infants who were breastfed for less than 12 months had higher rates of communicable disease, URTI, LRTI AGE and UTI history (p=0.047, 0.855, p=0.064, p=0.904, and p=0.390, respectively), but the difference was significant only in communicable disease history. The comparison of the health outcomes according to breastfeeding duration is presented in Table 3.

Table 2. The comparison of health outcomes among infants aged 12-24 months according to breastfeeding at least 6 months of age

		Breastfeeding <6 months		Breastfeeding ≥6 months		p-value
		n	%	n	%	
General health status	Good	51	86.4	270	95.1	**0.021***
	Fair/Bad	8	13.6	14	4.9	
Oral and Dental health status	Good	56	94.9	275	96.8	0.341*
	Fair/Bad	3	5.1	9	3.2	
Chronic disease	Yes	9	15.3	20	7.0	**0.042***
	No	50	84.7	264	93.0	
Restriction in daily life	Yes	10	16.9	20	7.0	**0.014**
	No	49	83.1	264	93.0	
Communicable disease history	Yes	5	8.5	6	2.1	**0.026***
	No	54	91.5	278	97.9	
URTI history	Yes	7	11.9	40	14.1	0.652
	No	52	88.1	240	85.9	
LRTI history	Yes	9	15.3	16	5.6	**0.010**
	No	50	84.7	268	94.4	
AGE history	Yes	23	39.0	107	37.7	0.851
	No	36	61.0	177	62.3	
UTI history	Yes	1	1.7	8	2.8	0.624
		58	98.3	276	97.2	

URTI: Upper respiratory tract infections, LRTI: Lower respiratory tract infections, AGE: Acute gastroenteritis, UTI: Urinary tract infections, *Fischer exact test

Table 3. The comparison of health outcomes rates among infants aged 12-24 months according to breastfeeding at least 12 months of age

		Breastfeeding <12 months		Breastfeeding ≥12 months		p-value
		n	%	n	%	
General health status	Good	109	90.8	212	95.1	0.127
	Fair/Bad	11	9.2	11	4.9	
Oral and Dental health status	Good	113	94.2	218	97.8	0.081*
	Fair/Bad	7	5.8	5	2.2	
Chronic disease	Yes	13	10.8	16	7.2	0.245
	No	107	89.2	207	92.8	
Restriction in daily life	Yes	15	12.5	15	6.7	0.071
	No	105	87.5	208	93.3	
Communicable disease history	Yes	7	5.8	4	1.8	**0.047***
	No	113	94.2	219	98.2	
URTI history	Yes	17	14.2	30	13.5	0.855
	No	103	85.8	193	86.5	
LRTI history	Yes	13	10.8	11	5.4	0.064
	No	107	89.2	211	94.6	
AGE history	Yes	46	38.3	84	37.7	0.904
	No	74	61.7	139	62.3	
UTI history	Yes	4	3.3	5	2.2	0.390*
	No	116	96.7	218	97.8	

URTI: Upper respiratory tract infections, LRTI: Lower respiratory tract infections, AGE: Acute gastroenteritis, UTI: Urinary tract infections, *Fischer exact test

Table 4. Mean differences in breastfeeding duration according to the health status of the children (n=343)

		Breastfeeding duration				
		Mean	Standard deviation	Median	IQR	p-value
General health status	Good	12.52	0.35	13	9	0.190
	Fair/Bad	10.59	1.55	11	22	
Oral and Dental health status	Good	12.48	0.35	13	9	0.144
	Fair/Bad	10	1.75	10.5	9	
Chronic disease	Yes	11.38	7.60	12	14	0.323
	No	12.49	6.22	13	9	
Restriction in daily life	Yes	9.70	1.25	11.5	8	**0.019**
	No	12.65	0.35	13	10	
Communicable disease history	Yes	7.09	1.72	6	10	**0.005**
	No	12.57	0.35	13	10	
URTI history	Yes	12.36	0.83	12	9	0.951
	No	12.40	0.38	13	9	
LRTI history	Yes	10.72	1.53	11	15	0.198
	No	12.53	0.35	13	9	
AGE history	Yes	12.16	0.55	13	11	0.836
	No	12.54	0.44	13	8	
UTI history	Yes	12.56	2.12	15	11	0.816
	No	12.39	0.35	13	9	

URTI: Upper respiratory tract infections, LRTI: Lower respiratory tract infections, AGE: Acute gastroenteritis, UTI: Urinary tract infections, IQR: Iterquartile range

DISCUSSION

The present study found that breastfeeding for at least 6 months and long-term breastfeeding (at least 12 months) in children from different segments of Türkiye was effective in reducing the rates of infectious diseases and LRTIs in children aged 1-2 years. The findings are consistent with the recommendations of the World Health Organization, Ministries of Health, and many child health organizations, which emphasize the protective effects of breastfeeding on child health[3,8-11].

In the study, mothers who breastfed their babies for six months and above had significantly better-perceived health of their children. This finding is in accordance with established literature showing that breastfeeding promotes overall health status and further reduces child morbidity worldwide[3]. There is a growing body of evidence showing that breastfeeding might play a protective role against chronic diseases such as asthma, obesity, hypertension, dyslipidemia, and type II diabetes mellitus[12-14]. It has been suggested that the anti-inflammatory and immunomodulatory effects of breastfeeding may explain the reduced prevalence of chronic diseases[11]. Duration-dependent protective associations have been shown between breastfeeding and chronic diseases such as childhood asthma, diabetes and rheumatoid arthritis[2,12,14]. In our study, we have shown a similar finding that breastfeeding for less than six months was significantly associated with higher rates of chronic diseases and restrictions in daily life due to illness. While this association can be attributed to the shortness of breastfeeding duration, it can also be possible that existing chronic illness and restrictions in daily life might have limited the breastfeeding process. Nevertheless, our finding shows the importance of breastfeeding during the first six months of life in improving health outcomes.

In this study, mothers who breastfed for more than 12 months showed a higher level of perceived health status of their child. However, the observed difference was not statistically significant. The loss of statistical significance may reflect the influence of other factors, such as environmental exposures, on child health beyond the first year, which can diminish the positive view of the parents on their children's health status[15,16]. It is also possible that mothers who have breastfed for less than six months may have more negative perceptions of their infants' health status because of the well-known recommendation of breastfeeding duration of up to two years. Breastfeeding for at least 12 months showed a similar trend towards less chronic disease and daily restriction history, with no statistical significance. A literature review revealed a paucity of studies investigating the associations between prolonged breastfeeding (>1 year) and infants' health and well-being. Therefore, further studies applying a variety of methodologies, such as prospective, mixed-method, or cross-cultural, are required to rigorously investigate the issue[17].

Breast milk provides the infant with essential nutrients and immunological components that strengthen the immune system and protect the infant from various infectious diseases[18,19]. Li et al.[20] showed that breastfeeding beyond six months was associated with a lower risk of infections even up to six years of age, reinforcing the importance of sustained breastfeeding for long-term health benefits. Similar findings have been reported by Victora et al.[3], where longer durations of breastfeeding were associated with a lower incidence of child infection, especially in early childhood. These beneficial effects have been documented in developed and less developed countries[10,21]. Our findings also showed a significant reduction in communicable disease history in children breastfed for at least six months. This aligns with the World Health Organization's recommendation that exclusive breastfeeding for the first six months of life enhances immune function and reduces the risk of infectious diseases[1].

LRTIs represent a significant public health concern, accounting for a considerable proportion of hospitalizations among infants and children[4]. A positive correlation has been identified in the literature between the use of the formula and an increased incidence of hospitalization due to LRTI[22]. In our study, extended breastfeeding was particularly associated with lower rates of LRTIs. Infants who breastfed for more than 6 months had a significantly lower rate of LRTIs than for shorter periods. In line with these findings, the study by Potharajula and Kadke[23] demonstrated a correlation between breastfeeding and a reduced risk of respiratory issues, including wheezing in children, further supporting the protective role of breast milk against respiratory infections. A study by Abdulla et al.[10] investigated the association between Respiratory Syncytial virus (RSV)-associated LRTI and breastfeeding and found that not breastfeeding infants was associated with a significant risk of severe RSV associated LRTI and hospitalisation.

Although breastfeeding duration was found to be shorter in those with a history of AGE, this difference was not statistically significant in our study. The analysis revealed no association between the history of UTI and the duration of breastfeeding. It is possible that the results are influenced by the fact that the questionnaire inquired about the infection history over the past six months rather than severity such as information about hospitalizations, duration of stay in hospital etc. Nevertheless, the existing literature examining the relationship between AGE and UTI and breastfeeding focuses on hospitalizations due to these infections. It has been documented that breastfeeding contributes to a reduction in hospitalizations due to AGE and UTI[3,8,24].

The breastfeeding rates observed in this study are, on the whole, relatively high according to another national study, the

Türkiye Demographic and Health Survey 2018, or international rates; however, they still do not meet the global breastfeeding targets of 80 % by 2030, concerning children breastfed at 1 year of age[6,11]. As indicated by the Global Breastfeeding Scorecard (2019), there is a need for increased commitment to breastfeeding through enhanced funding and improved policies and programmes[5]. In Türkiye, socio-economic, cultural, and systemic barriers play a significant role in the relatively low rates of exclusive breastfeeding. Maternal education, employment and support significantly influence breastfeeding duration in Türkiye, suggesting that increasing community-level support could enhance breastfeeding practices[25-27]. Baker et al.[7] emphasize the role of structural supports, such as parental leave policies and public awareness campaigns, in promoting breastfeeding, which could help increase these rates in Türkiye as well. Integrating breastfeeding support into the national health strategy may help improve child health indicators by increasing exclusive breastfeeding rates and extending breastfeeding duration.

Study Limitations

Our study has certain limitations. Firstly, the cross-sectional design of the study limits the capacity of the research to assert causal relations. Secondly, the survey questionnaire was designed by the TurkStat, and the quality of the questions sometimes limited our understanding of the phenomenon under investigation. For example, the dataset included two variables that overlapped (the past 6 months' history of communicable diseases and infectious diseases). Therefore, we only used the communicable disease variable to avoid confusion. Also, we could not show a relationship based on age due to the measurement of age based on years rather than months in the dataset. Another issue with the survey was that the child questionnaire of the dataset was not linked with the household questionnaire, which also limited our analysis regarding regional or socio-economic status. Lastly, the information on disease history was based on parental reports, which may have introduced a degree of subjectivity. Nevertheless, the dataset is a representative survey of Türkiye, which is one of the strong aspects of this study.

CONCLUSION

Our study highlights the significant benefits of breastfeeding, particularly breastfeeding during the first six months of life was associated with improved infant health outcomes and the history of reduced burden of both acute and chronic diseases in early childhood. Infants breastfed for at least six months exhibited better general health, fewer chronic illness histories and restrictions in daily life, and a lower incidence of communicable and LRTI than those breastfed for shorter durations. The results align with global evidence on the protective effects of breastfeeding, reinforcing the importance of promoting breastfeeding through public health initiatives.

Ethics

Ethics Committee Approval: Approval for the use of anonymized secondary data from the Turkish Statistical Institute Presidency, Department of Information Distribution and Communication dataset was obtained (decision no: 27964695-622.03-E.9930, date: 20.04.2018). This study was conducted under the ethical standards outlined in the Declaration of Helsinki.

Informed Consent: This study relies on the TurkStat, Türkiye Health Survey-2022 (THS-2022) data, which was collected in cross-sectional survey design research.

Footnotes

Authorship Contributions

Concept: Ö.Ö.A., Design: Ö.Ö.A., İ.K., Data Collection or Processing: İ.K., Analysis or Interpretation: Ö.Ö.A., İ.K., Literature Search: Ö.Ö.A., İ.K., Writing: Ö.Ö.A., İ.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. World Health Organization. Breastfeeding. 2023. Access date: 2024, December. Available from: https://www.who.int/health-topics/breastfeeding#tab=tab_1
2. Hossain S, Mihrshahi S. Exclusive breastfeeding and childhood morbidity: a narrative review. International journal of environmental research and public health 2022;19:14804. Available from: https://www.mdpi.com/1660-4601/19/22/14804
3. Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387:475-90.
4. Turkish Statistical Institute (TÜİK) (2023). Available from: https://data.tuik.gov.tr/Bulten/Index?p=Turkiye-Saglik-Arastirmasi-2022-49747
5. World Health Organization. Global breastfeeding scorecard, 2019: increasing commitment to breastfeeding through funding and improved policies and programmes. World Health Organization. Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-19.22
6. Hacettepe University Institute of Population Studies. (2018). Turkey Demographic and Health Survey. Nutrition of Children and Women. Ankara, Turkey: T.R. Presidency of Turkey Directorate of Strategy and Budget and TÜBİTAK 2019;145-6.
7. Baker P, Smith JP, Garde A, Grummer-Strawn LM, Wood B, Sen G, et al. The political economy of infant and young child feeding: confronting corporate power, overcoming structural barriers, and accelerating progress. Lancet. 2023;401:503-24.
8. Christensen N, Bruun S, Søndergaard J, Christesen HT, Fisker N, Zachariassen G, et al. Breastfeeding and infections in early childhood: a cohort study. Pediatrics. 2020;146:20191892.

9. Mineva GM, Purtill H, Dunne CP, Philip RK. Impact of breastfeeding on the incidence and severity of respiratory syncytial virus (RSV)-associated acute lower respiratory infections in infants: a systematic review highlighting the global relevance of primary prevention. BMJ Global Health 2023;8:e009693.

10. Abdulla F, Hossain MM, Karimuzzaman M, Ali M, Rahman A. Likelihood of infectious diseases due to lack of exclusive breastfeeding among infants in Bangladesh. PLoS One. 2022;17:0263890.

11. North K, Gao M, Allen G, Lee AC. Breastfeeding in a global context: epidemiology, impact, and future directions. Clin Ther. 2022;44:228-44.

12. Wilson K, Gebretsadik T, Adgent MA, Loftus C, Karr C, Moore PE, et al. The association between duration of breastfeeding and childhood asthma outcomes. Ann Allergy Asthma Immunol. 2022;129:205-11.

13. Kelishadi R, Farajian S. The protective effects of breastfeeding on chronic non-communicable diseases in adulthood: A review of evidence. Adv Biomed Res. 2014;3:3.

14. Alotiby AA. The role of breastfeeding as a protective factor against the development of the immune-mediated diseases: A systematic review. Front Pediatr. 2023;11:1086999.

15. Breton CV, Landon R, Kahn LG, Enlow MB, Peterson AK, Bastain T, et al. Exploring the evidence for epigenetic regulation of environmental influences on child health across generations. Commun Biol. 2021;4:769.

16. Maurage C. Children's nutrition and health in adulthood. Appetite. 2008;51:22-4.

17. Lackey KA, Fehrenkamp BD, Pace RM, Williams JE, Meehan CL, McGuire MA, et al. Breastfeeding Beyond 12 Months: Is There Evidence for Health Impacts? Annu Rev Nutr. 2021;41:283-308.

18. Caba-Flores MD, Ramos-Ligonio A, Camacho-Morales A, Martínez-Valenzuela C, Viveros-Contreras R, Caba M. Breast milk and the importance of chrononutrition. Front Nutr. 2022;9:867507.

19. Lawrence RA. Biochemistry of human milk. breastfeeding: a guide for the medical profession. Philadelphia: Elsevier. 2022;9:93-144.

20. Li R, Dee D, Li C-M, Hoffman HJ, Grummer-Strawn LM. Breastfeeding and risk of infections at 6 years. Pediatrics. 2014;134:13-20.

21. Ware JL, Li R, Chen A, Nelson JM, Kmet JM, Parks SE, et al. Associations between breastfeeding and post-perinatal infant deaths in the US. Am J Prev Med. 2023;65:763-74.

22. McNiel ME, Labbok MH, Abrahams SW. What are the risks associated with formula feeding? A re-analysis and review. Birth. 2010;37:50-8.

23. Potharajula S, Kadke A. Study of correlation between breast feeding and wheezing in children. Int J Contemp Pediatr 2019;6:191-4.

24. Ajetunmobi OM, Whyte B, Chalmers J, Tappin DM, Wolfson L, Fleming M, et al. Breastfeeding is associated with reduced childhood hospitalization: evidence from a Scottish Birth Cohort (1997-2009). J Pediatr. 2015;166:620-5.

25. Yılmaz T, Dinç H, Günaydın S, Kaçar N. Breastfeeding in Turkey: a systematic review. Clin Exp Health Sci. 2021;11:604-16.

26. Göktepe ST, Öztürk O. Duration of mothers breastfeeding and possible factors affecting it. TJFMPC. 2023;17:470-8.

27. Kök H, Şenol E, Keskindemirci G, Selver MB, Gökçay G. Breastfeeding rates among 4 and 6 month-old Çocuk Dergisi- Journal of Child 2020;20:43-47.

Supplementary Table. The contents of the questions and answers

Question contents	Answers
Gender	Male/Female
Age	..... (in month)
Breastfeeding duration	..... (in month)
Description of the child's health in general	Very good/Good/Fair/Bad/Very Bad
Any longstanding (at least 6 months or more) illness or health problem	Yes/No
Limitation because of a health problem in activities most children of the same age usually do	Severely limited/Limited, but not severely/Not limited at all
Description of the child 's oral and dental health	Very good/Good/Fair/Bad/Very bad
History of any communicable diseases (varicella, mumps etc.) in the past six months	Yes/No
Any upper respiratory tract infection (tonsillitis, middle ear infections, pharyngitis) history in the past six months	Yes/No
Any lower respiratory tract infection (pneumonia) history in the past six months	Yes/No
Any diarrhea history in the past six months	Yes/No
Any urinary tract infection history in the past six months	Yes/No

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A Novel Approach for Arteriovenous Fistula Maturation; Effects of Melatonin Loaded PLGA Nanofibers in Rats

Deneysel Arteriyovenöz Fistüller için Melatonin İçeren Matriks Geliştirilmesi ve Etkinliğinin Araştırılması

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ABSTRACT

Aim: Arteriovenous fistula (AVF) is a cannulation method that is accessed by a peripheric vein and an artery. AVF provides vascular access for chronic kidney disease patients so they can receive hemodialysis. AVF could be created by surgical intervention and facilitates arterial to venous circulation for rapid recovery. However, AVF maturation depends on venous proliferation and luminal diameter which allows the optimum flow rate for continuing circiulation and hemodialysis. Due to multiple unexpected conditions, non-maturation of AVFs limits the efficacy of the hemodialysis so patients must receive another surgery for AVF cannulation.

Materials and Methods: In this study, we aimed to utilize the effects of melatonin (MT), which is known to have antioxidant, anti-inflammatory, and antiapoptotic effects, to provide longer and more effective use of AVFs via a novel technique. For this purpose, firstly by electrospinning method, polylactic-co-glycolic acid (PLGA) nanofiber membranes were developed. After MT is loaded into the PLGA and characterized. Biodegradation and drug release profiles were analyzed. An *in vivo* study was performed in Wistar Albino male rats (n=18). Rats were randomly divided into three experimental groups; Sham, PLGA, and MT/PLGA respectively (n=6). AVF model was established in all groups between arteria carotica and vena jugularis under general anesthesia. The Sham group did not receive any treatment or biomaterial application. The developed membranes were placed onto the AVFs in PLGA and MT/PLGA groups. All rats were sacrificed on the 28th of the experiment. The anastomosis sites of all animals were harvested for histopathological analysis.

Results: Our results showed MT/PLGA group indicated increased maturation levels conmpared to Sham group ($p<0.05$).

Conclusion: The results showed that PLGA/MEL may be a promising material for early AVF maturation.

Keywords: Arteriovenous fistula, melatonin, PLGA, biocompatible materials, maturation, venous proliferati

ÖZ

Amaç: Arteriyovenöz fistül (AVF), periferik bir ven ve bir arterden erişilen bir kanülasyon yöntemidir. AVF, kronik böbrek hastalığı hastalarının hemodiyaliz alabilmeleri için vasküler erişim sağlar. AVF cerrahi müdahale ile oluşturulabilir ve hızlı iyileşme için arteriyel-venöz dolaşımı kolaylaştırır. Ancak AVF'nin olgunlaşması, sirkülasyonun ve hemodiyalizin devamı için optimum akış hızına izin veren venöz proliferasyona ve lümen çapına bağlıdır. Birçok beklenmedik durum nedeniyle, AVF'lerin olgunlaşmaması hemodiyalizin etkinliğini sınırlar, bu nedenle hastaların AVF kanülasyonu için başka bir ameliyat olması gerekir.

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Gereç ve Yöntem: Bu çalışmada, antioksidan, anti-enflamatuvar ve anti-apoptotik etkileri olduğu bilinen melatoninin (MT) etkilerinden yararlanarak yeni bir teknikle AVF'lerin daha uzun süre ve daha etkin kullanımını sağlamayı amaçladık. Bu amaçla ilk olarak elektrospinning yöntemi ile polilaktik-ko-glikolik asit (PLGA) nanofiber membranlar geliştirilmiştir. Daha sonra PLGA içine MT yüklenmiş ve karakterize edilmiştir. Biyobozunum ve ilaç salınım profilleri analiz edilmiştir. Wistar Albino erkek sıçanlarda (n=18) bir *in vivo* çalışma gerçekleştirilmiştir. Sıçanlar rastgele üç deney grubuna ayrılmıştır; sırasıyla Sham, PLGA ve MT/PLGA (n=6). Tüm gruplarda genel anestezi altında karotis arter ve jugular ven arasında AVF modeli oluşturulmuştur. Sham grubuna herhangi bir tedavi veya biyomateryal uygulaması yapılmadı. Geliştirilen membranlar PLGA ve MT/PLGA gruplarındaki AVF'lerin üzerine yerleştirildi. Tüm sıçanlar deneyin 28. gününde sakrifiye edildi. Tüm hayvanların anastomoz bölgeleri histopatolojik analiz için toplandı.

Bulgular: Sonuçlarımıza göre, MT/PLGA grubunda histopatolojik olarak vasküler proliferasyon ve fibroblastik hücre çoğalması açısından değerlendirildiğinde matürasyon düzeyinin Sham grubuna göre anlamlı düzeyde arttığı belirlendi (p<0,05).

Sonuç: Elde ettiğimiz veriler MT/PLGA nanoliflerinin erken AVF olgunlaşması için umut verici bir materyal olabileceğini göstermiştir.

Anahtar Kelimeler: Artetiyovenöz fistül, melatonin, PLGA, biyouyumlu materyaller, matürasyon, venöz proliferasyon

INTRODUCTION

Chronic kidney disease (CKD) treatment in the early stages is primarily aimed at preserving the existing kidney function and includes methods such as diet, adequate fluid intake, medical treatments such as the use of angiotensin inhibitors, lifestyle changes, and increased physical activity. In advanced renal failure, hemodialysis and peritoneal dialysis are the main treatments applied, while kidney transplantation is the last resort for end-stage renal failure patients[1]. According to the Global Diagnosis Burden 2017 data, CKD affects approximately 700 million people worldwide. Due to the extreme increase in the number of patients and the shortage of organ donors, CKD patients can only survive with hemodialysis[2]. Hemodialysis is indispensable for CKD patients to balance minerals in circulating blood and control blood pressure[3]. For long-term hemodialysis, patients need permanent vascular access established between arteries in a venous line, called an arteriovenous fistula (AVF). AVF is the most common cannulation method for long-term hemodialysis patients[4]. Primary maturation failure is the main limiting factor for AVF use due to internal remodeling and lumen stenosis. Previous studies have claimed that vein luminal diameter below 2 mm is prone to deterioration. In addition, it is widely accepted that the vein diameter should be higher than the anastomotic artery diameter[5]. Therefore, early maturation is urgent for long-term use of AVF. Otherwise, patients have to undergo several surgeries to have another AVF cannulation for hemodialysis treatment.

The immaturity of AVFs is mostly due to thickening of the vascular intima due to underlying pathological mechanisms consisting of oxidative stress, inflammation or hypoxia, which leads to abundant migration and proliferation of smooth muscle cells and accumulation of extracellular matrix[6]. Studies have reported that AVF failure mostly occurs in the early weeks after surgery[7]. At approximately 4 to 6 weeks, if blood flow is less than 500 mL/min. and the vessel diameter is less than 4 mm, urgent reoperation is required to reestablish a new AVF access[8]. Therefore, establishing successful AVF cannulation as well as ensuring AVF maturity are essential for long-term hemodialysis. Despite the use of improved surgical techniques and new pharmacotherapy agents, there is no gold standard method to prevent AVF failure. Therefore, in this study, we aimed to prevent early maturation of AVF and avoid stenosis to prevent insufficient blood flow between artery and vein.

At this point, tissue engineering offers promising results for the treatment of various pathologies with its biocompatibility properties[9]. Synthetic polymers are the most widely used carriers in the field of tissue engineering studies. Polylactic-co-glycolic acid (PLGA) is one of the preferred synthetic polymers for carrying various agents, cells or molecules. It consists of lactic acid and glycolic acid that can activate cell migration, proliferation and adhesion[10]. Due to its biocompatibility and biodegradability, PLGA provides beneficial effects for tissue healing or regeneration[11]. In our study, the effects of melatonin (MT) loaded PLGA nanofibers on AVF maturation were investigated. MT loaded PLGA biomaterials are frequently preferred in the field of tissue engineering. Since MT and PLGA are Food and Drug Administration (FDA)-approved molecules, it is stated that they are quite suitable for clinical translation[12-14]. In addition, the anti-inflammatory and antioxidant properties of MT provide a treatment-enhancing feature in the treatment of many pathogenesis[15]. On the other hand, the feature of PLGA, which mimics the extracellular matrix of cells, strengthens this synergy and provides various positive results in experimental studies[16]. MT is a hormone secreted from the pineal gland, with a hydrophilic structure that allows all cell compartments to show their effects. The antioxidant and anti-inflammatory effects of MT are well known[17,18]. In addition, MT has healing effects on the cardiovascular system by reducing iNOS and eNOS synthesis and increasing angiogenesis[19]. Controlled release studies of MT have gained importance in recent years 15. The reasons for this may be that oral or parenteral use of MT limits its effect in the first pass through the liver and does not provide sufficient bioavailability[20].

In an experimental abdominal adhesion model where MT-loaded PLGA nanofibers were applied, it was reported that

MT-loaded nanofibers prevented adhesion in the early period and reduced inflammatory cell infiltration and fibrosis[21]. In another study investigating the effects of MT-loaded PLGA nanoparticles on radiation-induced lung injury, it was reported that they reduced lung inflammation and apoptosis in rats[22]. In an experimental *in vivo* study in which MT-loaded PLGA biomaterials were applied on carbon tetrachloride-induced liver injury, it was observed that MT exhibited protective effects on the liver and reduced the adverse effects of toxicity[23].

In this study, the effects of MT on the maturation of AVF were investigated by controlled release application to the anastomosis region where AVF was created. To our knowledge, this is the first study on the controlled local release of MT-loaded PLGA to the AVF region.

MATERIALS AND METHODS

Production of Biomaterial

Production of PLGA Nanofibers

PEG6000 was used to optimize PLGA nanofibers to achieve optimum biodegradation. For this purpose, nanofibers were developed by electrospinning at 0.8 mL/h flow rate, 20 cm distance and 20 kV voltage 21. The electrospinning method used for this purpose is spraying the prepared solution onto a collector surface known as Taylor Cone with the help of an injector and obtaining nanofiber membranes with a thickness of less than approximately 1 mm[24]. All preparation procedures were carried out under red light to prevent MT weight loss.

Production of MT Loaded PLGA Nanofibers

The MT dose loaded into the nanofibers was started as 50% MT by weight of the PLGA nanofibers and continued until a homogeneous fiber distribution was obtained. The MT dose in the final composition was optimized to be 1 mg MT in the 1x3 cm rectangular material to be applied to rats.

Determination of Biodegradation Profile of MT Loaded Nanofibers

The biodegradation of MT-loaded PLGA nanofibers was optimized to undergo at least 90% biodegradation in 28 days to provide early AVF maturation. For this purpose, the prepared MT-loaded PLGA nanofibers were first incubated in 10 mL phosphate-buffered saline (PBS) (0.1M, pH 7.4). The material was followed up to 30 days to plot the biodegradation curve. PBS was replaced with new solution every 3 hours. All samples were weighed every 24 hours and weight loss was calculated as percentage (%)[25].

Determination of Drug Release Profile

For drug release evaluation, MT-loaded PLGA nanofibers were exposed to 50 ml of pH 7.4 PBS. MT release was monitored at 300-190 nm wavelength with UV spectrophotometer (Shimadzu, Japan) for 3 h periods[26].

In vivo Experiments

Animal Material

All experimental studies were carried out in the Experimental Research Application and Research Center of Çanakkale Onsekiz Mart University. Eighteen Wistar Albino male rats (3-4 months old; 250-300 g) were used in this study. Rats were housed at 22±2 °C, 12 h dark/light cycle. Rats were fed ad libitum and had free access to water. Ethical approval for this study was obtained from the Local Ethics Committee for Animal Experiments at Çanakkale Onsekiz Mart University (decision number: 2022/01-03, date: 21.01.2022). All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals". Rats were randomly divided into three groups (n=6) as follows;

Sham (n=6): An AVF model was created between the carotid artery and jugular vein. The surgical site and anastomosis line were washed with 100 IU/mL heparin.

PLGA (n=6): After creating the AVF model between the carotid artery and jugular vein, the surgical area and anastomosis line were washed with 100 IU/mL heparin and the anastomosis area was covered with pure PLGA matrix.

MT/PLGA: (n=6): After creating the AVF model between the carotid artery and jugular vein, the surgical site and anastomosis line were washed with 100 IU/mL heparin and then the anastomosis site was covered with pure MT/PLGA matrix.

Surgical Procedure

AVF was created as previously described[27]. Briefly, all rats were anesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (7 mg/kg). After the neck skin was shaved, an incision was made in the right neck. The submandibular tissue and muscles were clamped, and the jugular vein was seen. To prevent massive bleeding, all vascular branches were ligated with 6.0 proline sutures. Then, the jugular vein was irrigated with 100 IU/mL heparin-containing saline solution. The entire surgery was performed with loop goggles. Then, the carotid artery was dissected and clamped. A minimal incision was made at the midpoint of the carotid artery and then connected to the jugular vein end and sutured with 8.0 proline. Blood flow

was observed with an intravascular cannula. In the biomaterial group, 1×3 cm rectangular membranes were covered around the anastomosis (Figure 1). The AVF model was also created in the Sham group, but no biomaterial was given. All rats were then taken to recovery and 0.9% NaCl was applied to prevent hypovolemic shock.

On day 28 of the experiment, all rats were sacrificed by cervical dislocation under general anesthesia. Anastomotic lines were immediately collected for histopathological analysis and kept in 10% buffered formaldehyde until analysis.

Statistical Analysis

Histopathological Analysis

After euthanasia, AVF region samples obtained under general anesthesia were stored in paraformaldehyde for 48 hours. Then, they were washed under water overnight and exposed to increasing alcohol series. Finally, the samples were washed 3 times with toluene for transparency and embedded in paraffin (Slee, MPS, Mainz, Germany). Four µm sections were taken from paraffin blocks and stained with hematoxylin-eosin according to the kit protocol. Stained slides were evaluated with an Olympus BX40 (Olympus, Japan) microscope for vascular proliferation, fibroblastic cell proliferation and fibrosis parameters (0=none, 1=low, 2=moderate, 3=high) by a blinded investigator[28]. Vascular proliferation and fibroblastic cell scores were considered together as maturation scores and were included in statistical evaluation.

The obtained data were analyzed within the scope of SPSS 24.0 package program. Differences between groups were determined by Kruskal-Wallis, and comparisons between two groups were determined by post-hoc Tukey HSD tests. Statistical significance was accepted as $p<0.05$. Data were summarized with mean ± standard deviation (mean ± SD).

RESULTS

Our study findings include material characterization and tissue analysis results performed on the AVF anastomosis line obtained from rats *in vivo*. There was no loss of rats during the entire study.

In vitro and *In vivo* Biodegradation Findings

In vitro biodegradation was approximately 85-90% complete on day 28 of the assay. *In vivo* macroscopic observation showed that the implanted biomaterial was not completely degraded in 2 animals from the PLGA group.

In vitro Drug Release Profile Findings

According to *in vitro* drug release assay, PLGA and MT were released at almost the same timepoint at 220 nm wavelength.

Hematoxylin-Eosin Staining Findings

Vascular proliferation and fibroblastic cell proliferation parameters were evaluated as the level of maturation with hematoxylin-eosin staining results. According to histopathological scoring, a significant increase was determined in the MT/PLGA (3.67±0.25) group compared to the Sham group ($p<0.05$). On the other hand, there was no significant difference between the MT/PLGA group and the PLGA (3.16±0.65) group ($p>0.05$). However, no significant difference was found between the Sham (2.4±0.4) and PLGA groups (Figure 2, Figure 3). The data of these examinations are given in Table 1.

Another parameter examined histopathologically was fibrosis. According to the results obtained, no significant difference was detected in terms of fibrosis between the MT/PLGA (1.25±0.25), PLGA (0.83±0.40) and Sham (0.2±0.2) groups ($p>0.05$).

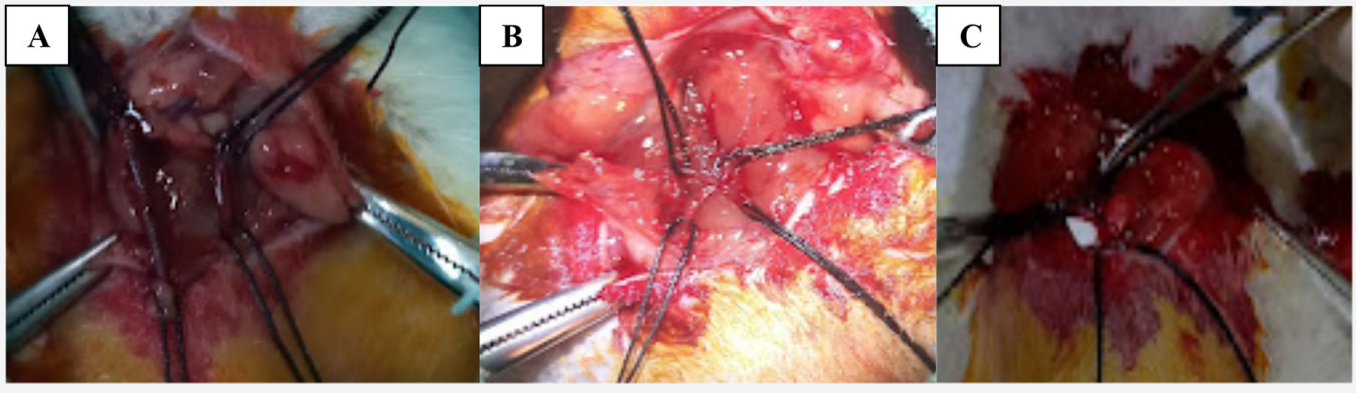

Figure 1. Performing the surgical procedure. (A) Retraction of the jugular vein and carotid artery, (B) performing the AVF, (C) Applying the biomaterial

AVF: Arteriovenous fistula

DISCUSSION

Functional vascular access is the lifeline for patients with terminal CKD. For this purpose, AVF anastomoses are commonly used by creating an arterial and venous junction. However, the tendency for AVFs to fail before and after hemodialysis makes it impossible to provide permanent vascular access[29]. Lack of vascular maturation significantly increases the risk of morbidity in patients with terminal kidney disease[30]. Therefore, in addition to new surgical methods to provide long-term usable and functional vascular access, the urgent need for supportive applications for rapid maturation and its permanence has increased. For this purpose, our study aimed to develop a new method for successful and permanent

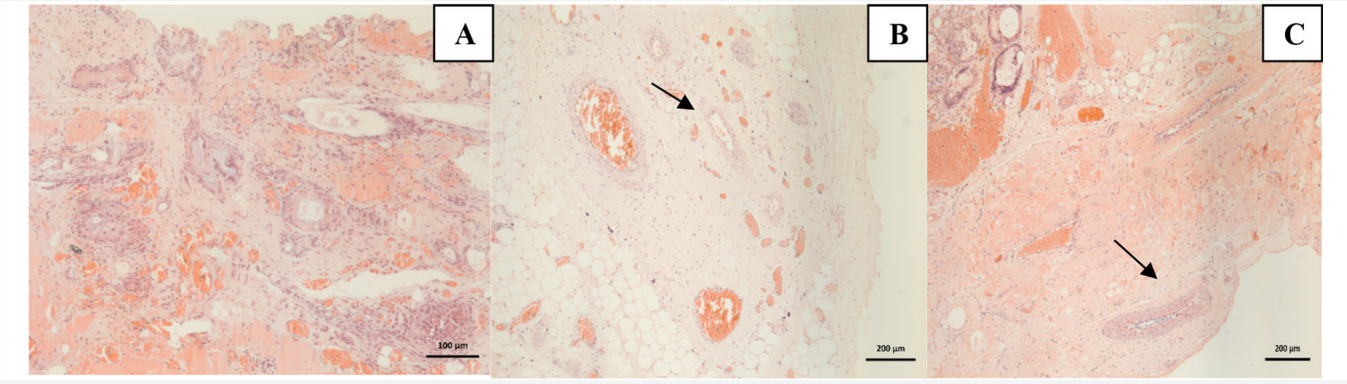

Figure 2. Hematoxylin-eosin staining. (A) Sham, (B) PLGA, (C) MT/PLGA. Results black arrows indicate vessel remodeling and vascular proliferation

MT/PLGA: Melatonin/Polylactic-co-glycolic acid

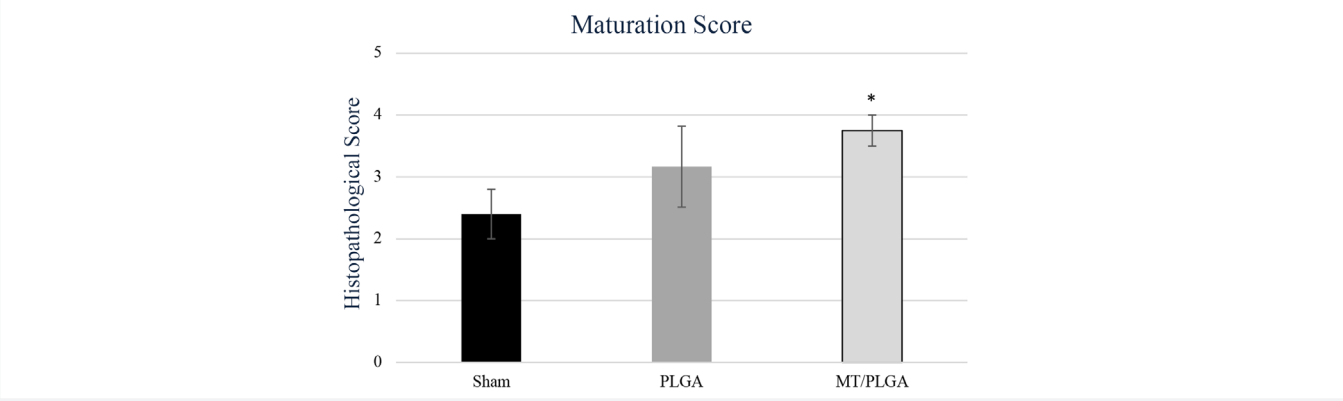

Figure 3. Statistical graphical representation of the maturation score based on the evaluation of vascular proliferation and fibroblastic cell proliferation in histopathological evaluation (*p<0.05)

MT/PLGA: Melatonin/Polylactic-co-glycolic acid

Table 1. Maturation score and fibrosis assessment according to groups

Sham			PLGA			MT/PLGA		
Rat number	Maturation score	Fibrosis	Rat number	Maturation score	Fibrosis	Rat number	Maturation score	Fibrosis
1	1	0	**1**	3	0	**1**	4	1
2	2	0	**2**	3	0	**2**	3	1
3	2	1	**3**	1	2	**3**	4	1
4	0	1	**4**	2	0	**4**	4	1
5	2	1	**5**	1	0	**5**	3	2
6	1	0	**6**	3	2	**6**	4	1

MT/PLGA: Melatonin/Polylactic-co-glycolic acid

vascular access by examining the effectiveness of MT-loaded PLGA nanofibers on AVF maturation.

In controlled release studies, it is possible to optimize the biomaterial produced in accordance with the healing process of the pathological mechanism. For this purpose, various synthetic or naturally sourced polymeric materials are used[31,32]. In recent years, there are biomaterials developed in the field of tissue engineering and biomaterials that have successfully completed experimental processes for use in the treatment of many diseases. Among these, synthetic biomaterials have become more preferred due to their lack of immunogenicity. Among these, PLGA is more preferred due to its biocompatibility, biodegradability and ability to mimic the extracellular matrix, which is higher than other natural and synthetic polymers[33]. PLGA is a polymeric material approved by the FDA and is a type of synthetic polymer widely used in the field of tissue engineering due to its biocompatibility, biodegradability and extracellular matrix mimicry properties[34]. PLGA provides antiadhesive effects and is a good choice for loading several different molecules and agents[35]. Biodegradation of PLGA nanofibers can be optimized according to the needs of specific tissue healing[36-38]. In our study, we aimed to develop PLGA nanofibers with a structure in the form of a covering membrane. For this purpose, a biodegradable barrier containing MT and providing controlled release of MT for AVF maturation and vascular proliferation was produced, and its *in vitro* release and degradation profile and *in vivo* efficacy were determined. For this purpose, firstly PLGA nanofibers were produced and a matrix containing 1 mg MT per 1x3 cm^2 was developed. *In vitro* experiments were conducted on this matrix to investigate MT release and biodegradation of its mass. As a result of *In vitro* studies, it was revealed that nanofibers lost 85-90% of their mass on the 28th day.

Maturation of AVF anastomoses involves complex steps including vascular repair, neointimal hyperplasia and inflammation. In these processes, the success of AVF depends on vascular proliferation and rapid fibroblastic cell proliferation. When vascular proliferation and fibroblastic cell proliferation processes are suppressed, abnormal vascular remodeling is observed. This leads to primary vascular access failure[39]. Therefore, it is critical to ensure vascular proliferation and fibroblastic cell proliferation in the AVF anastomosis line. In the *in vivo* part of our study, histopathological examinations were performed with hematoxylin-eosin staining on tissue samples taken from rats. Hematoxylin-eosin staining results and vascular proliferation and fibroblastic cell proliferation parameters were evaluated in terms of maturation level. According to the histopathological scoring, a significant increase was determined in the MT/PLGA group compared to the Sham group ($p<0.05$). It is possible to say that MT, which was released from MT-loaded PLGA nanofibers for 28 days, provides vascular proliferation and supports AVF maturation by increasing fibroblastic cell proliferation. Studies have shown that MT regulates vascular proliferation by suppressing abnormal proliferation of smooth muscle cells[40,41]. The histopathological findings of our study suggest that MT increases vascular proliferation to provide AVF maturation and prevents intimal hyperplasia. It is likely that MT achieves this effect by increasing nitric oxide release on vascular smooth muscle and reducing oxidative stress[42]. However, a signaling pathway or molecular mechanism in this direction was not examined in our study.

Among tissue engineering studies, considering the current literature, controlled release agents have been used to suppress intimal hyperplasia and increase vascular proliferation in biomaterial studies to ensure maturation of AVF anastomoses. In one of these studies, the vascular maturation effects of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) loaded PLGA nanofibers were evaluated. The results of this study showed that VEGF and PDGF loaded PLGA nanofibers increased vascular maturity in the first 21 days of the experiment[43]. In another study, angiogenic factor-loaded PLGA nanofibers were implanted subcutaneously in mice to study subcutaneous vascular formation. Application of nitric oxide-releasing nanomatrix gel to rats with AVFs provided better results in outward remodeling and inhibited intimal hyperplasia at 28 days[44]. Rapamycin-loaded nanofibrous membranes were used to evaluate the maturation effects of the material in an animal model of AVF anastomosis. After 4 weeks, the anastomosis sites were harvested and evaluated for further analysis. As a result of this study, it was stated that rapamycin-loaded nanofibrous membranes reduced intimal hyperplasia and facilitated AVF maturation[45]. The findings obtained from our study suggest that MT-loaded PLGA nanofibers increased vascular proliferation on day 28 and provided AVF maturation. The sustained release of MT in the AVF line with a biocompatible biomaterial for 28 days indicates that it contributes to vascular proliferation in addition to its anti-inflammatory and antioxidant effects.

In our study, another parameter investigated to investigate the effects of MT-loaded PLGA nanofibers on AVF maturation was fibrosis. Fibrosis is the accumulation of extracellular collagen matrix in the vascular intima[46]. The extent to which the presence of medial fibrosis indicates the success of the anastomosis for AVF fistula maturation is still a matter of debate in the literature. There are studies showing that increased arterial fibrosis and venous fibrosis ensure the success of AVF maturation, and there are study results showing that increased fibrosis causes AVF immaturity[47,48]. In our study, no significant results were found between the experimental groups in terms of fibrosis evaluations made from vascular sections.

In our study, we used MT-loaded PLGA nanofibers as a cover material around the AVF line. Our results revealed that MT increased vascular maturity in the early stages of remodeling. In addition, MT's excellent antioxidant andante-inflammatory properties suggest that it contributes to maturation by preventing oxidative and inflammatory damages at the surgical site and anastomosis line. PLGA seems to be a good option for loading different agents in vascular tissue engineering.

In summary, it was observed that the MT-loaded PLGA biomaterial developed in our project initiated AVF maturation on the 28th day by providing vascular proliferation at both macroscopic and microscopic levels.

Study Limitations

In our study, biomaterial production was optimized and the effectiveness of the produced biomaterial on AVF maturation was investigated with *in vivo* experiments. In our study, histopathological analysis was performed only on the tissue obtained from the anastomosis line.

CONCLUSION

However, it has not been determined by which molecular signaling mechanism MT exerts these effects. In addition, biodegradation after degradation of MT-loaded PLGA nanofibers has only been determined macroscopically. It is aimed to eliminate these limitations with further research and experimental planning.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Local Ethics Committee for Animal Experiments at Çanakkale Onsekiz Mart University (decision number: 2022/01-03, date: 21.01.2022).

Informed Consent: All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.A., S.Ş, C.A., S.A., M.U.J., Concept: A.O., S.S., Design: A.O., D.A., Data Collection or Processing: S.S., D.A., C.A., Analysis or Interpretation: Ö.Y., C.A., Literature Search: D.A., Writing: D.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Turner JM, Bauer C, Abramowitz MK, Melamed ML, Hostetter TH. Treatment of chronic kidney disease. Kidney Int. 2012;81:351-62.
2. Carminatti M, Tedesco-Silva H, Silva Fernandes NM, Sanders-Pinheiro H. Chronic kidney disease progression in kidney transplant recipients: a focus on traditional risk factors. Nephrology (Carlton). 2019;24:141-7.
3. Jadoul M, Aoun M, Masimango Imani M. The major global burden of chronic kidney disease. Lancet Glob Health. 2024;12:e342-e343.
4. Manov JJ, Mohan PP, Vazquez-Padron R. Arteriovenous fistulas for hemodialysis: brief review and current problems. J Vasc Access. 2022;23:839-846.
5. Fila B, Ibeas J, Tey RR, Lovčić V, Zibar L. Arteriovenous fistula for haemodialysis: the role of surgical experience and vascular access education. Nefrología. 2016;3689-94.
6. Farrington CA, Cutter G, Allon M. Arteriovenous fistula nonmaturation: what's the immune system got to do with it? Kidney360. 2021;2:1743.
7. Sabiu G, Gallieni M. Pathophysiology of arteriovenous fistula maturation and nonmaturation. Clin J Am Soc Nephrol. 2022;18:8.
8. Gameiro J, Ibeas J. Factors affecting arteriovenous fistula dysfunction: a narrative review. J Vasc Access. 2020;21:134-47.
9. Thomas AC, Campbell GR, Campbell JH. Advances in vascular tissue engineering. Cardiovascular Pathology. 2003;12:271-6.
10. Han J, Lazarovici P, Pomerantz C, Chen X, Wei Y, Lelkes PI. Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering. Biomacromolecules. 2011;12:399-408.
11. Jeong SI, Kim SY, Cho SK, Chong MS, Kim KS, Kim H, et al. Tissue-engineered vascular grafts composed of marine collagen and PLGA fibers using pulsatile perfusion bioreactors. Biomaterials. 2007;28:1115-22.
12. Gökçe ON, Aykora D, Danışman M, Demircan N, Ermiş H, Gürbüz M. Development of melatonin-embedded PLGA-PEG6000 nanofiber biomaterial, and investigation of the effects on abdominal adhesion formation. 2023;39:63-75.
13. Lv N, Hou M, Deng L, Hua X, Zhou X, Liu H, et al. A sponge-like nanofiber melatonin-loaded scaffold accelerates vascularized bone regeneration via improving mitochondrial energy metabolism. Mater Today Bio. 2024;26:101078.
14. Wang S, Li J, He Y, Ran Y, Lu B, Gao J, et al. Protective effect of melatonin entrapped PLGA nanoparticles on radiation-induced lung injury through the miR-21/TGF-β1/Smad3 pathway. Front Bioeng Biotechnol. 2021;602:120584.
15. Mirza-Aghazadeh-Attari M, Mihanfar A, Yousefi B, Majidinia M. Nanotechnology-based advances in the efficient delivery of melatonin. Cancer Cell Int. 2022;22:1-11.
16. Guzmán-Soria A, Moreno-Serna V, Canales DA, García-Herrera C, Zapata PA, Orihuela PA. Effect of Electrospun PLGA/Collagen scaffolds on cell adhesion, viability, and collagen release: potential applications in tissue engineering. Polymers. 2023;15:1079.
17. Lee S, Kim HJ, Cho HB, Kim HR, Lee S, Park JI, et al. Melatonin loaded PLGA nanoparticles effectively ameliorate the in vitro maturation of deteriorated oocytes and the cryoprotective abilities during vitrification process. Biomater Sci. 2023;11:2912-23.
18. Jarrar H, Çetin Altındal D, Gümüşderelioğlu M. Effect of melatonin/BMP-2 co-delivery scaffolds on the osteoclast activity. J Mater Sci Mater Med. 2021;32.
19. Mendes L, Queiroz M, Sena CM. Melatonin and vascular function. Antioxidants. 2024;13:747.
20. Ait Abdellah S, Gal C, Guinobert I, Bardot V, Raverot V, Vitacca A, et al. Melatonin bioavailability after oral administration of a new delayed-release form in healthy male volunteers. Drugs R D. 2024;24:415-23.
21. Gökçe ON, Aykora D, Danışman M, Demir U, Aydeğer C, Suner SC, et al. Development of melatonin-embedded PLGA-PEG6000 nanofiber biomaterial, and investigation of the effects on abdominal adhesion formation. J Bioact Compat Polym. 2023;39:63-75.

22. Wang S, Li J, He Y, Ran Y, Lu B, Gao J, et al. Protective effect of melatonin entrapped PLGA nanoparticles on radiation-induced lung injury through the miR-21/TGF-β1/Smad3 pathway. Int J Pharm. 2021;602:120584.

23. Farid A, Michael V, Safwat G. Melatonin loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles reduce inflammation, inhibit apoptosis and protect rat's liver from the hazardous effects of CCL4. Sci Rep. 2023;13:16424.

24. Das SK, Chakraborty S, Naskar S, Rajabalaya R. Techniques and methods used for the fabrication of bionanocomposites. Bionanocomposites in Tissue Engineering and Regenerative Medicine. Published online. 2021:17-43.

25. Ramchandani M, Pankaskie M, Robinson D. The influence of manufacturing procedure on the degradation of poly(lactide-co-glycolide) 85:15 and 50:50 implants. Journal of Controlled Release. 1997;43:161-73.

26. Jusu SM, Obayemi JD, Salifu AA, Nwazojie CC, Uzonwanne V, Odusanya OS, et al. Drug-encapsulated blend of PLGA-PEG microspheres: in vitro and in vivo study of the effects of localized/targeted drug delivery on the treatment of triple-negative breast cancer. Sci Rep. 2020;10:1-23.

27. Zheng C, Zhou Y, Huang C, Zhang Z, Liu Y, Xu Y. Establishment of a rat autogenous arteriovenous fistula model following 5/6 nephrectomy. Exp Ther Med. 2015;10:219-24.

28. Alpers CE, Imrey PB, Hudkins KL, Wietecha TA, Radeva M, Allon M, et al. Histopathology of veins obtained at hemodialysis arteriovenous fistula creation surgery. J Am Soc Nephrol. 2017;28:3076-88.

29. Applewhite B, Andreopoulos F, Vazquez-Padron RI. Periadventitial biomaterials to improve arteriovenous fistula and graft outcomes. J Vasc Access. 2024;25:713-27.

30. Vazquez-Padron RI, Duque JC, Tabbara M, Salman LH, Martinez L. Intimal hyperplasia and arteriovenous fistula failure: looking beyond size differences. Kidney360. 2021;2:1360.

31. Lee EJ, Kasper FK, Mikos AG. Biomaterials for tissue engineering. Ann Biomed Eng. 2013;42:323.

32. Hines DJ, Kaplan DL. Poly(lactic-co-glycolic) acid-controlled-release systems: experimental and modeling insights. Crit Rev Ther Drug Carrier Syst. 2013;30:257-76.

33. Liu S, Yu JM, Gan YC, Qiu XZ, Gao ZC, Wang H, et al. Biomimetic natural biomaterials for tissue engineering and regenerative medicine: new biosynthesis methods, recent advances, and emerging applications. Military Med Res. 2023;10:1-30.

34. Gentile P, Chiono V, Carmagnola I, Hatton P V. An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. Int J Mol Sci. 2014;15:3640-59.

35. Sadat Tabatabaei Mirakabad F, Nejati-Koshki K, Akbarzadeh A, Yamchi MR, Milani M, Zarghami N, et al. PLGA-based nanoparticles as cancer drug delivery systems. Asian Pac J Cancer Prev. 2014;15:517-35.

36. Wang B, Lu G, Song K, Chen A, Xing H, Wu J, et al. PLGA-based electrospun nanofibers loaded with dual bioactive agent loaded scaffold as a potential wound dressing material. Colloids Surf B Biointerfaces. 2023;231:113570.

37. Lee JY, Bashur CA, Goldstein AS, Schmidt CE. Polypyrrole-coated electrospun PLGA nanofibers for neural tissue applications. Biomaterials. 2009;30:4325.

38. Kim MJ, Kim JH, Yi G, Lim SH, Hong YS, Chung DJ. In vitro and in vivo application of PLGA nanofiber for artificial blood vessel. Macromol Res. 2008;16:345-52.

39. Siddiqui MA, Ashraff S, Santos D, Carline T. An overview of AVF maturation and endothelial dysfunction in an advanced renal failure. Ren Replace Ther. 2017;3:1-6.

40. Hyun BK, Kim MK, Park KG. Melatonin inhibits vascular smooth muscle cell proliferation and apoptosis through upregulation of Sestrin2. Endocrine Abstracts. 2020;70.

41. Lv D, Cui PL, Yao SW, Xu YQ, Yang ZX. Melatonin inhibits the expression of vascular endothelial growth factor in pancreatic cancer cells. Chin J Cancer Res. 2012;24:310.

42. Mendes L, Queiroz M, Sena CM. Melatonin and vascular function. Antioxidants. 2024;13:747.

43. Del Vento F, Poels J, Vermeulen M, Ucakar B, Giudice MG, Kanbar M, et al. Accelerated and ımproved vascular maturity after transplantation of testicular tissue in hydrogels supplemented with VEGF- and PDGF-loaded nanoparticles. Int J Mol Sci. 2021;22:5779.

44. Somarathna M, Hwang PT, Millican RC, Alexander GC, Isayeva-Waldrop T, Sherwood JA, et al. Nitric oxide releasing nanomatrix gel treatment inhibits venous intimal hyperplasia and improves vascular remodeling in a rodent arteriovenous fistula. Biomaterials. 2022;280:121254.

45. Rong D, Wang T, Liu X, Pan X. The study of rapamycin nanofibrous membrane for preventing arteriovenous fistula stenosis. Mater Des. 2024;245:113297.

46. Shiu YT, Litovsky SH, Cheung AK, Pike DB, Tey JCS, Zhang Y, et al. Preoperative vascular medial fibrosis and arteriovenous fistula development. Clin J Am Soc Nephrol. 2016;11:1615-23.

47. Martinez L, Duque JC, Tabbara M, Paez A, Selman G, Hernandez DR, et al. Fibrotic venous remodeling and nonmaturation of arteriovenous fistulas. J Am Soc Nephrol. 2018;29:1030-40.

48. Allon M, Litovsky S, Young CJ, Deierhoi MH, Goodman J, Hanaway M et al. Medial fibrosis, vascular calcification, ıntimal hyperplasia, and arteriovenous fistula maturation. Am J Kidney Dis. 2011;58:437-43.

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Aronia Melanocarpa Extract May Modulate Brain Oscillations and Functional Connectivity: Evidence from EEG Analysis

Aronia Melanocarpa Ekstresi Beyin Osilasyonlarını ve Fonksiyonel Bağlantısallığı Modüle Edebilir: EEG Analizinden Kanıtlar

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ABSTRACT

Aim: This study examines the acute and long-term effects of aronia melanocarpa extract (AME) on brain activity using electroencephalography (EEG). The goal is to determine its impact on neural oscillations, functional connectivity, and network efficiency across different frequency bands.

Materials and Methods: Fifteen healthy volunteers (8 males, 7 females) participated in the study. EEG recordings were conducted before the consumption of AME, one-hour post-consumption, and one week after consumption. Power spectral density (PSD), functional connectivity based on the imaginary part of coherence and network efficiency metrics [global efficiency (GE), local efficiency (LE), and transitivity (T)] were analyzed across Delta, Theta, Alpha, Beta I-III, and Gamma I frequency bands.

Results: Acute effects: PSD increased significantly in frontal and temporal regions across multiple frequency bands. Functional connectivity increased, especially in prefrontal-frontal and prefrontal-temporal pathways. Network efficiency was significantly increased in Beta I-III and Gamma bands ($p<0.05$). Long-term effects: one week later, no significant changes in PSD, GE, or T were found. However, LE increased in the left frontal and frontal midline channels ($p<0.05$).

Conclusion: The acute enhancements in PSD and functional connectivity suggest that AME may temporarily boost cognitive functions by promoting neuronal synchronization and network efficiency. The prominent increases in Beta and Gamma bands are consistent with improved attention, memory, and executive functions. However, the lack of sustained effects highlights the need for continuous intake to maintain cognitive benefits. AME acutely enhances brain activity, particularly in Beta and Gamma bands, suggesting improved cognitive processing and neural communication. However, these effects diminish over time, indicating that regular intake may be necessary for sustained cognitive benefits.

Keywords: Aronia extract, electroencephalography, functional connectivity, power spectral density

ÖZ

Amaç: Bu çalışma, aronia melanocarpa ekstresinin (AME) beyin aktivitesi üzerindeki akut ve uzun vadeli etkilerini elektroensefalografi (EEG) kullanarak incelemektedir. Çalışmanın amacı, farklı frekans bantlarında sinirsel osilasyonlar, fonksiyonel bağlantısallık ve ağ verimliliği üzerindeki etkilerini belirlemektir.

Gereç ve Yöntem: Çalışmaya 15 sağlıklı gönüllü (8 erkek, 7 kadın) katılmıştır. EEG kayıtları, AME tüketilmeden önce, tüketimden bir saat sonra ve bir hafta sonra gerçekleştirilmiştir. Güç spektral yoğunluğu (PSD), ve fonksiyonel bağlantısallık [küresel verimlilik (GE), yerel verimlilik (LE) ve geçişlilik (T)] Delta, Teta, Alfa, Beta I-III ve Gama I frekans bantlarında analiz edilmiştir.

Bulgular: Akut etkiler: Birçok frekans bandında, özellikle frontal ve temporal bölgelerde PSD anlamlı şekilde artmıştır. Fonksiyonel bağlantısallık, özellikle prefrontal-frontal ve prefrontal-temporal yollarında artış göstermiştir. Ağ verimliliği, Beta I-III ve Gama bantlarında anlamlı şekilde artmıştır

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(p<0,05). Uzun vadeli etkiler: Bir hafta sonra PSD, GE ve T değerlerinde anlamlı bir değişiklik gözlemlenmemiştir. Ancak, sol frontal ve frontal orta hat kanallarında LE artışı tespit edilmiştir (p<0,05).

Sonuç: PSD ve fonksiyonel bağlantısallıktaki akut artışlar, AME'nin nöronal senkronizasyonu ve ağ verimliliğini artırarak bilişsel işlevleri geçici olarak güçlendirebileceğini düşündürmektedir. Beta ve Gama bantlarındaki belirgin artışlar, dikkat, hafıza ve yürütücü işlevlerdeki iyileşmelerle tutarlıdır. Ancak, etkilerin kalıcı olmaması, bilişsel faydaların sürdürülebilmesi için düzenli tüketimin gerekli olabileceğini vurgulamaktadır. AME, özellikle Beta ve Gama bantlarında beyin aktivitesini akut olarak artırarak bilişsel işlemleme ve sinirsel iletişimin iyileştiğini göstermektedir. Ancak, bu etkiler zamanla azalmaktadır ve uzun vadeli bilişsel faydaların sürdürülebilmesi için düzenli tüketim gerekebilir.

Anahtar Kelimeler: Aronia ekstresi, elektroensefalografi, fonksiyonel bağlantısallık, güç spektral yoğunluğu

INTRODUCTION

The healthy functioning of brain activities depends on the balanced operation of neural network dynamics, functional connectivity, and cortical activity. Recent studies have revealed that dietary polyphenols may exhibit neuroprotective effects on the nervous system and improve cognitive functions[1-3]. Among polyphenols, anthocyanins are particularly known for their antioxidant, anti-inflammatory, and neuroprotective properties against neurodegenerative diseases[4,5]. By enhancing brain plasticity, they can strengthen synaptic connectivity and regulate neuronal activity.

In this context, aronia melanocarpa extract (AME) stands out as a functional food rich in polyphenols. Due to its polyphenols enriched with anthocyanins, aronia extract has been identified as containing bioactive compounds that may support brain functions[6]. The literature suggests that AME may enhance cognitive performance, reduce neuroinflammation, and exert positive effects on the nervous system through the gut-brain axis[7]. However, studies directly examining the acute and long-term effects of aronia extract on the brain are quite limited.

In this study, the effects of AME consumption on brain activity were evaluated using electroencephalography (EEG). EEG is a non-invasive method that measures the brain's electrical activity through the skull. Its high temporal resolution makes EEG an ideal tool for examining functional connections and frequency components. Since the electrical activity measured in EEG represents a local field potential projected onto the skull, the volume conduction effect may be a limitation for spatial analysis. To mitigate this effect, the Laplacian reference, one of the reference techniques, was applied in functional connectivity analysis. In this study, EEG power spectral density (PSD), and functional connectivity measurements were examined to investigate the effects of aronia extract on neural oscillations and brain network efficiency. The Welch method was used when calculating PSD, and the imaginary part of coherence (iCOH) was used to examine functional connectivity.

The aim of this study is to determine the short and long-term effects of AME on brain activity. The results of this study may contribute to our understanding of the potential effects of dietary polyphenols on brain functions and shed light on the development of neuroprotective strategies.

MATERIALS AND METHODS

Participants and Experimental Procedure

This study included 15 participants, consistent with previous EEG research that often uses 10-20 participants in exploratory neurophysiological studies[8]. The demographic information and clinical characteristics of the participants are presented in Table 1. The research protocol was conducted in accordance with the approvals granted by the Ethics Committee of Nişantaşı University.

Individuals with any physiological or psychiatric conditions that could potentially affect the study outcomes were excluded. Participants were screened to ensure they were not taking any medications that could influence metabolism related to such conditions.

The extract obtained from aronia berries was prepared as 250 mL per serving. EEG recordings were conducted two hours after the participants consumed the aronia berry extract. This timing was adjusted according to the half-life of the compound[9].

EEG signals were recorded at three different times to assess the acute and long-term effects of the consumption of AME. All of the EEG recordings were performed in a resting state. Participants were seated in a quiet, dimly lit room, with their eyes closed and in a relaxed position. Each session lasted at least 30 minutes to ensure a comprehensive analysis of resting-state brain activity. The first recording was conducted before AME consumption, and the second EEG recording followed two hours later to evaluate the acute effects of AME. During the 2-hour waiting period, participants did not consume any food

Table 1. Demographic and clinical characteristics of participants

Subject number	**15**
Laterality	93.3% right-handed and 6.6% left-handed
Age (mean ± SD)	23.93±1.79
Gender	46.6% women 53.3% men
Literacy rate	100%
SD: Standard deviation	

or beverages other than water, or smoke, and were instructed to follow their usual daily activities. For the assessment of long-term effects, the third EEG recording was performed one week after AME consumption in the same environment.

Ethical permission to conduct this study was approved by the Nişantaşı University Clinical Research Ethics Committee (decision no: 2020/17, date: 25.09.2020). Informed consent was obtained from all participants before inclusion in the study, and the necessary permissions were secured.

Aronia Melanocarpa Extraction

The AME berries originated from Rize, Kırklareli, Türkiye. They began forming in June and were harvested around October and November at their optimal ripeness for processing. After harvest, the berries were transported using vehicles equipped with cold storage facilities. Prior to extraction, they were stored at 4 °C to maintain their quality. The extraction process was conducted within one week without significant delay. Fresh berries were used in this study. AME berries were pureed using a grinder. The resulting pure was mixed with water containing 0.1% hydrochloric acid to form a homogeneous solution. This mixture was extracted using an ultrasonication method at 30 °C for 30 minutes. The ultrasonication process facilitated the release of bioactive compounds by breaking down the cell walls, allowing these compounds to be transferred into the solution.

Following the extraction process, the mixture was centrifuged at 10,000 rpm for 15 minutes. The resulting supernatant (upper liquid phase) was collected and stored at +4 °C to preserve the bioactive compounds.

Total Phenol Analysis

The total phenol content of the aronia fruit extract was determined using the Folin-Ciocalteu method, as described by Chen et al.[10]. Briefly, 0.2 mL of the sample (diluted if necessary) was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 minutes. Then, 1.2 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added and thoroughly mixed. The mixtures were incubated at room temperature for 60 minutes, after which the absorbance was measured at 765 nm using a Shimadzu UV-1800 spectrophotometer against a blank. The analyses were performed in triplicate, and the calibration curve was constructed using gallic acid in the concentration range of 0-250 ppm.

Total Antioxidant Capacity Analysis

The cupric ion reducing antioxidant capacity (CUPRAC) assay was applied to determine the antioxidant capacity of aronia fruit extract, following the procedure described by Apak et al.[11]. Briefly, 1 mL of 10^{-2} M $CuCl_2$, 1 mL of 7.5×10^{-3} M neocuproine, and 1 mL of 1 M NH_4Ac were mixed in a test tube. Then, the sample (or standard) solution (x mL) and H_2O (1.1 - x mL) were added to the initial mixture to obtain a final volume of 4.1 mL. The mixture was then incubated at room temperature for 30 minutes. Finally, absorbance was measured at 450 nm against a blank using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The calibration curve was constructed using Trolox in the range of 10-100 µmol/L.

EEG Recording and Processing

EEG signals were recorded using the 10-20 international electrode placement system, employing 19 EEG channels and mastoid referencing, with ANT Neuro sport EEG device. The recordings were conducted with a sampling frequency of 500 Hz and a finite impulse response band-pass filter applied between 1-45 Hz. For data analysis, PSD were calculated via Welch method[12] for all EEG channels in each participant. Functional connectivity was assessed using the iCOH[13], while coherence-based network metrics, including global efficiency (GE), local efficiency (LE), and transitivity (T) were evaluated. In functional connectivity analysis, the monopolar reference was replaced with the Laplacian reference[14] through re-referencing to enhance the spatial performance of the analysis. EEG recordings were analyzed across seven frequency bands: Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta I (12-18 Hz), Beta II (18-24 Hz), Beta III (24-30 Hz), and Gamma I (30-45 Hz), along with the full spectrum (1-45 Hz). These metrics were systematically examined to determine changes in brain network dynamics following AME.

Statistical Analysis

Differences between the initial and post-consumption recordings were statistically analyzed using the Wilcoxon signed-rank test.

Given the exploratory nature of this study and the relatively small sample size, we did not apply a formal correction for multiple comparisons.

A post-hoc power analysis was conducted to evaluate statistical power. Effect sizes (Cohen's d) were computed for each feature and frequency band that showed significant changes. Using G*Power, a paired t-test power analysis was performed with α=0.05, n=15, and the calculated effect sizes were used to determine statistical power.

RESULTS

The total phenol content of the aronia extract was found to be approximately 800 mg GAE/250 mL (gallic acid equivalents). The total antioxidant capacity of the aronia berry extract was determined using the CUPRAC method. The extract demonstrated a high antioxidant capacity of approximately 1.100 µmoL trolox equivalents per liter (µmol TE/L).

When comparing the PSD results between the initial recording and the two-hour post-consumption recording, a statistically significant increase in PSD was observed across the average of all EEG channels, as well as in specific brain regions such as the left frontal, right frontal, and right temporoparietal regions for the entire spectrum (1-45 Hz). In the Delta frequency band, PSD increased significantly across all channels, particularly in the left frontal, left temporal, and right temporal regions. A similar increase was observed in the Theta frequency band, with heightened activity in the entire frontal region and the right temporoparietal region. In the Alpha band, PSD values were notably higher in the frontal and right temporal regions, a pattern that was also present in the Beta I band. Additionally, in the Gamma I band, higher PSD values were observed across the entire frontal region.

The iCOH results revealed statistically significant increases in functional connectivity across the whole frequency spectrum (1-45 Hz). These connections were observed in prefrontal-frontal, interhemispheric frontal, and frontal-central pathways, as well as frontoparietal connections linking anterior and posterior regions. Enhanced connectivity was also present in central-parietal and central-occipital pathways, indicating integration between midline and posterior regions. Furthermore, interhemispheric connectivity was evident in both frontal and occipital regions, while temporoparietal and temporo-occipital interactions extended across lateral areas. These results demonstrated a widespread increase in connectivity, integrating anterior, midline, and posterior regions across both hemispheres. Mentioned connections can be seen in Figure 1A.

No significant connectivity changes were observed in the Delta frequency band given in Figure 1B. However, in the Theta band, stronger connections emerged in the left central-temporal and midline central-occipital pathways, suggesting increased

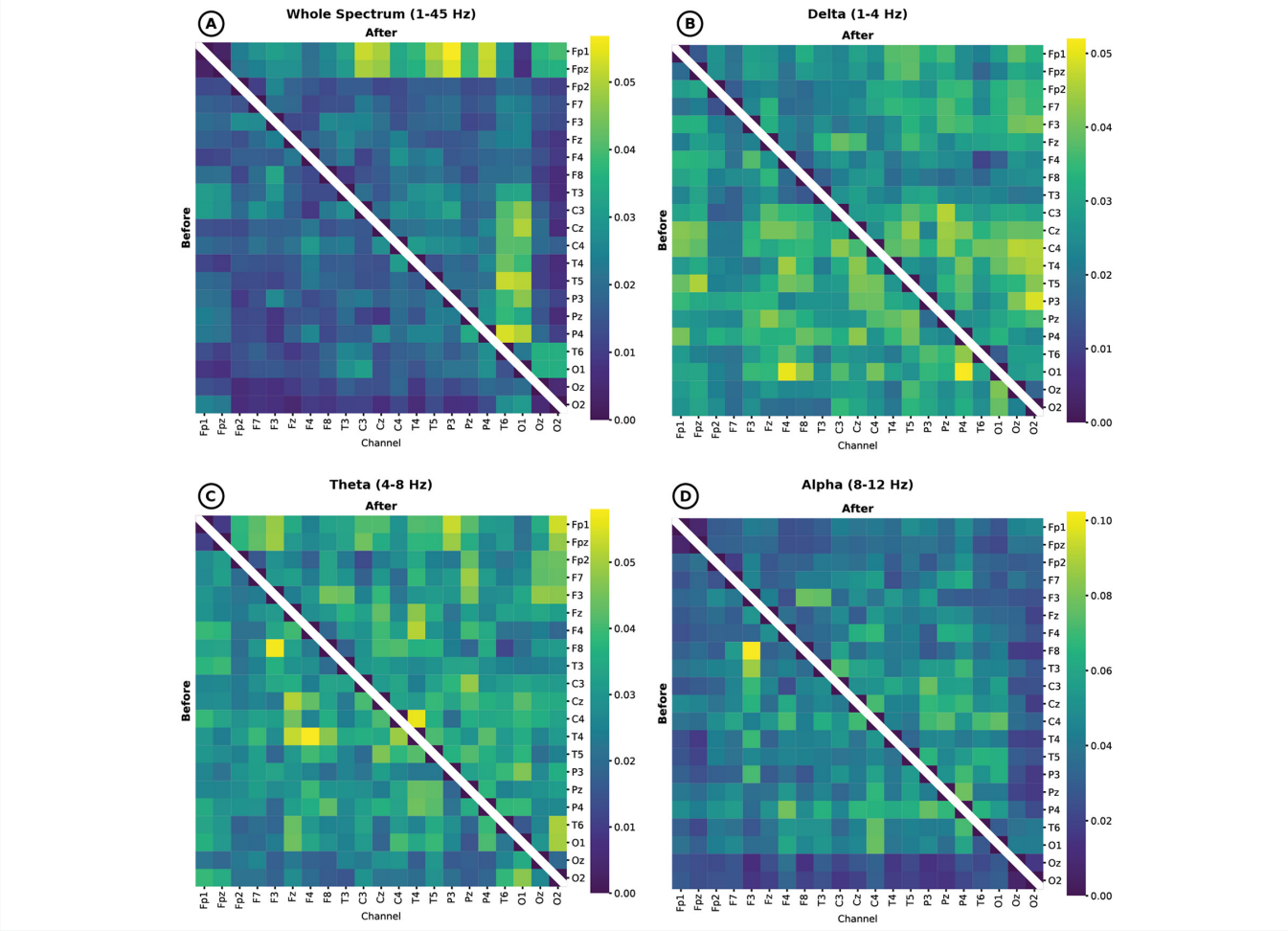

Figure 1. This figure illustrates the statistical comparison of iCOH values between the pre- and 2-hour post-consumption conditions of AME across different frequency bands. Panel A displays the results for the whole frequency spectrum (1-45 Hz), while Panels B, C, and D correspond to the delta (1-4 Hz), theta (4-8 Hz), and alpha (8-12 Hz) bands, respectively

iCOH: Imaginary part of coherence, AME: Aronia melanocarpa extract

integration between these regions. Those connections can be seen in Figure 1C. In the Alpha frequency band, notable connectivity increases were detected in the interhemispheric prefrontal-occipital, right prefrontal-occipital, left central-temporal, midline central-temporal, right temporal-occipital, and interhemispheric occipital pathways, shown in the Figure 1D. These findings indicate enhanced communication across anterior, midline, and posterior regions, as well as between hemispheres. In the Beta I frequency band, stronger connectivity was observed in prefrontal-frontal, prefrontal-parietal, and prefrontal-occipital pathways, demonstrating increased integration between anterior and posterior regions. Frontal-central and frontal-temporal interactions, along with connections extending from temporal and central regions to parietal and occipital areas, also showed increased strength. Interhemispheric occipital connectivity exhibited further enhancement, reinforcing posterior integration. Similar trends were observed in the Beta II frequency band, where stronger connections were noted in prefrontal-frontal, prefrontal-central, prefrontal-temporal, and prefrontal-occipital pathways, further linking anterior regions with midline and posterior areas. The Beta III frequency band exhibited stronger connectivity across prefrontal, frontal, temporal, parietal, and occipital regions, with pronounced prefrontal-posterior and temporal-occipital interactions. In the Gamma band, significant connectivity increases were observed in prefrontal-temporal, prefrontal-central, and prefrontal-posterior pathways. Frontal-temporal and frontal-posterior connections also displayed enhanced connectivity, alongside stronger interactions in central-posterior, central-occipital, temporal-occipital, and interhemispheric occipital pathways. These connections can be seen in (Figure 2. E, F, G, H), respectively.

When comparing GE before and after AME, a statistically significant increase was observed in the Beta I, Beta II, and Beta III bands (p-value=0.01, 0.02, and 0.02, respectively), as well as in

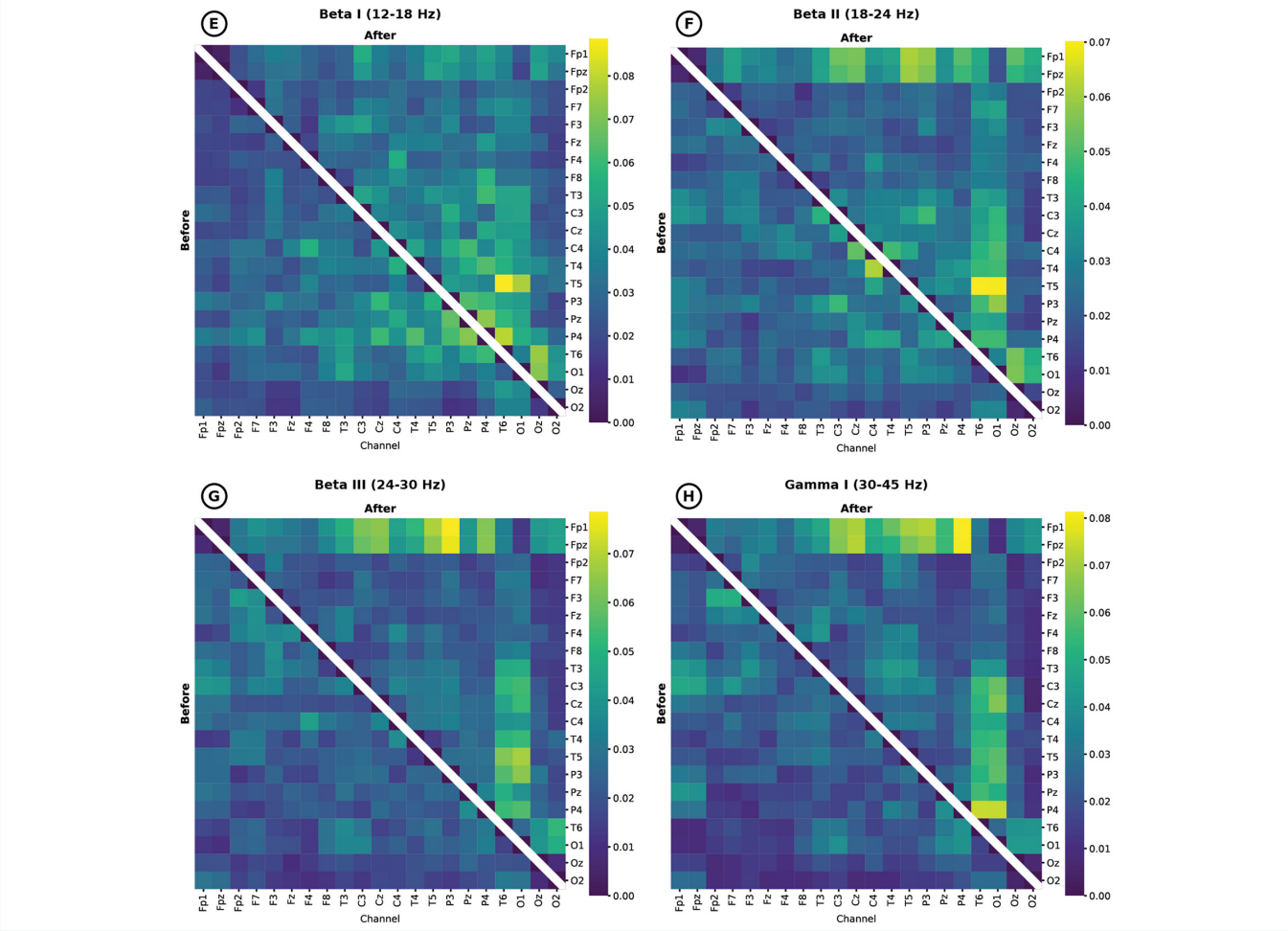

Figure 2. This figure illustrates the statistical comparison of iCOH values between the pre- and 2-hour post-consumption conditions of AME across higher frequency bands. Panels E, F, and G show results for the beta I (12-18 Hz), beta II (18-24 Hz), and beta III (24-30 Hz) bands, respectively, while Panel H presents results for the gamma band (30-45 Hz)

iCOH: Imaginary part of coherence, AME: Aronia melanocarpa extract

the Gamma band (p-value=0.01). A significant increase in GE was also detected across the entire frequency spectrum (1-45 Hz), with a p-value of 0.004. LE showed increases in the prefrontal, left frontal, midline frontal, central, right temporal, right temporoparietal, and occipital regions. Within specific frequency bands, Beta I displayed an increased LE in the same regions, while Beta II showed enhancements in a subset of these regions, excluding the left frontal region. Beta III demonstrated higher LE, particularly in the central and parietal regions, while Gamma activity was associated with increased LE similar to Beta III but without the occipital region. For T, significant increases were observed in Beta I, Beta II, and Beta III (p-value=0.004, 0.03, and 0.04, respectively), as well as in the Gamma band (p-value=0.01). Additionally, significant increases in GE were observed across the entire frequency spectrum, with a p-value of 0.001.

When comparing the PSD results between pre-consumption and one-week post-consumption, although some increases and decreases were observed, they were not statistically significant. Similarly, no significant differences were detected in GE or T. However, LE showed two significant increases in the left frontal and frontal midline channels (p-value=0.05 and 0.03, respectively) within the Beta I frequency band.

Post-hoc power analysis showed effect sizes ranging from approximately 0.82 to 1.17 across different EEG features and frequency bands. The corresponding statistical power ranged between 91.2% and 97.6%, indicating a high probability of detecting significant effects despite the sample size limitation.

DISCUSSION

This study investigated the acute and long-term effects of aronia extract consumption on brain activity using EEG spectral power analysis and functional connectivity measurements. Our findings suggest that aronia extract may modulate neural network dynamics, particularly in the Beta and Gamma frequency bands, which are associated with cognitive processes such as attention, memory, and executive functions. These effects may be closely related to the high polyphenol content and antioxidant capacity of the aronia extract.

Our results revealed a significant increase in PSD in the Delta, Theta, Alpha, Beta I, and Gamma I bands, particularly in the frontal and temporal regions. The total phenolic content of the aronia extract was found to be notably high, approximately 800 mg GAE/250 mL. These findings are consistent with previous studies suggesting that polyphenol-rich dietary interventions can enhance cortical excitability and neuronal synchronization[15,16]. The increase in Delta and Theta waves in the frontal and temporal regions indicates strengthened resting-state synchronization and cortical inhibition. This enhancement may contribute to improved cognitive stability and greater regularity in resting-state brain activity.

The power increase in Alpha and Beta I bands, particularly in the frontal and right temporal regions, suggests heightened cognitive awareness and readiness. Research indicates that anthocyanins can improve synaptic transmission, thereby enhancing cortical efficiency[17]. The increase in Gamma waves in the frontal regions suggests potential improvements in higher-order cognitive functions. Gamma oscillations are closely linked to cognitive integration and memory consolidation[18], suggesting that aronia extract may contribute to complex information processing in the brain.

In addition to EEG power analysis, iCOH-based functional connectivity analyses showed widespread increases in connectivity across the brain. This effect was particularly evident in the strengthened connections between the prefrontal, frontal, temporal, parietal, and occipital regions. The most significant increases in connectivity were observed in the prefrontal-temporal, prefrontal-central, and prefrontal-posterior pathways. These connections play a crucial role in working memory and cognitive control processes[19,20]. These findings suggest enhanced long-range cortical communication and increased integration capacity of brain networks. Previous studies have proposed that the anthocyanins found in aronia berries support synaptic plasticity, thereby strengthening communication between brain regions[5-17].

Following aronia extract consumption, significant increases in GE were observed, particularly in the Beta I, Beta II, Beta III, and Gamma bands. GE is a critical measure reflecting the efficiency of brain networks and their information processing capacity[21]. This increase suggests that cognitive processes have become more efficient and that the functionality of neural networks has improved. The enhancement of GE in the Beta and Gamma bands can be associated with faster information processing and improved cognitive performance[22,23].

In our study, the high antioxidant capacity of the aronia extract, measured at 1,100 µmol TE/L, may have played a significant role in reducing oxidative stress. Oxidative stress can cause damage to nerve cells, adversely affecting cognitive functions[24]. The strong antioxidant capacity of aronia extract may help neutralize free radicals, thereby protecting neuronal health and contributing to the observed increases in both global and local efficiency.

An increase in LE was also detected, particularly in the prefrontal, left frontal, central, right temporal, and occipital regions. The increase in LE in these regions indicates more efficient local neural processing.

T, which measures the density of connections between nodes within a network, is associated with cognitive integration[25]. The increase in T values in the Beta and Gamma bands indicates that brain networks have become more integrated and better organized.

In EEG analyses conducted one week after aronia extract consumption, less pronounced results were obtained compared to short-term changes. Although some increases and decreases were observed in the PSD analysis, these changes were not statistically significant. No significant changes were detected in GE and T values, suggesting that while aronia extract has strong short-term effects, its long-term effects may be more limited. However, a significant increase in LE was observed in the Beta I band, particularly in the left frontal and midline frontal regions, indicating that some regional adaptations may persist over time.

Study Limitations

Our study was limited to 15 healthy volunteers. This small sample size restricts the generalizability of the results. Studies with larger sample groups are essential for validating these findings. Additionally, only healthy individuals were included in the study. It remains unclear how the effects of aronia extract might differ in individuals with cognitive impairments or neurological disorders. This limitation affects the applicability of the results to clinical populations. Finally, our study focused on a group of young adults with an average age of 23.93 (±1.79); to enhance the applicability of these findings, future research should extend to other age groups.

It is important to note that the current study provides only a limited scope of the immediate effects of AME, as the evaluation of its long-term effects relies on a single measurement taken one week after consumption, not capturing how these effects change over time. More frequent or extended follow-up assessments would offer a clearer understanding of whether the effects of AME persist over time or gradually diminish with prolonged use.

In this study, EEG was used to assess brain activity through functional connectivity. Although EEG provides high temporal resolution, the spatial limitations of this technique make it challenging to identify the precise sources of brain activity. Even with improvements made through methods such as the Laplacian reference technique, localization remains imprecise. Future studies could benefit from combining EEG with other neuroimaging techniques, such as functional magnetic resonance imaging, to gain a more accurate understanding of the spatial aspects of brain function related to AME consumption.

CONCLUSION

This study has demonstrated that AME acutely enhances brain activity, particularly by increasing PSD and functional connectivity in the Beta and Gamma frequency bands. These enhancements suggest improvements in neuronal communication and cognitive processing. Additionally, significant increases in network efficiency, especially in the Beta I-III and Gamma bands, were observed, indicating a potential for faster information processing and better cognitive performance.

However, these effects were not sustained over the long term, as no significant changes were observed in GE and T one week after consumption. Nevertheless, a persistent increase in LE was detected in the left frontal and midline frontal regions, suggesting that some regional neural adaptations may continue over time.

Overall, the findings indicate that aronia extract has the potential to modulate neurological activity in the short term, but regular consumption may be required to achieve long-term benefits. These results support the potential role of polyphenol and antioxidant-rich dietary interventions in enhancing cognitive health and highlight the need for larger-scale, long-term studies to further explore these effects.

Ethics

Ethics Committee Approval: Ethical permission to conduct this study was approved by the Nişantaşı University Clinical Research Ethics Committee (decision no: 2020/17, date: 25.09.2020).

Informed Consent: Informed consent was obtained from all participants before inclusion in the study, and the necessary permissions were secured.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.M.K., İ.K., Concept: A.M.K., İ.K., Design: A.M.K., İ.K., Data Collection or Processing: A.M.K., A.Ö., M.Y.Ö., İ.K., Analysis or Interpretation: A.M.K., A.Ö., M.Y.Ö., Literature Search: A.M.K., A.Ö., M.Y.Ö., İ.K., Writing: A.M.K., İ.K.

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REFERENCES

1. Arias-Sánchez RA, Torner L, Fenton Navarro B. Polyphenols and neurodegenerative diseases: potential effects and mechanisms of neuroprotection. Molecules. 2023;28:5415.
2. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61:1361779.
3. Ahles S, Stevens YR, Joris PJ, Vauzour D, Adam J, de Groot E, et al. The effect of long-term aronia melanocarpa extract supplementation on cognitive performance, mood, and vascular function: a randomized controlled trial in healthy, middle-aged individuals. Nutrients. 2020;12:2475.
4. Silva RFM, Pogačnik L. Polyphenols from food and natural products: neuroprotection and safety. Antioxidants (Basel). 2020;9:61.

5. Zaa CA, Marcelo ÁJ, An Z, Medina-Franco JL, Velasco-Velázquez MA. Anthocyanins: molecular aspects on their neuroprotective activity. Biomolecules. 2023;11:1598.

6. Lee HY, Weon JB, Ryu G, Yang WS, Kim NY, Kim MK, et al. Neuroprotective effect of Aronia melanocarpa extract against glutamate-induced oxidative stress in HT22 cells. BMC Complement Altern Med. 2017;17:207.

7. Zhao Y, Liu X, Zheng Y, Liu W, Ding C. Aronia melanocarpa polysaccharide ameliorates inflammation and aging in mice by modulating the AMPK/SIRT1/NF-κB signaling pathway and gut microbiota. Sci Rep. 2021;11:20558.

8. Larson MJ, Carbine KA. Sample size calculations in human electrophysiology (EEG and ERP) studies: a systematic review and recommendations for increased rigor. Int J Psychophysiol. 2017;111:33-41.

9. Wiczkowski W, Romaszko E, Piskula MK. Bioavailability of cyanidin glycosides from natural chokeberry (Aronia melanocarpa) juice with dietary-relevant dose of anthocyanins in humans. Journal of Agricultural and Food Chemistry. 2010;58:12130-6.

10. Chen LY, Cheng CW, Liang JY. Effect of esterification condensation on the Folin-Ciocalteu method for the quantitative measurement of total phenols. Food chemistry. 2015;170:10-5.

11. Apak R, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektaşoğlu B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules. 2007;12:1496-547.

12. Solomon OM Jr. PSD computations using Welch's method. NASA STI/Recon Technical Report [Report]. 1991;92:23584.

13. Nolte G, Bai O, Wheaton L, Mari Z, Vorbach S, Hallett M. Identifying true brain interaction from EEG data using the imaginary part of coherency. Clin Neurophysiol. 2004;10: 2292-307.

14. Miljevic A, Bailey NW, Vila-Rodriguez F, Herring SE, Fitzgerald PB. Electroencephalographic connectivity: a fundamental guide and checklist for optimal study design and evaluation. Biol Psychiatry Cogn Neurosci Neuroimaging. 2022;7:546-54.

15. Vauzour D. Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. Oxid Med Cell Longev. 2012;2012:914273.

16. Ammar A, Trabelsi K, Boukhris O, Bouaziz B, Müller P, M Glenn J, et al. Effects of polyphenol-rich interventions on cognition and brain health in healthy young and middle-aged adults: systematic review and meta-analysis. J Clin Med. 2020;9:1598.

17. Vauzour D, Rendeiro C, D'Amato A, Waffo-Téguo P, Richard T, Mérillon JM, et al. Anthocyanins promote learning through modulation of synaptic plasticity related proteins in an animal model of ageing. Antioxidants (Basel). 2021;10:1235.

18. Basso E, Arai M, Dabaghian Y. Gamma synchronization influences map formation time in a topological model of spatial learning. PLoS Comput Biol. 2016;12:e1005114.

19. Kirchhoff BA, Wagner AD, Maril A, Stern CE. Prefrontal-temporal circuitry for episodic encoding and subsequent memory. J Neurosci. 2000;20:6173-80.

20. Sarnthein J, Petsche H, Rappelsberger P, Shaw GL, von Stein A. Synchronization between prefrontal and posterior association cortex during human working memory. Proc Natl Acad Sci U S A. 1998;95:7092-6.

21. van den Heuvel MP, Stam CJ, Kahn RS, Hulshoff Pol HE. Efficiency of functional brain networks and intellectual performance. J Neurosci. 2009;29:7619-24.

22. Bassett DS, Bullmore ET, Meyer-Lindenberg A, Apud JA, Weinberger DR, Coppola R. Cognitive fitness of cost-efficient brain functional networks. Proc Natl Acad Sci U S A. 2009;106:11747-52.

23. Cuesta P, Ochoa-Urrea M, Funke M, Hasan O, Zhu P, Marcos A, et al. Gamma band functional connectivity reduction in patients with amnestic mild cognitive impairment and epileptiform activity. Brain Commun. 2022;4:fcac012.

24. Ionescu-Tucker A, Cotman CW. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. Neurobiol Aging. 2021;107:86-95.

25. Garrison KA, Scheinost D, Finn ES, Shen X, Constable RT. The (in)stability of functional brain network measures across thresholds. Neuroimage. 2015;118:651-61.

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Insulin Glargine U-300 in Type 1 Diabetes Mellitus: Single-Center Experience

Tip 1 Diabetes Mellitus'ta İnsülin Glargin U-300: Tek Merkez Deneyimi

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ABSTRACT

Aim: This study aimed to evaluate the clinical outcomes of switching from first-generation basal insulins to insulin glargine U-300 (Gla-300) in individuals with type 1 diabetes mellitus (T1DM) under real-world conditions.

Materials and Methods: A retrospective analysis was conducted on 46 adult patients with T1DM who switched to Gla-300 due to frequent hypoglycemia or inadequate glycemic control. HbA1c, fasting plasma glucose (FPG), daily injection frequency, insulin dose, and hypoglycemia rates were evaluated over a 12-month period.

Results: A significant reduction in HbA1c (from 8.45±1.27% to 7.83±1.01% at 3 months, p<0.001) and FPG levels was observed. Injection frequency decreased, particularly in patients previously on detemir or neutral protamine hagedorn. Despite a statistically significant increase in basal insulin dose over time, the frequency of all hypoglycemia subtypes declined, with the most prominent reduction in nocturnal hypoglycemia (p<0.001). No severe hypoglycemia occurred after the third month. No cases of ketosis or hospitalizations were reported. The majority of improvements occurred within the first 3 months and were maintained throughout the follow-up.

Conclusion: In real-life clinical practice, switching to Gla-300 provided improved glycemic control and reduced hypoglycemia risk in adults with T1DM, particularly in those previously using multiple daily injections. Considering the limited national data and frequent use of older basal insulins in Türkiye, these findings support the potential value of Gla-300 as an alternative option in individualized treatment planning for T1DM.

Keywords: Type 1 diabetes mellitus, glargine U-300, hypoglycemia, basal insulin, real-world evidence

ÖZ

Amaç: Bu çalışmanın amacı, birinci nesil bazal insülinlerden insülin glargin U-300 (Gla-300)'e geçiş yapılan tip 1 diabetes mellitus (T1DM) hastalarında, gerçek yaşam koşullarında klinik sonuçları değerlendirmektir.

Gereç ve Yöntem: Hipoglisemi atakları ya da yetersiz glisemik kontrol nedeniyle Gla-300 tedavisine geçiş yapılan 46 erişkin T1DM hastası retrospektif olarak analiz edildi. HbA1c, açlık plazma glukozu (APG), günlük enjeksiyon sıklığı, insülin dozu ve hipoglisemi oranları 12 aylık takip süresince değerlendirildi.

Bulgular: HbA1c düzeylerinde anlamlı bir düşüş izlendi (%8,45±1,27'den %7,83±1,01'e; 3. ayda, p<0,001) ve APG düzeylerinde de belirgin bir azalma gözlendi. Özellikle daha önce detemir veya nötr protamin hagedorn kullanan hastalarda enjeksiyon sıklığı azaldı. Bazal insülin dozunda istatistiksel olarak anlamlı bir artış olmasına rağmen, tüm hipoglisemi alt tiplerinin sıklığında azalma görüldü; en belirgin düşüş ise gece hipoglisemilerinde saptandı (p<0,001). Üçüncü aydan sonra şiddetli hipoglisemiye rastlanmadı. Ketozis ya da hastaneye yatış gerektiren bir durum izlenmedi. İyileşmelerin büyük bölümü ilk 3 ay içerisinde gerçekleşmiş olup takip süresince korundu.

Sonuç: Gerçek yaşam koşullarında, Gla-300'e geçiş yapılan erişkin T1DM hastalarında glisemik kontrolde iyileşme ve hipoglisemi riskinde azalma sağlanmıştır. Bu fayda, özellikle günde birden fazla enjeksiyon uygulanan hastalarda daha belirgindir. Türkiye'de eski nesil bazal insülinlerin yaygın kullanımı ve ulusal veri eksikliği göz önüne alındığında, bu bulgular Gla-300'ün bireyselleştirilmiş tedavi planlamasında potansiyel bir alternatif olarak değerlendirilebileceğini desteklemektedir.

Anahtar Kelimeler: Tip 1 diabetes mellitus, glargin U-300, hipoglisemi, bazal insülin, gerçek yaşam verisi

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a clinical condition characterized by absolute insulin deficiency resulting from autoimmune or other causes leading to β-cell destruction[1]. The cornerstone of T1DM management consists of insulin therapy combining basal and preprandial short-acting insulin[1]. In recent years, basal insulin formulations have progressively evolved from intermediate-acting to long-acting and ultra-long-acting insulin analogs, aiming to improve fasting glucose control, reduce glycemic variability, and minimize the risk of hypoglycemia[2-3].

Although there have been significant therapeutic advancements, a substantial proportion of patients with T1DM continue to struggle with achieving recommended HbA1c targets, experience frequent glycemic fluctuations, and remain at risk of severe hypoglycemic episodes[4]. Furthermore, insulin regimen complexity, frequent injections, and fear of hypoglycemia negatively impact treatment adherence and decrease quality of life[5-6]. Thus, selecting basal insulin therapies capable of effectively addressing these clinical issues continues to represent a critical aspect of diabetes management[5].

Despite recent advancements, human intermediate-acting insulins [neutral protamine hagedorn (NPH)] insulin, detemir, and insulin glargine (Gla-100) remain widely used as basal insulin options for T1DM treatment in Türkiye, primarily due to established patient-physician habits, strong clinical evidence, and lower cost. Insulin glargine 300 U/mL (Gla-300), a long-acting basal insulin with distinct pharmacokinetic properties, represents a second-generation alternative, exhibiting different absorption and distribution characteristics compared to first-generation basal insulins[1].

As an ultra-long-acting basal insulin, Gla-300 has been increasingly utilized in diabetes management due to its prolonged duration of action, stable pharmacokinetic profile, and comparable glycemic efficacy with a lower risk of hypoglycemia compared to first-generation basal insulin analogs in individuals with T1DM[7-12]. However, real-world data on Gla-300 use in T1DM remain limited due to variations in patient populations, treatment protocols, and healthcare practices, particularly in Türkiye[13-15].

This study retrospectively evaluates the long-term efficacy of Gla-300 in T1DM patients transitioning from other basal insulins, analyzing one-year data on glycemic control, insulin requirements, and hypoglycemia risk in a single-center setting.

MATERIALS AND METHODS

The medical records of patients with T1DM who attended the diabetes outpatient clinic of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine between 2020 and 2023 were retrospectively analyzed.

Patients whose treatment was switched to Gla-300 and who had been on this therapy for at least one year were included in the present study. Additionally, only those who attended regular outpatient follow-ups, demonstrated full treatment adherence, and performed routine 7-point self-monitoring of blood glucose were enrolled. Individuals with type 2 diabetes, as well as those with type 1 diabetes who were insulin-naive, using an insulin pump, or not adhering to regular insulin use were not included in the study. The patients included in the study had C-peptide levels ≤0.20 nmol/L. Additionally, patients with acute coronary syndrome, acute cerebrovascular events, chronic liver disease, cancer, or those undergoing dialysis for end-stage renal disease were not included. Patients using glucose-elevating medications such as steroids, those with a history of alcohol or drug abuse, pregnant individuals, participants enrolled in another clinical trial, and non-compliant patients were also not included in the study. All participants were following a diabetic diet, as confirmed by their medical records.

The demographic and clinical characteristics of the patients, their current basal and bolus insulin types and doses, the number of basal insulin injections, as well as the reasons for treatment modification, were recorded from medical files. The changes in the daily number of insulin injections, basal insulin dose, fasting plasma glucose (FPG), HbA1c levels, and the occurrence of hypoglycemic episodes compared to the previous basal insulin regimen were retrospectively evaluated at the initiation of Gla-300 treatment, the third month, the sixth month, and one year.

Glycemic control was considered uncontrolled if HbA1c levels exceeded 8%. Hypoglycemia was defined based on ADA criteria as a blood glucose level of ≤70 mg/dL and/or the presence of symptoms requiring treatment with fast-acting carbohydrates or adjustments in glucose-lowering therapy[16]. Hypoglycemia

was classified as "mild" when the patient could self-manage the episode without assistance and "severe" when external help or medical intervention was required.

Hypoglycemic events occurring before and after the initiation of glargine U-300 were classified into three categories based on their occurrence in the last three months: nocturnal hypoglycemia, daytime hypoglycemia, and severe hypoglycemia. In follow-up assessments, the presence or absence of nocturnal, daytime, and severe hypoglycemia was recorded.

The study was approved by the local ethics committee of Cerrahpaşa Medical Faculty Dean's Office Clinical Research

Ethics Committee (decision no: 192547, date: 17.12.2019) the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice principles.

Statistical Analysis

Statistical analyses were performed using SPSS Version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation for normally distributed data and median (minimum-maximum) for non-normally distributed data, while categorical variables were presented as numbers and percentages. Normality was assessed using the Shapiro-Wilk test and skewness-kurtosis values. For group comparisons, the Student's t-test was used for normally distributed data, while the Mann-Whitney U test was applied for non-normally distributed data. Categorical variables were analyzed using the Fisher's exact test or Pearson's chi-square test, as appropriate. For repeated measurements, Repeated Measures ANOVA was used, and the LSD post-hoc test was applied when needed. The median values of quantitative variables were determined as cut-off points and added into the model. A p-value<0.05 was considered indicative of statistical significance. A p-value<0.05 was considered statistically significant.

RESULTS

Forty-six patients with T1DM were enrolled in the study. The mean duration of diabetes was 20.67±7.22 (4-36) years. The demographic and clinical characteristics of participants are presented in Table 1.

Table 1. Demographic and clinical characteristics of participants (n=46)

Variable	Value
Age mean ± SD	34.22±7.98
Sex n (%)	
Female	25 (54.3)
Male	21 (45.7)
Weight (kg) mean ± SD	64.3±10.9
BMI (kg/m²) mean ± SD	24.5±7.7
Macrovascular complications, n (%)	2 (4.3)
Microvascular complications, n (%)	
Nephropathy	4 (8.7)
Retinopathy	5 (10.9)
Neuropathy	3 (6.5)
Fasting blood glucose (mg/dL) mean ± SD	217.28±77.4
HbA1c (%) (mmol/mol) Mean ± SD	8.45±1.27

SD: Standard deviation, BMI: Body mass index

At baseline, all participants were receiving a basal-bolus insulin regimen, and none were on oral antidiabetic agents. The distribution of basal insulins used at study initiation is presented in Table 2. Before the initiation of the study, 56.5% of the patients (n=26) were on once-daily basal insulin regimens, while 43.5% (n=20) used basal insulin twice daily. The most frequently used basal insulin was insulin glargine U-100 (54.3%), predominantly administered once daily (92.0%). Insulin detemir was used by 41.3% of the patients, mainly in a twice-daily regimen (84.2%). Only two patients (4.3%) were using NPH insulin, both on a twice-daily schedule. All patients in this study administered insulin glargine U-300 as their basal insulin in the evening. Regarding bolus insulin therapy, 73.9% (n=34) of the patients were using insulin aspart, and 26.1% (n=12) were on insulin lispro.

Frequent hypoglycemic episodes with the previous basal insulin regimen were the reason for switching to glargine U-300 in 28 patients (60.9%), whereas in 18 patients (39.1%), glargine U-300 was initiated due to uncontrolled hyperglycemia resulting from inadequate glycemic control. No adjustments were made to rapid-acting insulin therapy.

When patients were compared based on their baseline basal insulin type, those using insulin detemir had a significantly higher initial injection frequency than those using insulin glargine U-100 (p<0.001). Among patients using insulin glargine U-100, the prevalence of daytime hypoglycemia before switching to glargine U-300 was 64.6% (16/25), while nocturnal hypoglycemia was 72% (18/25). These rates were significantly higher compared to the detemir group [31.6% (6/19) and 36.8% (7/19), respectively; p=0.033 and p=0.020]. The incidence of severe hypoglycemia was similar in both groups. The most common reason for switching basal insulin was hypoglycemia in patients using glargine U-100 (76%) and inadequate glycemic control in those using insulin detemir (63.1%). No significant differences were found between the groups in other baseline clinical parameters. Since only two patients were using NPH insulin, this group was not statistically compared with the detemir and glargine U-100 groups.

Table 2. Basal insulin regimens before study initiation

Treatment	n (%)	Once-daily basal insulin n (%)	Twice-daily basal insulin n (%)
Basal insulin regimen	46 (100%)	26 (56.5%)	20 (43.5%)
Basal insulin			
Insulin glargine U-100	25 (54.3%)	23 (92.0%)	2 (8.0%)
Insulin detemir	19 (41.3%)	3 (15.8%)	16 (84.2%)
NPH insulin	2 (4.3%)	0 (0%)	2 (100%)

NPH: Neutral protamine hagedorn insulin

However, these patients had a long diabetes duration (mean: 16.5 years) and frequent hypoglycemia. Both experienced daytime and nocturnal hypoglycemia, which was the reason for switching to glargine U-300. Additionally, as they administered basal insulin in divided morning and evening doses, their daily injection frequency was higher.

Table 3 presents the HbA1c levels, FPG, and total daily glargine U-300 doses at baseline, as well as at the third, sixth, and twelfth months of Gla-300 treatment. The analysis of repeated measures revealed statistically significant differences in all parameters over time (p<0.001). The reduction in HbA1c levels was most pronounced during the first three months, decreasing from 8.45±1.27 at initiation to 7.83±1.01 at the third month (p<0.001). However, in pairwise comparisons, although the rate of decline slowed between the third and sixth months and between the sixth and twelfth months, the decrease remained statistically significant (p<0.001). Similarly, the decrease in FPG was most prominent in the first three months (from 217.28±77.4 mg/dL at initiation to 180.07±57.4 mg/dL at the third month, p<0.001). Pairwise comparisons showed that the reduction continued significantly between the third and sixth months (p=0.003) and between the sixth and twelfth months (p=0.006), albeit with a diminished rate of decline. Regarding total daily basal insulin dose, there was a statistically significant increase from initiation to the third month, from the third to the sixth month, and from the sixth to the twelfth month (p<0.001). However, pairwise comparisons indicated that, unlike HbA1c and FPG, the increase in insulin dose did not show a distinct difference between these periods. For the number of insulin injections, a statistically significant reduction was observed from initiation to the third month (p<0.001), while pairwise comparisons demonstrated that no further changes occurred after the third month.

Throughout the study period, no episodes of ketosis or hospitalization were observed.

Detailed data on changes in hypoglycemia rates are presented in Table 4. In this study, a statistically significant decrease was observed in all hypoglycemia categories over time (p<0.05). The most pronounced reduction occurred between baseline and the third month. The greatest decline was observed in nocturnal hypoglycemia rates (p<0.001). Severe hypoglycemia was no longer observed after the third month (p=0.041).

In the subgroup analysis based on the type of basal insulin used at baseline, changes in clinical parameters from baseline to month 12 (HbA1c, FPG, basal insulin dose, and the incidence of daytime, nighttime, and severe hypoglycemia) were compared. Except for a reduction in the number of injections, no statistically significant differences were observed in other parameters among patients using detemir. The decrease in daily injection frequency was more pronounced in the detemir group (p<0.001). Since only two patients were using NPH insulin, they were not included in the subgroup analysis. However, in both patients, reductions in HbA1c and FPG levels, as well as a decrease in the frequency of daytime, nighttime, and severe hypoglycemia, were observed, consistent with the overall patient population.

DISCUSSION

In this study, patients with type 1 diabetes who switched to glargine U-300 due to hypoglycemia or inadequate glycemic control were retrospectively evaluated over one year. Regardless of the type of previous basal insulin, treatment with glargine U-300 resulted in a significant reduction in HbA1c and FPG. This improvement was accompanied by a statistically significant decrease in the number of daily injections. Notably,

Table 3. Changes in HbA1c, fasting plasma glucose, and total daily basal insulin dose over the course of Gla-300 treatment

	Initiation Mean ± SD	3. month Mean ± SD	6. month Mean ± SD	12. month Mean ± SD	p-value
HbA1c (%)	[a]8.45±1.27	[b]7.83±1.01	[c]7.68±0.95	[d]7.45±0.8	**<0.001***
Fasting plasma glucose (mg/dL)	[a]217.28±77.4	[b]180.07±57.4	[c]170.52±49.39	[d]158.57±39.23	**<0.001***
Total daily basal insulin dose (IU)	[a]27.41±8.5	[b]30.33±8.63	[c]32.35±9.34	[d]34.54±9.46	**<0.001***
Number of insulin injection (n)	[b]4.39±0.49	[a]4±0	[a]4±0	[a]4±0	**<0.001**[+]

*Repeated measures were analyzed using ANOVA test, [+]Friedman test, different superscript letters [(a, b, c, d)] in the rows indicate statistically significant differences according to the LSD post-hoc test, SD: Standard deviation, IU: International unit

Table 4. Changes in hypoglycemia episodes over time (*)

Hypoglycemia type	Baseline n (%)	3rd month n (%)	6th month n (%)	12th month n (%)	p-value
General hypoglycemia	24 (52.2)	12 (26.1)	9 (19.6)	11 (23.9)	**0.017**
Nocturnal hypoglycemia	27 (58.7)	6 (13.0)	6 (13.0)	2 (4.3)	**<0.001**
Severe hypoglycemia	4 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)	**0.041**

[*]All hypoglycemia episodes refer to events occurring in the last 3 months, p-value were obtained using Pearson's chi-square test

nearly half of the patients had previously required twice-daily basal insulin regimens particularly those on detemir or NPH contributing substantially to the overall injection burden. The most pronounced changes were observed within the first three months. Despite a statistically significant increase in basal insulin dose during follow-up, a significant reduction was observed in all categories of hypoglycemia, including daytime, nocturnal, and severe hypoglycemia, with the most evident decrease occurring by the third-month follow-up. The reduction in nocturnal hypoglycemia was particularly prominent, and no severe hypoglycemia events were reported after the third month. No cases of ketosis or hospitalizations were reported during the study period.

Our findings are largely consistent with three major prospective, open-label, randomized studies evaluating the efficacy of insulin glargine U-300 (Gla-300) in patients with T1DM - EDITION 4, EDITION JP1, and its 12-month extension study[8,9,17]. These studies showed that Gla-300 was non-inferior to Gla-100 in reducing HbA1c levels, although this effect was achieved with higher basal insulin doses. Significant reductions in nocturnal hypoglycemia were reported, especially in the first 8 weeks, and the JP1 study also showed a statistically significant decrease in daytime hypoglycemia. In the extension phase, hypoglycemia continued to decline even with stable insulin doses, and HbA1c non-inferiority was maintained. None of these trials showed a significant difference in severe hypoglycemia incidence. Similarly, our study showed the most prominent reduction in HbA1c at the third month, which was maintained throughout one year. This early response may be attributed to the pharmacological properties of Gla-300. Long-term glycemic control might also be associated with regular dose titrations despite the retrospective design. Furthermore, a significant reduction in nocturnal hypoglycemia was observed despite increasing basal insulin doses, and daytime hypoglycemia also decreased, consistent with JP1 and its extension. Unlike the previous studies, we observed a statistically significant decline in severe hypoglycemia. These results align with the long-acting and stable pharmacodynamic profile of Gla-300. In support of this, Becker et al.[18] demonstrated that Gla-300 had a flatter and longer action profile compared to Gla-100, and Bergenstal et al.[10] showed that Gla-300 provided a more stable 24-hour glucose profile with reduced glycemic variability in patients with T1DM. Therefore, the improvements observed in our study are compatible with the known pharmacodynamic advantages of Gla-300.

Although the findings of prospective randomized trials are valuable, real-world data provide additional insight into the effectiveness and safety of treatments in routine clinical settings. In a study by Oriot et al.[19] including 116 patients with T1DM, switching from Gla-100 to Gla-300 led to similar glycemic control in the short term, with a significant reduction in nocturnal hypoglycemia and a modest but significant HbA1c reduction after six months. In our study, although the baseline basal insulin types were more heterogeneous, a similar improvement was observed with higher doses of Gla-300. Unlike Oriot's study[11], the most evident improvements in HbA1c and hypoglycemia frequency occurred by the third month and were maintained throughout follow-up. The SPARTA study is a large, multicenter real-world study in T1DM patients. As in our study, patients had previously used various basal insulins, and many were on twice-daily regimens. In SPARTA, HbA1c decreased by 0.4% overall and 0.6% in the twice-daily group ($p<0.001$), consistent with our results. In both studies, Gla-300 was used at higher doses compared to previous basal insulins. Although the proportion of patients switching due to hypoglycemia was lower in SPARTA (19%) than in our study (60.9%), this may reflect differences in patient selection and referral reasons. However, both studies showed a significant reduction in hypoglycemia rates and treatment burden due to injection frequency. Gla-300's ability to provide similar or better glycemic control with fewer injections supports our findings. A single-center retrospective study from Türkiye reported HbA1c and hypoglycemia improvements in 35 younger T1DM patients who switched to Gla-300 solely due to hypoglycemia, without dose adjustments. However, hypoglycemia subtypes were not specified, and HbA1c improvement appeared later. In contrast, our study included a more diverse population and showed early and consistent improvements with dose titration. Therefore, our findings offer a more comprehensive contribution to national real-world evidence.

The most recent real-world data on Gla-300 use in T1DM come from the TOP1 (n=123) and COMET-T (n=94) studies, published in 2024 and 2025, respectively[7,20]. In the COMET-T study, patients were monitored using continuous glucose monitoring (CGM), and a significant improvement was observed in time in range, especially among those who had previously used insulin detemir. A reduction in hypoglycemia frequency was also reported across the entire study group[7]. The TOP1 study evaluated patients switching from twice-daily basal insulin to once-daily Gla-300. It showed reductions in injection frequency and hypoglycemia, along with improved patient satisfaction[20]. In both studies, Gla-300 was associated with clinical and patient-centered benefits, particularly in those who had been using first-generation basal insulins. In our study, which retrospectively included 46 T1DM patients, similar findings were observed. Switching from detemir or NPH to Gla-300 led to a decrease in the number of daily injections, a significant early reduction in hypoglycemia rates, and improved HbA1c levels. Although patient satisfaction could not be assessed through structured questionnaires due to the retrospective design, high follow-up adherence may reflect overall treatment acceptability.

Although first-generation basal insulins such as NPH, detemir, and glargine U-100 have similar glycemic efficacy in type 1 diabetes treatment, pharmacodynamic differences especially with NPH and detemir often require more frequent injections[21]. This increases injection burden and may negatively affect patient comfort, treatment adherence, and quality of life[5]. Our study reflects how these pharmacological differences translate into clinical practice and adds to the current real-world evidence in this area.

Study Limitations

This study has several limitations. First, the retrospective design and relatively small sample size may limit the generalizability of the findings. Hypoglycemic episodes were assessed based on patient self-reports, as CGM was not used due to its high cost and lack of reimbursement. Additionally, body weight data were missing in many patient records, so the effect of Gla-300 on weight could not be evaluated. Finally, since all patients in this study received Gla-300 in the evening, the effectiveness of morning administration could not be assessed.

CONCLUSION

Type 1 diabetes is a unique patient population that requires long-term and intensive insulin therapy, with a direct impact on quality of life. Therefore, choosing basal insulin should be evaluated not only in terms of glycemic control but also treatment sustainability, patient profile, healthcare delivery, and quality of life. In Türkiye, first-generation basal insulins are still widely used, creating a treatment burden both for patients and physicians due to multiple daily injections. Our study provides updated real-world data on basal insulin preferences and clinical outcomes in T1DM management in our country. These findings suggest that Gla-300 may be considered as a treatment option in individualized care plans, especially when factors like patient comfort, hypoglycemia safety, adherence, and quality of life are taken into account.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee of Cerrahpaşa Medical Faculty Dean's Office Clinical Research Ethics Committee (decision no: 192547, date: 17.12.2019), the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice principles.

Informed Consent: The medical records of patients with T1DM who attended the diabetes outpatient clinic of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine between 2020 and 2023 were retrospectively analyzed.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.P.K., Z.O.S., Concept: Ö.P.K., Design: Ö.P.K, Z.O.S., Data Collection or Processing: Ö.P.K., Analysis or Interpretation: Ö.P.K., Z.O.S., Literature Search: Ö.P.K., Writing: Ö.P.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Yılmaz A, Demir B. Diyabet tanı ve tedavi yöntemi. Diyabetin klinik yönetimi. Tıp Yayınevi. 2023-32-42.
2. Lajara R, Cengiz E, Tanenberg RJ. The role of the new basal insulin analogs in addressing unmet clinical needs in people with type 1 and type 2 diabetes. Curr Med Res Opin. 2017;33:1045-55.
3. Dawoud D, O'Mahony R, Wonderling D, Cobb J, Higgins B, Amiel SA. Basal insulin regimens for adults with type 1 diabetes mellitus: a systematic review and network meta-analysis. Value Health. 2018;21:176-84.
4. James S, Perry L, Lowe J, Harris M, Craig ME; ADDN study group. Suboptimal glycemic control in adolescents and young adults with type 1 diabetes from 2011 to 2020 across Australia and New Zealand: data from the Australasian Diabetes Data Network registry. Pediatr Diabetes. 2022;23:736-41.
5. Karter AJ, Subramanian U, Saha C, Crosson JC, Parker MM, Swain BE, et al. Barriers to insulin initiation: the translating research into action for diabetes insulin starts project. Diabetes Care. 2010;33:733-5.
6. Cryer PE. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med. 2004;350:2272-9.
7. Gölz S, Mader JK, Bilz S, Kenzler J, Danne T. Safety and effectiveness of glargine 300 U/mL after switching from basal insulins in patients with type 1 diabetes: COMET-T study. Diabetes Ther. 2025;16:121-34.
8. Home PD, Bergenstal RM, Bolli GB, Ziemen M, Rojeski M, Espinasse M, et al. New insulin glargine 300 U/mL versus glargine 100 U/mL in people with type 1 diabetes: a randomized, phase 3a, open-label clinical trial (EDITION 4). Diabetes Care. 2015;38:2217-25.
9. Matsuhisa M, Koyama M, Cheng X, Takahashi Y, Riddle MC, Bolli GB, et al. New insulin glargine 300 U/mL versus glargine 100 U/mL in Japanese adults with type 1 diabetes using basal and mealtime insulin: glucose control and hypoglycaemia in a randomized controlled trial (EDITION JP 1). Diabetes Obes Metab. 2016;18:375-83.
10. Bergenstal RM, Bailey TS, Rodbard D, Ziemen M, Guo H, Muehlen-Bartmer I, et al. Comparison of insulin glargine 300 U/mL and 100 U/mL in adults with type 1 diabetes: continuous glucose monitoring profiles and variability using morning or evening injections. Diabetes Care. 2017;40:554-60.
11. Pang T, Bain SC, Black RNA, Boyle JG, Elliott J, Holcombe A, et al. A multicentre, UK, retrospective, observational study to assess the effectiveness of insulin glargine 300 units/mL in treating people with type 1 diabetes mellitus in routine clinical practice (SPARTA). Diabet Med. 2019;36:110-9.
12. Matsuhisa M, Odawara M, Hirose T, Koshida R, Senda M, Tanaka Y, et al. Real-world data on the use of insulin glargine 300 U/mL in Japanese patients with type 1 diabetes: twelve-month results from a post-marketing surveillance study (X-STAR study). Expert Opin Pharmacother. 2021;22:249-56.
13. Kişioğlu SV, Demir AS, Tufekci D, Gunay YE, Coskun H, Ucuncu O, et al. Clinical research of insulin glargine U300 in type 1 diabetes mellitus

patients with frequent hypoglycaemia: real-world experience. Arch Med Sci Atheroscler Dis. 2021;6:102-8.

14. Satılmış M, Şendur SN, İlhan A, Erbaş T, Gürlek A, Dağdelen S. Kırılgan tip 1 diyabet tedavisinde insülin glarjin U300: tek merkez gerçek yaşam deneyimi. 54. Ulusal Diyabet Kongresi. 2018;P235.
15. Güngör Semiz G, Ünal M Ç, Selimoğlu İ, Kalender S, Erbay E, Arayıcı ME et al. İnsülin glarjin U300 tedavisinin glisemik kontrol ve hipoglisemi üzerine etkisinin retrospektif olarak değerlendirilmesi: gerçek yaşam verisi. Endokurs 6 – Mezuniyet Sonrası Eğitim Kursu. 2022.
16. American Diabetes Association Professional Practice Committee. 6. Glycemic goals and hypoglycemia: Standards of Care in Diabetes-2024. Diabetes Care. 2024;47:128-45.
17. Matsuhisa M, Koyama M, Cheng X, Sumi M, Riddle MC, Bolli GB, et al. Sustained glycaemic control and less nocturnal hypoglycaemia with insulin glargine 300 U/mL compared with glargine 100 U/mL in Japanese adults with type 1 diabetes (EDITION JP 1 randomized 12-month trial including 6-month extension). Diabetes Res Clin Pract. 2016;122:133-40.
18. Becker RH, Dahmen R, Bergmann K, Lehmann A, Jax T, Heise T. New insulin glargine 300 U/mL provides a more even activity profile and prolonged glycemic control at steady state compared with insulin glargine 100 U/mL. Diabetes Care. 2015;38:637-43.
19. Oriot P, Jérémie W, Buysschaert M. Outcomes of glycemic control in type 1 diabetic patients switched from basal insulin glargine 100 U/mL to glargine 300 U/mL in real life. Expert Rev Endocrinol Metab. 2018;13:167-71.
20. Dualib PM, Dib SA, Augusto GA, Truzzi AC, de Paula MA, Réa RR. Effect of switching from twice-daily basal insulin to once-daily insulin glargine 300 U/mL (Gla-300) in Brazilian people with type 1 diabetes. Diabetol Metab Syndr. 2024;16:152.
21. Rosselli JL, Archer SN, Lindley NK, Butler LM. U300 insulin glargine: a novel basal insulin for type 1 and type 2 diabetes. J Pharm Technol. 2015;31:234-42.

ORIGINAL ARTICLE

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Screening of Premature Ovarian Insufficiency-Associated Genes in Turkish Patients

Türk Hastalarda Prematür Over Yetmezliği ile İlişkili Genlerin Taranması

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ABSTRACT

Aim: Infertility is primarily caused by premature ovarian insufficiency (POI). Since multiple genes have been linked to the genetic foundation of POI, the genetic investigation of the condition needs to be a component of the clinical diagnosis. Analyzing the genetic background of POI in a Turkish cohort was the goal of our investigation.

Materials and Methods: The onset age ranged from 18 to 39 years old. Every patient was prescreened for the most common POI-associated fragile-X premutation premutation and had the karyotype 46,XX. Next-generation sequencing (NGS) of 26 genes previously linked to POI was performed on 68 unrelated individuals from Türkiye in order to detect genetic changes.

Results: We examined the DNA samples of 68 unrelated POI patients in order to use targeted panel sequencing to find possible causal variations of the disease. Three POI-related genes in our sample had three heterozygous variants of unclear significance and one heterozygous potentially pathogenic gene. These variants were related to 3 genes: *Newborn ovary homeobox (NOBOX),* GDF9 and STAG3.

Conclusion: POI is distinguished by a complex genetic background with an increasing number of genes and diverse phenotypic traits. This is the first genetic epidemiology study in Türkiye focusing on the effects of 26 genes related to POI. Among the variations we detected in our patient group, the variation we detected in the *STAG3* gene has not been reported before. Two separate variations were detected in the *NOBOX* gene in two patients. Finally, one variation was detected in the *GDF9* gene. The variation in the *STAG3* gene was classified as likely pathogenic. The variations in the *NOBOX* and *GDF6* genes are classified as of unknown clinical significance. Due to the intricate network governing human folliculogenesis, individual patients exhibit significant phenotypic diversity, necessitating the development of NGS sequencing methods to aid in POI diagnosis.

Keywords: Premature ovarian insufficiency, genetic investigation, next-generation sequencing

ÖZ

Amaç: İnfertilite esas olarak prematür over yetmezliğinden (POI) kaynaklanır. POI'nin genetik temelinde birden fazla gen bulunduğundan, durumun genetik incelemesi klinik tanının bir bileşeni olmalıdır. Araştırmamızın amacı, POI'nin genetik geçmişini bir Türk kohortunda analiz etmekti. **Gereç ve Yöntem:** Başlangıç yaşı 18 ila 39 arasında değişen hastalar çalışmaya dahil edildi. Her hasta POI ile ilişkili Frajil X ön mutasyonu premutasyonu için önceden tarandı ve karyotipler 46,XX idi. Genetik değişiklikleri tespit etmek için POI ile bağlantılı 26 genin yeni nesil dizilemesi (NGS) aralarında akrabalık bulunmayan 68 bireyde gerçekleştirildi.

Bulgular: Hastalığın olası nedensel varyasyonlarını bulmak için hedefli panel dizilemesini kullanmak amacıyla 68 akrabalık ilişkisi bulunmayan POI hastasının DNA örneklerini inceledik. Hasta grubunda POI ile ilişkili 3 gende 3 heterozigot klinik önemi bilinmeyen varyant ve bir heterozigot olası patojenik varyasyon saptanmıştır. Bu varyantlar 3 genle ilişkiliydi: *Yenidoğan yumurtalık homeoboks geni* (*NOBOX),* GDF9 ve STAG3.

Sonuç: POI, giderek artan sayıda gen ve çeşitli fenotipik özelliklerle karmaşık bir genetik geçmişe sahiptir. Bu, POI ile ilgili 26 adet genin etkisine odaklanan Türkiye'nin ilk genetik epidemiyoloji çalışmasıdır. Hasta grubumuzda tespit ettiğimiz varyasyonlar arasında, *STAG3* geninde tespit ettiğimiz varyasyon daha önce bildirilmemiştir. *NOBOX* geninde 2 hastada 2 ayrı varyasyon saptanmıştır. Son olarak *GDF9* geninde bir adet varyasyon

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saptanmıştır. STAG3 genindeki varyasyon olası patojenik olarak sınıflandırılmıştır. *NOBOX* ve GDF6 genlerindeki varyasyonlar klinik önemi bilinmeyen sınıfında yer almaktadır. İnsan folikülogenezini yöneten karmaşık ağ nedeniyle, bireysel hastalar önemli fenotipik çeşitlilik göstermektedir ve bu durum POI tanısına yardımcı olmak için dizileme yöntemlerinin geliştirilmesini gerekli kılmaktadır. NGS taramasının kapsamını daha önce infertilite ile ilişkilendirilen genleri de kapsayacak şekilde genişletmek, POI için daha kesin, hızlı ve uygun fiyatlı genetik tanılamalara olanak sağlayabilir. Hastaların genomik analizi klinik karar almada yardımcı olabilir ve yaklaşan klinik denemeler ve tedaviler için kapıyı açabilir.

Anahtar Kelimeler: Prematüre over yetmezliği, genetik araştırma, yeni nesil dizileme

INTRODUCTION

Premature ovarian insufficiency (POI), also referred to as premature ovarian failure, denotes the cessation of ovarian function before the age of 40 years. By the end of the 2000s, the term POI was adopted to describe this condition of premature ovarian ageing more accurately. This terminology highlights that women with this dysfunction may occasionally experience spontaneous follicular development, the return of menses, and even conception following the diagnosis[1,2]. POI is characterized by the depletion of ovarian follicles, leading to infertility before the age of 40 years, and presents with a diverse range of clinical phenotypes[3]. This condition is defined by the absence of menses (amenorrhea or oligomenorrhea) for a minimum of four months, elevated gonadotropin levels [follicle-stimulating hormone (FSH) > luteinizing hormone], and hypoestrogenism[4]. Primary amenorrhea, typically identified early in life in individuals with delayed puberty, absent breast development, and menarche, can be the first warning sign. The most common POI phenotype, secondary amenorrhea, however, manifests between the ages of 20 years and 40 years and is characterized by an irregular menstrual cycle followed by amenorrhea alongside normal pubertal development[3]. The studies showed that the global overall prevalence of POI among women was 3.5%. By subgroup analysis, the prevalence of POI among women with iatrogenic etiology was 11.2%, followed by autoimmunity (10.5%); the prevalence of POI by region was 11.3% at the highest in North America followed by South America (5.4%); and the prevalence of POI was 5.3% in a developing country, higher than 3.1% in a developed country. The trend of prevalence of POI over the past 20 years was on the rise (although $p>0.05$)[5].

Genetic flaws, autoimmune disorders, iatrogenic causes (such as chemotherapy or radiation therapy), viral infections, poisons, or even idiopathic conditions can all contribute to POI[5].

Nonetheless, many impacted women have a positive family history. POI with various genetic etiologies may suggest a hereditary status of the cases[6]. Surgical, medical, infectious or autoimmune ovarian damages are the other well-known reasons for POI[7].

Regarding genetic factors, the POI phenotype can be instigated by chromosomal abnormalities and monogenic disorders. Following the confirmation of a clinical diagnosis of POI, chromosome analysis, fragile-X premutation (FMR1) testing, evaluation of thyroid and adrenal (21-hydroxylase) antibodies, and pelvic ultrasonography should all be performed[3]. Approximately 10-13% of individuals exhibit chromosomal abnormalities[8]. In terms of chromosomal origins, X-chromosome abnormalities account for 12% of POI cases. These abnormalities encompass monosomy, trisomy, deletions, duplications, and X-autosome translocations[9]. Critical X chromosomal regions potentially correlating with POI have been identified through cytogenetic studies. POI has been associated with deletions in the Xq21.3-q27 region or X-autosome translocations in the Xq13.3-q21.1 region. Additionally, POI has been linked to the deletion of the p arm of the X chromosome. Cytogenetic analysis can be utilized to evaluate karyotypes for numerical alterations, and various methods, including array comparative genomic hybridization, have been developed to identify copy number variants within the context of POI[10].

Furthermore, expanding a cytosine-guanine-guanine repeat in the 5′ regulatory region of the *FMR1* gene, which results in Fragile-X syndrome, may also contribute to syndromic POI. Since the *FMR1* premutation is linked to POI in approximately 20% of affected women, its presence in those diagnosed with POI should be investigated[11]. Microdeletions in the *FMR2* gene may also significantly contribute to POI, as suggested by another study[12]. Most cases of POI remain without a clear underlying cause; however, this screening might be useful in identifying the etiology of POI. Genetic disorders involved include not only Turner syndrome and *FMR1* gene premutation, but also monogenic disorders. Karyotype analysis is insufficient to resolve all cases of POI due to low resolution. Since *FMR1* gene premutation analysis can only resolve some of the cases (3% to 15% of cases of POI), monogenic gene analysis appears to be the most accurate method choice[13]. Therefore, monogenic analysis should be the next step if the tests do not provide any positive findings substantiating the POI diagnosis.

Given that the intricate network governing human folliculogenesis leads to significant phenotypic variance in POI syndrome with a diverse genetic etiology, NGS analysis could offer a more accurate, rapid, and cost-effective genetic diagnosis for POI[14-2]. Furthermore, a theory positing oligogenic

origins for this condition has been proposed, highlighting the necessity for multigene panel sequencing[15,16]. Previous studies suggest that these genes may be clustered in the POI1 and POI2 loci on the female sex chromosome[17]. It is suggested that several X- and autosome-encoded genes are critical candidates for POI, as they may play a role in human folliculogenesis. A more effective diagnostic pathway could be developed by further investigating their functional contributions to the genetic etiology of POI in clinical settings.

Our understanding of the genetic causes of idiopathic POI has significantly increased in the Next-generation sequencing (NGS) era. Numerous novel pathogenic variants of well-known genes [*FSHR, GDF9, BMP15, FIGLA*, and *Newborn ovary homeobox (NOBOX*)] have been linked to POI through high-throughput sequencing approaches[3]. Due to their roles in sex chromosome remodeling, metabolism, autoimmune associations, meiosis and DNA repair, oogenesis, folliculogenesis, hormone signaling, and germ cell development, these genes have been proposed to play a part in the etiology of POI. Furthermore, extensive genomic research paves the way for discovering additional gene variants underlying currently unknown POI. Our expanding knowledge may lead to more promising results when analyzing the genetic makeup of POI patients and may unveil new pathways for discovering potential treatments for women with POI. This is the only comprehensive genomic investigation to date on Turkish POI patients that utilizes the potential of the NGS method.

MATERIALS AND METHODS

Subjects

Sixty-eight Turkish-unrelated patients diagnosed with POI, who experienced amenorrhea for at least six months before the age of 40 years and had FSH plasma levels exceeding 40 IU/L, were recruited. The age of onset ranged from 18 to 39 years. Each patient underwent an *FMR1* molecular analysis and had at least 20 cells karyotyped. We excluded patients found to have Turner syndrome based on the karyotype or any other karyotype abnormality, as well as patients with a FMR1 premutation. Patients who previously underwent a gonadotoxic treatment (chemotherapy or pelvic radiation) or extensive ovarian surgery were also excluded from the study. NGS analysis was performed after all these exclusion criteria were met. Each patient signed a paper granting informed consent. The local ethics committee approved the study. All procedures performed in the study involving human participants were approved by the Ethics Committee for Scientific Research, Faculty of Medicine, Trakya University (decision no: TÜTF-BAEK 2018/319, date: 01.10.2018) and followed the Declaration of Helsinki. Targeted panel sequencing was performed on 68 POI patients (P01∼P68).

Targeted Panel Sequencing

Our goal has been to cover every known POI risk locus. Based on information from the literature, a list of 26 genes under investigation was compiled (Table 1).

Sixty-eight samples were sequenced using the QIAseq Targeted DNA Custom Panel (Qiagen, Hilden, Germany). Two milliliters (2 mL) of peripheral blood were collected and preserved in anticoagulation tubes. Genomic DNA was isolated from peripheral whole blood using the EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany). After DNA extraction, target sequences were enriched using customized capture probe chips (Illumina, San Diego, CA). This kit included 26 genes associated with the disease. Libraries covering the target genes were prepared according to the QIAseq Targeted DNA Panel Protocol (Qiagen, Hilden, Germany). Following the target enrichment process, libraries were sequenced on the MiSeq system (Illumina, San Diego, CA, USA). Obsessive compulsive

Table 1. A panel of 26 candidate genes

Gene name	Transcript ID	Protein ID
BMP15	NM_005448.2	NP_005439.2
CYP17A1	NM_000102.3	NP_000093.1
CYP19A1	NM_000103.3	NP_000094.2
DIAPH2	NM_007309.3	NP_009293.1
ERCC6	NM_000124.3	NP_000115.1
FIGLA	NM_001004311.3	NP_001004311.2
FMR1	NM_002024.5	NP_002015.1
FOXL2	NM_023067.3	NP_075555.1
FSHR	NM_000145.3	NP_000136.2
GALT	NM_000155.3	NP_000146.2
GDF9	NM_005260.5	NP_005251.1
GNAS	NM_080425.3	NP_536350.2
HFM1	NM_001017975.4	NP_001017975.4
LHCGR	NM_000233.3	NP_000224.2
LMNA	NM_170707.3	NP_733821.1
MCM8	NM_182802.2	NP_877954.1
MSH5	NM_172165.3	NP_751897.1
NOBOX	NM_001080413.3	NP_001073882.3
NR5A1	NM_004959.4	NP_004950.2
POF1B	NM_024921.3	NP_079197.3
POLG	NM_002693.2	NP_002684.1
POR	NM_000941.2	NP_000932.3
PSMC3IP	NM_016556.3	NP_057640.1
STAG3	NM_001282716.1	NP_001269645.1
STAR	NM_000349.2	NP_000340.2
SYCE1	NM_130784.3	NP_570140.1
WT1	NM_024426.4	NP_077744.3

inventory analysis (Qiagen, Hilden, Germany) was employed to control quality and generate Variant Call Format files. In silico evaluation of the pathogenicity of nucleotide changes in exons was performed using Polymorphism Phenotyping v2 (PolyPhen-2, http:// genetics.bwh.harvard.edu/pph2/), Sorting Intolerant from Tolerant (SIFT, https://sift.bii.a-star.edu.sg/), and MutationTaster (http://www.mutationtaster.org). Minor allele frequencies were checked in the Genome Aggregation Database gnomAD (http://gnomad.broadinstitute.org/). Variant analysis was conducted using Ingenuity software (Qiagen, Hilden, Germany). Variants were interpreted according to the American College of Medical Genetics and Genomics (ACMG) recommended standard. Sanger sequencing was performed for confirmation when target region coverage was less than 15 reads. Nucleotide alterations were analyzed and validated by Sanger sequencing. After confirmation, each variant was classified as a pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, or benign, according to the ACMG guidelines. Coding genomic regions that were sequenced with coverage less than 15X were eventually re-sequenced using Sanger technology. All detected variations were confirmed to be accurate by Senger sequencing.

NGS assays produced ~14,5 GB data for 68 samples for each individual as pair-end reads, having up to 97 bp mean read length, and about 90% (0.2 Mb length) of the targeted bases were covered, thereby sufficiently passing our thresholds for calling single nucleotide polymorphisms and short insertions or deletions (indels).

Primer sets were created for all required areas in order to execute Sanger sequencing on an ABI 3130 (Applied Biosystems, USA) capillary electrophoresis machine and validate the variations and segregation analyses.

RESULTS

We examined the DNA samples of sixty-eight unrelated patients with POI using targeted panel sequencing to identify potential causal variations of the disease. Within our sample, three POI-related genes exhibited three heterozygous VUS and one heterozygous gene that may be pathogenic. With the assistance of Franklin (available online at https://franklin.genoox.com) and VarSome[18], we detailed the clinical interpretation and implications for the identified variants. All variants identified were heterozygous, with most classified as missense (Table 2).

A total of one likely pathogenic variant (STAG3) was identified in 1.47% (1 of 68) of POI patients, which is regarded as a molecular genetic diagnosis of POI. VUS were detected in two genes in three patients from the total patient group. The genes identified were *NOBOX* and *GDF9*. A frameshift was recognized in one of the 68 patients.

Statistical Analysis

Characteristics of patients were gathered through a review of electronic medical records. Descriptive statistics, such as medians, ranges, and frequencies, were employed.

DISCUSSION

POI is characterized by a robust genetic background with increasing genes and diverse phenotypic traits[3]. The POI phenotype is associated with detrimental gene variations affecting meiosis, DNA repair, gonadal development (oogenesis and folliculogenesis), hormone signaling, immunological function, and metabolism[3]. This study represents, to our knowledge, the first genetic epidemiology study in Türkiye focusing on 26 genes in a single panel associated with POI, and several genetic alterations have been identified in genes associated with this condition. Sixty-eight Turkish patients with a clinical diagnosis of POI, who had undergone prescreening for *FMR1* pathogenic expansion, were included in our investigation.

The NM_001282717.2 (*STAG3*):c.1237_1238insAA variation identified in one of our patients constitutes a frameshift alteration that has not previously been reported for POI.

The meiosis-specific subunit of the cohesin complex, encoded by *STAG3*, comprises SMC1α/SMC1β, SMC*3*, RAD21/REC8, or RAD21L[19]. Sister chromatids are held together during mitosis and meiosis by the multiprotein cohesin complex encircles them in a ring. SMC1β, REC8, and RAD21L are exclusively present in meiotic cells, while SMC1α and RAD21 are ubiquitously found[20]. Although *STAG3* is restricted to the testes and ovaries[21,22], *STAG1/2* is present in mitotic cells[23]. The phenotypic effect of the *STAG3* gene exhibits recessive inheritance.

Table 2. Potential causal variants found in 4 POI patients via targeted panel sequencing

Gene	ACMG	dbSNP ID	Sequence change	Type
GDF9	VUS (PM2, PP3)	-	NM_005260.7:c.1297G>A (p.E433K)	Missense
NOBOX	VUS (PM1, PP3)	rs749172175	NM_001080413.3: c.1067G>A (p.R356Q)	Missense
STAG3	Likely Pathogenic (PM2, PVS1)	-	NM_001282717.2:c.1237_1238insAA p. (Ile413LysfsTer10)	Frameshift
NOBOX	VUS (PM2, PP3)	-	NM_001080413.3:c.1788G>C (p.Trp596Cys)	Missense

POI: Premature ovarian insufficiency, VUS: Variant of unknown significance, ACMG: American College of Medical Genetics and Genomics

Table 3. Clinical features (patient group average)	
Actual age	**29**
Amenorrhea	Primary
FSH, IU/L	96.2
LH, IU/L	20.8
E2 pg/mL	16
FSH: Follicle-stimulating hormone, LH: Luteinizing hormone	

In the study by Caburet et al.[22], the identified homozygous STAG3 variant leads to a premature stop codon, and autosomal recessive inheritance was demonstrated in four sisters of consanguineous parents. In another more recent study, a homozygous donor splice-site variant of STAG3 was reported leading to POI in two females[25].

There was no anomaly other than POI in our patient, for whom we detected *STAG3* heterozygous frameshift variation. However, the contribution of variations in the *STAG3* gene to the POI has been reported in the literature. Our finding is consistent with this finding. However, due to the recessive inheritance of STAG3, we do not expect any phenotypic effects[13,26].

Variations in the *NOBOX* gene were identified in two patients, both classified as VUS. The *NOBOX* gene is believed to be one of the primary genetic contributors to POI[27,28]. This ovarian *homeobox* gene is involved in the early stages of folliculogenesis, with the phenotypic effect of the *NOBOX* gene exhibiting dominant inheritance. Although the variant detected in our patient (rs749172175) is defined as VUS according to ACMG criteria, it is evaluated as "pathogenic" in variant evaluation prediction tools and the allele frequency given as 0.00000881 in the gnomAD database supports this possibility. The variations we detected in the *NOBOX* gene are consistent with the studies in the literature. Another variation detected in *NOBOX*, c.1788G>C (p.(Trp596Cys), was evaluated as VUS. However, there are values in the databases suggesting pathogenicity (SIFT: Damaging, CADD (phred): 27.10). However, since both variations were reported as VUS, their clinical significance is unknown[29].

A variation classified as a VUS was identified in the *GDF9* gene in one of our patients. Clinical evaluation of the patient revealed POI. According to Qin et al.[28] and Jiao et al.[30], variations in *GDF9* are frequently cited among the top 20 common genetic causes of POI. Notably, various *GDF9* variations have been found globally, albeit with a slightly uneven distribution by geography and ethnicity. The phenotypic effect of the *GDF9* gene exhibits recessive inheritance. Since the *GDF9* variation in our patient was both heterozygous and classified as a VUS, we did not consider it likely to have a phenotypic effect. The c.1297G>A variation detected in GDF9 has not been reported before. Therefore, a comparison with the literature could not be made.

High-throughput methods have been essential for identifying novel variants in candidate genes and genes previously linked to POI. The encoded proteins' primary functions are meiosis and DNA repair, gonadal development (oogenesis and folliculogenesis), hormone signaling, immunological response, and metabolism. As shown in several animal models, changes in genes related to meiosis and DNA repair may result in distinct symptoms of ovarian insufficiency because of the resting state of oocytes[31].

Study Limitations

There are certain limitations in our study. One of them is the number of the patients. There may not be enough power to detect rare variants with a frequency of less than 5% in 68 patients. We could obtain more results with an increased number of patients. A setting of risk prediction, routine diagnosis, and early intervention will benefit greatly from targeted panels of hotspot mutations and/or validated causal genes, as well as broader analysis like whole-exome and whole genome sequencing in the future.

CONCLUSION

POI is a highly diverse disorder that may have a complex array of genetic variants due to its many contributing causes. Furthermore, phenotypic variability is complicated by the unclear definition and nomenclature of POI, which exacerbates the genetic heterogeneity of POI and vice versa. It is uncertain if different phenotypes-like ovarian dysgenesis, primary or secondary amenorrhea, or early or late onset POI-share the same genetic makeup but have differing cumulative effects. A very large number of variations with unclear significance will surface in the NGS era. Data analysis and filtering will be quite difficult but crucial. Mutation-directed transgenic models or transformation experiments should be used to confirm causal relevance. However, it is getting more and harder to identify a single genetic alteration in any one POI patient as "causative" because many of the known POI genes appear to work in concert while exhibiting characteristics of incomplete penetrance or variable expressivity on their own. The relationship between genetics and phenotype requires more investigation.

Determining who is at risk for POI is still difficult. The potential of NGS technologies in clinical practice is huge.

Ethics

Ethics Committee Approval: All procedures performed in the study involving human participants were approved by the Ethics Committee for Scientific Research, Faculty of Medicine, Trakya University (decision no: TÜTF-BAEK 2018/319, date: 01.10.2018) and followed the Declaration of Helsinki.

Informed Consent: Each patient signed a paper granting informed consent.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: H.G., S.Y., K.E., S.A., Concept: H.G., E.A., K.E., S.A., Design: E.İ.A., Data Collection or Processing: E.İ.A, H.G., S.Y., H.S.G., D.Z., E.A., Analysis or Interpretation: E.İ.A, S.D., Literature Search: E.İ.A., Writing: E.İ.A.

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REFERENCES

1. Rebar RW, Connolly HV. Clinical features of young women with hypergonadotropic amenorrhea. Fertil Steril. 1990;53:804-10.
2. Nelson LM, Anasti JN, Kimzey LM, Defensor RA, Lipetz KJ, White BJ, et al. Development of luteinized graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. J Clin Endocrinol Metab. 1994;79:1470-5.
3. França MM, Mendonca BB. Genetics of primary ovarian insufficiency in the next-generation sequencing era. J Endocr Soc 2019;4:37.
4. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, et al. ESHRE guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31:926-37.
5. Li M, Zhu Y, Wei J, Chen L, Chen S, Lai D. The global prevalence of premature ovarian insufficiency: a systematic review and meta-analysis. Climacteric. 2023;26:95-102; Conway GS. Premature ovarian failure. Curr Opin Obstet Gynecol. 1997;9:202-6.
6. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update. 2015;21:787-808.
7. Oral E, Toksoy G, Sofiyeva N, Celik HG, Karaman B, Basaran S, et al. Clinical and genetic investigation of premature ovarian insufficiency cases from Turkey. J Gynecol Obstet Hum Reprod. 2019;48:817-23.
8. Tamhankar PM, Tamhankar VP, Vaniawala S. Genetics of premature ovarian insufficiency. Fertility Science and Research. 2024;11:6.
9. Bestetti I, Castronovo C, Sironi A, Caslini C, Sala C, Rossetti R, et al. High-resolution array-CGH analysis on 46,XX patients affected by early onset primary ovarian insufficiency discloses new genes involved in ovarian function. Hum Reprod. 2019;34:574-83
10. Sullivan SD, Welt C, Sherman S. FMR1 and the continuum of primary ovarian insufficiency. Semin Reprod Med. 2011;29:299-307.
11. Murray A, Webb J, Dennis N, Conway G, Morton N. Microdeletions in FMR2 may be a significant cause of premature ovarian failure. J Med Genet. 1999;36:767-70.
12. França MM, Funari MFA, Lerario AM, Santos MG, Nishi MY, Domenice S, et al. Screening of targeted panel genes in Brazilian patients with primary ovarian insufficiency. PLoS ONE. 2020;15:0240795.
13. Eskenazi S, Bachelot A, Hugon-Rodin J, Plu-Bureau G, Gompel A, Catteau-Jonard S, et al. Next generation sequencing should be proposed to every woman with "idiopathic" primary ovarian insufficiency. J Endocr Soc. 2021;5:bvab032.
14. Frank M, Prenzler A, Eils R, Graf von der Schulenburg JM. Genome sequencing: a systematic review of health economic evidence. Health Econ Rev. 2013;3:29
15. Fonseca DJ, Patiño LC, Suárez YC, de Jesús Rodríguez A, Mateus HE, et al. Next generation sequencing in women affected by non-syndromic premature ovarian failure displays new potential causative genes and mutations. Fertil Steril. 2015;104:154-62.
16. Fassnacht W, Mempel A, Strowitzki T, Vogt P. Premature ovarian failure (POF) syndrome: towards the Molecular Clinical Analysis of its genetic complexity. Curr Med Chem. 2006;13:1397-410.
17. Knight AK. Explaining the unexplained: new genetic mutations in unexplained premature ovarian insufficiency. FS Rep. 2020;1:168-9
18. Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, et al. VarSome: the human genomic variant search engine. Bioinformatics. 2019;35:1978-80.
19. Nishiyama T. Cohesion and cohesin-dependent chromatin organization. Curr Opin Cell Biol. 2019;58:8-14.
20. Ward JD, Sutton PC, Werner AD, Costanza R, Mohr SH, Simmons CT. Is Decoupling GDP Growth from Environmental Impact Possible? PLoS One. 2016;11:164733.
21. Xiao WJ, He WB, Zhang YX, Meng LL, Lu GX, Lin G, et al. In-frame variants in STAG3 gene cause premature ovarian insufficiency. Front Genet. 2019;10:1016.
22. Caburet S, Arboleda VA, Llano E, Overbeek PA, Barbero JL, Oka K, et al. Mutant cohesin in premature ovarian failure. N Engl J Med. 2014;370:943-9.
23. Carramolino L, Lee BC, Zaballos A, Peled A, Barthelemy I, Shav-Tal Y, et al. SA-1, a nuclear protein encoded by one member of a novel gene family: molecular cloning and detection in hemopoietic organs. Gene. 1997;195:151-9.
24. Bouilly J, Bachelot A, Broutin I, Touraine P, Binart N. Novel NOBOX loss-of-function mutations account for 6.2% of cases in a large primary ovarian insufficiency cohort. Hum Mutat. 2011;32:1108-13.
25. He WB, Banerjee S, Meng LL, Du J, Gong F, Huang H, et al. Whole-exome sequencing identifies a homozygous donor splice-site mutation in STAG3 that causes primary ovarian insufficiency. Clin Genet. 2018;93:340-4.
26. Heddar A, Dessen P, Flatters D, Misrahi M. Novel STAG3 mutations in a Caucasian family with primary ovarian insufficiency. Mol Genet Genomics. 2019;294:1527-34.
27. Jiao X, Ke H, Qin Y, Chen ZJ. Molecular genetics of premature ovarian insufficiency. Trends Endocrinol Metab. 2018;29:795-807.
28. Luo W, Ke H, Tang S, Jiao X, Li Z, Zhao S, et al. Next-generation sequencing of 500 POI patients identified novel responsible monogenic and oligogenic variants. J Ovarian Res. 2023;16:39.
29. Qin Y, Choi Y, Zhao H, Simpson JL, Chen ZJ, Rajkovic A. NOBOX homeobox mutation causes premature ovarian failure. Am J Hum Genet. 2007;81:576-81.
30. Jiao SY, Yang YH, Chen SR. Molecular genetics of infertility: loss-of-function mutatioin humans and corresponding knockout/mutated mice. Hum Reprod Update. 2021;27:154-189.
31. Huhtaniemi I, Hovatta O, La Marca A, Livera G, Monniaux D, Persani L, et al. Advances in the Molecular Pathophysiology, Genetics, and treatment of primary ovarian insufficiency. Trends Endocrinol Metab. 2018;29:400-19.

ORIGINAL ARTICLE

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Prognostic Factors Influencing the Efficacy of Regorafenib in the Treatment of Metastatic Colorectal Cancer

Metastatik Kolorektal Kanser Tedavisinde Regorafenib Etkinliğini Etkileyen Prognostik Faktörler

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ABSTRACT

Aim: Metastatic colorectal cancer (mCRC) remains a significant clinical challenge for patients who have exhausted standard treatment options. Regorafenib, an oral multikinase inhibitor, is approved for use in refractory with mCRC patients; however, its real-world efficacy continues to be an area of ongoing research. This study aimed to evaluate the efficacy and clinical outcomes of regorafenib in mCRC patients.

Materials and Methods: This retrospective study assessed the efficacy of regorafenib in mCRC patients who had progressed after at least two lines of systemic therapy. A total of 120 patients were included in the study. Univariate and multivariate analyses of factors affecting survival were conducted using the Cox regression models.

Results: Of the patients, 46 (38.3%) were female and the median age was 58 years. The median progression-free survival (PFS) was 3.38 months and the median overall survival (OS) was 8.01 months. Age and BRAF mutation status were determined as important prognostic factors for PFS. Patients under 65 years of age had a shorter PFS compared to patients aged 65 years and older (p=0.045). Patients with BRAF mutations exhibited significantly shorter PFS compared to those without the mutation (1.84 vs. 3.41 months, p=0.014). In OS analysis, ECOG score (p=0.022), regorafenib dose reduction (p=0.005) and carbohydrate antigen 19-9 (CA19-9) level (p=0.004) were independent prognostic factors. KRAS and NRAS mutations, primary tumor localization and prior targeted therapies combined with chemotherapy did not significantly affect PFS or OS.

Conclusion: Regorafenib is an effective option for the treatment of mCRC in third-line and beyond. ECOG performance status, regorafenib dose adjustment and CA19-9 levels are significant factors influencing survival.

Keywords: Regorafenib, metastatic colorectal cancer, survival

ÖZ

Amaç: Metastatik kolorektal kanser (mCRC), standart tedavi seçeneklerini tüketmiş hastalarda önemli bir klinik zorluk olmaya devam etmektedir. Oral bir multikinaz inhibitörü olan regorafenib, refrakter mCRC hastalarında kullanım için onaylanmıştır, ancak gerçek yaşamdaki etkinliği hala araştırılmaktadır. Bu çalışmanın amacı, regorafenibin mCRC hastalarındaki etkinliğini ve klinik sonuçlarını değerlendirmektir.

Gereç ve Yöntem: Bu retrospektif çalışma, en az iki sıra sistemik tedaviden sonra progresyon gösteren mCRC hastalarında regorafenibin etkinliğini değerlendirmektedir. Çalışmaya toplam 120 hasta dahil edilmiştir. Sağkalımı etkileyen faktörlerin tek değişkenli ve çok değişkenli analizleri Cox regresyon modelleri kullanılarak oluşturulmuştur.

Bulgular: Hastaların 46'sı (38,3) kadındı ve ortanca yaş 58 bulundu. Medyan progresyonsuz sağkalım (PFS) 3,38, medyan genel sağkalım (GS) ise 8,01 ay olarak bulundu. Yaş ve BRAF mutasyon durumu PFS için önemli prognostik faktörler olarak belirlendi. 65 yaş altı hastalarda PFS 65 yaş ve üstü hastalara kıyasla daha kısaydı (p=0,045). BRAF mutasyonu olan hastalar, mutasyonu olmayanlara göre anlamlı derecede daha kısa PFS gösterdi (1,84 vs. 3,41 ay, p=0,014). GS analizinde, ECOG skoru (p=0,022), regorafenib dozunun azaltılması (p=0,005) ve karbonhidrat antijen 19-9 (CA19-9)

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düzeyi (p=0,004) bağımsız prognostik faktörlerdi. KRAS ve NRAS mutasyonları, primer tümör lokalizasyonu ve kemoterapi ile kombine edilen önceki hedefe yönelik tedaviler, PFS veya GS'yi anlamlı şekilde etkilemedi.

Sonuç: Regorafenib üçüncü basamak ve sonrasında mCRC tedavisinde etkili bir seçenektir ve ECOG performans skoru, regorafenib doz ayarlamaları ve CA19-9 düzeyleri sağkalımı belirleyen önemli faktörlerdir.

Anahtar Kelimeler: Regorafenib, metastatik kolorektal kanser, sağkalım

INTRODUCTION

Colorectal cancer (CRC) is a widespread malignancy and a high contributor to cancer-related mortality[1-2]. Although the incidence is increasing, mortality is decreasing, probably due to earlier diagnosis, surgical success and treatment options[3]. Fluoropyrimidine-based therapies combined with oxaliplatin or irinotecan have been the backbone of metastatic colorectal cancer (mCRC) treatment for many years[4]. However, there is a burgeoning clinical demand for efficient tertiary and beyond therapeutic choices for patients who have exhausted standard first- and second-line treatment alternatives. In mCRC patients with good performance status (PS) and potential to respond to treatment, resistance development and exhaustion of effective options in earlier lines complicate disease management in third-line and beyond. Regorafenib is a tyrosine kinase inhibitor and is a treatment option used in line 3 and beyond for this purpose[5]. Clinical trials, in particular the REFLECT and CONCUR studies, have shown a clear survival advantage of regorafenib[6]. Although its efficacy has been demonstrated in phase 3 trials, the prognostic and predictive factors determining the clinical efficacy of regorafenib have not been fully clarified. Age, gender, tumor localization (right or left colon), and molecular mutation profiles such as KRAS, NRAS, and BRAF are among the factors that may influence treatment response. In particular, due to biological differences between right and left colon tumors, whether the efficacy of regorafenib is different in these groups is not yet clear.

We investigated the role of regorafenib in overall survival (OS) and progression-free survival (PFS) in mCRC patients in the third-line setting and beyond. Given the limited real-world data on regorafenib use beyond second-line treatment in mCRC, this study seeks to provide clinically relevant observations regarding patient characteristics that may influence survival outcomes.

MATERIALS AND METHODS

Patients diagnosed with colorectal adenocarcinoma by the pathology unit and seen in the medical oncology outpatient clinic between January 2017 and November 2024 were retrospectively analyzed. Patients over 17 years of age were included. The project was approved by Marmara University Faculty of Medicine Ethical Committee for Non-Pharmaceutical and Non-Medical Device Research Ethics Committee (decision no: 09.2024.1591, date: 24.12.2024). All patients received regorafenib in any line during the metastatic period. In this study, regorafenib was selected as a third-line or later therapy in patients with treatment potential who had exhausted chemotherapy options. The choice of third-line treatment was based on prior response to chemotherapy. Specifically, in patients who had achieved remission for more than six months with chemotherapy, chemotherapy rechallenge was preferred as the initial third-line option. However, in patients who experienced rapid progression under chemotherapy, regorafenib was prioritized in the third-line setting. These selection criteria ensured that treatment decisions were tailored to disease dynamics and individual patient response patterns.

Radiologic imaging methods were used to evaluate response to treatment. Response to regorafenib was defined according to radiological assessment. Patients achieving complete response (CR), partial response (PR), or stable disease (SD) were classified as responders, whereas those with progressive disease (PD) were categorized as non-responders. Clinicopathologic and demographic characteristics and laboratory parameters of the patients were obtained from patient files and the electronic database of the hospital. The association of parameters such as age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), tumor location, targeted therapies used in combination with chemotherapy, presence of RAS and BRAF mutations with OS and PFS in patients receiving regorafenib was analyzed. Although dose reductions were recorded in the dataset, detailed documentation of adverse events was not consistently available in-patient records due to the retrospective nature of the study. Therefore, a comprehensive analysis of toxicity profiles could not be conducted. However, based on available notes, the most frequently reported reasons for regorafenib dose modification were anorexia, fatigue, and dermatologic toxicity, such as hand-foot skin reaction.

Statistical Analysis

Statistical analysis was performed using SPSS software program version 26.0. Continuous variables were summarized as median interquartile range (IQR), while categorical variables were presented as frequencies and percentages. The comparison of continuous variables between groups was performed using the Mann-Whitney U test (for two groups) or the Kruskal-Wallis test (for three or more groups), depending on the number of categories. Categorical variables were analyzed using chi-

square or Fisher's exact tests as appropriate. Survival curves for each subgroup were constructed using the Kaplan-Meier method with 95% confidence intervals (CI). Between-group survival differences were evaluated using the log-rank test. Prognostic factors were initially assessed by univariate analysis and factors with a value of p less than 0.05 were then included in the multivariate analysis. Hazard ratios (HRs) were calculated using the Cox proportional hazards model. A p-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS

Population Characteristics: Demographic and Clinical Insights

The study included a total of 120 mCRC patients who received regorafenib in third-line or later lines. The median age was 58 years (IQR: 50.2-65.7). 74 (61.7%) of 120 patients were male. The ECOG-PS indicated that 101 (84.2%) of patients had a score of 0-1. 80 (66.7%) had a body mass index (BMI) of ≥25 kg/m^2. Most patients had left-sided primary tumors (75%) and synchronous metastases (58.3%). Liver-limited metastases were present in 20% of patients. KRAS mutation was detected in 50%, NRAS mutation in 16.7%, and BRAF mutation in 3.3% of cases. Regarding targeted therapies, 38.3% of patients received anti-epidermal growth factor receptor (EGFR) therapy in combination with chemotherapy at any step, while 80% received anti-vascular endothelial growth factor (VEGF) therapy. Regorafenib was administered as third-line treatment in 71.7% of patients and required dosage decrement in 70.8%. The best response to regorafenib was PR in 11.7% of patients, SD in 17.5% and PD in 70.8%. No CR was observed in any patient (Table 1).

Table 1. Population characteristics: demographic and clinical insights

Age, year Median (IQR)	**58 (50.2-65.7)**
Age group, n (%)	
<65	84 (70)
≥65	36 (30)
Gender, n (%)	
Female	46 (38.3)
Male	74 (61.7)
ECOG-PS, n (%)	
0/1	101 (84.2)
≥2	19 (15.8)
BMI group, n (%)	
<25 kg/ m^2	40 (33.3)
≥25 kg/m^2	80 (66.7)
Type of tumor, n (%)	
Colon	84 (70)
Rectum	36 (30)
The side of primary tumor	
Right side	30 (25)
Left side	90 (75)
Metastatic status*, n (%)	
Metachronous	50 (41.7)
Synchronous	70 (58.3)
Metastatic site, n (%)	
Single site	29 (24.2)
Multiple sites	91 (75.8)
Liver metastasis only&, n (%)	
Yes	24 (20)
No	96 (80)
Surgery for primary tumor, n (%)	
Yes	97 (82.2)
No	21 (17.8)
KRAS mutation, n (%)	
Yes	60 (50)
No	60 (50)
NRAS mutation, n (%)	
Yes	20 (16.7)
No	100 (83.3)
BRAF mutation, n (%)	
Yes	4 (3.3)
No	116 (96.7)
Anti-EGFR treatment, n (%)	
Yes	46 (38.3)
No	74 (61.7)
Anti-VEGF treatment, n (%)	
Yes	96 (80)
No	24 (20)
Line of regorafenib treatment, n (%)	
3rd	86 (71.7)
4th or above	34 (28.3)

Analysis of Survival

In the whole population, median PFS was 3.38 months, while median OS was 8.01 months, respectively (Figure 1).

Progression-Free Survival Outcomes and Analysis

Age was found to be a significant factor for PFS, with patients aged <65 years having a slightly shorter PFS compared to those aged ≥65 years (3.35 vs. 3.41 months, p=0.045). Patients with BRAF-mutated tumors had significantly worse PFS compared to those without the mutation (1.84 vs. 3.41 months, p=0.014). Furthermore, responders to regorafenib demonstrated a significantly longer PFS compared to non-responders (4.96 vs. 3.02 months, p<0.001), reinforcing the clinical relevance of achieving disease control with regorafenib. Other factors, including gender (0.496), ECOG-PS (0.390), BMI (0.718), primary tumor location (0.299), metastatic status at diagnosis (0.460), prior targeted therapies (anti-VEGF

Table 1. Continued	
Age, year Median (IQR)	**58 (50.2-65.7)**
Regorafenib dose reduction, n (%)	
Yes	85 (70.8)
No	35 (29.2)
Best response to regorafenib, n (%)	
Partial response	14 (11.7)
Stable disease	21 (17.5)
Progressive disease	85 (70.8)

IQR: Interquartile range, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, BMI: Body mass index, EGFR: Epidermal growth factor receptor, VEGF: Vascular endothelial growth factor

p=0.682, anti-EGFR p=0.692), regorafenib dose reduction (p=0.423), carcinoembryonic antigen (CEA) level (p=0.145) and carbohydrate antigen 19-9 (CA19-9) level (p=0.496) did not significantly impact PFS (Table 2).

Overall Survival Outcomes and Analysis

Results of univariate analysis revealed that ECOG-PS 1-2 (p=0.022), regorafenib dose reduction (p=0.005), CEA level (p=0.005) and CA19-9 level (p=0.004) were significantly associated with OS. Additionally, responders to regorafenib had significantly longer OS compared to non-responders (10.84 vs. 6.47 months, p<0.001). Although patients with BRAF mutations showed numerically worse OS compared to non-mutated cases (2.43 vs. 7.95 months), this difference was not statistically significant (p=0.242). Other clinical and molecular characteristics, including age (p=0.240), gender (p=0.856), tumor location (p=0.245), metastatic site (p=0.096), prior anti-EGFR (p=0.644) or anti-VEGF therapies (0.762) and treatment line (p=0.530) were not significantly associated with OS (Table 2). In multivariate analysis, the results indicate that ECOG-PS (HR: 1.82; 95% CI: 1.08-3.06), regorafenib dose reduction (HR: 1.58; 95% CI: 1.04-2.41), CA19-9 level (HR: 2.14; 95% CI: 1.43-3.22), and regorafenib response (HR: 0.38; 95% CI: 0.24-0.60, p<0.001) remained significant predictors of OS (Table 3).

DISCUSSION

This study evaluated the real-life efficacy of regorafenib for mCRC in settings in tertiary care and beyond, and explored the clinical and molecular factors that influence treatment outcomes. Our findings demonstrated a median PFS of 3.38 months and a median OS of 8.01 months, which are consistent with previously reported data. In particular, while age and BRAF mutation were prognostic factors for PFS, ECOG-PS, regorafenib dose reduction and CA19-9 levels have been determined as prognostic factors for OS. Additionally, our analysis revealed that patients who responded to regorafenib had significantly longer PFS and OS compared to non-responders, further supporting the clinical significance of achieving disease control with regorafenib. These findings support the role of regorafenib as a viable treatment option in the later lines of therapy for mCRC.

The pivotal CORRECT study evaluating regorafenib in heavily treated mCRC patients reported a median PFS of 1.9 and median OS of 6.4 months, reinforcing its therapeutic benefit in this challenging patient population[7]. Similarly, the CONCUR trial conducted in Asian patients found comparable results, with regorafenib improving PFS and OS over placebo[6]. Notably, the median OS observed in our study was longer compared to the CORRECT (6.4 months) and CONCUR (8.4 months) trials[6,7]. Several factors may explain this difference. First, our cohort predominantly consisted of patients with an ECOG-PS of

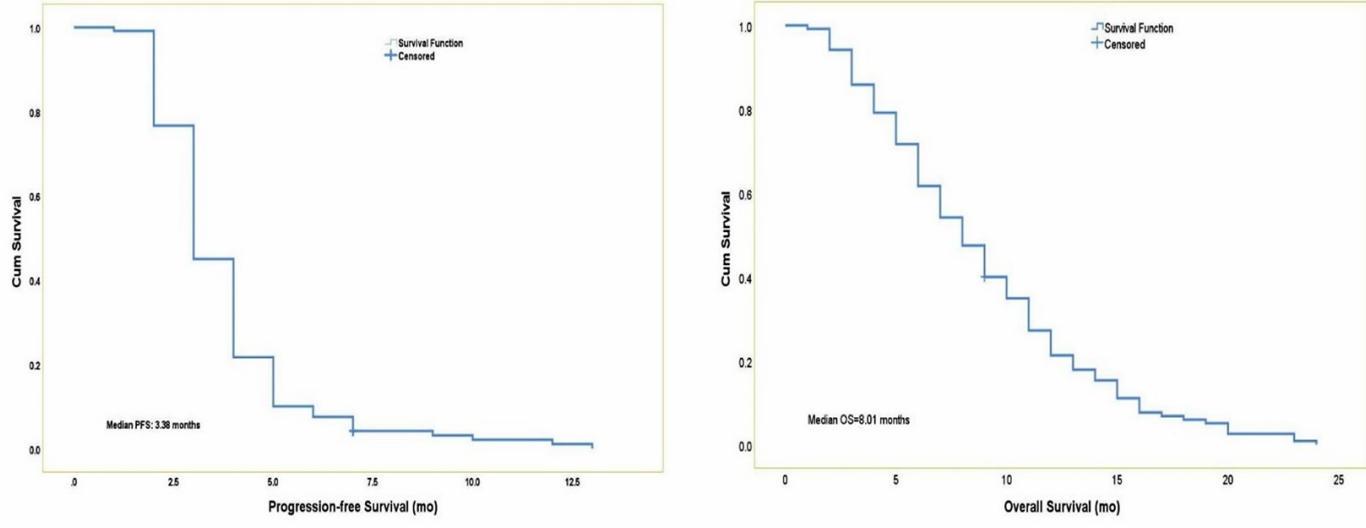

Figure 1. Survival outcomes: Kaplan-Meier estimates for PFS and OS
PFS: Progression-free survival, OS: Overall survival

Table 2. Univariate analysis of determinants for progression-free survival and overall survival

	Median PFS (months)	HR (95% CI)	p	Median OS (months)	HR (95% CI)	p-value
Age						
<65	3.35 (3.08-3.62)	0.66 (0.44-0.99)	0.045	7.75 (6.36-9.14)	0.78(0.52-1.17)	0.240
≥65	3.41 (2.69-4.14)			9.03 (6.13-11.93)		
Gender						
Male	3.41 (3.10-3.72)	0.88 (0.60-1.27)	0.496	7.82 (6.00-9.63)	1.03 (0.71-1.50)	0.856
Female	3.31 (3.06-3.56)			8.08 (7.02-9.14)		
ECOG-PS						
0/1	3.41 (3.24-3.60)	1.23 (0.75-2.02)	0.390	8.70 (7.65-9.75)	1.78 (1.08-2.96)	0.022
≥2	2.92 (2.22-3.62)			5.81 (4.18-7.45)		
BMI group						
<25 kg/ m^2	3.58 (3.11-4.05)	1.07 (0.71-1.62)	0.718	7.36 (5.81-8.90)	0.71 (0.47-1.08)	0.114
≥25 kg/m^2	3.31 (3.01-3.62)			8.90 (7.31-10.50)		
Type of tumor, n (%)						
Colon	3.35 (3.02-3.67)	0.80 (0.54-1.21)	0.299	8.08 (6.46-9.70)	0.78 (0.51-1.18)	0.245
Rectum	3.34 (3.04-3.66)			7.82 (5.84-9.8)		
The side of primary tumor						
Right side	3.35 (2.75-3.95)	1.07 (0.95-1.21)	0.375	6.37 (4.39-8.35)	1.10 (0.97-1.24)	0.129
Left side	3.35 (3.15-3.54)			8.77 (7.58-9.96)		
Metastatic status						
Metachronous	3.41 (3.14-3.68)	1.14 (0.79-1.66)	0.460	7.95 (6.16-9.73)	1.43 (0.97-2.11)	0.068
Synchronous	3.35 (3.12-3.57)			7.75 (6.07-9.43)		
Metastatic site						
Single site	3.48 (3.02-3.94)	1.18 (0.77-1.81)	0.426	7.65 (5.75-9.56)	1.43 (0.93-2.20)	0.096
Multiple sites	3.35 (3.12-3.57)			8.34 (7.03-9.65)		
Liver metastasis only						
Yes	3.48 (2.81-4.15)	1.10 (0.70-1.72)	0.676	7.65 (5.9-9.35)	1.28 (0.81-2.01)	0.283
No	3.35 (3.15-3.55)			8.14 (6.72-9.56)		
Surgery for primary tumor						
Yes	3.35 (3.15-3.54)	1.17 (0.72-1.90)	0.719	8.08 (6.90-9.26)	0.87 (0.53-1.42)	0.582
No	3.41 (2.80-4.03)			9.59 (4.62-14.56)		
RAS mutation						
Yes	3.48 (3.24-3.72)	0.92 (0.663-1.32)	0.648	8.70 (7.05-10.35)	0.95 (0.66-1.38)	0.819
No	3.28 (3.06-3.51)			7.65 (6.82-8.49)		
BRAF mutation						
Yes	1.84 (0.74-2.93)	3.26 (1.18-8.96)	0.014	2.43 (1.10-9.12)	1.80 (0.66-4.92)	0.242
No	3.41 (3.23-3.60)			7.95 (6.76-9.13)		
Anti-EGFR treatment						
Yes	3.48 (3.26-3.70)	0.93 (0.64-1.35)	0.692	7.89 (6.50-9.26)	1.09 (0.75-1.59)	0.644
No	3.22 (2.87-3.56)			8.14 (6.17-10.12)		
Anti-VEGF treatment						
Yes	3.41 (3.18-3.64)	1.13 (0.62-2.07)	0.682	8.14 (6.83-9.46)	1.09 (0.60-2.01)	0.762
No	3.31 (3.20-3.43)			7.82 (3.80-11.83)		
Line of regorafenib treatment						
3rd	3.31 (3.10-3.53)	0.84 (0.56-1.26)	0.394	7.81 (6.14-9.45)	1.05 (0.75-1.47)	0.530
4th or above	3.64 (3.36-3.92)			8.08 (6.62-9.53)		

Table 2. Continued

	Median PFS (months)	HR (95% CI)	p	Median OS (months)	HR (95% CI)	p-value
Regorafenib dose reduction						
Yes	3.35 (3.14-3.56)	1.17 (0.78-1.76)	0.423	7.65 (6.17-9.14)	1.78 (1.18-2.70)	0.005
No	3.41 (2.94-3.89)			9.59 (7.31-11.88)		
Regorafenib response						
Non-responders	3.02 (2.78-3.26)	0.05 (0.02-0.10)	<0.001	6.47 (5.48-7.46)	0.40 (0.26-0.62)	<0.001
Responders	4.96 (4.61-5.31)			10.84 (9.46-12.22)		
CEA						
<58	3.48 (3.26-3.70)	1.31 (0.90-1.90)	0.145	8.77 (6.82-10.72)	1.72 (1.17-2.53)	0.005
≥58	3.08 (2.79-3.38)			6.73 (4.99-8.48)		
CA19-9						
<74	3.35 (3.13-3.57)	1.14 (0.77-1.67)	0.496	9.03 (7.12-10.95)	1.75 (1.19-2.58)	0.004
≥74	3.48 (2.85-4.11)			6.37 (4.18-8.57)		

PFS: Progression-free survival, HR: Hazard ratio, CI: Confidence interval, OS: Overall survival, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, BMI: Body mass index, EGFR: Epidermal growth factor receptor, VEGF: Vascular endothelial growth factor, CEA: Carcinoembryonic antigen, CA19-9: Cancer antigen 19-9

0-1 (84.2%), indicating a relatively better functional status, whereas the CORRECT and CONCUR trials included a broader range of ECOG-PS scores, which could negatively impact survival. Second, a higher proportion of our patients had received prior targeted therapies, particularly anti-VEGF agents, which may have contributed to improved OS. Additionally, as a real-world study, flexible dosing strategies and individualized patient management may have led to better tolerability and prolonged treatment duration, ultimately enhancing survival. Lastly, advancements in supportive care over time could also be a contributing factor to the improved OS observed in our study. In a different large randomized trial, mCRC patients receiving regorafenib as treatment had a median OS of 5.6 months and a 12-month survival rate of 22%[8]. Our study demonstrated aligns with these findings, further validating regorafenib's effectiveness in this patient group. Notably, our results also highlight the impact of factors, such as ECOG-PS and regorafenib dose reduction, on survival outcomes, suggesting the potential for more personalized treatment strategies in mCRC management.

Table 3. Multivariate analysis of predictors for overall survival

	HR (95% CI)	p-value
ECOG-PS		
0-1	Ref	0.025
≥2	1.82 (1.08-3.06)	
Regorafenib dose reduction		
No	Ref	0.030
Yes	1.58 (1.04-2.41)	
CEA		
<58	Ref	0.225
≥58	1.29 (0.85-1.97)	
CA19-9		
<74	Ref	<0.001
≥74	2.14 (1.43-3.22)	
Regorafenib response		
Non-responders	Ref	<0.001
Responders	0.38 (0.24-0.60)	

HR: Hazard ratio, CI: Confidence interval, ECOG-PS: Eastern Cooperative Oncology Group Performance Status, CEA: Carcinoembryonic antigen, CA19-9: Cancer antigen 19-9

To date, there are no biomarkers to predict the response to regorafenib in mCRC, but evidence suggests that prior exposure to targeted therapies is associated with worse outcomes. In particular, in the CORRECT trial, all patients had previously received bevacizumab, and 52% of patients had been exposed to anti-EGFR therapy. In the CONCUR trial, these rates were 41% and 35%, respectively[9]. The better OS observed in the CONCUR trial may have been influenced by these results. Similar results were reported in a single-arm, phase 2b study evaluating regorafenib in patients with chemotherapy-resistant, antiangiogenic-naive mCRC[10], consistent with the CONCUR findings. Similarly, in our study, 80% of patients had prior anti-VEGF therapy, while 38.3% had received anti-EGFR therapy at some point during their treatment course. Despite this high rate of prior targeted therapy exposure, our findings demonstrated a median OS of 8.01 months, which is numerically longer than that reported in CORRECT and CONCUR. This suggests that patient selection, treatment sequencing, and additional prognostic factors may influence survival outcomes with regorafenib.

Dose modification is a critical aspect of regorafenib treatment, as adverse events often necessitate dose reductions to maintain tolerability without compromising efficacy. In our study, 70.8% of patients required dose reduction, and multivariate analysis identified it as an independent predictor of OS. These findings are consistent with real-world data from the REBECCA study, a large observational cohort evaluating regorafenib in routine

clinical practice[8]. Starting at a lower dose and adjusting based on tolerance is a common approach in clinical practice to enhance treatment adherence. Furthermore, emerging evidence suggests that initiating regorafenib at a reduced dose with subsequent titration, as explored in the ReDOS trial, can improve tolerability and overall treatment success[11]. These findings highlight the importance of personalized dosing strategies to enhance the clinical benefit of regorafenib in heavily treated mCRC patients.

The role of molecular alterations in the efficacy of regorafenib remains controversial. In the CORRECT trial, KRAS, NRAS, and BRAF mutation statuses were not significantly associated with treatment outcomes, indicating that regorafenib exerts its antitumor effects independently of RAS mutation status[7]. The subgroup analysis of the CONCUR study also found no significant difference in OS between RAS mutant and wild-type tumors[6]. However, smaller retrospective studies have associated BRAF mutations with worse outcomes in patients treated with regorafenib, likely reflecting the inherently poor prognosis of BRAF mutant mCRC[12]. In our study, the results were consistent with the literature, with KRAS and NRAS mutations not significantly affecting PFS or OS. Nevertheless, this lack of statistical significance should be interpreted with caution, as these mutations represent only a limited aspect of tumor biology. Other factors-such as pathway crosstalk, epigenetic regulation, and tumor-stroma interactions-may contribute to therapeutic resistance and response variability, especially in real-world settings where patient heterogeneity is high. However, in our cohort, patients with BRAF mutations had a trend toward shorter OS, but the difference was not statistically significant, potentially due to the limited sample size. Moreover, the prognostic significance of tumor markers like CEA and CA19-9 continues to be a contention of discussion in clinical practice. Studies have shown that elevated CA19-9 levels may be associated with poor prognosis[13]. Associations between treatment outcomes and various laboratory parameters have also been documented in the literature, with elevated platelet counts and high neutrophil-to-lymphocyte ratios being linked to poorer OS, while higher lymphocyte counts have been associated with improved OS[14,15]. In our study, elevated CA19-9 levels were found to be an independent predictor of regorafenib efficacy, whereas CEA levels were not significantly associated with survival outcomes. CA19-9 is a sialylated Lewis antigen expressed on epithelial cells and secreted by mucin-producing adenocarcinomas. Its elevation may reflect not only higher tumor burden or biliary tract involvement, but also a more biologically aggressive phenotype characterized by enhanced mucin production, desmoplastic reaction, and increased metastatic capacity. Prior studies in gastrointestinal malignancies have demonstrated that elevated CA19-9 is associated with reduced treatment responsiveness and inferior survival outcomes. Accordingly, in the context of regorafenib therapy, baseline CA19-9 levels may reflect both tumor burden and biological aggressiveness, potentially contributing to the observed differences in survival outcomes. These findings suggest that CA19-9 could be considered a prognostic biomarker in clinical practice. Identifying reliable predictive biomarkers for regorafenib could enable more personalized treatment strategies and warrants further investigation.

Our findings highlight the importance of patient selection in regorafenib treatment for mCRC, as ECOG-PS and CA19-9 levels were significant prognostic factors for survival. The high rate of dose reductions emphasizes the need for careful toxicity management to improve treatment adherence. Although BRAF-mutant tumors showed a trend toward worse outcomes, the limited sample size precludes definitive conclusions, warranting further investigation. Future studies should focus on identifying biomarkers predictive of regorafenib response, optimizing treatment sequencing, and evaluating its role in combination strategies to enhance clinical benefit.

Study Limitations

Some limitations of this study need to be acknowledged. The retrospective design introduces the potential for selection bias, as patients were not randomly assigned to treatment groups. This may have led to an overrepresentation of patients with better PS or those who tolerated treatment longer, while patients with poorer prognosis may have been underrepresented. Additionally, our analysis lacked detailed data on adverse events, which is a critical aspect of regorafenib treatment. While dose reductions were recorded in our dataset, the specific reasons, severity grading, and timing of these modifications were not systematically documented. This limits our ability to assess the direct relationship between adverse events and dose adjustments, as well as their impact on treatment adherence and clinical outcomes. Given that a significant proportion of patients required dose reduction, it is likely that toxicity played a crucial role in treatment modifications. However, the absence of detailed adverse event profiles prevents us from determining whether specific toxicities had a greater influence on survival outcomes. The relatively small sample size may also limit the applicability of the results. Although the data were retrieved from medical records, detailed information on dose reduction and patient tolerance was not comprehensively collected. Dose reductions are a critical aspect of regorafenib therapy, and the lack of detailed information on the reasons and timing of dose adjustments may limit our understanding of how these factors affect treatment outcomes. Although we were able to assess OS and PFS, other relevant factors such as adverse event profiles could not be assessed. Furthermore, our dataset does not include treatment regimens after regorafenib in detail. Since most patients did not receive further treatment due to disease progression or clinical deterioration, and the available data on chemotherapy rechallenge in those who

underwent retreatment were insufficient for a comprehensive analysis, we were unable to assess the impact of subsequent therapies on survival outcomes.

CONCLUSION

Regorafenib is an efficient therapeutic choice for heavily pretreated mCRC patients and improves both OS and PFS. Our findings, consistent with prior studies, emphasize the importance of patient selection and dose modification in optimizing treatment outcomes. Despite the absence of definitive predictive biomarkers, our results suggest that factors such as ECOG-PS, treatment dose and CA19-9 levels may influence survival outcomes in patients treated with regorafenib. Given the limited evidence on genetic mutations and their impact on regorafenib efficacy, further investigation into molecular profiling and the role of specific biomarkers is warranted. Future prospective studies should focus on integrating clinical and molecular profiling to better elucidate why established prognostic factors such as ECOG PS and CA19-9 levels remain predictive of regorafenib outcomes, while common mutational markers fail to demonstrate consistent associations.

Ethics

Ethics Committee Approval: The project was approved by Marmara University Faculty of Medicine Ethical Committee for Non-Pharmaceutical and Non-Medical Device Research Ethics Committee (decision no: 09.2024.1591, date: 24.12.2024).

Informed Consent: Patients diagnosed with colorectal adenocarcinoma by the pathology unit and seen in the medical oncology outpatient clinic between January 2017 and November 2024 were retrospectively analyzed. Patients over 17 years of age were included.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.S., Concept: N.S., İ.V.B., Design: N.S., İ.V.B., Data Collection or Processing: N.S., Analysis or Interpretation: N.S., İ.V.B., Literature Search: N.S., İ.V.B., Writing: N.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386-422.
2. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144:1941-53.
3. Leto SM, Trusolino L. Primary and acquired resistance to EGFR-targeted therapies in colorectal cancer: impact on future treatment strategies. J Mol Med (Berl). 2014;92:709-22.
4. Grothey A, Sargent D. Overall survival of patients with advanced colorectal cancer correlates with availability of fluorouracil, irinotecan, and oxaliplatin regardless of whether doublet or single-agent therapy is used first line. J Clin Oncol. 2005;23:9441-2.
5. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19:329-59.
6. Li J, Qin S, Xu R, Yau TC, Ma B, Pan H, et al. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2015;16:619-29.
7. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013;381:303-12.
8. Adenis A, de la Fouchardiere C, Paule B, Burtin P, Tougeron D, Wallet J, et al. Survival, safety, and prognostic factors for outcome with Regorafenib in patients with metastatic colorectal cancer refractory to standard therapies: results from a multicenter study (REBECCA) nested within a compassionate use program. BMC Cancer. 2016;16:412.
9. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Characteristics and outcomes of patients enrolled in the CORRECT and CONCUR phase 3 trials of regorafenib for metastatic colorectal cancer (mCRC). Annals of Oncology. 2015;26:303-12.
10. Riechelmann RP, Leite LS, Bariani GM, Glasberg J, Rivelli TG, da Fonseca LG, et al. Regorafenib in patients with antiangiogenic-naive and chemotherapy-refractory advanced colorectal cancer: results from a phase IIb trial. Oncologist. 2019;24:1180-7.
11. Bekaii-Saab TS, Ou FS, Ahn DH, Boland PM, Ciombor KK, Heying EN, et al. Regorafenib dose-optimisation in patients with refractory metastatic colorectal cancer (ReDOS): a randomised, multicentre, open-label, phase 2 study. Lancet Oncol. 2019;20:1070-82.
12. Chu JE, Johnson B, Kugathasan L, Morris VK, Raghav K, Swanson L, et al. Population-based Screening for BRAF (V600E) in metastatic colorectal cancer reveals increased prevalence and poor prognosis. Clin Cancer Res. 2020;26:4599-605.
13. Komori A, Taniguchi H, Hamauchi S, Masuishi T, Kito Y, Narita Y, et al. Serum CA19-9 Response is an early predictive marker of efficacy of regorafenib in refractory metastatic colorectal cancer. Oncology. 2017;93:329-35.
14. Del Prete M, Giampieri R, Loupakis F, Prochilo T, Salvatore L, Faloppi L, et al. Prognostic clinical factors in pretreated colorectal cancer patients receiving regorafenib: implications for clinical management. Oncotarget. 2015;6:33982-92.
15. Arai H, Miyakawa K, Denda T, Mizukami T, Horie Y, Izawa N, et al. Early morphological change for predicting outcome in metastatic colorectal cancer after regorafenib. Oncotarget. 2017;8:110530-9.

ORIGINAL ARTICLE

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Treatment Outcomes with the Optic Neuritis Treatment Trial Protocol in Typical Optic Neuritis and Prognostic Factors Associated with Final Visual Acuity: Real-Life Data

Tipik Optik Nöritte Optik Nörit Tedavi Denemesi Protokolü ile Tedavi Sonuçları ve Sonuç Görme Keskinliği ile İlişkili Prognostik Faktörler: Gerçek Yaşam Verileri

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ABSTRACT

Aim: This study aims to evaluate the etiological, demographic, and clinical characteristics of patients diagnosed with typical optic neuritis and treated according to the optic neuritis treatment trial (ONTT) protocol, as well as to assess prognostic factors associated with long-term visual acuity.

Materials and Methods: A retrospective analysis was conducted on 106 patients treated with the ONTT protocol at a tertiary eye clinic between January 2010 and June 2023. The patients' age, gender, affected eye, initial and final best-corrected visual acuity (BCVA), anterior or retrobulbar form of optic neuritis, presence of relative afferent pupillary defect, and visual field defects were evaluated. Factors influencing long-term visual acuity after treatment were analyzed.

Results: Of the patients, 57.5% were female, with a mean age of 32.25±12.79 years. Optic neuritis was observed in 53.7% of right eyes and 46.3% of left eyes. Multiple sclerosis (MS) was diagnosed in 13.2% of patients. The mean initial BCVA was 0.33±0.41, while the final BCVA was 0.65±0.38. A higher initial BCVA (p=0.01) and the presence of retrobulbar optic neuritis (p=0.04) were significantly associated with better long-term visual prognosis.

Conclusion: Most patients with typical optic neuritis treated with the ONTT protocol experienced improved visual acuity. Higher initial BCVA and retrobulbar optic neuritis were identified as positive prognostic factors. Long-term follow-up of patients with potential MS and larger-scale studies are necessary.

Keywords: Anterior, neuritis, optic, prognosis, retrobulbar, visual acuity

ÖZ

Amaç: Bu çalışma, tipik optik nörit tanısı konulan ve optik nörit tedavi denemesi (ONTT) protokolüne göre tedavi edilen hastaların etiyolojik, demografik ve klinik özelliklerini inceleyerek, uzun dönem görme keskinliği ile ilişkili prognostik faktörleri değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Üçüncü basamak bir göz kliniğinde Ocak 2010 - Haziran 2023 tarihleri arasında ONTT protokolü ile tedavi edilen 106 hasta retrospektif olarak incelendi. Hastaların yaş, cinsiyet, etkilenen göz, başlangıç ve sonuç en iyi düzeltilmiş görme keskinliği (EİDGK), optik nöritin anterior veya retrobulber formda olması, rölatif afferent pupil defekti varlığı ve görme alanı defektleri değerlendirildi. Tedavi sonrası uzun dönem görme keskinliği ile ilişkili faktörler analiz edildi.

Bulgular: Hastaların %57,5'i kadın olup yaş ortalaması 32,25±12,79 olarak hesaplandı. Optik nörit, hastaların %53,7'sinde sağ gözde, %46,3'ünde sol gözde görüldü. Hastaların %13,2'sinde multipl skleroz (MS) tanısı mevcuttu. Başlangıç EİDGK ortalaması 0,33±0,41, sonuç EİDGK ortalaması ise

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0,65±0,38 idi. Başlangıç EİDGK'nin yüksek olması (p=0,01) ve retrobulber optik nörit varlığı (p=0,04), uzun dönem görme prognozunun daha iyi olmasıyla anlamlı ilişki gösterdi.

Sonuç: ONTT protokolü ile tedavi edilen tipik optik nörit hastalarının büyük çoğunluğunda görme keskinliği olumlu etkilenmektedir. Başlangıç EİDGK ve optik nöritin retrobulber formda olması, olumlu prognostik faktörler olarak belirlenmiştir. MS ile ilişkili olabilecek hastaların uzun vadeli takibi ve daha geniş ölçekli çalışmalar gerekmektedir.

Anahtar Kelimeler: Anterior, nörit, optik, prognoz, retrobulber, görme keskinliği

INTRODUCTION

Optic neuritis is characterized by inflammation of the optic nerve, causing acute, unilateral, painful vision loss[1]. Only 0.4% of patients develop symptoms in both eyes simultaneously[2]. The presumed pathophysiology of optic neuritis is inflammation and demyelination of the optic nerve. Activated peripheral T-cells cross the blood-brain barrier and release cytokines and other inflammatory mediators, leading to neuronal cell death and axonal degeneration[3]. It occurs most commonly in young adults and more frequently in women[4]. The incidence of optic neuritis is greater at higher latitudes compared with geographic locations closer to the equator[5]. The worldwide incidence of unilateral optic neuritis ranges from 0.94 to 2.18 per 100,000 per year[6].

There is no consensus on a systematic classification of optic neuritis. Studies often use different classification systems. Optic neuritis is traditionally divided into typical and atypical forms on clinical grounds. Typical optic neuritis is considered to be a clinically isolated demyelinating syndrome that presents as idiopathic optic neuritis and is at risk of developing into multiple sclerosis (MS) in the white population, or is associated with MS[7]. Thus, most cases of optic neuritis are due to idiopathic inflammatory demyelination, which can occur in isolation or as a manifestation of MS[8]. In atypical optic neuritis, recovery of visual acuity may be poorer and the risk of developing optic nerve atrophy may be greater in patients with idiopathic or MS-related optic neuritis than in patients with typical optic neuritis[9]. Atypical cases are characterized by the absence of eye pain, the presence of exudates and hemorrhages on examination, very severe, bilateral, or progressive visual loss, or failure to regain vision. These atypical optic neuritis cases include neuromyelitis optica, autoimmune optic neuropathy, chronic recurrent inflammatory optic neuropathy, idiopathic recurrent neuroretinitis and optic neuropathy associated with systemic diseases[10]. Typical optic neuritis is the initial symptom of MS in 25% of cases, and long-term follow-up studies have reported conversion to clinically definite MS in 34-75% of patients presenting with optic neuritis in the UK and USA[11].

Treatment of typical optic neuritis has been investigated in several studies, the results of which have shown that corticosteroids accelerate recovery of vision without affecting the final visual outcome[12]. Atypical optic neuritis usually requires corticosteroid therapy but often requires aggressive immunosuppression in addition[10]. In 1988, Spoor and Rockwell[13] reported excellent results in a study evaluating high-dose intravenous (IV) steroids for the treatment of optic neuritis, and the optic neuritis treatment trial (ONTT) was initiated following this report. The ONTT was designed to help answer whether treatment with oral or IV steroids improves vision or improves vision more quickly after acute optic neuritis. One group received high-dose IV methylprednisolone followed by oral steroids, the second group received low-dose oral steroids only, and the third group received placebo. The results show that 3 days of high-dose IV methylprednisolone did not alter the overall visual acuity results after 6 months, but did accelerate visual recovery after optic neuritis. However, lower dose oral steroids increase the incidence of recurrent optic neuritis for reasons that remain unclear. In addition, contrast sensitivity, visual fields, and color vision in the ONTT group showed sustained improvement after 6 months compared to placebo in the IV methylprednisolone group. The treatment protocol for a typical optic neuritis attack in ONTT was to give 250 mg methylprednisolone IV four times daily, totaling 1000 mg, followed by oral steroid reduction (1 mg/kg/day for 11 days). ONTT provides the literature with the basis for evidence-based discussions on treatment and prognosis[14].

The aim of this study is to evaluate the etiological data, demographic characteristics, long-term follow-up results and prognostic factors related to final visual acuity of patients diagnosed with typical optic neuritis and treated according to the ONTT treatment protocol by presenting real-life data.

MATERIALS AND METHODS

This study adhered to the principles of the Declaration of Helsinki and was approved by Trakya University Faculty of Medicine Non-Interventional Scientific Research Ethics Committee (desicion no: TÜTF-GÖBAEK 2024/28, date: 05.02.2024). The rights of all participants were protected, and written informed consent was obtained from the participants before the procedure according to the Declaration of Helsinki.

In the study, the files of patients who were treated with the treatment protocol recommended by ONTT, considering them to have typical optic neuritis in a tertiary ophthalmology clinic, were retrospectively reviewed. 106 patients who received

treatment with the current diagnosis between January 2010 and June 2023 were included in the study. The patients' ages, genders, and the eye from which the attack occurred were recorded. The best corrected visual acuities (BCVA) of all patients were evaluated before treatment and at 10 days, 1-3.6, and 12 months after treatment and at the last follow-up visit. BCVA at the first application was recorded as the initial BCVA, and BCVA at the last visit was recorded as the final BCVA. The study included patients with at least 12 months of follow-up. All patients underwent detailed biomicroscopic and fundoscopic examinations, and intraocular pressures were measured and noted using an applanation tonometer.

All patients included in the study were questioned about the presence of diagnosed MS disease. In the current optic neuritis attack, the neuritis types observed in the fundoscopic examination, including anterior optic neuritis accompanied by optic disc edema and retrobulbar optic neuritis without optic disc edema, were noted. The presence of a relative afferent pupillary defect (RAPD) at the time of the attack was examined. The presence of a visual field defect in the perimetry test performed at the first application and the type of defect (scotoma, altitudinal defect or concentric constriction) were recorded. All patients who were decided to have typical optic neuritis were hospitalized and received IV methylprednisolone treatment. As maintenance treatment, oral steroids were tapered off, and it was observed that the duration of oral steroid tapering was longer in some patients, and this period was grouped as shorter than 1 month and longer than 1 month and recorded. Which of these parameters had an effect on the final BCVA was examined.

Patient Selection

Inclusion criteria for the study:

Symptoms present for less than 1 week

Complaints of unilateral, sudden loss of vision or loss of contrast sensitivity

Full Ophthalmological Examinations Available

For the eye with the complaint, absence of any previous trauma or ocular surgery.

Absence of signs and symptoms suggestive of atypical optic neuritis (no increased pain with eye movements or hemorrhages in the optic nerve head or peripapillary area)

Absence of systemic or infectious diseases that may accompany atypical optic neuritis

Treatment Protocol

All patients were treated as recommended by ONTT for typical optic neuritis. Hospitalized patients were treated with 250 mg methylprednisolone 4 times a day (06:00-12:00-18:00-00:00) for 3 days, and a total of 1000 mg methylprednisolone IV per day. On the morning of the 4th day, the patients were discharged with a 1mg/kg oral steroid treatment as maintenance treatment. During this treatment process, the dose was reduced considering the visual gain and oral steroids were gradually discontinued.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (IBM, USA) version 20.0. The Kolmogorov-Smirnov test was used to assess the normality of the data. For numerical descriptive statistics, normally distributed variables were presented as means and standard deviations. Descriptive statistics for categorical variables were presented as percentages. Chi-square test was used to compare categorical data. The relationship between numerical variables was assessed using the Pearson correlation coefficient test. The significance level was accepted as $p<0.05$.

RESULTS

Of the patients included in the study, 61 were female (57.5%) and 45 were male (42.5%). The mean age of the patients was 32.25±12.79 years. When the eyes with attacks were examined, 57 were right eyes (53.7%) and 49 were left eyes (46.3%) (Table 1).

The patients included in the study were consulted by the Neurology unit during hospitalization and it was seen that there were 14 patients who had MS diagnosis or were diagnosed at the time of the attack. This corresponded to 13.2% of the patients. In 65 patients (61.3%), a visual field defect was present in the perimetry test performed at the time of the attack. When these visual field defects were grouped within themselves, it was seen that 28 (26.4%) had scotoma, 17 (16%) had altitudinal defect and 20 (18.9%) had concentric narrowing. The number of optic neuritis attacks detected in the form of anterior optic neuritis accompanied by optic disc edema seen in fundoscopic examination was 67 (63.2%) and the number of those detected in the form of retrobulbar optic neuritis without optic disc edema was 39 (36.7%). RAPD was found to be positive in 56 patients (52.8%) and negative in 50 patients (47.2%) (Table 2).

The mean BCVA of the patients at the time of the attack was determined as 0.33±0.41 (0.003-1.0), while the mean final BCVA was determined as 0.65±0.38 (0.05-1.0). The patients' age, gender, side of the eye experiencing the attack, whether or not they were diagnosed with MS, presence/absence of

Table 1. Demographic features of the patients

Female/Male	61 (57.5%)	45 (42.5%)
Right eye/Left eye	57 (53.7%)	49 (46.3%)
Mean age (year)	32.25±12.79 (18-50)	

Table 2. Optic neuritis attack presentation findings of the patients	
Existence of MS	14 (13.2%)
Presence of visual field defect	65 (61.3%)
Type of visual field defect	Scotoma: 28 (26.4%) Altitudinal defect: 17 (16%) Concentric narrowing: 20 (18.9%)
Optic neuritis form	Anterior: 67 (63.2%) Retrobulbar: 39 (36.7%)
RAPD	Yes: 56 (52.8%) No: 50 (47.2%)
MS: Multiple sclerosis, RAPD: Relative afferent pupillary defect	

Table 3. Initial and final BCVA of patient subgroups	
MS	**BCVA**
Yes (14)	Initial 0.24±0.24 (0.001-0.70) Final 0.67±0.37 (0.02-1.0)
No (92)	Initial 0.33±0.33 (0.001-1.0) Final 0.65±0.39 (0.001-1.0)
MS: Multiple sclerosis, BCVA: Best-corrected visual acuity	

visual field defect or its type if any, type of optic neuritis attack, initial BCVA, and the rate of discontinuation of oral steroids (longer or shorter than 1 month) were evaluated in terms of their effects on the final BCVA (Table 3). It was found that two parameters had a statistically significant effect on the final BCVA. The first of these was the initial BCVA, and it was found that the high initial BCVA was positively correlated with the final BCVA (p=0.01). In addition, it was found that the previous optic neuritis attack being retrobulbar optic neuritis was a positive factor on the final BCVA (p=0.04).

DISCUSSION

This study evaluated the demographic characteristics, clinical findings, and long-term visual acuity of patients diagnosed with typical optic neuritis, characterized by inflammation of the optic nerve, and treated according to the ONTT protocol, and the prognostic factors affecting these results. The study findings are consistent with previous literature showing that optic neuritis is more common in young adults and female patients[1,4,7,15]. The proportion of female patients in this study was 57.5%, which is in line with previous epidemiological studies. Similarly, a single-center study was recently conducted in a tertiary hospital in Eastern India to determine the clinical and demographic profile of optic neuritis, and it was reported that 64.3% of patients diagnosed with optic neuritis were female[16].

In this study, a significant relationship was found between initial BCVA and final BCVA. It was observed that high initial BCVA had a positive effect on the long-term visual acuity of the patients (p=0.01). This finding suggests that, as reported in previous studies, early detection of visual acuity in optic neuritis patients may be a determining factor in terms of long-term prognosis[17]. Küchlin et al.[18] reported that they examined clinical predictors in acute optic neuritis and concluded that being older, being male, and having worse visual function at the beginning posed a risk for worse clinical outcomes. This study also predicts that the higher the initial BCVA, the better the final BCVA, in line with the literature; however, no clinically significant correlation was observed between age and gender and final BCVA. This study also found that patients with an attack of retrobulbar optic neuritis had a better visual prognosis than patients with anterior optic neuritis (p=0.04). This may suggest that retrobulbar optic neuritis may generally be associated with milder inflammatory processes.

When examined in terms of visual field defects, 61.3% of the patients in this study had any visual field defects detected in the perimetry test. Different patterns such as scotoma, altitudinal defect and concentric narrowing were observed. In the study published by ONTT, it was reported that 68.8% of the optic neuritis patients they followed had visual field involvement at the beginning, 48.2% of them had diffuse visual field loss, 8.3% had central or centrocecal scotoma, 20.1% had altitudinal or other nerve fiber bundle type defects and 23.4% had various other defects[19]. Similar visual field defects have been reported in patients with optic neuritis in previous studies[20]. This finding emphasizes that optic neuritis has different effects on the optic nerve and that patients' visual field losses should be taken into account in clinical management. In a study, it was shown with electrodiagnostic evidence [visual evoked potential (VEP)] that the combined corticosteroid regimen recommended by ONTT improved conduction in the visual pathways of patients with first-attack optic neuritis earlier than conservative treatment[21]. In this study, the patients' VEP results were not evaluated because they could not be accessed completely.

In addition, in this study, the ONTT protocol was applied and a significant improvement in visual acuity was observed in the majority of patients treated with this protocol. ONTT was a multicenter randomized clinical trial that was established in the 1980s in the USA with the support of the National Eye Institute and developed to evaluate corticosteroid treatment for optic neuritis. Four hundred fifty-seven patients were included in the ONTT study between 1988-1991 and the first report was reported in the 6th month[22], and the last examinations were made in 2006 and the results of approximately 15 years were published[23]. It is stated that the dose adjustments of corticosteroids in the ONTT study were selected based on data currently used in clinical practice when designing the study[24,25]. The ONTT results showed that IV high-dose methylprednisolone accelerated early visual recovery but had no significant effect on final visual acuity[26]. In this study, similar to the results of the ONTT study, the majority of patients showed improvement

in visual acuity after treatment. However, the effect of steroid treatment on long-term MS development is still controversial, and in the study reporting ONTT results, it was stated that only low-dose oral steroid use may temporarily increase the risk of optic neuritis recurrence[23]. Therefore, future studies can evaluate the long-term results of treatment protocols more comprehensively. According to the same study, those receiving IV corticosteroids followed by oral corticosteroids have a temporary lower risk of developing a second demyelinating event consistent with MS compared to subjects treated with oral placebo or oral corticosteroids alone[23]. In light of this information, the rationale for implementing the treatment protocol recommended in the ONTT is primarily based on the aim of rapidly reversing vision loss, suppressing inflammation, and preventing relapses and possible neurological progression in the long term. Future studies may evaluate the long-term outcomes of treatment protocols more comprehensively.

In a study conducted by Rodriguez et al.[27] to determine the prevalence and incidence of optic neuritis, the rates of optic neuritis patients developing MS were also evaluated and it was determined that 39% of the patients progressed to clinically definite MS in a 10-year follow-up. It was also reported that 49% of the patients progressed to MS in 20 years, 54% in 30 years and 60% in 40 years. In this study, 13.2% of the patients had a diagnosis of MS. While the lack of communication between the patients in their neurology consultations may be the reason for this, the lack of sufficient follow-up periods may also be considered as another reason.

Study Limitations

This study has some limitations. First of all, due to the retrospective nature of the study, data were obtained from past records, which may have caused some clinical and demographic information to be missing. Follow-up periods, long-term activations, or MS diagnoses that may have been received later in another center may have been missed. In addition, since patient selection was made based on existing recorded data, the possibility of selection bias in the sample cannot be ruled out. The lack of standardization of clinical data at the time of initial recording also increases the risk of information bias. Considering all these limitations, prospective studies with large patient populations are needed to obtain more information about the clinical course of optic neuritis, response to treatment, and factors affecting prognosis.

CONCLUSION

In conclusion, the data obtained in this study support the effectiveness of the ONTT treatment protocol in patients with typical optic neuritis. The female predominance and the mean age of 32.25 years are consistent with previous reports. Initial BCVA and retrobulbar form of optic neuritis were determined as positive factors in terms of long-term visual prognosis. However, it is thought that long-term follow-up and treatment options of patients who may have MS should be examined in more detail.

Ethics

Ethics Committee Approval: This study adhered to the principles of the Declaration of Helsinki and was approved by Trakya University Faculty of Medicine Non-Interventional Scientific Research Ethics Committee (desicion no: TÜTF-GÖBAEK 2024/28, date: 05.02.2024).

Informed Consent: The rights of all participants were protected, and written informed consent was obtained from the participants before the procedure according to the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.C.Ç., A.K.Ç., A.K.S., R.G., E.K., H.G., Concept: A.C.Ç, A.K.Ç., A.K.S., R.G., E.K., H.G., Design: A.C.Ç, A.K.Ç., A.K.S., R.G., E.K., H.G., Data Collection or Processing: A.C.Ç, A.K.Ç., A.N.M.D., T.B., Analysis or Interpretation: A.C.Ç., A.K.Ç., Literature Search: A.C.Ç., A.K.Ç., A.N.M.D., T.B., A.K.S., R.G.,E.K., H.G., Writing: A.C.Ç., A.K.Ç.

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REFERENCES

1. Bennett JL. Optic neuritis. Continuum (Minneap Minn). 2019;25:1236-64.
2. Morrow MJ, Wingerchuk D. Neuromyelitis optica. J Neuroophthalmol. 2012;32:154-66.
3. Söderström M, Link H, Sun JB, Fredrikson S, Wang ZY, Huang WX, et al. Autoimmune T-cell repertoire in optic neuritis and multiple sclerosis: T-cells recognising multiple myelin proteins are accumulated in cerebrospinal fluid. J Neurol Neurosurg Psychiatry. 1994;57:544-51.
4. Optic Neuritis Study G. Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Arch Neurol. 2008;65:727-32.
5. Kurtzke JF. Optic neuritis or multiple sclerosis. Arch Neurol. 1985;42:704-10.
6. Aylor BV, Lucas RM, Dear K, Kilpatrick TJ, Pender MP, van der Mei IA, et al. Latitudinal variation in incidence and type of first central nervous system demyelinating events. Mult Scler. 2010;16:398-405.
7. Pau D, Al Zubidi N, Yalamanchili S, Plant GT, Lee AG. Optic neuritis. Eye (Lond). 2011;25:833-42.
8. Optic Neuritis Study G. The 5-year risk of MS after optic neuritis. Experience of the optic neuritis treatment trial. Neurology. 1997;49:1404-13.
9. Bennett JL, Costello F, Chen JJ, Petzold A, Biousse V, Newman NJ, et al. Optic neuritis and autoimmune optic neuropathies: advances in diagnosis and treatment. Lancet Neurol. 2023;22:89-100.
10. Malik A, Ahmed M, Golnik K. Treatment options for atypical optic neuritis. Indian J Ophthalmol. 2014;62:982-4.

11. Francis DA, Compston DA, Batchelor JR, Mcdonald WI. A reassessment of the risk of multiple sclerosis developing in patients with optic neuritis after extended follow-up. J Neurol Neurosurg Psychiatry. 1987;50:758-65.

12. Hickman SJ, Dalton CM, Miller DH, Plant GT. Management of acute optic neuritis. Lancet. 2002;360:1953-62.

13. Spoor TC, Rockwell DL. Treatment of optic neuritis with intravenous megadose corticosteroids. A consecutive series. Ophthalmology. 1988;95:131-4.

14. Volpe NJ. The optic neuritis treatment trial: a definitive answer and profound impact with unexpected results. Arch Ophthalmol. 2008;126:996-9.

15. Abel A, McClelland C, Lee MS. Critical review: typical and atypical optic neuritis. Surv Ophthalmol. 2019;64:770-9.

16. Biswas PS, Antara; Islam, Md Nazarul. Clinico-demographic profile of optic neuritis: a single-center study at a tertiary care hospital in Eastern India. Medical Journal of Dr DY Patil Vidyapeeth. 2025;18:270-6.

17. Beck RW, Cleary PA, Backlund JC. The course of visual recovery after optic neuritis: experience of the optic neuritis treatment trial. Ophthalmology. 2020;127:174-81.

18. Küchlin S, Ihorst G, Heinrich SP, Farassat N, Márquez Neila P, Hug MJ, et al. Clinical predictors in acute optic neuritis: analysis based on clinical trial data. Ophthalmology. 2025;132:631-43.

19. Keltner JL, Johnson CA, Spurr JO, Beck RW. Baseline visual field profile of optic neuritis. The experience of the optic neuritis treatment trial. Optic Neuritis Study Group. Arch Ophthalmol. 1993;111:231-4.

20. Gerling J, Meyer JH, Kommerell G. Visual field defects in optic neuritis and anterior ischemic optic neuropathy: distinctive features. Graefes Arch Clin Exp Ophthalmol. 1998;236:188-92.

21. Dahanayake P, Dassanayake TL, Pathirage M, Senanayake S, Sedgwick M, Weerasinghe VS. The effect of optic neuritis treatment trial (ONTT) combined corticosteroid regimen on pattern reversal visual evoked potentials: a prospective follow-up study. BMC Ophthalmol. 2021;21:324.

22. Beck RW. The optic neuritis treatment trial. Arch Ophthalmol. 1988;106:1051-3.

23. Beck RW, Gal RL. Treatment of acute optic neuritis: a summary of findings from the optic neuritis treatment trial. Arch Ophthalmol. 2008;126:994-5.

24. Biousse V, Calvetti O, Drews-Botsch CD, Atkins EJ, Sathornsumetee B, Newman NJ. Optic Neuritis Survey Group. Management of optic neuritis and impact of clinical trials: an international survey. J Neurol Sci. 2009;276:69-74.

25. Hickman SJ, Petzold A. Update on optic neuritis: an international view. Neuroophthalmology. 2022;46:1-18.

26. Beck RW, Cleary PA, Backlund JC. The course of visual recovery after optic neuritis. Experience of the Optic Neuritis Treatment Trial. Ophthalmology. 1994;101:1771-8.

27. Rodriguez M, Siva A, Cross SA, O'Brien PC, Kurland LT. Optic neuritis: a population-based study in Olmsted County, Minnesota. Neurology 1995;45:244-50.

CASE REPORT

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The Effectiveness of Slow Deep Breathing as a Pain Management Intervention in Coronary Heart Disease: A Case Report

Koroner Kalp Hastalığında Bir Ağrı Yönetimi Müdahalesi Olarak Yavaş ve Derin Nefes Almanın Etkinliği: Bir Olgu Raporu

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ABSTRACT

Patients with coronary heart disease often experience pain as their primary complaint. One effective nursing intervention to alleviate this pain is the practice of slow, deep breathing. This case study focuses on Mrs. A, a 57-year-old woman diagnosed with coronary heart disease five years ago. To help manage her pain, a slow, deep breathing relaxation technique was administered for four consecutive days. This nursing intervention not only reduced her pain but also calmed her down, highlighting its positive impact on pain management.

Keywords: Coronary disease, pain management, breathing exercise, relaxation therapy

ÖZ

Koroner kalp hastalığı olan hastalar genellikle birincil şikayetleri olarak ağrıyı deneyimlerler. Bu ağrıyı hafifletmek için etkili olan hemşirelik müdahalelerinden birisi de, yavaş ve derin nefes alma uygulamasıdır. Bu olgu çalışmasında, beş yıl önce koroner kalp hastalığı teşhisi konulan 57 yaşındaki Bayan A değerlendirildi. Ağrısını yönetmesine yardımcı olmak için, evinde dört gün boyunca yavaş ve derin nefes alarak gevşeme tekniği uygulandı. Bu hemşirelik müdahalesi sadece hastanın ağrısını azaltmakla kalmayıp, aynı zamanda sakinleştirerek ağrı yönetimi üzerindeki olumlu etkisini vurguladı.

Anahtar Kelimeler: Koroner hastalık, ağrı yönetimi, nefes egzersizi, gevşeme terapisi

INTRODUCTION

Slow, deep breathing exercises use diaphragmatic breathing, where the abdomen rises slowly and the chest expands as the patient inhales deeply[1-4]. This method is commonly employed as a non-pharmacological intervention because it helps reduce pain by promoting relaxation[5,6]. It alleviates stress and anxiety, lowers blood pressure, and improves lung function and oxygen supply to the heart[7-9]. Together, these effects contribute to pain reduction, making this technique particularly beneficial as a nursing intervention for patients with coronary heart disease, who experience pain[2,3].

Slow, deep breathing relaxation and psychoeducation were provided at the patient's home over four consecutive days. Patient data were collected through face-to-face interviews, observation, and physical examination. At the first meeting, the researchers assessed the patient's pain intensity using

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the Numeric Rating Scale (NRS) and then administered the intervention. During the second to fourth meetings, the patient practiced slow, deep breathing relaxation for 15 minutes or until the pain subsided. On the fourth day, the pain intensity was measured again to evaluate the effectiveness of the therapy. This study aims to analyze the effectiveness of slow, deep breathing techniques in reducing pain and to educate families as a support system for patients undergoing therapy.

CASE REPORT

Mrs. A, a 57-year-old woman, was diagnosed with coronary heart disease in February 2022. Her medical history revealed that she had been suffering from hypertension and heart disease for the past five years. Despite regularly taking antihypertensive medication, her heart condition continued to require special attention, particularly following her diagnosis of coronary heart disease.

In March 2022, Mrs. A experienced severe chest pain, leading to hospitalization. A percutaneous coronary intervention, including angiography, revealed a blockage in her coronary arteries, necessitating the placement of a stent. Despite this procedure, Mrs. A's lifestyle remained a contributing risk factor. She continued to consume salty and fatty foods and rarely exercised. Additionally, she often forgot to take her prescribed 5 mg dose of amlodipine, which was essential for managing her hypertension.

In April 2022, Mrs. A began experiencing left-sided chest pain that radiated to her back and left hand. These symptoms were especially pronounced during physical activities, such as lifting heavy objects or talking for extended periods. At the time of assessment, Mrs. A was fully conscious and able to comprehend questions but reported that strenuous activities or long conversations often triggered chest tightness and pain. Vital signs showed a blood pressure of 154/92 mmHg, a pulse of 96 beats per minute, and a respiratory rate of 21 breaths per minute. In addition to her verbal complaints, physical signs such as a furrowed brow, grimacing expression, and clutching the left side of her chest indicated significant discomfort. This condition interfered with her daily activities, causing her to feel anxious and uncomfortable.

After being diagnosed with coronary heart disease, Mrs. A was prescribed isosorbide dinitrate as an antianginal medication to manage her angina pectoris symptoms, particularly the chest pain radiating to her back and left arm. Despite taking the medication regularly, she continued to experience angina attacks several times a month, especially during physical activities. Furthermore, she suffered from a persistent headache after taking the medication, which exacerbated her anxiety and diminished her quality of life.

Given the lack of improvement, Mrs. A consulted her cardiologist once again. After further evaluation and considering her ongoing symptoms, the doctor decided to change her antianginal therapy from isosorbide dinitrate to a nitroglycerin transdermal patch. This new treatment was chosen because it provided a more stable antianginal effect with a lower risk of side effects.

Following the change in therapy, Mrs. A reported a significant reduction in the frequency of angina attacks, from 4-5 times per week to only 1-2 times per week. The severity of her pain also decreased, with the average intensity dropping from a scale of 7 to 4-5. Her pain subsided more quickly, typically lasting only 5-10 minutes, and did not always necessitate stopping her activities. The anxiety that had previously accompanied her angina attacks also diminished. Additionally, the side effects of headaches that she had experienced with isosorbide dinitrate were drastically reduced. With the decrease in both the frequency and severity of her angina attacks, as well as the reduction in drug-related side effects, Mrs. A experienced a significant improvement in her quality of life. She could now perform daily activities more comfortably and with fewer disruptions.

For Mrs. A, repeat tests such as myocardial perfusion scintigraphy, stress test, or coronary angiography were not necessary as her symptoms remained stable and under control. Usually, these tests are only repeated if the patient experiences worsening symptoms or if standard treatment does not relieve her pain. As Mrs A's chest pain and other signs of ischemia could be effectively managed with lifestyle changes, medication, and slow deep breathing, further tests might not be necessary at that time.

In addition, since coronary angiography is an invasive test with some risks, doctors usually avoid it unless new symptoms arise. The positive effect of slow deep breathing on her pain suggested that her symptoms were responding well to this nursing intervention, thus reducing the need for more intensive testing. Clinical guidelines also recommend avoiding expensive tests, such as myocardial perfusion and coronary angiography unless they are necessary. In single-case report research, ethical approval is obtained by securing the patient's and their family's permission. The researcher provides a detailed explanation of the study's objectives, procedures, and methods. Following this, approval is formalized through informed consent from the patient and family. This study adhered to the principles of honesty, ensuring patient and family privacy, and maintaining anonymity.

Methods and Implementation

The slow, deep breathing nursing intervention was implemented in the patient's home over four days. Before the intervention,

the patient was given a NRS questionnaire to measure the level of pain experienced (Figure 1). The NRS is a pain measurement tool that uses a scale from 0 to 10. This scale is effective in assessing pain both before and after the intervention. A score of 0 indicates "no pain," 1-3 indicates "mild pain," 4-6 indicates "moderate pain," and 7-10 indicates "severe pain" (Figure1).

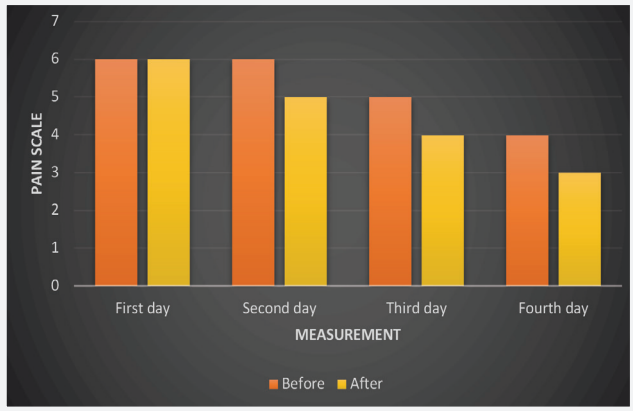
The patient was guided through a slow deep breathing relaxation technique with the following steps:

1) Position the patient in a semi-Fowler's position, 2) Ask the patient to place one hand on their chest and the other on their abdomen, 3) Instruct the patient to take a deep breath through their nose, counting to three while keeping their mouth closed, 4) Encourage the patient to notice the expansion of their abdomen as they inhale, 5) Have the patient hold their breath for three seconds, 6) Instruct the patient to exhale slowly, counting to three, through their mouth as if blowing, 7) Repeat steps 1-6 for 15 minutes.

Slow, deep breathing relaxation was carried out with the active involvement of the patient's family, who were educated on how to support the patient during pain episodes. Nurses educated the patient and their family about the benefits of slow, deep breathing techniques for alleviating chest pain. They also highlighted the crucial role of family support, explaining how it could boost the patient's motivation to follow medical therapy and help reduce their symptoms.

The bar chart (Graph 1) illustrates changes in pain scale measurements over four days, comparing pain levels before and after a specific intervention. On the first day, before the intervention, the pain scale is at its highest, recorded at 6. After the intervention, the pain level remains unchanged at 6, indicating no immediate relief. By the second day, the pain scale before the intervention remains at 6, but after the intervention, there is a slight decrease to 5, suggesting the intervention begins to have some effect. On the third day, the pain scale before the intervention decreases to 5, and after the intervention, it further drops to 4, reflecting a continued positive impact. By the fourth day, the pain scale before the intervention is 4, while after the intervention, it decreases to 3, marking the most significant improvement in pain relief over the four days. The chart reveals a progressive decrease

Figure 1. Numeric Rating Scale



Graph 1. Characteristics of pain levels before and after slow deep breathing intervention

in pain levels following the intervention, with no change on the first day but a steady reduction from the second day onward. By the fourth day, the significant difference between pain levels before and after the intervention demonstrates the effectiveness of the treatment in reducing pain over time

DISCUSSION

The results of this study indicate that implementing slow, deep breathing techniques can effectively reduce pain in patients with coronary heart disease. This is achieved because slow, deep breathing enhances alveolar ventilation, maintains gas exchange, prevents lung atelectasis, improves cough efficiency, and reduces physical and emotional stress[10-12]. Consequently, it decreases pain intensity and anxiety. Patients who practice deep breathing relaxation techniques experience notable benefits, including pain relief, mental calmness, and reduced anxiety[13-15].

The results of this research are supported by findings from Gholamrezaei et al.[1], who observed a reduction in chest pain levels among patients with coronary heart disease in Belgium after implementing deep and slow breathing relaxation techniques compared to those practicing uncontrolled breathing. Although the overall difference in pain intensity between controlled and unchecked breathing was approximately 0.5 points on a 10-point scale, this difference was more pronounced, about 1 point, in patients with higher pain scores. Additionally, research by Shao et al.[16] demonstrated significant improvements in chest pain characteristics in the study group after applying deep breathing techniques over two days. All aspects of pain-severity, quality, and expression-showed improvement, with more participants reporting no pain, compared to a higher percentage in the control group, who continued to experience moderate pain, persistent pain quality, and restlessness. These findings confirm the

positive impact of deep breathing technique training on pain reduction[7,10,13,14].

Deep breathing relaxation techniques reduce pain levels and effectively alleviate the stress experienced by patients during painful episodes[17]. The benefits of these techniques include a sense of calm, reduced anxiety, and diminished feelings of worry and restlessness. Additionally, deep breathing helps lower blood pressure, decrease heart rate, and enhance disease resistance[18]. Beyond these physiological effects, it contributes to better mental health[19], improved sleep quality[12], enhanced memory[20], and increased creativity and confidence[21].

Deep breathing exercises have a significant impact on reducing anxiety levels, improving quality of life, and reducing medication use in patients with coronary heart disease[22]. By optimizing oxygen exchange and reducing the stress response, these exercises can lower the anxiety that often accompanies coronary heart conditions[16]. In addition, patients who regularly perform deep breathing exercises tend to report improved quality of life, including improvements in sleep, energy, and emotional well-being[5]. Along with reduced anxiety and increased self-control, patients' need for sedatives or painkillers may decrease, potentially reducing dependence on pharmacotherapy and lowering the risk of side effects related to long-term drug use[14].

Deep breathing techniques have a profound long-term impact on the quality of life and well-being of patients, particularly those with chronic conditions such as coronary heart disease. Regular practice of these techniques not only aids in managing physical pain but also enhances patients' mental and emotional well-being.

Physiologically, deep breathing improves oxygenation efficiency, lowering blood pressure and enhancing cardiovascular function. This improvement helps to reduce the frequency and intensity of angina attacks, enabling patients to be more active and independent in their daily lives. Additionally, deep breathing is effective in alleviating anxiety and stress, which often exacerbate heart conditions. By managing stress more effectively, patients experience better moods, improved sleep, and a reduced need for sedatives or analgesics.

The psychological benefits of deep breathing are equally significant. Patients who consistently engage in deep breathing exercises often feel more empowered and in control of their condition. This sense of control fosters increased self-confidence and emotional well-being, further enhancing overall quality of life. Thus, incorporating deep breathing techniques into long-term care provides holistic benefits, addressing patient health's physical, mental, and emotional aspects.

Although deep breathing techniques provide various benefits, their application in managing chronic diseases like coronary heart disease is not always straightforward and can encounter several challenges and limitations. One significant challenge is ensuring patient motivation and compliance with consistent practice. Patients may struggle to incorporate these techniques into their daily routine, especially if they do not experience immediate benefits or lack understanding of their importance. Additionally, physical limitations such as severe breathlessness or fatigue, common in patients with serious health conditions or comorbidities, can hinder the effective practice of these exercises. Education and support limitations also pose barriers, as constrained time and resources in healthcare settings often make it difficult to provide comprehensive guidance. Without proper support, patients may not know how to execute these techniques correctly or appreciate the need for consistent practice. Environmental factors, such as environments that do not promote relaxation, and social factors, such as a lack of family support, can further diminish the effectiveness of deep breathing techniques. Moreover, responses to these techniques may vary among patients due to age, disease severity, and mental state, potentially leading to varied outcomes despite correct adherence to the exercise regimen. Addressing these challenges requires a holistic and integrated approach, including ongoing educational support, regular monitoring by healthcare professionals, and tailoring techniques to meet individual patient needs.

CONCLUSION

Deep breathing exercises reduce pain, improve quality of life, and decrease medication use in patients with coronary heart disease. These exercises help lower pain intensity by increasing alveolar ventilation and reducing stress while enhancing patients' mental and emotional well-being, improving overall quality of life. Moreover, as anxiety and pain diminish, patients become less reliant on sedative and analgesic medications, thereby lowering the risk of side effects and the costs associated with long-term treatment. As a non-pharmacological intervention, deep breathing exercises offer holistic benefits and should be integrated into the routine care of coronary heart patients.

Ethics

Informed Consent: In single-case report research, ethical approval is obtained by securing the patient's and their family's permission. The researcher provides a detailed explanation of the study's objectives, procedures, and methods. Following this, approval is formalized through informed consent from the patient and family. This study adhered to the principles of honesty, ensuring patient and family privacy, and maintaining anonymity.

Footnotes

Authorship Contributions

Concept: Y.B.P., T.P.W., Design: Y.B.P., Data Collection or Processing: Y.B.P., T.P.W., Analysis or Interpretation: Y.B.P., Literature Search: Y.B.P., T.P.W., Writing: Y.B.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Gholamrezaei A, Van Diest I, Aziz Q, Pauwels A, Tack J, Vlaeyen JWS, et al. Effect of slow, deep breathing on visceral pain perception and its underlying psychophysiological mechanisms. Neurogastroenterol Motil. 2022;34:14242.
2. Jarrah MI, Hweidi IM, Al-Dolat SA, Alhawatmeh HN, Al-Obeisat SM, Hweidi LI, et al. The effect of slow deep breathing relaxation exercise on pain levels during and post chest tube removal after coronary artery bypass graft surgery. Int J Nurs Sci. 2022;9:155-61.
3. Shang H, Zhang K, Guan Z, Li Y, Wang X, Liu H, et al. Optimization of evidence-based research in the prevention and treatment of coronary heart disease with traditional Chinese medicine: a comprehensive review. J Tradit Chinese Med Sci. 2022;9:100-7.
4. Gholamrezaei A, Van Diest I, Aziz Q, Pauwels A, Tack J, Vlaeyen JWS, et al. Effect of slow, deep breathing on visceral pain perception and its underlying psychophysiological mechanisms. Neurogastroenterol Motil. 2022;34:14242.
5. Doi SK, Isumi A, Yamaoka Y, Shakagori S, Yamazaki J, Ito K, et al. The effect of breathing relaxation to improve poor sleep quality in adults using a huggable human-shaped device: a randomized controlled trial. Sleep Breath. 2024;28:429-39.
6. Volberg C, Schmidt-Semisch H, Maul J, Nadig J, Gschnell M. Pain management in German hospices: a cross-sectional study. BMC Palliat Care. 2024;23:7.
7. Jarrah MI, Hweidi IM, Al-Dolat SA, Alhawatmeh HN, Al-Obeisat SM, Hweidi LI, et al. The effect of slow deep breathing relaxation exercise on pain levels during and post chest tube removal after coronary artery bypass graft surgery. Int J Nurs Sci. 2022;9:155-61.
8. de Souto Barbosa JV, do Nascimento Sales Figueiredo Fernandes AT, da Silva JL, da Silva Leal L, de Aquino Santos MLB, de Albuquerque Cacique New York BS, et al. Effectiveness of paced breathing guided by biofeedback on clinical and functional outcomes patients with chronic obstructive pulmonary disease: an uncontrolled pilot study. Appl Psychophysiol Biofeedback. 2023;48:423-32.
9. Ramalingam V, Cheong SK, Lee PF. Effect of six-week short-duration deep breathing on young adults with chronic ankle instability-a pilot randomized control trial. BMC Sports Sci Med Rehabil. 2023;15:155.
10. Joseph AE, Moman RN, Barman RA, Kleppel DJ, Eberhart ND, Gerberi DJ, et al. Effects of slow deep breathing on acute clinical pain in adults: a systematic review and meta-analysis of randomized controlled trials. J Evidence-Based Integr Med. 2022;27:2515690X2210780.
11. Birdee G, Nelson K, Wallston K, Nian H, Diedrich A, Paranjape S, et al. Slow breathing for reducing stress: the effect of extending exhale. Complement Ther Med. 2023;73:102937.
12. Niu S, Zhang T, Li W, Wen S, Dong L, Wang S, et al. Positive effect of deep diaphragmatic breathing training on gastroesophageal reflux-induced chronic cough: a clinical randomized controlled study. Respir Res. 2024;25:169.
13. Joseph AE, Moman RN, Barman RA, Kleppel DJ, Eberhart ND, Gerberi DJ, et al. Effects of slow deep breathing on acute clinical pain in adults: a systematic review and meta-analysis of randomized controlled trials. J Evid Based Integr Med. 2022;27:2515690X2210780.
14. Tavoian D, Craighead DH. Deep breathing exercise at work: potential applications and impact. Front Physiol. 2023;14:1040091.
15. Utami S. Efektifitas relaksasi napas dalam dan distraksi dengan latihan 5 jari terhadap nyeri post laparatomi. Univ Riau. 2016;4:1-13.
16. Shao R, Man ISC, Lee TMC. The effect of slow-paced breathing on cardiovascular and emotion functions: a meta-analysis and systematic review. Mindfulness. 2024;15:1-18.
17. Vlemincx E, Van Diest I, Van den Bergh O. A sigh of relief or a sigh to relieve: the psychological and physiological relief effect of deep breaths. Physiol Behav. 2016;165:127-35.
18. Misra S, Smith J, Wareg N, Hogdes K, Gandhi M, Mcelroy JA, et al. Take a deep breath: a randomized control trial of Pranayama breathing on uncontrolled hypertension. Adv Integr Med. 2019;6:66-72.
19. Fincham GW, Strauss C, Cavanagh K. Effect of coherent breathing on mental health and wellbeing: a randomised placebo-controlled trial. Sci Rep. 2023;13:22141.
20. Girin B, Juventin M, Garcia S, Lefèvre L, Amat C, Fourcaud-Trocmé N, et al. The deep and slow breathing characterizing rest favors brain respiratory-drive. Sci Rep. 2021;11:7044.
21. Kamboj M, Kadian K, Dwivedi V, Wary Alongbar, Ojha Swastika. Advanced detection techniques for driver drowsiness: a comprehensive review of machine learning, deep learning, and physiological approaches. Multimed Tools Appl. 2024;83:90619-82.
22. Reafaat Abdelkader Atia R. Deep breathing exercise application: it's effect on physiological parameters among patients with acute coronary syndrome. Egypt J Heal Care. 2024;15:52-62.

CASE REPORT

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Cerebral Toxoplasmosis Presenting with Confusion and Headache: A Case Report

Konfüzyon ve Baş Ağrısı ile Prezente Olan Serebral Toksoplazmoz: Bir Olgu Sunumu

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ABSTRACT

Toxoplasmosis is an infectious disease caused by a protozoan parasite called *Toxoplasma gondii*. Rarely, undiagnosed Human Immunodeficiency virus patients may be diagnosed with cerebral toxoplasmosis as the etiology of confusion and headache. Our case is a 26-year-old man who presented with a disturbance of consciousness and headache for a week and was admitted to the hospital. There were no significant features in the patient's medical and family history. Magnetic resonance imaging and CD4 positive T-cell blood level revealed a high suspicion of toxoplasma infection. Polymerase chain reaction test for *Toxoplasma gondii* deoxyribonucleic acid in his cerebrospinal fluid was positive. The patient was diagnosed with cerebral toxoplasmosis and acquired immunodeficiency syndrome. Trimethoprim-sulfamethoxazole (TMP-SMX) was initiated at 1600 mg SMX/320 mg TMP two times a day and clindamycin 900 mg three times a day. Tenofovir, emtricitabine and efavirenz were added. His symptoms gradually improved over 72 hours and after 21 days, the patient was discharged from the hospital. While toxoplasmosis is often curable, it poses a serious health threat to immunocompromised individuals and can be fatal if not promptly treated.

Keywords: Cerebral toxoplasmosis, confusion, headache, neuroinfection

ÖZ

Toksoplazmoz, *Toksoplasma gondii* adı verilen bir protozoan parazitin neden olduğu bulaşıcı bir hastalıktır. Nadiren tanısı konulmamış İnsan İmmün Yetmezlik virüsü hastalarında, konfüzyon ve baş ağrısının etiyolojisi olarak serebral toksoplazmoz tanısı konulabilir. Olgumuz, bir haftadır bilinç bozukluğu ve baş ağrısı şikayetiyle hastaneye yatırılan 26 yaşında bir erkek hastadır. Hastanın tıbbi ve aile geçmişinde önemli bir özellik yoktu. Manyetik rezonans görüntüleme ve CD4 pozitif T-hücre kan seviyesi, serebral toksoplazmozis yüksek şüphesi uyandırdı. Beyin omurilik sıvısında *Toksoplasma gondii* deoksiribonükleik asit için polimeraz zincir reaksiyon testi pozitifti. Hastaya serebral toksoplazmoz ve edinilmiş immün yetmezlik sendromu tanısı konuldu. Günde iki kez trimetoprim-sulfametoksazol (TMP-SMX)1600 mg SMX/320 mg TMP ve günde üç kez 900 mg klindamisin başlandı. Tenofovir, emtrisitabin ve efavirenz eklendi. Semptomları 72 saat içinde kademeli olarak düzeldi ve hasta 21 gün sonra hastaneden taburcu edildi. Toksoplazmoz genellikle tedavi edilebilir olsa da, bağışıklık sistemi baskılanmış bireyler için ciddi bir sağlık tehdidi oluşturur ve derhal tedavi edilmezse ölümcül olabilir.

Anahtar Kelimeler: Serebral toksoplazmoz, konfüzyon, baş ağrısı, nöroenfeksiyon

INTRODUCTION

Toxoplasmosis is an infectious disease caused by a protozoan parasite called *Toxoplasma gondii*. It occurs by consuming contaminated food sources such as water, undercooked meat, and cat feces[1]. Severe toxoplasmosis causing damage to the brain is most likely in immunocompromised patients such as those with Human Immunodeficiency Virus[2] (HIV). The

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incidence of toxoplasmosis in HIV-infected patients increases with a CD4+ count below 200 cells/μL. The greatest risk is in patients with CD4+ counts less than 50 cells/μL[3]. Central nervous system (CNS) toxoplasmosis rarely results from primary infection[4]. Seroprevalence of anti-toxoplasma antibody varies substantially among different geographic locales, with a prevalence of approximately 11% in the United States, versus 50% to 80% in certain European, Latin American, and African countries[5]. In toxoplasmosis seroprevalence studies conducted in Türkiye, it was reported that IgG antibody positivity was 28.8% in pregnant women, 31.9% in the population with HIV infection and even 65.2% in women living in rural areas[6]. Cerebral toxoplasmosis is observed in 10-34% of autopsies conducted on patients with HIV[7]. Common symptoms of toxoplasma encephalitis are flu-like symptoms, headache, seizures, hemiparesis, and mental disorders. Extracerebral involvement can also be seen including but not limited to pneumonitis, chorioretinitis, etc.[8,9].

CASE REPORT

Our case is a 26-year-old man of Turkish ethnicity, who presented with a disturbance of consciousness and headache for a week and was admitted to the hospital. There were no significant features in the patient's medical and family history. Written informed consent was obtained from the patient to present this case. This study was performed in line with the principles of the Declaration of Helsinki.

Patient's systemic examination was normal. Neurological examination revealed apathy, somnolence, bilateral positive Babinski's sign, and increased deep tendon reflexes. Magnetic resonance imaging (MRI) revealed a high index of suspicion of a toxoplasma infection (Figure 1). His CD4+ count was 87 cells/μL and his HIV1-RNA viral load was 6.2×10^5 copies/mL. The serum *Toxoplasma gondii* IgG antibody measured with enzyme-linked immunosorbent assay was over 250 IU/mL (Table 1). Polymerase chain reaction test for *Toxoplasma gondii* DNA in his cerebrospinal fluid was positive. The patient was diagnosed with cerebral toxoplasmosis and Acquired Immunodeficiency syndrome (AIDS). Trimethoprim-sulphametoxazole (TMP-SMX) was initiated at 1600 mg SMX/320 mg TMP two times a day and clindamycin 900 mg three times a day. Tenofovir, emtricitabine, and efavirenz were started. His symptoms gradually improved over 72 hours and after 21 days, the patient was discharged from the hospital.

DISCUSSION

Toxoplasma gondii is the leading cause of brain lesions in patients with AIDS[10]. Diagnosis of the cerebral toxoplasmosis requires the presence of three components; clinical symptoms corresponding to toxoplasma infection, specific CNS lesions detected by computed tomography or MRI scans and positive *Toxoplasma gondii* serological tests[8]. All of these criteria are present in the described case. Considering differential diagnoses, cerebral cysticercosis is associated with epileptic seizures, headache, meningeal irritation, cognitive disorders, hemiparesis and palsy. Mononuclear pleocytosis, eosinophils, elevated levels of protein, and normal or lower levels of blood glucose are detected in the cerebrospinal fluid. The diagnosis is set on grounds of the ELISA, which is used for the detection of specific IgM antibodies[11]. The changes demonstrated by the laboratory test results in our patient were mild proteinemia and pleocytosis with a high prevalence of the lymphocytes and normal glucose levels. In adult patients, tuberculosis (TB) of the CNS is secondary, developing after the primary TB infection located most often in the lungs. The imaging results reveal a large number of small-sized foci situated in the basal region of the brain[12]. In the described case, lung imaging does not suggest TB. Multiple cerebral abscesses are associated with various infectious diseases (aspergillosis, cryptococcosis), neoplasms, and vasculitis. The imaging tests show a significant number of ring-like lesions similar to those observed in our case. However, clinically, brain abscesses are typically associated with persistent fever, recurrent seizures, visual disturbances, focal neurological deficits, and hemiparesis[13]. However, our patient did not have fever. Multiple brain tumor metastases result from a primary cryptogenic malignant process. 40% of the gliomas and blastomas are demonstrated by similar ring-like lesions[14]. In our case, we found no evidence of a primary neoplastic process. In multiple sclerosis, demyelinating lesions do not look like a closed ring[15]. In our patient, the clinical criteria for this diagnosis were not present.

The standard treatment involves a combination of pyrimethamine, sulfadiazine, and folinic acid. TMP-SMX can be used as an alternative regimen. Clindamycin is an option for patients allergic to sulfa drugs. Effective antiretroviral therapy is equally important[16,17]. In our study, treatment success was achieved with the combination of TMP-SMX and clindamycin. With antibiotic therapy, 74% of patients improve by day 7 and 91% improve by day 14. Imaging studies are performed every 4-6 weeks until the complete resolution of the lesion or stabilization after partial resolution. The primary therapy is administered for 6 weeks, after which long-term suppressive therapy at lower doses is continued, with the duration based on the response to Highly Active Antiretroviral Therapy (HAART). HAART typically involves a combination of three or more antiretroviral drugs. The most common treatment combination is two nucleoside reverse transcriptase inhibitors (tenofovir-emtricitabine) plus a non-nucleoside reverse transcriptase inhibitor or integrase strand transfer inhibitor[18]. In our case, tenofovir, emtricitabine, and efavirenz were started. The long-term suppressive therapy can be discontinued in patients with persistent elevation of CD4+ counts greater than 200 cells/μL and resolution of lesions on MRI.

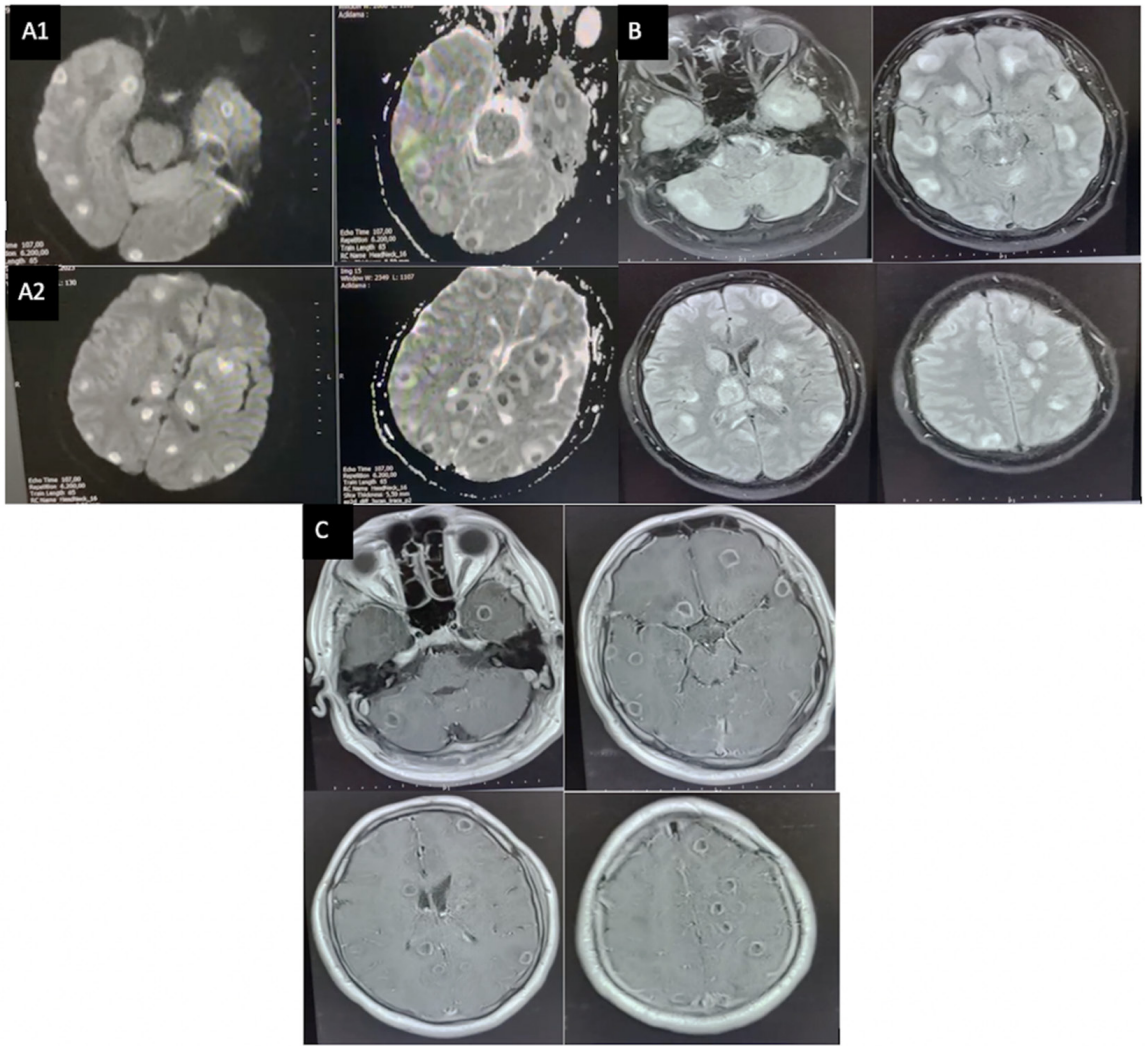

Figure 1. (A1, A2) Diffusion weighted MRI showed multiple, focal diffusion restricted areas. (B) Fluid-attenuated inversion recovery MRI showed multiple hyperintense lesions with surrounding edema in both the supratentorial and infratentorial. (C) Gadolinium-enhanced T1-weighted MRI showed multiple, focal heterogeneous ring-enhancing lesion around supratentorial and infratentorial area

MRI: Magnetic resonance imaging

Table 1. Cerebrospinal fluid findings

Appearance	Clear
Color	Colorless
Cells	10/mm^3 leukocytes (lymphocytes)
Total protein	120 mg/dL
Glucose	63 mg/dL

To prevent primary toxoplasmosis, patients should refrain from consuming undercooked meat and ensure thorough handwashing after handling soil or coming into contact with cats. Patients who are seropositive for toxoplasma should be started on prophylaxis against CNS toxoplasmosis if their CD4+ count drops below 100 cells/µL^3.

The use of antiretroviral therapy markedly decreases the incidence of cerebral toxoplasmosis. Nevertheless, this disease continues to be the leading cause of expansive brain lesions, resulting in high morbidity and mortality among individuals with advanced immunosuppression, especially in low- and middle-income countries. Anti-toxoplasma therapy is an important component of the diagnostic approach to expansive brain lesions in AIDS. Local neuroepidemiology, degree of immunosuppression, and individual clinical, laboratory and neuroradiological features are extremely important in making differential diagnoses. TMP-SMX can be used for primary prophylaxis, initial therapy, and secondary prophylaxis of HIV-associated cerebral toxoplasmosis. Early initiation of antiretroviral therapy is currently possible because more effective and safer treatment options are available.

CONCLUSION

Cerebral toxoplasmosis is a common and treatable condition in HIV-positive patients; therefore, we aimed to present our case. Due to logistical challenges, a follow-up brain MRI could

not be performed on the patient presented in our article after clinical improvement. Only clinical improvement was observed, and the patient failed to adhere to follow-up appointments. The lack of follow-up imaging and the patient's non-compliance with follow-up visits are the limitations of our article, as the clinical outcome of the case remains unknown. While toxoplasmosis is often curable, it poses a serious health threat to immunocompromised individuals and can be fatal if not promptly treated.

Ethics

Informed Consent: Written informed consent was obtained from the patient to present this case. This study was performed in line with the principles of the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.K., Concept: İ.K., Design: İ.K., T.K.A., Data Collection or Processing: İ.K., Analysis or Interpretation: İ.K., Literature Search: İ.K., T.K.A, Writing: İ.K., T.K.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, et al. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ. 2000;321:142-7.
2. Bowen LN, Smith B, Reich D, Quezado M, Nath A. HIV-associated opportunistic CNS infections: pathophysiology, diagnosis and treatment. Nat Rev Neurol. 2016;12:662-74.
3. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58:1-207.
4. Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, et al. Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. J Clin Microbiol. 2002;40:4037-44.
5. National Institutes of Health. Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV: Toxoplasma gondii Encephalitis. Available from: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-opportunistic-infections/toxoplasmosis?guideline%5B0%5D=title_bookpart%3AHIV+Clinical+Guidelines%3A+Adult+and+Adolescent+Opportunistic+Infectio ns&redirected=1
6. Senoglu S, Yesilbag Z, Aydin OA, Karaosmanoglu HK, Yasar KK. Toxoplasma gondii IgG seroprevalence in patients with HIV/AIDS. Turkiye Parazitol Derg. 2018;42:175-9.
7. Kornienko VN, Pronin IN. Diagnostic neuroradiology. AJNR Am J Neuroradiol. 2009;30:E136.
8. Porter SB, Sande MA. Toxoplasmosis of the central nervous system in the acquired immunodeficiency syndrome. N Engl J Med. 1992;327:1643-8.
9. Pereira-Chioccola VL, Vidal JE, Su C. Toxoplasma gondii infection and cerebral toxoplasmosis in HIV-infected patients. Future Microbiol. 2009;4:1363-79.
10. Rothman RE, Marco CA, Yang S. Human immunodeficiency virus infection and acquired immunodeficiency syndrome in: Tintinalli JE, Stapczynski JS, Cline DM, Ma OJ, Cydulka RK, Meckler GD. Tintinalli's emergency medicine: a comprehensive study guide. 7th ed. Chapter 149, New York; McGraw- Hill; 2011.
11. Kimura-Hayama ET, Higuera JA, Corona-Cedillo R, Chávez-Macías L, Perochena A, Quiroz-Rojas LY, et al. Neurocysticercosis: radiologic-pathologic correlation. Radiografics. 2010;30:1705-19
12. Khatri GD, Krishnan V, Antil N, Saigal G. Magnetic resonance imaging spectrum of intracranial tubercular lesions: one disease, many faces. Pol J Radiol. 2018;83:524-35.
13. Krishna K, Sada E, Vikram A, Gupta A. Multiple brain abscesses - diagnostic dilemma and therapeutic nightmare. J Neurosci Rural Pract. 2013;4:234-6.
14. Fink KR, Fink JR. Imaging of brain metastases. Surg Neurol Int. 2013;4:209-19.
15. Ge Y. Multiple sclerosis: the role of MR imaging. Am J Neuroradiol. 2006;27:1165-76.
16. Dedicoat M, Livesley N. Management of toxoplasmic encephalitis in HIV-infected adults (with an emphasis on resource-poor settings). Cochrane Database Syst Rev. 2006;19:5420.
17. Bertschy S, Opravil M, Cavassini M, Bernasconi E, Schiffer V, Schmid P, et al. Discontinuation of maintenance therapy against toxoplasma encephalitis in AIDS patients with sustained response to anti-retroviral therapy. Clin Microbiol Infect. 2006;12:666-71.
18. Eggleton JS, Nagalli S. Highly Active Antiretroviral Therapy (HAART). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.

REVIEW

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The Scope of Nurturing Care in Early Childhood and Its Applications in Our Country

Erken Çocukluk Döneminde Geliştiren Bakımın Kapsamı ve Ülkemizdeki Uygulamalar

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ABSTRACT

It is thought that supporting the early childhood period, which is of critical importance in terms of cognitive, social, emotional and physical development in children, with the components of the developmental care scope will increase the peace and welfare levels of future generations. In addition to the applications carried out in this context in our country, there is also a need for applications that we need to add to our health practice. The purpose of the review is to talk about the scope of nurturing care and nurturing care practices in our country in the light of current information and to offer suggestions to further develop the scope.

Keywords: Nurturing care, early childhood, Türkiye

ÖZ

Çocuklarda bilişsel, sosyal, duygusal ve fiziksel gelişim açısından kritik öneme sahip olan erken çocukluk döneminin geliştiren bakım kapsamı bileşenleri ile desteklenmesinin, gelecek nesillerin huzur ve refah seviyelerini artıracağı düşünülmektedir. Ülkemizde bu bağlamda yapılan uygulamaların yanı sıra, sağlık pratiğimize eklememiz gereken uygulamalara da ihtiyaç vardır. Derlemenin amacı güncel bilgiler ışığında geliştiren bakımın kapsamından ve ülkemizdeki geliştiren bakım uygulamalarından bahsetmek ve kapsamı daha ileriye taşımak için öneriler sunmaktır.

Anahtar kelimeler: Geliştiren bakım, erken çocukluk dönemi, Türkiye

INTRODUCTION

Early childhood, which covers children's first eight years of life, constitutes the cornerstones of cognitive, social, emotional and physical development. In this critical period, the development of children, poverty, nutrition insecurity, gender inequality, violence, environmental harmful substances and the mental health of parents or caregivers are influenced by many factors[1]. Starting from the pregnancy process, these risk factors are known to have significant effects on early childhood development. Studies on a global scale reveal that at least 250 million children under the age of 5 years are in danger of not reaching the development potential[2]. In order to address this situation, a model has been developed by the WHO, UNICEF and other common organizations to ensure that children can healthly exist, grow and realize all their potential in life[3]. This scope was introduced at the 71st World Health Council meeting in Geneva in May 2018[4]. In this study, the concept of nurturing care and its components were handled, nurturing care practices in Türkiye were examined, and it was aimed to provide suggestions for the development of these applications.

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The Scope of Nurturing Care

Early childhood plays a fundamental role in terms of life-long health, welfare, learning skills and productivity of the individual. In this period, in order to support the healthy growth and development of children, parents or caregivers should be supported within the framework of nurturing care[2]. It consists of five basic components: health, adequate nutrition, sensitive care, early learning opportunities and safety[3]. In this context, starting from the pregnancy period, a child -oriented and family- centered approach should be adopted in the first three years of life[5]. In order to meet the developmental needs of children, UNICEF and other stakeholders have identified the nurturing care indicators. These indicators have been implemented in 197 countries since 2018 in order to follow up the development data of early childhood in the countries and evaluated through 42 indicators. These indicators within the scope of nurturing care include: demography (country population, population of children under the age of five, the number of annual births, the rate of death under the age of five), the factors that threaten child development (child poverty, dwarf ratio, low birth weight, preterm births, mother mortality, adolescent pregnancy rate, violence and neglect) and rate of children at risk (risk distributions according to gender and locations). Furthermore, the economic cost of the growth deficit of children at risk for development, early childhood development index and their functional problems are among the criteria of evaluation. The indicators of sub-components such as health, nutrition, early learning, sensitive care and safety are monitored at national and global levels[6].

Health

Regular monitoring of children's physical and emotional health, response to their daily needs with love and appropriately, protection from home and environmental hazards are among the priority responsibilities of parents or caregivers. With hygiene applications, minimizing the risk of infection, utilizing preventive health services and timely treatment of children is of great importance. The nurturing care approach aims to support the health and welfare of not only children, but also parents or caregivers[3].

Adequate Nutrition

Nutrition of the pregnant mother during pregnancy has a critical role not only for its own health, but also for the growth and nutrition of the developing baby. For the first six months from birth, only breastfeeding should be supported with skin-to-skin contact. After the sixth month, in addition to breast milk, a nutrition process is required with balanced and various complementary foods suitable for the age of the child. Adequate nutrition, which is one of the main components of nurturing care, is directly related to the nutritional safety of both the child and the family[3].

Sensitive Care

Sensitive care involves careful observation of children's gestures, sounds, movements and other communication signals and responding appropriately to them. This understanding makes it easier to recognize and meet the basic needs of children. This approach, which also includes sensitive nutrition, supports children's growing in healthy way[3].

Early Learning Opportunities

In a loving and safe family environment, it is very important for children to reach learning opportunities with daily life activities and interpersonal interactions. Such guidance and mutual interactions are necessary for children to develop social skills and understand their relationship with others. Early learning opportunities are one of the main components that support children's cognitive and social development[3].

Safety

Children are quite vulnerable to physical injuries, environmental dangers and emotional stress. Safety, another important element of nurturing care, includes physical and emotional protection mechanisms that will make children feel safe. This approach is critical to support children's healthy growth and development[3].

Nurturing Care Practices in Türkiye

In Türkiye, the "Early Childhood Development Policies Project" has been implemented in cooperation with the Association of Child Development and Educators and UNICEF within the scope of policies for early childhood development. Early Childhood Development Platform was established as an output of this project and various non-governmental organizations operating in order to support early childhood development were brought together[5]. 2023 data on nurturing care indicators in Türkiye are summarized as follows:

Türkiye's population is 85,341,241 and the annual number of births is 1,236,900. The number of children under the age of five was calculated as 6,421,178, which corresponds to 8% of the total population. The death rate under the age of five is 9 per thousand. The mother mortality rate was recorded as 17 at every 100,000 births. Low birth weight ratio was reported as 13%, preterm birth rate as 12%, the rate of dwarfness under the age of five as 6%. While data on child poverty are unavailable, the rate of neglect related to violence and disciplinary methods is 6%. The proportion of small children with developmental risk was recorded as 18% in 2005, 15% in 2010 and 4% in 2015 and this rate has decreased over the years. There are not

enough data in the risks categories by settlement and gender. Early childhood growth deficit is estimated to have a 48% effect of the individual's annual income loss in adulthood. However, there is not enough information about children with functional problems. On the other hand, 74% of children of 36-59 months were evaluated as developmentally normal[7].

The proportion of children applying to health institutions on suspicion of pneumonia in Türkiye is 45%. The proportion of four or more antenatal care areas during pregnancy is 90% and the rate of those who benefit from postnatal care services is 79%. There are no data on pregnant women receiving Human Immunodeficiency Virus treatment (health). While 71% of infants begin to be breastfed immediately after birth, the rate of those fed only with breast milk for the first six months is 41%. There are no data about minimum acceptable diet (nutrition). While 65% of children are supported by early learning opportunities at home, the rate of those who have toys is 76% and the rate of those who have books is 29%. There is no information about the rate of attendance in preschool education (early learning opportunities). The birth recording rate is 98%, access to basic drinking water is 97%, and the basic sanitation rate is over 99%. There are no data on positive disciplinary applications (safety). There are not enough data on the quality of parental support, mental health, community information and child day care services (sensitive care)[7]. In Türkiye, while the duration of paid maternity leave varies between 14 and 18 weeks, paternity leave is shorter. The law for the marketing of breast milk substitutes is partially applied. Although there is a national minimum wage, social protection policies for children and family are limited. International Agreements: Türkiye has been party to the Convention on the Rights of the Child, Convention on the Rights of Persons with Disabilities, and Convention on Protection of Children and Cooperation in Respect of Intercountry Adoption[7].

Health

In order for pregnant women in Türkiye to have a healthy pregnancy and to provide more conscious care for their newborns, the Ministry of Health launched the "Pregnancy Schools" practice in public and private hospitals with the Circular (2018/23) published on October 2, 2018. The four-week training in these schools is provided by expert health personnel. The training covers topics such as pregnancy physiology, nutrition during pregnancy, preparation for birth, types of birth, pregnancy exercises, breathing awareness, methods of coping with pain during pregnancy, newborn care, the importance of breast milk, breastfeeding techniques, postpartum period and family planning[8]. A study has shown that pregnancy schools are effective and that the cesarean section rates are lower than in control groups regardless of the education level, income level or employment status of the participating women[9]. In this context, pregnancy schools are considered an important tool for reaching the WHO prenatal care and education standards and the recommended cesarean section rates. In line with the "Prenatal and Postnatal Management Guide" published by the Ministry of Health, it is aimed for pregnant women to be monitored at least four times in a qualified manner and for postnatal care to be provided both in the hospital and at home. In this process, the frequency of postnatal monitoring includes three home visits after the hospital. In addition, deliveries occur in hospitals, and, if necessary, stabilized patients are referred to higher-level facilities[10]. Before and during pregnancy, women are screened in family health centers for problems such as maternal infections, thyroid diseases, gestational diabetes, preeclampsia, eclampsia, anemia, and asymptomatic bacteriuria. In addition, women are given free diphtheria-tetanus vaccine and iron, vitamin D, and folic acid supplements are provided. However, it is recommended that the diphtheria, tetanus and pertussis (Tdab) vaccine should also be offered free of charge to pregnant women. Given that pertussis infection can cause serious respiratory distress in young infants, the Tdab vaccine provides passive immunity to both mother and baby when administered between the 27th and 36th weeks of pregnancy[11]. Regular monitoring of children's growth and development from birth is of great importance in terms of taking preventive measures. In this context, the Ministry of Health's infant, child and adolescent monitoring guide provides detailed screening, examination and vaccinations specific to each age group. This guide aims to protect children's health and to raise awareness and empower families on this issue[12]. Screening examinations provide an opportunity for early diagnosis of physical, emotional, developmental and behavioral problems in children. Such interventions can reduce the burden of disease that is carried into adulthood[13]. In Türkiye, the "Regulation on Special Needs Assessment for Children" published in 2019 for children with special needs adopted a non-stigmatizing and holistic approach. The Special Needs Report for Children aims to ensure that at-risk infants and children with developmental delays benefit from early intervention services[14].

Nutrition

Starting from pre-pregnancy, during pregnancy and breastfeeding, the mother's nutritional habits have a significant impact not only on the mother's health but also on the baby's development. In recent years, it has been emphasized that these processes determine the child's long-term predisposition to health problems such as obesity, diabetes and cardiovascular disease, and that they reveal an approach called "early metabolic programming of long-term health"[15]. Therefore, it is important to correctly determine the nutritional needs of women in primary health care services and to evaluate these needs at each follow-up. It is also recommended that

micronutrient deficiencies be identified and the necessary support be provided.

WHO recommends that babies be exclusively breastfed for the first six months of their lives and that breastfeeding should continue until at least two years of age[16]. Initiating breastfeeding, especially within the first half hour after birth, increases the mother's confidence in her milk and strengthens the reflexes that support milk production and secretion. During this period, mother and baby staying together is critical for the sustainability of breastfeeding[17]. In order to increase breastfeeding rates in Türkiye, the "Breastfeeding Consultancy" and "Baby-Friendly Hospitals" programs were launched in 1991. In addition to these programs, practices such as "Mother Support Groups", "Mother to Mother Support Groups", "Baby-Friendly Province", "Golden Baby-Friendly Provinces", "Baby-Friendly Family Medicine" and "Baby-Friendly Workplace" were developed. With the increasing importance given to kangaroo care in neonatal intensive care units, "Baby-Friendly Neonatal Intensive Care Centers" were launched. It is possible to further increase breastfeeding rates with policies such as providing 1.5 hours of daily breastfeeding leave for working mothers until the child is one year old, and extending the paid leave period after birth until the child is two years old. In addition, the regulations of the "International Code of Marketing of Breast-milk Substitutes" should be fully implemented and formula advertisements should be monitored[18]. Complementary feeding should be started from the sixth month onwards, as breast milk does not meet all the nutritional needs of the baby. During this process, it is important to choose foods that are appropriate for the baby's age, prepare them safely and store them appropriately. Complementary foods should be introduced in gradual transitions such as puree, lumpy and solid consistencies. In addition, factors such as meal frequency, energy density, vitamin-mineral support and continuing breastfeeding during illness should be taken into consideration[19]. In a study evaluating the complementary feeding practices of babies aged 6-12 months, it was concluded that in order to prevent deficiencies in infant feeding, it should be done individually, practically, and in a way that will provide solutions to mothers' questions and problems, taking into account their education levels. The "Baby-Friendly Complementary Feeding" practice in Türkiye should be widespread, especially in primary health care services[20].

In order to prevent obesity, the "Türkiye Healthy Nutrition and Active Life Program (2010-2014)" was launched in cooperation with the Ministry of Health and other sectors and this program was updated between 2018-2023 as a result of the WHO evaluation. The program includes various strategies aiming at providing children with healthy eating habits and encouraging physical activity[21]. In addition, it is recommended that hyperlipidemia screenings be applied to all children in the risk group starting from the age of two[12].

Early Learning

In the early years of life, the inadequacy of environmental stimuli and the limited learning opportunities can lead to disruptions in both emotional and physical development of the child. These deficiencies can cause development delays in language, cognitive, motor, social and emotional fields in addition to forming the basis of behavioral problems[22].

Regular book reading activities with babies have positive effects on language development, social-emotional skills, early literacy skills, parent-child communication, and the quality of the home environment. Therefore, during child health monitoring, early and regular book reading recommendations should be made to families[23].

One of the most effective methods for children to learn is to use tools that support sensory perception. Especially age-appropriate, well-designed and accessible toys contribute significantly to the development process of children. Cheap but functional toys can provide more benefits than expensive but inadequately functional ones[24]. It is very important for those who care for the child to communicate with the child by playing and to turn playtimes into education and fun. Play supports the child in gaining the skills he/she will need in daily life and in developing basic rules and behavioral habits. It can also help him/her cope with stress[25]. As the famous play therapist Garry Landreth put it, "play is the child's language, and toys are his/her words".[26]

Toy libraries, which all children can benefit from regardless of socioeconomic inequalities, are resource centers that provide support, counseling, information about play, educational materials, and toys to young children and their families. These libraries operate on a toy lending system and offer materials appropriate to the development of children[27]. Expanding the existing toy libraries in Türkiye will be an important step in creating early learning opportunities especially for disadvantaged children and strengthening developmental care services[28,29].

The preschool period is a critical period in terms of the speed of brain development and the density of synaptic connections. During this period, the brain is most sensitive to environmental factors. The child's development should be supported by rich cognitive stimuli, quality language experiences and positive social-emotional interactions. A quality preschool education develops the child's sense of independence and creates a positive attitude towards learning. According to the regulations of the Ministry of National Education in Türkiye, the care and education of children aged 0-36 months is provided in kindergarten, while the education of children aged 36-72 months is provided in preschool[30]. However, in Türkiye, children from high-income families can generally

attend preschool education, while children from lower-income families, those with less educated mothers and those with many siblings directly start primary school[31]. Public policies should be developed to prevent such inequalities and ensure that disadvantaged children have equal access to educational opportunities with their peers.

Safety

Every child born in Türkiye must be reported to the birth registration office within thirty days from the date of birth. Birth notification is made with an official document. Children born in marriage are registered in the household where their father is officially registered with the father's surname, while illegitimate child is registered with the mother's maiden surname. When paternity is determined by recognition or court decision, the child is registered with the father's surname[32]. Worldwide, unsafe drinking water, poor hygiene, air pollution, infectious diseases such as diarrhea, and consumption of contaminated food are among the traditional environmental threats that negatively affect children's health. Modern risks such as industrialization, unplanned urbanization, and the accumulation of toxic chemicals in the environment, especially in low- and middle-income countries, further increase these threats[33]. In this context, all children should have access to a home and school environment where they can breathe clean air, consume safe water and food, and have hygienic practices. In addition, safe family and play areas should be established in both urban and rural areas[3].

Extraordinary situations, such as natural disasters, wars, environmental pollution or technological accidents require special measures for the care of children. In such cases, the lists of children remaining orphaned should be issued and notified to the relevant units. Safety measures can be increased by attaching arm tapes containing identity information to children. In addition, families should be informed about child safety in these cases[34].

Child maltreatment can take the form of physical, sexual and psychological violence, neglect or abuse. In most cases, such treatment is perpetrated by parents, caregivers or authority figures and occurs in the home, school or care setting. Maltreatment can have long-term negative effects on children's mental and physical health, social life and educational performance[34]. During child health monitoring, the child and family should be assessed for maltreatment.

In some cases, children may become in need of protection and care due to reasons such as death, illness, divorce or abandonment. In Türkiye, the "Social Services Law" dated 24 May 1983 and numbered 2828 provides institutional care, foster family and adoption services for children in need of protection[35].

Sensitive Care

Practices such as skin-to-skin contact between mother and baby immediately after birth and kangaroo care for low-birth-weight babies are an important part of sensitive care[3]. A systematic review has shown that skin-to-skin contact initiated early in newborns accelerates the breastfeeding process, reduces the frequency of hypothermia, and facilitates the baby's adaptation to the outside world. In addition, positive effects on the mother's health have been observed, such as shortening the third stage of labor, reducing the risk of postpartum hemorrhage and reducing pain perception. This practice strengthens the bond between mother and baby and also reduces rates of maternal stress, anxiety and depression[36].

Responsive feeding refers to a feeding style in which the parent or caregiver is sensitive to the needs of the child. While providing responsive feeding within the framework of nurturing care, the baby should be expected to stop breastfeeding himself/herself, and feeding habits should be combined with reading books from the sixth month onwards. In addition, it is important to encourage young children to feed themselves and to pay attention to hunger and satiety signals. It should not be forgotten that meal times are an opportunity for both learning and sharing love. Making eye contact with the child during this process can strengthen emotional bonds[37].

Screen time refers to the time spent on devices such as television, computers, smartphones or tablets. The WHO and the Canadian Paediatric Association do not recommend screen exposure for children under the age of two, while the American Academy of Paediatrics sets this limit at 18 months. For older children, it has been stated that screen time should be limited and age-appropriate[38,39]. Excessive screen use can lead to problems such as musculoskeletal disorders, eating disorders, vision problems, sleep disorders, anxiety and depression in children. At the same time, negative effects such as attention deficit, hyperactivity and developmental delays are also observed[40].

Even background screen exposure can have negative effects on children's language and cognitive development. This can lead to a decline in executive function skills[41]. Technoference, where technological devices interrupt communication between parent and child, also has negative effects on parenting processes[42]. In one study, mothers of children under the age of three reported that technological devices negatively affected their interactions with their children during play, reading, and mealtimes[43].

In order to reduce screen time, parents' awareness of the negative effects of screen exposure needs to be increased. In addition, activities such as reading books and playing games should be encouraged with family-based approaches. These interventions provide successful results[44]. Healthcare

professionals should counsel families about screen exposure and technoference during child health monitoring.

Footnotes

Authorship Contributions

Concept: G.G., Design: E.Y.K., Data Collection or Processing: E.Y.K., Analysis or Interpretation: G.G., Literature Search: E.Y.K., Writing: E.Y.K.

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REFERENCES

1. Early childhood development: the formative early years of a child's life demand a nurturing environment and attentive care. Available from: ///C:/Users/asus/Downloads/Formative-Years-ECD-Brochure-EN%20(2).pdf
2. Daelmans B, Manji SA, Raina N. Nurturing care for early childhood development: global perspective and guidance. Indian Pediatr. 2021;58 (Suppl 1):11-5.
3. Implementing the Nurturing care framework. Available from: https://iris.who.int/bitstream/handle/10665/371352/9789240072350-eng.pdf
4. Nurturing care for early childhood development: Nurturing Care Hand Book. Available from: https://nurturing-care.org/ncf-for-ecd
5. Deniz S. Nurturing care: a holistic approach to early childhood development. Journal of Child. 2023;23:179-84.
6. UNICEF, Countdown to 2030. Thrive nurturing care for early childhood development country profiles for early childhood development. Available from: https://nurturing-care.org/wp-content/uploads/2023/10/ECD_Countdown2030_Global.pdf
7. Thrive nurturing care for early childhood development: country profiles for early childhood development. Available from: https://iris.who.int/bitstream/handle/10665/272603/9789241514064-eng.pdf?sequence=1&isAllowed=y
8. T.C. Sağlık Bakanlığı Sağlık Hizmetleri Genel Müdürlüğü Sağlık Meslekleri Dairesi Başkanlığı. Sağlık hizmetlerinde gebe bilgilendirme sınıfı, gebe okulu, doğuma hazırlık ve danışmanlık merkezlerinin çalışma usul ve esasları. Available from: https://www.mevzuat.gov.tr/MevzuatMetin/yonetmelik/7.5.41098.pdf
9. Özceylan G, Toprak D. Effect of pregnant schools on birth types. Kocaeli Sağlık Bilimleri Dergisi. 2020;6:145-9.
10. T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. Doğum Sonu Yönetim Rehberi. Available from: https://hsgm.saglik.gov.tr/depo/Yayinlarimiz/Rehberler/DOGUM_SONU_BAKIM_08-01-2019_1.pdf
11. Halperin BA, Morris A, Mackinnon-Cameron D, Mutch J, Langley JM, McNeil SA, et al. Kinetics of the antibody response to tetanus-diphtheria-acellular pertussis vaccine in women of childbearing age and postpartum women. Clin ınfect Dis. 2011;53:885-92.
12. T.C. Sağlık Bakanlığı Türkiye Halk Sağlığı Kurumu Çocuk ve Ergen Sağlığı Dairesi Başkanlığı. Bebek, Çocuk, Ergen İzlem Protokolleri. Available from: https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Dokumanlar/Kitaplar/Bebek_Cocuk_Ergen_Izlem_Protokolleri_2018.pdf?utm_source=chatgpt.com
13. Orhon F. Taramalar; tanım, amaçlar ve genel ilkeler. Çocuk sağlığı izleminde taramalar. Türkiye Klinikleri Çocuk Sağlığı ve Hastalıkları - Özel Sayı. 2021;1:1-8.
14. Özalp Akın E, Sandıkçı İskenderli H, Atasoy SC, Yağbasan B, Keleş C, Bingöller Pekcici EB. Experence differences reported by the familes of chlidren with special needs who had disability eligibility report and report for children with spiecal needs. Ankara Eğitim ve Araştırma Hastanesi Tıp Dergisi. 2022;55:156-60.
15. Koletzko B, Godfrey KM, Poston L, Szajewska H, van Goudoever jB, de Waard M, et al. Nutrition during pregnancy, lactation and early childhood and its implications for maternal and long-term child health: the early nutrition project recommendations. Ann Nutr Metab. 2019;74:93-106.
16. WHO (World Health Organization). Global strategy on infant and young child feeding. Infant and young child nutrition, Geneva, Switzerland, 2003a, rewieved: 2009. Report No: WS 120.
17. Gür E. Lactatıonal counseling and breastfeeding problems. Çocuk Beslenmesi. Türkiye Klinikleri. 2020;1:18-22.
18. Yalçın SS. Anne sütü ile beslenmenin yaygınlaştırılması ve desteklenmesi. İlk beş yaşta çocuk sağlığı izlemi. Nobel Tıp Kitabevi. 2021;2:284-95.
19. Koç F, Akşit S. Complementary feeding in infants. Çocuk Beslenmesi. Türkiye Klinikleri. 2020;1:29-34.
20. Yoldaş İlktaç H, Hızlı Güldemir H, Garipağaoğlu M. Evaluation of breast milk nutrition and complementary feeding practices of 6-12 month-old babies. SBÜHD. 2023;5:115-22.
21. T.C. Sağlık Bakanlığı. (2013). *Türkiye Sağlık Beslenme ve Hareketli Hayat Programı Eylem Planı (2010-2014)* (Yayın No. 773). Sağlık Bakanlığı. ISBN: 978-975-590-311-8.
22. Aksoy B. Stimulus deficiency and autism in child development. Pearson Journal of Social Sciences and Humanities. 2023;8:158-63.
23. Tercanlı Metin G, Gökçay G. Reading book during infancy and early childhood: an effective recommendation for well-child care. Çocuk Dergisi. 2014;14:89-94.
24. Yazıcı S, İnce T, Aydın A. Çocuklar için oyuncak seçimi. İlk beş yaşta çocuk sağlığı izlemi. Nobel Tıp Kitabevi. 2021;2:443-9.
25. Polat B, Kara M. The effect of toys and playing on child development. Kastamonu Üniversitesi Sağlık Bilimleri Fakültesi Dergisi. 2023;2:1-10.
26. Sarıkaya N, Kaya G. A study on attitudes, perceptions and behaviors of parents towards toy choice. European Journal of Managerial Research (EUJMR). 2022;6:1-14.
27. Kırman A, Çetin Z. Studies on toy libraries for early childhood in the world and in Turkey. IntJCES. 2016;2:249-260.
28. Samancı H, Çetintaş HB. Toy libraries in the world and Turkey: samples of alipaşa and bergama toy libraries. Bilgi Dünyası. 2018;19:145-66.
29. Deniz S, Güner B, Kural B. A developmental care practice: toy library – a cross-sectional study. Türkiye Klinikleri Pediatri Dergisi. 2022;3:45-52.
30. Vatandaş N. Okul öncesi eğitim ve kreş sağlığı ilk beş yaşta çocuk sağlığı izlemi. Nobel Tıp Kitabevi. 2021;2:437-42.
31. Anne Çocuk Eğitim Vakfı. Eğitim Reformu Girişimi. Türkiye'de Erken Çocukluk Bakımı ve Okul Öncesi Eğitime Katılım. Available from: https://www.acev.org/wp-content/uploads/2018/01/Tu%CC%88rkiyede-Erken-C%CC%A7ocukluk-Bak%C4%B1m%C4%B1-ve-Okul-O%CC%88ncesi-Eg%CC%86itime-Kat%C4%B1l%C4%B1m-30.10.17.pdf
32. T.C. İçişleri Bakanlığı Nüfus ve Vatandaşlık İşleri Genel Müdürlüğü. Doğum İşlemleri/Bildirim Zorunluluğu ve Süreleri. Available from: https://www.nvi.gov.tr/dogum-islemleri/
33. Yurdakök K. Çevre kirliliği ve çocuk. İlk beş yaşta çocuk sağlığı izlemi. Nobel Tıp Kitabevi. 2021;2:181-9.
34. Responding to child maltreatment: a clinical handbook for health professionals, World Health Organization 2022. Available from: https://iris.who.int/bitstream/handle/10665/361272/9789240048737-eng.pdf?sequence=1
35. 2828 Sayılı Sosyal Hizmetler Kanunu, Sayı: 18059/ Tertip: 5/ Cilt: 22/ Sayfa:355. Available from: https://www.mevzuat.gov.tr/MevzuatMetin/1.5.2828.pdf
36. Yerlikaya A, İldan Çalım S. The effect of early skin to skin contact on mother and newborn health: a systematic review. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi. 2021;24:117-30.

37. Yılmazbaş P, Gökçay G. Healthy nutrition and ımproving the habit of healthy nutrition in early two years. Çocuk Dergisi. 2013:147-53.

38. Ponti M. Screen time and preschool children: promoting health and development in a digital world. Paediatrics & Child Health. 2023;28:184-92.

39. World Health O. Guidelines on physical activity, sedentary behaviour and sleep for children under 5 years of age. Geneva: World Health Organization. 2019.

40. Kuş G, Ulukol B. Akılcı medya kullanımı. İlk beş yaşta çocuk sağlığı izlemi. Nobel Tıp Kitabevleri. 2021;2:221-44.

41. Swider-Cios E, Vermeij A, Sitskoorn M. Young children and screen-based media: the impact on cognitive and socioemotional development and the importance of parental mediation. Cognitive Development. 2023;66:101319.

42. Akbağ M, Sayıner B. The reflections of digital technology: parental technoference and phubbing. Humanistic Perspective. 2021;3:753-78.

43. McDaniel BT, Coyne SM. Technology interference in the parenting of young children: Implications for mothers' perceptions of coparenting. The Social Science Journal. 2016;53:435-43.

44. Downing KL, Hnatiuk JA, Hinkley T, Salmon J, Hesketh KD. Interventions to reduce sedentary behaviour in 0-5-year-olds: a systematic review and meta-analysis of randomised controlled trials. Br J Sports Med. 2018;52:314-21.