ISSN 1309-9833
e-issn 1308-0865

Pamukkale Medical Journal

Pamukkale Tıp Dergisi

Vol: 18 Issue: 2 April 2025

https://youtu.be/aWfo55x0UX8

ISSN 1309-9833
e-ISSN 1308-0865

Pamukkale Medical Journal

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Vol: 18 Issue: 2 April 2025

Pamukkale Medical Journal, TR Index, Turkey Citation Index, Turkish Medline, SOBIAD, Semantic Scholar, Crossref, EBSCO, Google Scholar, WoldCat, BASE, EuroPub, İdealonline, MIAR, DRJI, Dimensions, ResearchBib, Scilit, CiteFactor, OUCI, ProQuest, Academindex, OpenAIRE/EXPLORE, CNKI Scholar, ResearchGate, ROOTINDEXING, ACARINDEX, Paperity, J-Gate, CAS, GALE Cengage Learning, SCOPUS, Journals Insights, Harvard Library, Scinapse, ASCI, ZENDY and OpenAlex it is indexed by.

Name of the Journal: Pamukkale Medical Journal
Web Address: https://dergipark.org.tr/tr/pub/patd
Publication Type: Periodical
Publishing Period: 4 Issues per Year
ISSN: 1309-9833 **e-ISSN:** 1308-0865

Address: Pamukkale Medical Journal, Pamukkale University Faculty of Medicine Dean's Office, Yunusemre Street, no: 3/F, Kınıklı, 200070 Pamukkale, Denizli.
E-mail: tipdergisi@pau.edu.tr
Tel: +902582961619
Fax: +902582961765

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Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1510589

Evaluation of quality of life, health belief model and insomnia in obese and overweight individuals: a cross-sectional study

Obez ve kilolu bireylerde yaşam kalitesi, sağlık inanç modeli ve uykusuzluğun değerlendirilmesi: kesitsel bir çalışma

Selin Davun, Mehmet Akif Sezerol

Posted date:08.07.2024 Acceptance date:01.10.2024

Abstract

Purpose: This study was conducted to evaluate the quality of life, insomnia severity, and approaches according to the health belief model of obese and overweight individuals who applied to the dietician polyclinic of the district health directorate in a district of Istanbul.

Material and methods: This cross-sectional study was conducted between September 2022 and March 2023 among 300 obese and overweight individuals aged above 18 who applied to the dietician polyclinic of the district health directorate in a district of Istanbul. The survey used the insomnia severity index, quality of life short form, and health belief model scale. To compare variables, the Chi-Square test, Mann Whitney U, and Kruskal Wallis tests were used for statistical analysis of the data. $p<0.05$ was considered statistically significant.

Results: 97.3% of the participants in the research were female and 2.7% were male. The mean scores of those who were overweight on the quality of life scale were found to be significantly higher than those of the obese ($p<0.001$). A significant difference was found between the obese and overweight in terms of insomnia severity index scores ($p=0.003$). Also, a significant correlation was found between the perceived benefit subscale and the perceived seriousness, sensitivity, importance of health sub-dimensions, and quality of life scale scores.

Conclusions: This study demonstrated the health beliefs, quality of life, and insomnia levels of obese and overweight individuals. Qualitative studies to be conducted should address the health belief model in more detail by using the data of this study.

Keywords: Obese, overweight, quality of life, insomnia.

Davun S, Sezerol MA. Evaluation of quality of life, health belief model and insomnia in obese and overweight individuals: a cross-sectional study. Pam Med J 2025;18:232-243.

Öz

Amaç: Bu çalışma, İstanbul'un bir ilçesinde ilçe sağlık müdürlüğüne bağlı diyetisyen polikliniğine başvuran obez ve fazla kilolu bireylerin yaşam kalitesi, uykusuzluk şiddeti ve sağlık inanç modeli yaklaşımlarını değerlendirmek amacıyla yapılmıştır.

Gereç ve yöntem: Bu kesitsel çalışma, Eylül 2022 ile Mart 2023 tarihleri arasında, İstanbul'un bir ilçesinde ilçe sağlık müdürlüğüne bağlı diyetisyen polikliniğine başvuran 18 yaş üstü 300 obez ve fazla kilolu birey arasında gerçekleştirilmiştir. Anket kapsamında uykusuzluk şiddeti indeksi, yaşam kalitesi kısa formu ve sağlık inanç modeli ölçeği kullanılmıştır. Değişkenleri karşılaştırmak için Ki-Kare testi, Mann Whitney U ve Kruskal Wallis testleri kullanılmış olup, $p<0,05$ istatistiksel olarak anlamlı kabul edilmiştir.

Bulgular: Araştırmaya katılanların %97,3'ü kadın ve %2,7'si erkekti. Yaşam kalitesi ölçeğinden alınan puanların ortalamaları fazla kilolu bireylerde obez bireylerden anlamlı derecede yüksek bulunmuştur ($p<0,001$). Obez ve fazla kilolu bireyler arasında uykusuzluk şiddeti indeksi açısından anlamlı bir fark bulunmuştur ($p=0,003$). Ayrıca, algılanan yarar alt ölçeği ile algılanan ciddiyet, duyarlılık, sağlık önemi alt boyutları ve yaşam kalitesi ölçeği puanları arasında anlamlı bir korelasyon bulunmuştur.

Sonuç: Bu çalışma, obez ve fazla kilolu bireylerin sağlık inançlarını, yaşam kalitelerini ve uykusuzluk seviyelerini ortaya koymuştur. Gelecekte yapılacak niteliksel çalışmalar, bu çalışmanın verilerini kullanarak sağlık inanç modelini daha detaylı bir şekilde ele almalıdır.

Anahtar kelimeler: Obez, fazla kilolu, yaşam kalitesi, uykusuzluk.

Davun S, Sezerol MA. Obez ve kilolu bireylerde yaşam kalitesi, sağlık inanç modeli ve uykusuzluğun değerlendirilmesi: kesitsel bir çalışma. Pam Tıp Derg 2025;18:232-243.

Selin Davun, M.D. Department of Public Health, Department of Public Health, Istanbul Medipol University, Istanbul, Türkiye, e-mail: slnblc@hotmail.com (https://orcid.org/0000-0001-5765-7757) (Corresponding Author)

Mehmet Akif Sezerol, M.D. Department of Public Health, School of Medicine, Istanbul Medipol University, Istanbul, Türkiye, e-mail: masezerol@gmail.com (https://orcid.org/0000-0001-6744-1343)

Introduction

Obesity and overweight are health problems that indicate excessive and abnormal accumulation of body fat, leading to adverse health effects [1]. Globally, the prevalence of obesity and overweight among adults has been increasing over the past few decades. According to the World Health Organization, the prevalence of obesity nearly tripled worldwide between 1975 and 2016. In 2016, 1.9 billion adults were overweight, and 650 million of them were obese [2]. Worldwide, the prevalence of obesity has increased dramatically during the last four decades, and if this trend continues, a majority of the world's adult population will be either overweight or obese by 2030 [3].

Obesity and overweight are serious public health problems that are increasing in prevalence in Türkiye and all over the world. Türkiye has the highest obesity rates among European countries. According to the WHO European Regional Obesity Report 2022, the age-standardized obesity rate in adults in Türkiye is 32.1%, while the overweight rate is 66.8% [4]. According to the Türkiye Health Survey 2019 data, the obesity rate among individuals aged 15 and over was 19.6% in 2016 and increased to 21.1% in 2019. In terms of gender, in 2019, 24.8% of females were obese and 30.4% were pre-obese, while 17.3% of men were obese and 39.7% were pre-obese [5].

Studies have shown that obesity and overweight are public health problems, as they are risk factors for many other health issues. In addition to the increased risk of death associated with excess body fat, obesity and overweight increase the risk of developing various diseases, such as obstructive sleep apnea, type II diabetes mellitus, coronary heart disease, and certain cancers [6-8]. Apart from their physical effects, obesity and overweight can also cause psychological problems such as depression, stress, anxiety, and insomnia, which are as significant as physical problems [9, 10]. Therefore, it has become increasingly clear that obesity-related issues do not simply cause or aggravate medical conditions [11]. Moreover, all factors affecting a person's private, professional, and daily life will directly impact their quality of life. Studies show that being obese or overweight has a significant impact on a person's functional capacity and quality of life [12, 13].

It is also believed to be linked to the psychological effects of increased obesity and the impact of sleep loss on hormones such as leptin and ghrelin, which play a crucial role in the central regulation of appetite and energy expenditure [14, 16]. The significant decrease in average sleep duration over the last 50 years, which aligns with the rise in obesity rates in the population, suggests that recurrent partial sleep deprivation could have significant implications for public health due to the adverse effects observed in metabolic and hormonal processes [14].

Studies have shown that being obese or overweight reduces quality of life and increases the severity of insomnia. However, few studies have evaluated these effects together with the health belief model. This study examines individuals' levels of insomnia according to the Health Belief Model and their quality of life, as well as the impact of sociodemographic factors such as marital status, gender, and education level on quality of life.

To investigate the relationships between these factors, this study was conducted to evaluate the quality of life, insomnia severity, and approaches according to the health belief model of obese and overweight individuals who applied to the dietician polyclinic of a district health directorate in a district of Istanbul.

Material and methods

Type of research

This is a descriptive and cross-sectional study that has used a *face-to-face* survey design.

Study population

This study was conducted between September 2022 and March 2023. When the daily dietitian application was calculated as 18 individuals, the universe of this study was determined as 1440 adults aged above 18 years. The sample size for the study was calculated using the OpenEpi website [15]. When the sample size was calculated with a 50% anticipated frequency and a 5% margin of error, it was aimed to reach 304 individuals. In this study, the convenience sampling method, one of the non-probability sampling techniques, was used for sample selection.

The study was completed with 300 individuals. Inclusion criteria were to be over 18 years old, obese, overweight, and not having any disease that could cause weight gain.

Measuring tools

Data collection was carried out face-to-face. A survey form consisting of 91 questions in total was directed to the participants. In the first part of the questionnaire, there are questions about sociodemographic characteristics, whether there is a chronic or psychiatric disease, height, weight, fat measurements, and the number of applications to a dietitian. The BMI was calculated using the standard formula: weight in kilograms divided by the square of height in meters (kg/m²). According to World Health Organization (WHO) guidelines, individuals were classified as follows: Overweight: BMI 25-29.9 kg/m², Obesity: BMI ≥30 kg/m². Weight was measured using a 'Tanita BC-418MA' scale. The scale was calibrated before each session to ensure accuracy.

Height was measured using a standard stadiometer (ADE Assembly Note Column Scale). Participants were asked to stand upright without shoes, with their backs against the stadiometer, and their heads in the Frankfort plane position.

All measurements were conducted by a single dietitian to maintain consistency across the data collection process.

In the second part, there is a 32-item health belief model scale, the Turkish validity and reliability of which was developed by Dedeli et al. (2011) [17].

The health belief model scale

This scale consists of a total of 32 items, each scored on a 5-point Likert scale. The first 7 items are rated as follows: 1: Never, 2: Sometimes, 3: Often, 4: Very Often, 5: Always. The subsequent 25 items are rated as follows: 1: Strongly Disagree, 2: Disagree, 3: Neutral, 4: Agree, 5: Strongly Agree. The 16th item is reverse-coded. The Obesity Health Belief Model Scale does not calculate an overall score. Each subscale is calculated by summing the item scores within the subscale and dividing by the number of items in that subscale. It consists of the importance of health, the perception of seriousness, the perception of sensitivity, the perception of obstacles, and the perception of benefit. The averages of the scores obtained by the individuals from each sub-dimension are given, and it is interpreted that the one with the higher average has more perception of that sub-dimension. The importance of health consists of 8 items; it shows the level of importance that individuals give to their health. Perceived severity evaluates to what extent individuals see obesity as a serious disease and consists of 4 items (9-11, 17). Perceived sensitivity evaluates the extent to which individuals find themselves prone to complications and health problems that may develop due to obesity and to what extent they find the interventions related to obesity useful and consists of 4 items (12-14, 16). Perceived benefit shows the level of awareness of the benefits that individuals will gain in terms of their health when they can manage obesity and consists of 8 items (21, 22, 24-28, 32). The detected obstacle shows the level of barriers that individuals perceive in applying health recommendations for obesity and consists of 8 items (15, 18, 19, 20, 23, 29-31). The Cronbach alpha coefficient of the scale is 0.80. In this study, it was found to be 0.72.

In the third part, the Quality of Life Scale Short Form was used.

Quality of life scale short form

This scale consists of 34 questions. It was validated and found to be reliable in Turkish by Yagma et al. [18]. A 5-point Likert scale (1-5) is used for each question; with each item rated as follows: 1: always/enormously; 2: often/a lot; 3: sometimes/ moderately; 4: rarely/a little; 5: never/not at all. A score was then calculated for each dimension by adding together its constituent items. The total scores for each dimension were derived by adding the responses (graded from 1 to 5) for all items within that dimension. These scores were then transformed to reflect the range of possible outcomes. The lowest possible score on the quality of life scale is 34 points, and the highest possible score is 170 points. Higher scores indicate a better quality of life. The Cronbach alpha coefficient of the scale in the original validation study is 0.80. In this study, the Cronbach's alpha coefficient was calculated to be 0.70.

In the fourth part, the Insomnia Severity Index was used.

Insomnia severity index

The 7-item Insomnia Severity Index, whose validity and reliability were established by Boysan et al. (2010) [19], was used. The survey is a 5-point Likert scale, rated as follows: '0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Very Severe'. In the Insomnia Severity Index, higher scores indicate a deterioration in sleep quality. If the total score is between 0 and 7, there is no clinical insomnia; a score of 8–14 is considered the lower threshold for insomnia, 15–21 indicates moderate insomnia, and 22–28 represents severe insomnia. The Cronbach alpha coefficient of the scale is 0.79. In this study, it was found to be 0.71.

Permission was obtained from the Istanbul Medipol University Clinical Research Ethics Committee for the study (permission date: 26.08.2022, and permission number 746). Before commencing the research, permission to use each scale was obtained from the authors responsible for the scales.

Statistical analysis

Descriptive data are presented as standard deviation values, means, and frequency tables. The chi-square test was used for statistical analysis of the data to compare variables. The normal distribution of variables was examined using histogram and Kolmogorov-Smirnov/ Shapiro-Wilk tests. Independent samples were compared with the t-test for continuous variables with parametric distribution, and those without normal distribution were compared with the Mann-Whitney U and Kruskal-Wallis tests. Relationships between non-normally distributed continuous variables were evaluated with the Spearman correlation test. The SPSS Statistics 20.0 (Armonk, New York: IBM Corp.) statistical program trial version was used. $p<0.05$ was considered statistically significant.

Results

This study was completed with a total of 300 individuals. 97.3% of the participants in the research were female and 2.7% were male. All of the participants are over the age of 18, and their average age is 38.00±8.96. 28% of the participants were overweight (BMI 25.0 kg/m^2-29.9 kg/m^2), and 72% were obese (BMI>30.0 kg/m^2). 53.3% of the participants had previously consulted a dietitian from another institution. 41% of the participants applied to our institution for the first time, 26.7% for the second time, 9.6% for the third time, and 22.7% for the fourth or more times. 44% of the participants stated that they had any chronic disease, and the most frequently mentioned chronic disease was diabetes mellitus.

The sociodemographic characteristics of the participants in the study were examined according to the insomnia severity index and are shown in detail in Table 1. Accordingly, it was determined that obese people had significantly more severe insomnia symptoms than overweight people (p=0.003), and those with chronic diseases had significantly more severe insomnia symptoms than those without chronic diseases (p=0.034).

In Table 2, the sociodemographic characteristics of the participants and the scores they got from the quality of life scale were compared. The participants' quality of life scale average scores were 128.61±24.71, with a minimum of 36 points and a maximum of 170 points. When quality of life scores were evaluated according to gender, males' quality of life scores were higher than females, and the difference was not significant (p=0.560). The mean scores of those who were overweight on the quality of life scale were found to be significantly higher than those of the obese (p=0.000047). There was a significant difference between the patients with chronic (p=0.022) or psychiatric diseases and those with lower quality of life scale scores (p=0.012).

When the quality of life of the participants was evaluated according to the severity of insomnia in Table 3, the scores of the participants with moderate and severe insomnia were found to be significantly lower (p=0.000103).

The comparison of the scores they got from the health belief model subscales according to their sociodemographic characteristics is shown in Table 4 in detail. The difference between the scores of the participants' perceived barriers (p=0.002) and benefits sub-scales according to their educational status was found to be significant (p=0.011). The presence of obesity in first-degree relatives and the score they got from the perceived disability subscale

were also found to be significant (p=0.008). A significant difference was found between the obese and overweight in terms of perceived benefit scores (p=0.029). A comparison of the insomnia severity of the participants according to the sub-dimensions of the belief model in health is shown in Table 5 in detail. There was a significant difference between insomnia severity scores and both the importance of the health subscale (p=0.007) and the perceived barriers subscale (p=0.014).

The sub-dimensions of the health belief model, the scores obtained from the short form of the quality of life scale, and the correlation dimensions between the age variable were evaluated (Table 6). Accordingly, a significant correlation was found between the perceived benefit subscale and the perceived seriousness, sensitivity, importance of health sub-dimensions, and quality of life scale scores. While there was a positive correlation between the perceived benefit subscale and the other subscales of the scale, a negative correlation was observed with the quality of life. Perceived disability and perceived susceptibility subscales also showed a significant negative correlation with the quality of life scale. A significant positive correlation was observed between the age variable and the importance of the health subscale.

Table 1. Characteristics of participants according to the Insomnia Severity Index

		Insomnia Severity Index								
		Clinically insignificant		Insomnia threshold		Moderate insomnia		Severe insomnia		*p* value
		n	%	n	%	n	%	n	%	
Gender	Female	100	38.2	111	42.4	40	15.3	11	4.2	0.873 (cs=0.701)
	Male	4	50.0	3	37.5	1	12.5	0	0.0	
Education	Illiterate	1	25.0	1	25.0	1	25.0	1	25.0	0.109 (cs=18.211)
	Literate	1	9.1	6	54.5	4	36.4	0	0.0	
	Primary Education	55	39.0	57	40.4	22	15.6	7	5.0	
	Secondary Education	36	46.2	29	37.2	11	14.1	2	2.6	
	University	11	30.6	21	58.3	3	8.3	1	2.8	
Marital Status	Married[1]	95	38.9	100	41.0	38	15.6	11	4.5	0.039 ([1-3])* (cs=13.249)
	Single[2]	8	44.4	10	55.6	0	0.0	0	0.0	
	Divorced[3]	0	0.0	2	40.0	3	60.0	0	0.0	
Psychiatric disease	Yes	3	17.6	9	52.9	4	23.5	1	5.9	0.355 (cs=3.249)
	No	96	39.0	103	41.9	37	15.0	10	4.1	
Chronic Disease	Yes	35	31.0	47	41.6	23	20.4	8	7.1	0.034* (cs=8.683)
	No	60	42.0	63	44.1	17	11.9	3	2.1	
BMI	Overweight	42	51.2	34	41.5	4	4.9	2	2.4	0.003* (cs=13.960)
	Obese	62	33.0	80	42.6	37	19.7	9	4.8	

* p<0.05, cs: Chi-Square Test [1-3] Variables with significant differences

Table 2. Comparison of the sociodemographic characteristics of the participants according to the short form of the quality of life scale

		Quality of Life Scale Short Form				*p* value
		Mean	Standard deviation	Minimum	Maximum	
Gender	Female	128.36	25.05	36.00	170.00	0.560 (z=-0.58)
	Male	135.67	11.78	124.00	151.00	
Education	Illiterate	129.00	.	129.00	129.00	0.348 (h=4.29)
	Literate	115.71	30.01	68.00	148.00	
	Primary Education	130.36	23.85	64.00	170.00	
	Secondary Education	125.34	26.57	36.00	170.00	
	University	134.92	20.17	73.00	163.00	
Marital Status	Married	128.86	24.77	36.00	170.00	0.419 (h=1.74)
	Single	135.00	7.87	126.00	146.00	
	Divorced	106.00	39.34	64.00	142.00	
Have a child	Yes	128.57	24.85	36.00	170.00	0.836 (z=-0.20)
	No	126.17	24.39	64.00	150.00	
Obesity in first degree relatives	Yes	122.47	26.16	63.00	169.00	0.028* (z=-2.20)
	No	131.64	23.77	36.00	170.00	
Applying to a dietitian at another institution before	Yes	122.36	25.69	36.00	170.00	0.00051* (z=-3.26)
	No	134.93	22.69	63.00	170.00	
Psychiatric Disease	Yes	113.54	26.01	63.00	169.00	0.012* (z=-2.50)
	No	130.31	24.07	36.00	170.00	
Chronic Disease	Yes	124.45	22.47	64.00	169.00	0.022* (z=-2.29)
	No	131.39	26.28	36.00	170.00	
Use of any psychiatric drugs	Yes	108.50	30.85	63.00	169.00	0.021* (z=-2.30)
	No	129.67	24.24	36.00	170.00	
BMI	Overweight	138.24	24.90	36.00	170.00	0.00047* (z=-4.07)
	Obese	124.02	23.37	63.00	165.00	

* $p<0.05$, z: Mann Whitney U, h: Kruskal-Wallis H

Table 3. Comparison of the insomnia severity of the participants according to the short form of the quality of life scale

		Quality of Life Scale Short Form				*p* value[a]
		Mean	Standard deviation	Minimum	Maximum	
Insomnia Severity Index	Clinically insignificant[1]	137.48	21.76	36.00	170.00	0.000103 ([1-4])* (h=21.04)
	Insomnia threshold[2]	126.49	23.74	63.00	170.00	
	Moderate insomnia[3]	114.20	23.78	64.00	155.00	
	Severe insomnia[4]	115.71	34.48	70.00	155.00	

*$p<0.05$, h: Kruskal-Wallis H [1-4] Variables with significant differences

Table 4. Comparison of the sociodemographic characteristics of the participants according to the sub-dimensions of the belief model in health

		Importance of health		Perceived severity		Perceived sensitivity		Perceived barriers		Perceived benefits	
		Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
Gender	Female	2.98	0.76	4.75	0.69	4.18	0.94	2.22	0.91	4.67	0.64
	Male	3.11	0.92	4.78	0.41	4.31	0.58	1.56	0.41	4.70	0.33
***p* value**		0.685 (z=-0.40)		0.746 (z=-0.32)		0.865 (z=-0.17)		0.077 (z=-1.77)		0.448 (z=-0.75)	
Education	Illiterate[1]	3.13	1.02	4.67	0.58	4.44	0.55	3.04	0.69	4.50	0.38
	Literate[2]	3.21	0.94	4.92	0.21	4.48	0.73	3.19	0.95	4.74	0.39
	Primary Education[3]	3.05	0.79	4.65	0.86	4.23	0.98	2.23	0.96	4.68	0.67
	Secondary Education[4]	2.79	0.64	4.86	0.44	4.19	0.85	2.12	0.76	4.74	0.54
	University[5]	3.03	0.76	4.91	0.24	3.89	0.96	1.84	0.74	4.49	0.73
***p* value**		0.162 (h=6.46)		0.332 (h=4.59)		0.097 (h=7.85)		0.002 ([1-3])* (h=17.40)		0.011 ([2-4])* (h=12.98)	
Marital Status	Married	3.00	0.75	4.74	0.71	4.20	0.93	2.24	0.92	4.67	0.65
	Single	2.73	0.92	4.86	0.23	3.94	1.03	1.73	0.44	4.66	0.35
	Divorced	2.56	0.22	5.00	0.00	4.45	0.67	1.94	0.77	4.95	0.11
***p* value**		0.322 (h=2.26)		0.364 (h=2.02)		0.488 (h=1.43)		0.129 (h=4.09)		0.098 (h=4.65)	
Obesity in first degree relatives	Yes	2.97	0.75	4.77	0.69	4.24	0.89	2.39	0.88	4.67	0.54
	No	2.98	0.77	4.74	0.69	4.16	0.94	2.09	0.91	4.67	0.69
***p* value**		0.847 (z=-0.19)		0.301 (z=-1.03)		0.580 (z=-0.55)		0.008* (z=-2.63)		0.376 (z=-0.88)	
BMI	Overweight	2.99	0.83	4.66	0.86	4.10	1.04	2.11	0.91	4.52	0.82
	Obese	2.98	0.73	4.79	0.60	4.22	0.89	2.23	0.90	4.73	0.53
***p* value**		0.782 (z=-0.27)		0.465 (z=-0.73)		0.661 (z=-0.43)		0.295 (z=-1.04)		0.029* (z=-2.18)	

*$p<0.05$, z: Mann Whitney U h: Kruskal-Wallis H [1-3, 2-4] Variables with significant differences

Table 5. Comparison of the insomnia severity of the participants according to the sub-dimensions of the belief model in health

		Importance of health		Perceived severity		Perceived sensitivity		Perceived barriers		Perceived benefits	
		Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
Insomnia Severity Index	Clinically insignificant[1]	3.18	0.72	4.84	0.46	4.11	0.93	2.05	0.84	4.69	0.49
	Insomnia threshold[2]	2.83	0.71	4.74	0.66	4.15	0.97	2.12	0.83	4.64	0.72
	Moderate insomnia[3]	2.94	0.76	4.80	0.58	4.36	0.78	2.63	0.93	4.73	0.56
	Severe insomnia[4]	2.90	0.96	4.70	0.50	4.39	0.50	2.35	1.04	4.83	0.31
***p* value**		0.007 ([1-2])* (h=12.04)		0.375 (h=3.11)		0.609 (h=1.82)		0.014 ([3-4])* (h=10.63)		0.448 (h=2.65)	

* $p<0.05$, h: Kruskal-Wallis H [1-2, 3-4] Variables with significant differences

Table 6. Correlations between sub-dimensions of belief model in health, quality of life scores, and age

		Quality of Life Scale Short Form	Age
Perceived benefits	Spearman Correlation	-0.211**	0.028
	p value	0.006	0.656
Perceived barriers	Spearman Correlation	-0.289**	0.063
	p value	0.0001	0.347
Perceived severity	Spearman Correlation	-0.148	-0.024
	p value	0.061	0.702
Perceived sensitivity	Spearman Correlation	-0.172*	0.078
	p value	0.032	0.217
Importance of health	Spearman Correlation	-0.095	0.171**
	p value	0.232	0.006

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed)

Discussion

The participants' views on obesity, their quality of life, and their insomnia indices were assessed in this study of overweight and obese people. Ninety-seven point three percent (97.3%) of the individuals in this study who made dietetic applications were females. WHO statistics from 2016 showed that 39.2% of obese people in Türkiye who were 18 years of age or older were female and 24.4% were male; Turkish Statistical Institute (TUIK) data from 2019 showed that 24.8% of obese people who were 15 years of age or older were female and 17.3% were male [2, 5]. The higher incidence of obesity in females can be attributed to several factors, such as living a more stressful lifestyle than men, engaging in less physical activity, and experiencing hormonal or obstetric issues unique to females.

After assessing the participants' degree of insomnia, it was shown that those who were obese had more severe insomnia than those who were overweight. Furthermore, it has been observed that moderate to severe symptoms of sleeplessness are more common among obese or overweight people with chronic illnesses. Greater primary sleep disruptions and daytime tiredness were similarly linked to higher body mass, according to a study by Duraccio et al. [20] involving 1.133 participants. Muhammad et al. [1] did a cross-sectional study on 31.358 older persons and discovered that widows and singles experienced more severe insomnia than married people. This study demonstrated a strong correlation between the severity of insomnia and marital status, with divorced people reporting more mild insomnia. In the study they conducted, Vorona et al. [21] discovered a strong correlation between obesity and sleeplessness. Logue et al. [22] found in another study that obese patients had lower sleep duration and lower quality sleep. Additionally, Buscemi et al. [23] observed that among a sample of 200 obese patients with chronic illnesses, there was a strong correlation between obesity and shorter sleep durations.

The short form of the quality of life scale was used to evaluate participants' obese and overweight participants' quality of life. It was shown that those who were obese had poorer quality of life scores than those who were overweight. A substantial inverse association between obesity and quality of life was discovered in Jia et al. [24], which is consistent with the findings of this study. They also stated that the quality of life is low in those who are overweight, but the quality of life decreases as the BMI level increases, and the quality of life increases with weight loss. In a study conducted by Sach et al. [25] with a sample of 1865 individuals, it was stated that the quality of life of obese individuals was lower than those of normal weight and that their obesity-related chronic diseases may also be effective. In this study, the quality of life of obese and overweight individuals with comorbidities such as chronic illnesses and psychiatric disorders was found to be significantly lower than those who do not have any psychiatric or chronic diseases. In a cohort study involving 64.631 individuals that evaluated the relationship between obesity and quality of life, it was found that obese and overweight individuals had lower quality of life compared to those with normal weight. Additionally, those with three or more mental or physical illnesses were also associated with lower quality of life. The findings of this study, which have a higher reliability than ours, support our findings [26]. In Thommasen et al. [27], it was concluded that the presence of chronic disease is the strongest determinant among the health-related parameters affecting quality of life. In this study, quality of life was found to be significantly lower in individuals with more severe levels of insomnia. Similarly, a study conducted in São Paulo that evaluated the relationship between insomnia and quality of life also found that the severity of insomnia had a significant impact on quality of life [28].

It was found in this study that individuals who had applied to dietitians at various institutions had a lower quality of life. This might be the result of situations brought on by their despondency, the fact that these people apply to more institutions and do not receive the answers they want, or a combination of psychological or genetic diseases. The scores of the participants from the health belief model scale in obesity are not calculated as the total score, and the scores of the sub-dimensions were evaluated separately. Accordingly, the participants got the highest score from the perceived seriousness sub-dimension and the lowest score from the perceived obstacle sub-dimension. According to the obesity health belief model scale; the increase in the individual's perception of seriousness is related to their awareness of the seriousness of the situation and its consequences. Individuals with a high perception of seriousness towards a health problem are more likely to engage in health-promoting behavior because they feel prone to contracting the disease. In the studies, it was determined that the most effective determinants of behavior change were Perception of Benefit and Perception of Obstacles, while the least effective determinants were Perception of Severity, and it was stated that the effect of the perceived obstacle should be reduced and the effect of other sub-dimensions should be increased for the realization of the health behavior related to the disease [29, 30]. Barakat et al. [31] stated in a study carried out that the increase in perceived benefit also improves preventive health behaviors.

In this study, when the relationships between the sub-dimensions of the health belief model scale and the variables were examined, a significant relationship was found between the education level of the individuals and the perceived barrier and benefit sub-dimensions. As the level of education increases, the perceived disability score decreases, while the perceived benefit score increases. Kahraman et al. [32] stated in their study that there was a significant difference between education level and perceived disability dimensions. Darvishpour et al. [33] on the other hand, it is necessary to develop educational interventions to improve health behaviors, and in this way, perceived benefits should be understood, and barriers to health behaviors should be removed. When obese and overweight individuals were compared according to the health belief model, it was determined that the perceived benefit scores of obese individuals were significantly higher. This may be related to the awareness that obese individuals will gain more benefits from losing weight than overweight individuals. However, no significant difference was observed in terms of perceived severity between obese and overweight individuals. A study conducted in Iran with students reported that overweight students scored lower on perceived severity compared to those with normal weight. The lack of a significant difference in our study may be because we did not compare these findings with normal-weight individuals [34].

There are also some limitations of the research. First, the use of a non-probability sampling method and the fact that patients were recruited from a single center limit the generalizability of the study results. Additionally, as a cross-sectional study, it has the disadvantage of not being able to establish causality. Secondly, the length of the questionnaire was reflected in the answers of the participants and caused reluctance to answer some of the questions, resulting in missing data. Thirdly, the fact that the research was conducted in a low socioeconomic region caused the participants to have difficulty understanding and answering the questions, and it may tend to show differently.

Despite these, the research is a powerful one, as it has not been done before in the region. In addition, in the literature, these scales have been evaluated separately in obese individuals, and studies in which all of them are evaluated together are limited.

Accordingly, it was found that the level of insomnia was more severe, and the quality of life was lower in obese individuals than in overweight individuals. According to the health belief model, it was determined that obese individuals scored higher on the perceived benefit subscale than those who were overweight. In addition, the relationship between education level and perceived disability and benefit has been revealed. Qualitative studies to be conducted should address the health belief model in more detail by using the data of this study. In addition, these individuals continue their continue their follow-up consultations with dietitians, and changes after weight loss should be recorded, or prospective studies should be planned

Funding: No funding was received for this study.

Authors contributions: M.A.S. and S.D. constructed the main idea and hypothesis of the study. M.A.S. and S.D. developed the theory and arranged/edited the material and method section. S.D. has evaluated the data in the Results section. Discussion section of the article was written by S.D. M.A.S. reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Muhammad T, Gharge S, Meher T. The associations of BMI, chronic conditions and lifestyle factors with insomnia symptoms among older adults in India. *PLoS One*. 2022;17(9):e0274684. Published 2022 Sep 15. doi:10.1371/journal.pone.0274684
2. World Health Organization 2019; Available at: https://www.who.int/news-room/fact- sheets/detail/obesity-and-overweight#:~:text=%2Fm2).,Adults,than%20or%20equal%20to%2030. Accessed March 29, 2023
3. Haththotuwa RN, Wijeyaratne CN, Senarath U. Worldwide epidemic of obesity. Obesity and obstetrics. Elsevier 2020;3-8. https://doi.org/10.1016/B978-0-12-817921-5.00001-1
4. World Health Organization Report. Available at: https://apps.who.int/iris/bitstream/handle/10665/353747/9789289057738-eng.pdf. Accessed March 29, 2023

5. Türkiye Sağlık Araştırmaları, 2019. Available at: https://data.tuik.gov.tr/Bulten/Index?p=Turkey-Health-Survey-2019-33661. Accessed March 29, 2023
6. Chatterjee A, Gerdes MW, Martinez SG. Identification of Risk Factors Associated with Obesity and Overweight-A Machine Learning Overview. *Sensors (Basel)*. 2020;20(9):2734. Published 2020 May 11. doi:10.3390/s20092734
7. Sarma S, Sockalingam S, Dash S. Obesity as a multisystem disease: Trends in obesity rates and obesity-related complications. *Diabetes Obes Metab*. 2021;23(Suppl 1):3-16. doi:10.1111/dom.14290
8. Waddell IS, Orfila C. Dietary fiber in the prevention of obesity and obesity-related chronic diseases: From epidemiological evidence to potential molecular mechanisms. *Crit Rev Food Sci Nutr*. 2023;63(27):8752-8767. doi:10.1080/10408398.2022.2061909
9. Vogelzangs N, Kritchevsky SB, Beekman AT, et al. Obesity and onset of significant depressive symptoms: results from a prospective community-based cohort study of older men and women. *J Clin Psychiatry*. 2010;71(4):391-399. doi:10.4088/JCP.08m04743blu
10. Simon GE, Von Korff M, Saunders K, et al. Association between obesity and psychiatric disorders in the US adult population. *Arch Gen Psychiatry*. 2006;63(7):824-830. doi:10.1001/archpsyc.63.7.824
11. Hecker J, Freijer K, Hiligsmann M, Evers SMAA. Burden of disease study of overweight and obesity; the societal impact in terms of cost-of-illness and health-related quality of life. *BMC Public Health*. 2022;22(1):46. Published 2022 Jan 7. doi:10.1186/s12889-021-12449-2
12. Stephenson J, Smith CM, Kearns B, Haywood A, Bissell P. The association between obesity and quality of life: a retrospective analysis of a large-scale population-based cohort study. *BMC Public Health*. 2021;21(1):1990. Published 2021 Nov 3. doi:10.1186/s12889-021-12009-8
13. Busutil R, Espallardo O, Torres A, Martínez-Galdeano L, Zozaya N, Hidalgo-Vega Á. The impact of obesity on health-related quality of life in Spain. *Health Qual Life Outcomes*. 2017;15(1):197. Published 2017 Oct 10. doi:10.1186/s12955-017-0773-y
14. Van Cauter E, Spiegel K, Tasali E, Leproult R. Metabolic consequences of sleep and sleep loss. *Sleep Med*. 2008;9 Suppl 1(0 1):S23-S28. doi:10.1016/S1389-9457(08)70013-3
15. OpenEpi Sample Size. Available a t : https://www.openepi.com/SampleSize/SSPropor.htm. Accessed August 14, 2022
16. Rahe C, Czira ME, Teismann H, Berger K. Associations between poor sleep quality and different measures of obesity. *Sleep Med*. 2015;16(10):1225-1228. doi:10.1016/j.sleep.2015.05.023
17. Dedeli Ö, Fadıloğlu Ç. Obezitede sağlik inanç modeli ölçeği'nin geliştirilmesi. TSK Koruyucu Hekimlik Bülteni 2011;10:533-542. doi:10.5455/pmb.20110118022318
18. Yağma NM, Ünal E, Gökler ME, Mollahaliloğlu S. Cross-cultural adaptation, reliability, and validity of the Turkish version of the obesity-specific quality of life questionnaire: quality of life, obesity, and dietetics (QOLOD) rating scale. *Turk J Med Sci*. 2021;51(5):2369-2376. Published 2021 Oct 21. doi:10.3906/sag-2005-412
19. Boysan M, Güleç M, Besiroğlu L, Kalafat T. Uykusuzluk Şiddeti Indeksi'nin Türk örneklemindeki psikometrik özellikleri. Anadolu Psikiyatri Dergisi 2010;11:248-252.
20. Duraccio KM, Simmons DM, Beebe DW, Byars KC. Relationship of overweight and obesity to insomnia severity, sleep quality, and insomnia improvement in a clinically referred pediatric sample. *J Clin Sleep Med*. 2022;18(4):1083-1091. doi:10.5664/jcsm.9806
21. Vorona RD, Winn MP, Babineau TW, Eng BP, Feldman HR, Ware JC. Overweight and obese patients in a primary care population report less sleep than patients with a normal body mass index. *Arch Intern Med*. 2005;165(1):25-30. doi:10.1001/archinte.165.1.25
22. Logue EE, Scott ED, Palmieri PA, Dudley P. Sleep duration, quality, or stability and obesity in an urban family medicine center. *J Clin Sleep Med*. 2014;10(2):177-182. Published 2014 Feb 15. doi:10.5664/jcsm.3448
23. Buscemi D, Kumar A, Nugent R, Nugent K. Short sleep times predict obesity in internal medicine clinic patients. *J Clin Sleep Med*. 2007;3(7):681-688.
24. Jia H, Lubetkin EI. The impact of obesity on health-related quality-of-life in the general adult US population. *J Public Health (Oxf)*. 2005;27(2):156-164. doi:10.1093/pubmed/fdi025
25. Sach TH, Barton GR, Doherty M, Muir KR, Jenkinson C, Avery AJ. The relationship between body mass index and health-related quality of life: comparing the EQ-5D, EuroQol VAS and SF-6D. *Int J Obes (Lond)*. 2007;31(1):189-196. doi:10.1038/sj.ijo.0803365
26. Stephenson J, Smith CM, Kearns B, Haywood A, Bissell P. The association between obesity and quality of life: a retrospective analysis of a large-scale population-based cohort study. *BMC Public Health*. 2021;21(1):1990. Published 2021 Nov 3. doi:10.1186/s12889-021-12009-8
27. Thommasen HV, Zhang W. Impact of chronic disease on quality of life in the Bella Coola Valley. *Rural Remote Health*. 2006;6(2):528.
28. Lucena L, Polesel DN, Poyares D, et al. The association of insomnia and quality of life: Sao Paulo epidemiologic sleep study (EPISONO). *Sleep Health*. 2020;6(5):629-635. doi:10.1016/j.sleh.2020.03.002

29. Khorsandi M, Fekrizadeh Z, Roozbahani N. Investigation of the effect of education based on the health belief model on the adoption of hypertension-controlling behaviors in the elderly. *Clin Interv Aging*. 2017;12:233-240. Published 2017 Jan 27. doi:10.2147/CIA.S117142
30. Sulat JS, Prabandari YS, Sanusi R, Hapsari ED, Santoso B. The validity of health belief model variables in predicting behavioral change: a scoping review. *Health Educ.* 2018;118:499-512. doi:10.1108/HE-05-2018-0027
31. Barakat AM, Kasemy ZA. Preventive health behaviors during the coronavirus disease 2019 pandemic based on the health belief model among Egyptians. *Middle East Curr Psychiatr.* 2020;27:43(e1-9). doi:10.1186/s43045-020-00051-y
32. Kahraman G, Türker B, Akbolat M. Obeziteye yönelik tutum ve inançların geliştirilmesinde sağlık programlarının etkisi. *Acıbadem Univ Sağlık Bilim Derg.* 2015;6:89-98.
33. Darvishpour A, Vajari SM, Noroozi S. Can Health Belief Model Predict Breast Cancer Screening Behaviors?. *Open Access Maced J Med Sci*. 2018;6(5):949-953. Published 2018 May 19. doi:10.3889/oamjms.2018.183
34. Saghafi-Asl M, Aliasgharzadeh S, Asghari-Jafarabadi M. Factors influencing weight management behavior among college students: An application of the Health Belief Model [published correction appears in PLoS One. 2021 May 20;16(5):e0252258. doi: 10.1371/journal.pone.0252258]. *PLoS One*. 2020;15(2):e0228058. Published 2020 Feb 7. doi:10.1371/journal.pone.0228058

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1535671

Sex estimation by morphometric analysis of the intracranial volume and foramen magnum on three-dimensional volume rendering computed tomography

Üç boyutlu volume rendered bilgisayarlı tomografi görüntülerinde intrakranial hacim ve foramen magnum morfometrik analizinden cinsiyetin değerlendirilmesi

Ayşe Kurtuluş Dereli, Ergin Sağtaş, Alper Akça, Hakan Abdullah Özgül, Hande Şenol, Kemalettin Acar

Posted date:19.08.2024 Acceptance date:01.10.2024

Abstarct

Purpose: Identification of decomposed human bodies and bone remains is very important in medicolegal examinations. The cranium has an important place in sex estimation due to its dimorphic features. Recent studies in this field have used radiological methods. The present study aimed to examine sexual dimorphism through morphometric analysis of the intracranial volume and Foramen Magnum on Three-Dimensional (3D) Volume Rendering Computed Tomography (CT) images.

Material and methods: For this purpose, 3D images were generated after the reconstruction of CT Angiography scans of 87 female and 107 male cases. The length, width, circumference, area, and intracranial volume of the foramen magnum were measured on these 3D images.

Results: All measurements except foramen magnum index were greater in males than in females (length of foramen magnum p=0.0001; breadth of foramen magnum p=0.0001; foramen magnum area p=0.0001; foramen magnum circumference p=0.0001). The single best sex-discriminatory measurement was intracranial volume, with an accuracy rate of 84.5%. The best sex-discriminatory parameter in foramen magnum measurements was the length of the foramen magnum, with a rate of 74.2%. Intracranial volume was positively correlated with all parameters in male except for the foramen magnum index (length p=0.000; breadth p=0.000; area p=0.000; circumference p=0.000; index p=0.747).

Conclusion: Three-dimensional volume rendering CT images showed sexual dimorphism in the intracranial volume and foramen magnum. The use of radiological methods in forensic investigations may allow for examinations of decomposed human bodies without the need for maceration procedures. It may also help create databases by examining population-specific differences in today's societies.

Keywords: Volume rendering CT, 3D modeling, foramen magnum, intracranial volume, sex estimation.

Kurtulus Dereli A, Sagtas E, Akca A, Ozgul HA, Senol H, Acar K. Sex estimation by morphometric analysis of the intracranial volume and foramen magnum on three-dimensional volume rendering computed tomography. Pam Med J 2025;18:244-253.

Öz

Amaç: Medikolegal incelemelerde çürümüş cesetlerde ve kemik kalıntılarında kimliklendirme çok önemlidir. Kranium dimorfik özelliklere sahip olduğu için cinsiyet tahmininde önemli yer tutar. Son zamanlarda bu alandaki araştırmalarda radyolojik yöntemlerin kullanıldığı görülmektedir. Bu çalışmada Volume Rendered Bilgisayarlı Tomografi 3B görüntülerinde intrakranial hacim ve Foramen Magnum morfometrik analizinden seksüel dimorfizmin araştırılması amaçlanmıştır.

Gereç ve yöntem: Bu amaçla 87 kadın ve 107 erkek olguya ait BT Anjiografi görüntülerinden rekonstrüksiyon sonrası üç boyutlu (3B) görüntüler elde edilmiştir. Elde edilen görüntülerde foramen magnum uzunluğu, genişliği, çevresi, alanı ve intrakranial hacim ölçülmüştür.

Bulgular: Foramen magnum indeksi dışındaki tüm ölçümlerin erkeklerde kadınlardan daha büyük olduğu görülmüştür (foramen magnum uzunluğu p=0,0001; foramen magnum genişliği p=0,0001; foramen magnum alanı p=0,0001; foramen magnum çevresi p=0,0001). Cinsiyeti tek başına en iyi ayırt eden ölçüm %84,5

Ayşe Kurtuluş Dereli, Prof. Pamukkale University, Faculty of Medicine, Department of Forensic Medicine, Denizli, Türkiye, e-mail: akurtulus@pau.edu.tr (https://orcid.org/0000-0002-0592-585X) (Corresponding Author)

Ergin Sağtaş, Assoc. Prof. Pamukkale University, Faculty of Medicine, Department of Radiology, Denizli, Türkiye, e-mail: esagtas@pau.edu.tr (https://orcid.org/0000-0001-6723-6593)

Alper Akça, M.D. Pamukkale University Faculty of Medicine, Department of Forensic Medicine, Denizli, Türkiye, e-mail: replacka@gmail.com (https://orcid.org/0000-0002-0239-4432)

Hakan Abdullah Özgül, M.D. Atatürk Training and Research Hospital, Department of Radiology, İzmir, Türkiye, e-mail: haozgul@hotmail.com (https://orcid.org/0000-0001-6123-0668)

Hande Şenol, Assoc. Prof. Pamukkale University, Faculty of Medicine, Department of Biostatistics, Denizli, Türkiye, e-mail: hsenol@pau.edu.tr (https://orcid.org/0000-0001-6395-7924)

Kemalettin Acar, Prof. Pamukkale University, Faculty of Medicine, Department of Forensic Medicine, Denizli, Türkiye, e-mail: kacar@pau.edu.tr (https://orcid.org/0000-0002-0200-4764)

doğruluk oranı ile intrakranial hacimdi. Foramen magnum ölçümlerinde cinsiyeti en iyi ayırt eden parametre ise %74,2 oranı ile foramen magnum uzunluğuydu. Erkeklerde intrakranial hacim foramen magnum indeksi hariç tüm parametrelerle pozitif korelasyon gösterdi (foramen magnum uzunluğu *p*=0,000; foramen magnum genişliği *p*=0,000; foramen magnum alanı *p*=0,000; foramen magnum çevresi *p*=0,000; foramen magnum indeksi *p*=0,747).
Sonuç: Volume rendered BT 3B görüntülerinde intrakranial hacim ve foramen magnumda cinsel dimorfizm olduğu görülmüştür. Radyolojik yöntemlerin adli incelemelerde kullanılması çürümüş cesetlerde maserasyon işlemlerine gerek kalmadan incelemelerin yapılmasına olanak sağlayabilir. Ayrıca günümüz toplumlarındaki popülasyona özgü farklılıkların araştırılarak veri tabanlarının oluşturulmasına yardımcı olabilir.

Anahtar kelimeler: Volume rendering BT, 3B modelleme, foramen magnum, intrakranial hacim, cinsiyet tahmini.

Kurtuluş Dereli A, Sağtaş E, Akça A, Özgül HA, Şenol H, Acar K. Üç boyutlu volume rendered bilgisayarlı tomografi görüntülerinde intrakranial hacim ve foramen magnum morfometrik analizinden cinsiyetin değerlendirilmesi. Pam Tıp Derg 2025;18:244-253.

Introduction

Sex estimation in human skeletal remains in forensic anthropology studies is critical for identification and medicolegal studies. Since body and bone fragments with lost integrity will be encountered, especially in mass disasters and explosions, identification of these bone fragments will be required. The assessment of the skull, which has dimorphic features, using morphometric and morphological methods has an important place in sex estimation. The skull base of the cranial skeleton in the occipital bones is the most resistant to physical and environmental factors due to its anatomically preserved localization. The Foramen Magnum (FM) in this region is a suitable anatomical region for forensic anthropological studies [1-3]. It was first reported by Teixeria in 1982 [4] that FM could be used for sex estimation. Several studies found FM dimensions to differ significantly in sex estimation [5-8].

Since the intracranial volume shows sexual dimorphism, it is an important parameter used in identification [9-11]. It also helps in the assessment of growth and development and the examination of cranial pathologies [12]. The intracranial volume is assessed using anthropological methods such as linear dimensions and packing methods or radiological methods [12-14].

In forensic anthropology, imaging methods such as computed tomography (CT) may be useful, especially in decomposed human bodies, as it allows for the examination of bone tissues without the need for maceration. Three-dimensional (3D) reconstruction of bony structures can be made, especially on volume-rendering CT images. This enables 1:1 landmark measurements and other morphometric and morphological examinations in bony structures [15-19]. A comparative study by Franklin et al. [20] demonstrated that direct bone measurements and 3D volume rendering CT imaging-derived measurements were concordant and there was no statistically significant difference.

A limited number of studies evaluated foramen magnum measurements and intracranial volume on 3D volume rendering CT images [21-23]. The present study aimed to examine sexual dimorphism through morphometric analysis of the intracranial volume and FM on 3D volume-rendering CT images.

Materials and methods

This study was initiated following the granting of approval by the ethics committee for non-interventional clinical research. Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (permission date: 27.10.2020 and permission number: E-60116787-020-3505). All procedures performed in studies 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 who had cranial CT angiography at the Radiology Department of the Medical Faculty between January 2017 and December 2018 were evaluated in our study. Among these cases, after excluding those under the age of 18, with motion artefacts, head traumas and bone pathologies, 194 cases remained were included in the study.

CT angiography scans were acquired by a 16-detector MSCT device (Brilliance CT 16 V2.00 Philips Medical Systems, Cleveland, OH) with 1 mm thickness. These images in the archive were reuploaded to the standard workstation (MxViewexp; release 4.01; Philips Medical Systems), and three-dimensional images were generated after reconstruction.

On the three-dimensional images, the skull was rotated horizontally to get an en face view of the foramen magnum, and the maximum length (anteroposterior diameter) and the maximum width of the foramen magnum (transverse diameter) were measured. The distance between the basion and the opisthion was used for the maximum length of the foramen magnum (LFM). The largest distance between the lateral margins of the foramen magnum was measured for the maximum width of the foramen magnum (WFM) (Figure 1). In the same imaging plane, the entire contour of the foramen magnum was drawn using the manual segmentation method in such a way that it would not protrude into the surrounding bone tissue and be the widest, and the area (FMA) and circumference (FMC) of the foramen magnum were determined. The maximum length, maximum width, circumference, and area of the foramen magnum were expressed in millimeters.

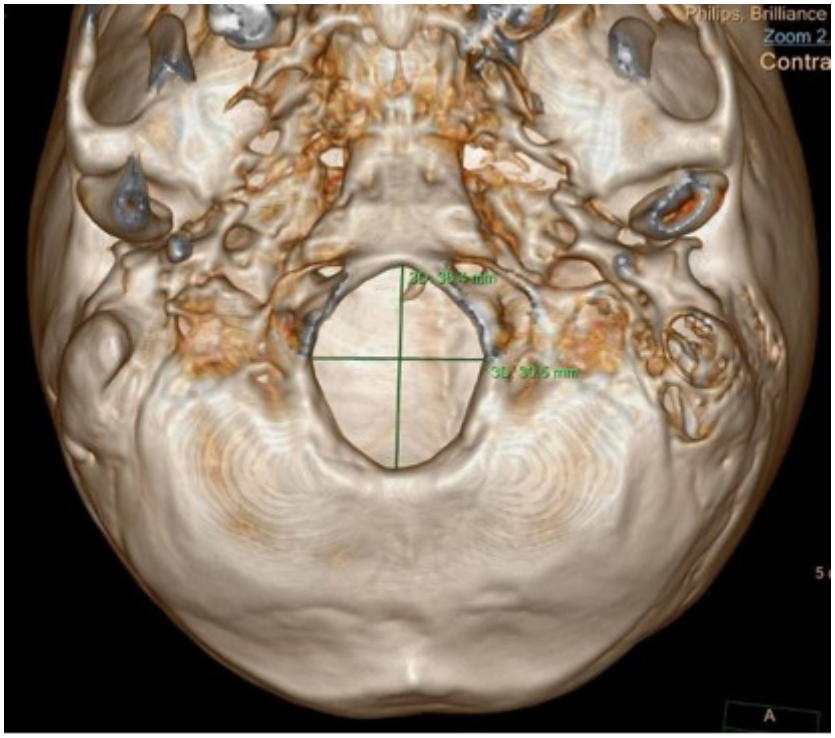


Figure 1. Measurement of foramen magnum dimensions on a three-dimensional volume rendering CT image of the skull base

The following formula was used to calculate the Foramen Magnum Index (FMI): FMI = (Width / Length) x 100

Volumetric measurement was made using IntelliSpace Portal 8.0 software on the workstation. The region of interest (ROI) was selected via the semi-automatic segmentation method, mainly using axial CT images, and then the entire intracranial volume was included using the region augmentation method (Figure 2). After observing that the ROI did not protrude into the bone tissue and completely filled the intracranial area in each axial section, a visual assessment was also performed in the sagittal and coronal planes, and improvements were made to the ROI contour when necessary. Following this process, the software automatically gave us the volume in cubic centimeters (cc).

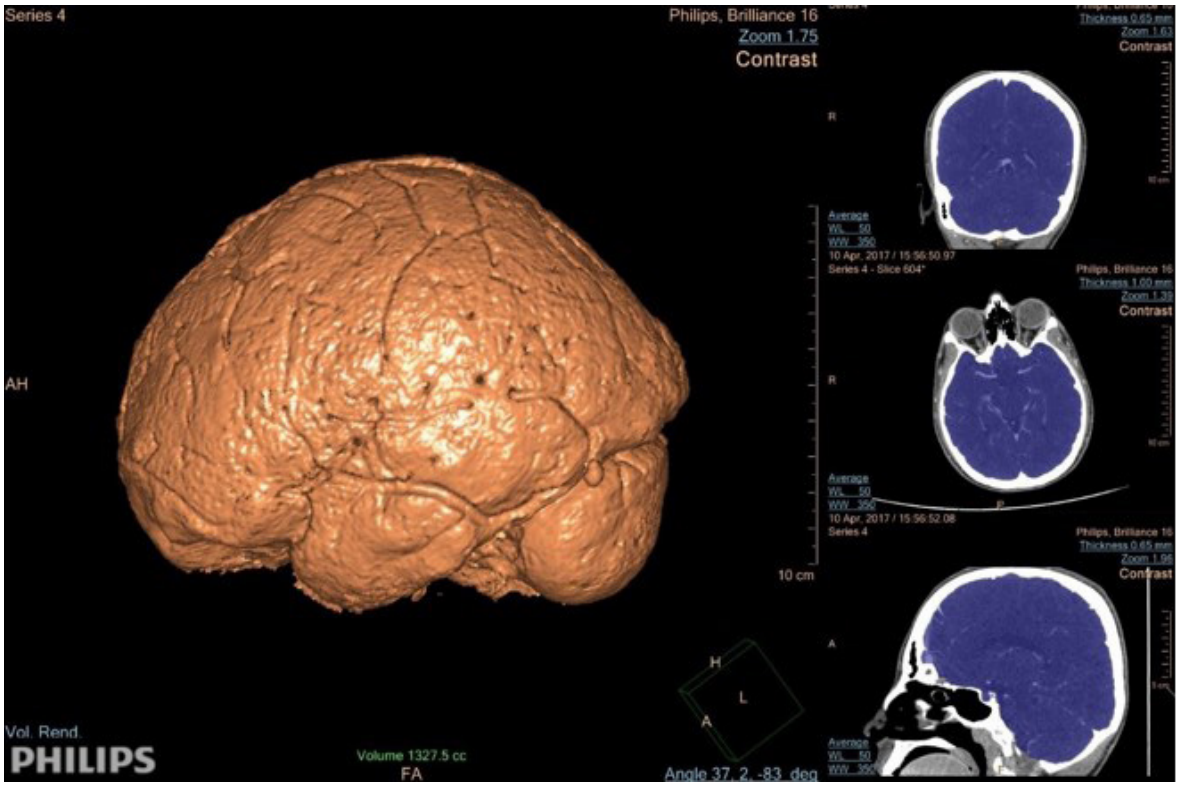


Figure 2. The entire intracranial space is marked in purple on coronal, axial, and sagittal CT images from top to bottom on the right, through the semi-automatic segmentation method. The measurement data and the three-dimensional image of the selected volume are displayed on the left

All statistical analyses were conducted with SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)). Continuous variables were defined by the mean ± standard deviation, median (minimum–maximum values), and categorical variables were defined by frequencies and percent. Normal distribution was determined using the Kolmogorov-Smirnov test. For independent group comparisons, Independent samples t test was used when parametric test assumptions were provided, and Mann Whitney U test was used when parametric test assumptions were not provided. Pearson correlation coefficient was used for investigating the relationships between continuous variables. We used Binary Logistic Regression Analysis and Linear Discriminant Analysis to test the power of predicting sex classification. Statistical significance was determined as $p<0.05$.

Results

This study evaluated the foramen magnum morphometric parameters and intracranial volume on 194 “volume-rendering” CT scans from 87 females and 107 males. The mean values, minimum and maximum values, and standard deviation for the length, width, circumference, and area of the foramen magnum, the foramen magnum index, and the intracranial volume are presented in Table 1. The examinations revealed statistically significant differences in all variables between the sexes. In all measurements except the FMI, the values were statistically significantly higher in males than in females ($p<0.01$). The FMI, in turn, was significantly higher in females than in males ($p<0.05$).

In females, there was no statistically significant association between intracranial volumes and other study variables. In males, in turn, intracranial volumes had a statistically significant moderate positive correlation with all variables, except for the FMI (Table 2).

In both females and males, the LFM had a statistically significant positive correlation with the WFM, FMA, and circumference and a statistically significant negative correlation with the FMI (Table 2).

In both females and males, the WFM had a statistically significant positive correlation with the FMA, circumference, and FMI (Table 2).

In both females and males, the FMA had a statistically significant positive correlation with the circumference. While the FMA had a statistically significant positive correlation with FMI in females, such a correlation was not statistically significant in males (Table 2).

Table 1. Comparison of foramen magnum parameters and intracranial volume by sex

		Total (n=194)	Female (n=87)	Male (n=107)	*p*
Intracranial volume	Mean±S.D.	1374.71±159.09	1263.77±110.26	1464.91±133.8	0.0001*
	Med (min-max)	1371.3 (937.2-2025.5)	1263.2 (1000.9-1504.6)	1460.8 (937.2-2025.5)	(z=-9.543)
LFM	Mean±S.D.	34.4±2.69	32.81±2.19	35.69±2.35	0.0001*
	Med (min-max)	34.15 (28.5-42)	32.8 (28.5-37.8)	35.9 (29-42)	(t=-8.737)
WFM	Mean±S.D.	29.29±2.52	28.27±2.36	30.12±2.34	0.0001*
	Med (min-max)	29.15 (23.3-37.5)	28.4 (23.3-35)	30.1 (24.3-37.5)	(t=-5.462)
FMA	Mean±S.D.	762.64±123.99	705.83±109.85	808.84±115.76	0.0001*
	Med (min-max)	767.95 (468.4-1170.4)	712.6 (468.4-951.5)	799 (519-1170.4)	(t=-6.307)
FMC	Mean±S.D.	110.2±9.26	105.98±8.82	113.63±8.16	0.0001*
	Med (min-max)	110.1 (87.3-141.9)	106.2 (87.3-128.4)	114.3 (93-141.9)	(t=-6.259)
FMI	Mean±S.D.	85.3±5.86	86.26±6.07	84.51±5.58	0.038*
	Med (min-max)	85.27 (69.33-98.84)	85.76 (74.6-97.61)	84.51 (69.33-98.84)	(t=2.087)

LFM: maximum length of foramen magnum, WFM: maximum breadth of foramen magnum, FMA: foramen magnum area
FMC: foramen magnum circumference, FMI: foramen magnum index, SD: standart deviation
*p<0.05, t: Independent samples t test; z: Mann Whitney U test

Table 2. Correlations between the intracranial volume and the parameters of the foramen magnum

		Female					Male				
		LFM	**WFM**	**FMA**	**FMC**	**FMI**	**LFM**	**WFM**	**FMA**	**FMC**	**FMI**
Intracranial volume	**r**	0.068	0.180	0.178	0.042	0.151	0.411*	0.372*	0.363*	0.357*	0.032
	p	0.529	0.096	0.099	0.696	0.163	0.000	0.000	0.000	0.000	0.747
LFM	**r**	1	0.576*	0.743*	0.694*	-0.267*	1	0.585*	0.827*	0.681*	-0.320*
	p		0.000	0.000	0.000	0.013		0.000	0.000	0.000	0.001
WFM	**r**		1	0.851*	0.792*	0.632*		1	0.756*	0.733*	0.580*
	p			0.000	0.000	0.000			0.000	0.000	0.000
FMA	**r**			1	0.856*	0.296*			1	0.788*	0.050
	p				0.000	0.005				0.000	0.609
FMC	**r**				1	0.274*				1	0.163
	p					0.010					0.094

*p<0.05 statistically significant correlation, r: Pearson Correlation Coefficient, LFM, maximum length of foramen magnum; WFM, maximum breadth of foramen magnum; FMA, foramen magnum area; FMC, foramen magnum circumference; FMI, foramen magnum index

The results of the discriminant analysis for the intracranial volume and the parameters of the foramen magnum showed that the variable with the highest success in sex discrimination was intracranial volume. It was followed by LFM, circumference, FMA, and WFM, respectively. The FMI had the lowest rate of accuracy (Table 3).

The results of the classification according to the logistic regression model using intracranial volume and foramen magnum measurements revealed that the most effective variable in sex estimation was intracranial volume, with a rate of 84.5%. It was followed by LFM (74.2%), circumference (70.1%), FMA (68%), and WFM (64.9%), respectively, while the least effective variable was FMI, with a rate of 61.3% (Table 4).

Table 3. Results of the discriminant analysis for the intracranial volume and the parameters of the foramen magnum

	CCC	Wilks' Lambda	Predicted Group Membership (F/M)%	Total
Intracranial volume	0.63	0.603	80.5 / 87.9	164 (84.5%)
LFM	0.533	0.716	69 / 78.5	144 (74.2%)
WFM	0.367	0.866	54 / 73.8	126 (64.9%)
FMA	0.414	0.828	60.9 / 73.8	132 (68%)
FMC	0.412	0.831	59.8 / 77.6	135 (69.6%)
FMI	0.149	0.978	35.6 / 82.2	119 (61.3%)

CCC: Canonical Correlation Coefficient; Discriminant Analysis

Table 4. Logistic regression models using intracranial volume and parameters of the foramen magnum

		Wald	*p*	O.R.	95% C.I.for O.R.		CCP (%)
					Lower	Upper	
Univariate models	**Intracranial volume**	47.483	0.0001*	1.015	1.011	1.019	84.5
	LFM	40.753	0.0001*	1.74	1.468	2.062	74.2
	WFM	22.694	0.0001*	1.412	1.225	1.627	64.9
	FMA	28.214	0.0001*	1.008	1.005	1.011	68
	FMC	27.807	0.0001*	1.116	1.072	1.163	70.1
	FMI	4.223	0.04*	0.949	0.903	0.998	61.3

*p<0.05 statistically significant; O.R: Odds Ratio; C.I: Confidence Interval; CCP: Correct Classification Percent; Logistic Regression Analysis. LFM, maximum length of foramen magnum; WFM, maximum breadth of foramen magnum; FMA, foramen magnum area; FMC, foramen magnum circumference; FMI, foramen magnum index

Discussion

In cases where the entire skeleton is not available, sex can be estimated using dimorphic features of the cranial bones. The sexual dimorphism-associated features of bones are affected by genetic, social, and environmental factors and are population-specific [24]. With new technologies, radiological methods are quite commonly used in skeletal sex estimation studies. Radiological methods are useful tools in detecting population-specific differences in modern societies, as they also allow research in living populations with known variables such as gender, age, geographical origin, and diseases [24]. Moreover, it allows morphometric measurements on radiological images without the need for maceration procedures in decomposed human bodies in forensic medicine and forensic anthropology practice [24, 25]. Since three-dimensional images of bones can be generated from reconstructed images in volume-rendering CT, measurements can be made as if working on real bones.

The present study determined that all parameters of the foramen magnum differed significantly between the two sexes at the measurements on 3D volume-rendering CT scans of the cranium. In all measurements except the FMI, the values were statistically significantly higher in males than in females. This finding is consistent with most of the previous studies [7, 22, 25-32]. However, some authors reported no sexual dimorphism in FML and FMW [6]. The studies by Meral et al. [29] and Chovalopoulou and Bertsatos [30] did not observe sexual dimorphism in the FM index.

Our study found that the length, circumference, area, and width of the foramen magnum estimated sex with an accuracy rate of 74.2%, 70.1%, 68%, and 64.9%, respectively. The study by Uysal et al. [33] using 3D CT reported that the foramen magnum estimated sex with an accuracy rate of 81%. The study by Tambawala et al. [34] using cone-beam CT (CBCT) established that the sex discriminatory power of FM measurements was 70.3% in males and 62.6% in females, with a mean of 66.4%. In an Iraqi population, Uthman et al. [26] reported an accuracy rate of 81.8% for foramen magnum parameters in sex estimation. Meral et al. [29] reported that the best sex-discriminatory parameter was the FM area (calculated by Radinsk'y formula) with a rate of 74.5% and the rate of accurate sex estimation was 75% when multiple parameters were used.

In this study, the mean FML and FMW were 35.69 mm and 30.12 mm in males, and 32.81 mm and 28.27 mm in females, respectively. A CT study by Toneva et al. [28] in a Bulgarian population reported that the mean FML and FMW were 36.63 mm and 31.47 mm in males, and 35.19 mm and 29.25 mm in females, respectively. A CBCT study by Akay et al. [25] in a Turkish population found the sagittal and transverse diameters and FMC to be statistically significantly higher in males than in females. The reported mean FML and FMW were 36.43 mm and 31.26 mm in males, and 34.66 mm and 29.78 mm in females, respectively [25]. A CT study by Meral et al. [29] in a Turkish population reported that the mean FML and FMW were 37.54 mm and 32.75 mm in males, and 34.76 mm and 29.98 mm in females, respectively. A 3D volume rendering CT study by Abdel Karim et al. [22] reported that the mean FML and FMW were 42.17 mm and 33.98 mm in males, and 38.75 mm and 31.38 mm in females, respectively. The study by Jain et al. [32] in an Indian population reported that the length and width were 31.3 mm and 36.2 mm in males, and 28.3 mm and 34 mm in females. The study by Gapert et al. [31] in a British population found the length and width of the FM to be 35.91 mm and 30.51 mm in males, and 34.71 and 29.36 mm in females, which is consistent with our study. The study by Uthman et al. [26] found the length and width to be 34.9 mm and 29.5 mm in males, and 32.9 and 27.3 mm in females, which is consistent with our study.

In our study, FMA and FMC were calculated automatically. The mean FMC was 113.63 mm in males and 105.98 mm in females, respectively, and the FM area was 808.4 mm^2 in males and 705.83 mm^2 in females. The study by Akay et al. [25] reported the mean FMC as 107.94 mm in males and 102.67 mm in females. The study by Ilguy et al. [35] found the mean FMC to be 108.10 mm in males and 102.21 mm in females. The study by Bayrak and Goller Bulut [36] reported the mean FMA as 906.05 mm^2 in males and 850.07 mm^2 in females. Meral et al. [29] found that the mean FMA, which was calculated by the Radinsk'y formula, was 967.66 mm^2 in males and 820.49 mm^2 in females. Toneva et al. [28] in turn, found the FMA to be 851.60 mm^2 in males and 763.64 mm^2 in females. Our results for FMA and FMC are lower than those reported in the literature. These differences in mean foramen magnum dimensions may be due to population-specific differences and/or different anatomical and radiological methods used in studies.

Meral et al. [29] identified the strongest correlations between LFM and FMA and between WFM and FMA in males and females. Tambawala et al. [34] found the strongest correlations between Area 1 (Routal formula) and Area 2 (Teixeira formula). Uthman et al. [26] established the strongest correlations between FMC and FMA in males and females. In our study, on the other hand, the length and width of the foramen magnum were strongly correlated with both area and circumference in males and females.

There are studies in the literature reporting the measurement of intracranial volume on CT scans [21, 37]. Our study measured intracranial volume on 3D volume-rendering CT scans

and found a higher mean intracranial volume in males than in females. These results are consistent with those reported by other studies in the literature [9-11, 38-41]. The sex estimation rate of intracranial volume alone was 84.5%. A comparison of our results on intracranial volume with various studies is presented in Table 5. It is believed that the differences in the mean values may result from the methods used and the population-specific differences. The present study identified a statistically significant positive correlation between intracranial volume and foramen magnum parameters (except FMI) in males. Gapert and Last [42] demonstrated that endocranial capacity was significantly correlated with LFM, FMA, and FMC. Shepur et al. [43] and Acer et al. [10] reported a significantly positive correlation between endocranial capacity and the area of the foramen magnum.

Table 5. Comparison of the intracranial volume determined in our study with those reported by various studies

Population	Male (cm^3)	Female (cm^3)	Method	Reference
Nigerian Ibo	1298.44±90.67	1186.73±79.05	CT (Stereology)	Onwuzu
South India	1030.050±35.648	850.24±40.944	Skull	Rasidi
India	1367.3±127.8	1255.2±113.3	Skull	Shepur
	1347.1±90.7	1130±111.9	CT	
Korea	1470±107	1317±117	Skull	Hwang
Türkiye	1474±93	1252±72	CT (point-counting)	Acer 2007
Türkiye	1389.50±96.50	1134.5±94.30	Skull (Water filling)	Sahin 2007
Türkiye	1382.5±104.5	1165.5±111.6	Skull (Water filling)	Emirzeoğlu M 2011
Türkiye	1464.91±133.8	1263.77±110.26	CT	Present study

In conclusion, according to the findings of our study, 3D volume-rendering CT images allow the measurement of foramen magnum parameters and intracranial volume. The foramen magnum parameters, except for FMI, had higher values in males than in females and may be useful in sex estimation since they show sexual dimorphism. Intracranial volume was higher in males than in females and was positively correlated with foramen magnum parameters. The use of radiological methods in medicolegal examinations will provide advantages such as the examination of decomposed human bodies without the need for time-consuming maceration procedures. Due to the archival storage of the DICOM digital data, examinations can be performed easily when re-assessment is required. The transfer of 3D volume-rendering CT images or the transfer of DICOM digital data will enable performing morphometric analyses after the 3D modeling of these data. Thereby, cases where the skulls cannot be transferred to other centers or where the experts in the field cannot be immediately available will be easily resolved. Furthermore, it will help to create databases by examining population-specific differences in today's societies and to use such data for sex estimation in forensic investigations, as it allows studying in the cranium of living people with known gender, age, and medical history.

Funding: None.

Authors contributions: A.K.D. and K.A. constructed the main idea and hypothesis of the study. E.S. and A.H.Ö. developed the theory and arranged/edited the material and method section. A.K.D, H.Ş. and A.A. have done the evaluation of the data in the Results section. Discussion section of the article was written by A.K.D. K.A. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Saukko P, Knight B. Knight's forensic pathology. 4th ed. London: CRC Press, 2016:95-132.
2. Krogman WM, Iscan MY. The human skeleton in forensic medicine. 2nd ed. Springfield IL USA: Charles C Thomas Publishers, 1986.
3. Krüger GC, L'Abbé EN, Stull KE, Kenyhercz MW. Sexual dimorphism in cranial morphology among modern South Africans [published correction appears in Int J Legal Med. 2015 Nov;129(6):1285. doi: 10.1007/s00414-015-1233-z]. *Int J Legal Med*. 2015;129(4):869-875. doi:10.1007/s00414-014-1111-0
4. Teixeira WR. Sex identification utilizing the size of the foramen magnum. *Am J Forensic Med Pathol*. 1982;3(3):203-206. doi:10.1097/00000433-198209000-00003
5. Günay Y, Altinkök M. The value of the size of foramen magnum in sex determination. *J Clin Forensic Med*. 2000;7(3):147-149. doi:10.1054/jcfm.2000.0430
6. Gruber P, Henneberg M, Böni T, Rühli FJ. Variability of human foramen magnum size. *Anat Rec (Hoboken)*. 2009;292(11):1713-1719. doi:10.1002/ar.21005
7. Catalina-Herrera CJ. Study of the anatomic metric values of the foramen magnum and its relation to sex. *Acta Anat (Basel)*. 1987;130(4):344-347. doi:10.1159/000146468
8. Acer N, Sahin B, Ekinci N, Ergür H, Basaloglu H. Relation between intracranial volume and the surface area of the foramen magnum. *J Craniofac Surg*. 2006;17(2):326-330. doi:10.1097/00001665-200603000-00020
9. Onwuzu SWI, Idigo FU, Okeji MC. Assessment of the total intra-cranial volume of the Igbo population of Nigeria using computed tomography. *Radiation Science and Technology.* 2017;3(2):8-12. doi:10.11648/j.rst.20170302.11
10. Acer N, Sahin B, Baş O, Ertekin T, Usanmaz M. Comparison of three methods for the estimation of total intracranial volume: stereologic, planimetric, and anthropometric approaches. *Ann Plast Surg*. 2007;58(1):48-53. doi:10.1097/01.sap.0000250653.77090.97
11. Shepur MP, Magi M, Nanjundappa B, Havaldar PP, Gogi P, Saheb SH. Morphometric analysis of endocranial capacity. *Int J Anat Res.* 2014;2(1):242-248.
12. Manjunath KY. Estimation of cranial volume an overview of methologies. *J Anat Soc India.* 2002;51(1):85-91.
13. Manjunath KY. Estimation of cranial volume in dissecting room cadavers. *J Anat Soc India.* 2002;51(2):168-172.
14. Breakey W, Knoops PGM, Borghi A, et al. Intracranial Volume Measurement: A Systematic Review and Comparison of Different Techniques [published correction appears in J Craniofac Surg. 2019 Jul;30(5):1596. doi: 10.1097/SCS.0000000000005818]. *J Craniofac Surg*. 2017;28(7):1746-1751. doi:10.1097/SCS.0000000000003929
15. Dereli AK, Zeybek V, Sagtas E, Senol H, Ozgul HA, Acar K. Sex determination with morphological characteristics of the skull by using 3D modeling techniques in computerized tomography. *Forensic Sci Med Pathol*. 2018;14(4):450-459. doi:10.1007/s12024-018-0029-0
16. Lagravère MO, Major PW. Proposed reference point for 3-dimensional cephalometric analysis with cone-beam computerized tomography. *Am J Orthod Dentofacial Orthop*. 2005;128(5):657-660. doi:10.1016/j.ajodo.2005.07.003
17. Franklin D, Cardini A, Flavel A, Kuliukas A. Estimation of sex from cranial measurements in a Western Australian population [published correction appears in Forensic Sci Int. 2013 Oct 10;232(1-3):153]. *Forensic Sci Int*. 2013;229(1-3):158.e1-158.e1588. doi:10.1016/j.forsciint.2013.03.005
18. Ramsthaler F, Kettner M, Gehl A, Verhoff MA. Digital forensic osteology: morphological sexing of skeletal remains using volume-rendered cranial CT scans. *Forensic Sci Int*. 2010;195(1-3):148-152. doi:10.1016/j.forsciint.2009.12.010
19. Cavalcanti MG, Rocha SS, Vannier MW. Craniofacial measurements based on 3D-CT volume rendering: implications for clinical applications. *Dentomaxillofac Radiol*. 2004;33(3):170-176. doi:10.1259/dmfr/13603271
20. Franklin D, Cardini A, Flavel A, et al. Concordance of traditional osteometric and volume-rendered MSCT interlandmark cranial measurements. *Int J Legal Med*. 2013;127(2):505-520. doi:10.1007/s00414-012-0772-9
21. Agarwal HK, Setia PS, Pandey S. Virtual determination of sex: estimating cut off value of digital metric traits of foramen magnum on three-dimensional computed tomography with receiver operating characteristic and logistic regression analysis. *J Forensic Sci Med.* 2021;7(1):1-8. doi:10.4103/jfsm.jfsm_59_20
22. Abdel Karim RI, Housseini AM, Hashish RK. Adult sex estimation using three dimensional volume rendering multislice computed tomography of the foramen magnum and occipital condyles: a study in Egyptian population. *Int J Advanced Res.* 2015;3(May):1212-1215.

23. François PM, Sandoz B, Laporte S, Decq P. Intra cranial volume quantification from 3D reconstruction based on CT-scan data. *Comput Methods Biomech Biomed Engin*. 2017;20(sup1):81-82. doi:10.1080/10255842.2017.1382870
24. Isaza J, Díaz CA, Bedoya JF, Monsalve T, Botella MC. Assessment of sex from endocranial cavity using volume-rendered CT scans in a sample from Medellín, Colombia. *Forensic Sci Int*. 2014;234:. doi:10.1016/j.forsciint.2013.10.023
25. Akay G, Güngör K, Peker İ. Morphometric analysis of the foramen magnum using cone beam computed tomography. *Turk J Med Sci*. 2017;47(6):1715-1722. Published 2017 Dec 19. doi:10.3906/sag-1607-127
26. Uthman AT, Al Rawi NH, Al Timimi JF. Evaluation of foramen magnum in gender determination using helical CT scanning. *Dentomaxillofac Radiol*. 2012;41(3):197-202. doi:10.1259/dmfr/21276789
27. Murshed KA, Çiçekcibaşi AE, Tuncer I. Morphometric evaluation of the foramen magnum and variations in its shape: a study on computerized tomographic images of normal adults. *Turk J Med Sci.* 2003;33(5):301-306.
28. Toneva D, Nikolova S, Harizanov S, et al. Sex estimation by size and shape of foramen magnum based on CT imaging. *Leg Med (Tokyo)*. 2018;35:50-60. doi:10.1016/j.legalmed.2018.09.009
29. Meral O, Belkıs Toklu B, Meydan R, Kaya A, Karadayı B, Acar T. Sex estimation from foramen magnum parameters in adult Turkish population: A computed tomography study. *Leg Med (Tokyo)*. 2020;47:101775. doi:10.1016/j.legalmed.2020.101775
30. Chovalopoulou ME, Bertsatos A. Estimating sex of modern Greeks based on the foramen magnum region. *J Anthropol.* 2017;1(1):1-7. doi:10.1155/2017/9129801
31. Gapert R, Black S, Last J. Sex determination from the foramen magnum: discriminant function analysis in an eighteenth and nineteenth century British sample. *Int J Legal Med*. 2009;123(1):25-33. doi:10.1007/s00414-008-0256-0
32. Jain D, Jasuja OP, Nath S. Evaluation of foramen magnum in sex determination from human crania by using discriminant function analysis. *El Med J.* 2014;2:89-92. doi:10.18035/emj.v2i2.82
33. Uysal S, Gokharman D, Kacar M, Tuncbilek I, Kosa U. Estimation of sex by 3D CT measurements of the foramen magnum. *J Forensic Sci*. 2005;50(6):1310-1314.
34. Tambawala SS, Karjodkar FR, Sansare K, Prakash N, Dora AC. Sexual dimorphism of foramen magnum using Cone Beam Computed Tomography. *J Forensic Leg Med*. 2016;44:29-34. doi:10.1016/j.jflm.2016.08.005
35. İlgüy D, İlgüy M, Ersan N, Dölekoğlu S, Fişekçioğlu E. Measurements of the foramen magnum and mandible in relation to sex using CBCT. *J Forensic Sci*. 2014;59(3):601-605. doi:10.1111/1556-4029.12376
36. Bayrak S, Goller Bulut D. Assessment of foramen magnum and clivus for esimation of age and gender using cone-beam CT. *Atatürk Univ Diş Hekimliği Fak Derg.* 2019;29(2):244-251. doi:10.17567/ataunidfd.482950
37. Mazonakis M, Karampekios S, Damilakis J, Voloudaki A, Gourtsoyiannis N. Stereological estimation of total intracranial volume on CT images. *Eur Radiol*. 2004;14(7):1285-1290. doi:10.1007/s00330-004-2253-0
38. Rasidi MQZBM, Kumar SS. Sexual dimorphism from cranial capacity of adult south India skulls. *Res J Pharm Tech.* 2016;9(9):1389-1392. doi:10.5958/0974-360X.2016.00267.5
39. Hwang YI, Lee KH, Choi BY, et al. Study on the Korean adult cranial capacity. *J Korean Med Sci*. 1995;10(4):239-242. doi:10.3346/jkms.1995.10.4.239
40. Sahin B, Acer N, Sonmez OF, et al. Comparison of four methods for the estimation of intracranial volume: a gold standard study. *Clin Anat*. 2007;20(7):766-773. doi:10.1002/ca.20520
41. Emirzeoğlu M, Şahin B, Uzun A, Bilgiç S. Evaluation of the volumetric relation between cranial cavity and orbits. *Türkiye Klinikleri J Med Sci.* 2011;31(2):297-299. doi:10.5336/medsci.2009-15635
42. Gapert R, Last J. P45: Endocranial capacity correlates with the size of the foramen magnum in human adult crania. *J Anat.* 2004;205(6):542-543.
43. Shepur MP, Magi M, Nanjundappa B, Havaldar PP, Gogi P, Saheb SH. Correlation between endocranial capacity and size of foramen magnum with special reference to sex. *Int J Anat Res.* 2014;2(1):273-278. https://www.ijmhr.org/ijar_articles_vol2_1/IJAR-2014-418.pdf

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1500706

Effect of peroperative local tranexamic acid admistration on bleeding in total knee arthroplasty performed with general or spinal anesthesia

Genel veya spinal anestezi ile yapılan total diz artroplastisinde peroperatif lokal traneksamik asit uygulamasının kanamaya etkisi

Bahadır Balkanlı, İsmail Tüter, Enver İpek

Posted date:13.06.2024 Acceptance date:15.10.2024

Abstract

Purpose: This retrospective study aimed to compare the effects of local tranexamic use on postoperative bleeding in total knee arthroplasty performed under general or spinal anesthesia.

Materials and methods: This study analyzed the amount of postoperative bleeding in 95 primary osteoarthritis knees that underwent total knee arthroplasty between 2017 and 2023. Patients who underwent general or spinal anesthesia were divided into four groups according to whether or not local tranexamic acid was administered perioperatively. Tranexamic acid was administered locally to the surrounding soft tissues after arthroplasty as 1 g. Postoperative hemogram changes, drain amounts, and transfusion needs were evaluated in the study.

Results: In Group 4, a significant decrease in hemoglobin levels was observed within the first 24 hours postoperatively (*p*1:0.001; *p*2:0.000; *p*3:0.001). There was no statistically significant difference in hemoglobin values on the third day between Groups 1-4 (*p*:0.117). Postoperative hemoglobin values in Group 3 were statistically higher than the other groups (*p*1:0.019; *p*2:0.003). Significantly higher drain volumes were seen in Group 4 compared to Groups 1-3 (*p*=0.000). The amount coming from the drain in Group 1 was statistically significantly lower (*p*1:0.001; *p*2:0.032). When the replacement amount of 2 units (6.1%) was evaluated, blood replacement rates in Group 3 (6.1%) were lower than in the other groups (*p*1:0.002; *p*2:0.000).

Discussion: The study found that locally applied tranexamic acid in total knee arthroplasty reduced postoperative bleeding, maintained hemoglobin levels, and reduced drainage volumes and transfusion requirements. It may contribute to reducing potential bleeding-related complications and costs.

Keywords: Tranexamic acid, knee arthroplasty, blood transfusion, anesthesia, surgical drain.

Balkanli B, Tuter I, Ipek E. Effect of peroperative local tranexamic acid admistration on bleeding in total knee arthroplasty performed with general or spinal anesthesia. Pam Med J 2025;18:256-269.

Öz

Amaç: Bu retrospektif çalışmada, genel veya spinal anestezi altında yapılan total diz artroplastisinde lokal traneksamik kullanımının postoperatif kanama üzerine etkisinin karşılaştırması amaçlandı.

Gereç ve yöntem: Bu çalışmada 2017-2023 tarihleri arasında total diz artroplastisi uygulanan primer osteoartritli 95 dizdeki postoperatif kanama miktarları analiz edildi. Genel veya spinal anestezi uygulanan hastalar perioperatif lokal traneksamik asit verilip verilmemesine göre dört gruba ayrıldı. Traneksamik asit uygulaması 1 gr olarak artroplastisi sonrası çevre yumuşak dokulara lokal olarak yapıldı. Çalışmada postoperatif hemogram değişiklikleri, dren miktarları ve transfüzyon ihtiyaçları değerlendirildi.

Bulgular: Grup 4'te, postoperatif ilk 24 saat içinde hemoglobin düzeylerinde anlamlı düzeyde bir düşüş görüldü (*p*1:0,001; *p*2:0,000; *p*3:0,001). Üçüncü gün hemoglobin değerlerinde Grup 1-4 arasında istatistiksel farklılık yoktu (*p*:0,117). Grup 3'te postoperatif hemoglobin değerleri diğer gruplara göre istatistiksel olarak yüksekti (*p*1:0,019; *p*2:0,003). Grup 4'te Grup 1-3'e göre anlamlı derecede yüksek dren hacimleri görüldü (*p*=0,000). Grup 1'deki drenden gelen miktar istatistiksel olarak anlamlı derecede düşüktü (*p*1:0,001; *p*2:0,032). 2 ünite (%6,1) replasman miktarı değerlendirildiğinde, Grup 3'te kan replasman oranları (%6.1) diğer gruplara göre düşüktü (*p*1:0,002; *p*2:0,000).

Sonuç: Çalışma, total diz artroplastisinde lokal olarak uygulanan traneksamik asidin postoperatif kanamayı azalttığını, hemoglobin seviyelerini koruduğunu ve drenaj hacimlerini ve transfüzyon gereksinimlerini azalttığını buldu. Potansiyel kanama ile ilişkili komplikasyonları ve maliyetleri azaltmaya katkıda bulunabilir.

Anahtar kelimeler: Traneksamik asit, diz artroplastisi, kan transfüzyonu, anestezi, cerrahi dren.

Balkanlı B, Tüter İ, İpek E. Genel veya spinal anestezi ile yapılan total diz artroplastisinde peroperatif lokal traneksamik asit uygulamasının kanamaya etkisi. Pam Tıp Derg 2025;18:256-269.

Bahadır Balkanlı, Specialist, Şişli Hamidiye Etfal Training and Research Hospital, Department of Orthopedics and Traumatology, Istanbul, Türkiye, e-mail: balkanlibahadir@gmail.com (https://orcid.org/0000-0003-4501-9090) (Corresponding Author)

İsmail Tüter, Specialist, Şişli Hamidiye Etfal Training and Research Hospital, Department of Orthopedics and Traumatology, Istanbul, Türkiye, e-mail: tuterismail@gmail.com (https://orcid.org/0000-0002-8766-981X)

Enver İpek, Specialist, Şişli Hamidiye Etfal Training and Research Hospital, Department of Orthopedics and Traumatology, Istanbul, Türkiye, e-mail: enveripek88@gmail.com (https://orcid.org/0000-0001-6205-1207)

Introduction

One of the orthopaedic surgical procedures that is most frequently carried out is total knee arthroplasty (TKA). The necessity of TKA increases throughout various nations as the population ages. TKA is performed to increase function and decrease pain in patients with end-stage gonarthrosis**.** Blood loss during and after this surgical procedure is a serious problem. In total knee arthroplastic surgery, the percentage of transfusions ranged from 18% to 68% [1]. Allogenic blood product transfusions increase the risk of morbidity. Several studies have identified causes that increase the risk of transfusion-related morbidities, such as allergic reactions, hemolytic reactions, disease transmission, surgical area infection, and venous thromboembolism [1-4]. Various strategies have been developed to limit peroperative and postoperative blood loss. The use of tourniquets, autologous blood transfusion, surgical clamping of the drain, controlled hypotensive anaesthesia, and the use of various drugs such as tranexamic acid can be examples of these strategies [5, 6]. Tranexamic acid (TXA) is an amino acid derivative of synthetic lysine that has been found in numerous studies to minimise bleeding and blood transfusions after total knee arthroplasty [7, 8]. The use of spinal anaesthesia in total knee arthroplasty has been associated with lower complication rates [9]. However, the relationship between these benefits and the type of anaesthetic should be studied further. The purpose of this study is to determine whether the local use of TXA during total knee arthroplasty (TKA) under various forms of anaesthesia leads to significantly different outcomes in terms of bleeding volume and transfusion requirements.

Materials and methods

The patient information from the hospital's data system is for patients who underwent total knee arthroplasty due to gonarthrosis between January 2017 and January 2023. Permission was received for the study from the Health Sciences University Şişli Hamidiye Etfal Training and Research Hospital SUAM Clinical Research Ethics Committee (permission date: 13/06/2023, and permission number: 3971). The investigation was conducted at a single location and followed a retrospective technique.

The demographic characteristics of patients will be reviewed retrospectively. The study comprised individuals aged 60 to 90 who received total knee arthroplasty for primary gonarthrosis. During surgery, patients were separated into two groups: general anaesthesia and spinal anaesthesia. In addition, patients were separated into two subgroups: those who got 1 g of local tranexamic acid during surgery and those who did not. Tranexamic acid administration was performed locally as 1 g injection into the surrounding soft tissues after cemented total knee arthroplasty. The incision site was then sutured according to the anatomical plan by applying a surgical drain. Intravenous tranexamic acid was not administered to the patients. Patients under 60 and over 90 who had received total knee arthroplasty were eliminated from the research. Patients who underwent revision arthroplasty due to reasons such as infection, trauma, and aseptic loosening were eliminated from the research. Additionally, patients with rheumatologic and systemic diseases involving joint issues were also dismissed from the research. The preoperative and postoperative hemogram results, the amount of bleeding from drains, the drain durations, and the blood transfusion requirements of patients who had total knee arthroplasty surgery in our clinic will be compared. In total knee arthroplasty, the effect of local tranexamic acid on bleeding and the requirement for transfusions in the postoperative phase will be studied in connection with the present anaesthesia type. Patients undergoing total knee arthroplasty had their hemograms taken preoperatively and on the first, third, and fifth days following surgery.

A tourniquet was applied to all patients during the surgery. Surgical drains were applied to all patients, and the volumes of drainage were recorded from medical records. The surgical drains were extracted at the 24th postoperative hour. Bleeding volumes from the drain and transfusion amounts were obtained from patient records. Two methods were evaluated in the study. Group 1: 1 g of tranexamic acid was applied locally to the knee joint after cemented TKA with general anesthesia. Group 2: local tranexamic acid was not applied to the knee joint after cemented TKA with general anesthesia. Group 3: 1 g of tranexamic acid was applied locally to the knee joint after cemented TKA with

spinal anesthesia. Group 4: Local tranexamic acid was not applied to the knee joint after cemented TKA with spinal anaesthesia.

Statistical analysis

The study's findings were analysed using the IBM SPSS Statistics 22 (IBM SPSS, Türkiye) statistical analysis programme. The Shapiro-Wilk test was used to determine whether the parameters had a normal distribution. A One-Way ANOVA test was employed to compare statistical methods such as mean, standard deviation, and frequency, as well as groups with normally distributed parameters, in the study data evaluation. For comparisons between groups with non-normally distributed parameters, the Kruskal-Wallis test and Dunn's test were utilised to identify the group responsible for the differences. The Wilcoxon signed-rank test was used to analyse non-normally divided parameters within groups. To analyse qualitative data, we employed the Fisher-Freeman-Halton and Chi-square tests. A *p*-value of <0.05 indicated the significance level.

Results

The research included 95 patients who underwent TKA between January 2017 and January 2023. The patients were between 50 and 89 years of age, with an average of 67.78±8.23. 17.9% of cases were male, and 82.1% were female. 51.57% of patients have been operated on with gonarthrosis on the right and 48.42% on the left (Table 1). In the study, they were examined in 4 groups: 15 patients (15.8%) in Group 1, 11 patients (11.6%) in Group 2, 33 patients (34.7%) in Group 3, and 36 patients (37.9%) in Group 4. The first 24 hours of haemoglobin (HG) decrease (the change in HG1 compared to HG0) ranges from -0.1 to 4.8 g/dl, with an average of 1.69±1.13 g/dl and a median of 1.5 g/dl. Total HG decrease (variation in HG4 compared to HG0) ranges from 0 to 5.7 g/dl, with an average of 2.81±1.33 g/dl and a median of 2.9 g/dl. (Table 2). The blood loss from the drain ranges from 40 to 220 ml, with an average of 95.11±32.55 ml and a median of 90 ml. 50.5% of patients were given peroperative local tranexamic acid, while 49.5% were not given tranexamic acid. 27.4% of the patients received general anaesthesia, and 72.6% test spinal anaesthesia. In 54.7% of patients, no blood replacement was performed; in 22.1%, 1 unit; in 21.1%, 2 units; and in 2.1%, 3 units (Table 3). There were no statistically notable variations between the groups in terms of average age or gender distribution (*p*:0.678; *p*:0.150)

Table 1. Distribution of general features

		Min-Max	Mean±Sd
Age		50-89	68.78±8.23
		n	**%**
Gender	**Male**	17	17.9
	Female	78	82.1
Side	**Right**	49	51.57
	Left	46	48.42

SD: Standard deviation

Table 2. Information on HG levels

	Min-Max	Mean±Sd (median)
HG0	10-15.8	13.11±1.18 (13.1)
HG1	8.6-15.2	11.42±1.3 (11.4)
HG3 (n=94)	8.2-14.3	10.5±1.38 (10.2)
HG5 (n=90)	7.6-14.2	10.25±1.27 (10.1)
HG decreases in the first 24 hours	(-0.1)-4.8	1.69±1.13 (1.5)
Total HG decrease	0-5.7	2.81±1.33 (2.9)

SD: Standard deviation

Table 3. Distribution of operating parameters

		Min-Max	Mean±Sd (median)
The amount of the drain (ml)		40-220	95.11±32.55 (90)
		n	**%**
Tranexanic acid usage	**TXA unused**	47	49.5
	TXA used	48	50.5
Type of Anesthesia	**General**	26	27.4
	Spinal	69	72.6
Blood replacement	**0**	52	54.7
	1	21	22.1
	2	20	21.1
	3	2	2.1

SD: Standard deviation

Furthermore, there was little change in the distribution of operated sides across Groups 1-3, and 4 (*p*:0.770) (Table 4).

There were substantial distinctions between groups in terms of HG1 (haemoglobin control value at 24 hours) (*p*=0.002). Binary comparisons revealed that Group 4 had significantly lower HG1 values compared to Groups 1-3 (*p*1:0.018; *p*2:0.046; *p*3:0.000*). Nevertheless, there were not any significant differences in HG1 values between the other groups (*p*1:0.144; *p*2:0.713; *p*3:0.671, *p*>0.05) (Table 5, Table 5.1). There was no statistically important difference between Groups 1-4 in terms of HG3 (haemoglobin control value on day 3) (*p*:0.117). There were important distinctions comparing each group in terms of HG5 (the 5th day haemoglobin control value) (*p*=0.010). Binary comparisons revealed that Group 3 had considerably higher HG5 values than Groups 1, 2, and 4 (*p*1:0.019, *p*2:0.003). There is not a significant variation in HG5 readings between the other groups (*p*1:0.224; *p*2:0.195; *p*3:0.329; *p*4:0.536 *p*>0.05) (Table 5.1). After the Bonferroni correction, the significance level for pairwise comparisons within the groups was set at *p*=0.0167 (Table 5). In Groups 1, 3, and 4, the decreases observed in HG1, HG3, and HG5 levels compared to the HG0 values were found to be statistically significant (Group 1: HG0-HG1 *p*:0.001*, HG0-HG3 *p*:0.001*, HG0-HG5 *p*:0.001*; Group 3: HG0-HG1 *p*:0.001*, HG0-HG3 *p*:0.001*, HG0-HG5 *p*:0.001*; Group 4: HG0-HG1 *p*:0.001*, HG0-HG3 *p*:0.001*, HG0-HG5 *p*:0.001*; *p*=0.0167). In Group 2, the decreases observed in HG1, HG3, and HG5 levels compared to HG0 values were statistically significant (*p*1:0.004*; *p*2:0.009*; *p*=0.0167). However, the decrease observed at the HG5 level compared to HG0 was not statistically significant (*p*:0.018; *p*=0.0167) (Table 5, Figures 1 and 2).

The first 24-hour decreases in haemoglobin (HG) levels differed significantly between groups (*p*=0.000). Comparisons were made to determine from which group the differences stem. In group 4, the first 24 hours of HG decreases were statistically significantly higher compared to Groups 1-3 (*p*1:0.001; *p*2:0.000; *p*3:0.001). The first 24-hour haemoglobin (HG) declines did not differ significantly from the other groups (*p*1:0.632; *p*2:0.567; *p*3:0.290; *p*>0.05) (Table 6, Table 6.1).

There are numerically significant variations between the groups in terms of total haemoglobin (HG) reductions (*p*=0.007). Pairwise comparisons revealed that Group 4 experienced a substantially larger total HG drop than Groups 1-3 (*p*1:0.002; *p*2:0.014). Although no statistically significant difference in overall HG reduction values was seen between the groups (*p*1:0.144; *p*2:0.776; *p*3:0.103; *p*4:0.158 *p*>0.05) (Table 6.1).

Table 4. Evaluation of general characteristics between groups

	General anesthesia Txa used Group 1	General anesthesia Txa unused Group 2	Spinal anesthesia Txa used Group 3	Spinal anesthesia Txa unused Group 4	Test Name	Test	*p*
	(Min-Max)-(Median±SD)	**(Min-Max)-(Median±SD)**	**(Min-Max)-(Median±SD)**	**(Min-Max)-(Median±SD)**			
Age	(57-84)-(68.87±7.36)	(57-76)-(66.36±6.34)	(55-89)-(69.85±9.06)	(50-86)-(68.5±8.43)	**F**	0.507	[a]0.678
	n (%)	**n (%)**	**n (%)**	**n (%)**			
Gender							
Male	1 (6.7%)	0 (0%)	9 (27.3%)	7 (19.4%)	**χ^2**	5.158	[b]0.150
Female	14 (93.3%)	11 (100%)	24 (72.7%)	29 (80.6%)			
Side							
Right	11 (73.33%)	5 (45.45%)	16 (48.48%)	17 (47.22%)	**χ^2**	4.108	[c]0.770
Left	4 (26.66%)	6 (54.54%)	17 (51.51%)	19 (52.77%)			

[a]One-Way Anova Test, [b]Fisher Freeman Halton Test, [c]Chi-Square analysis test, *p<0.05

Table 5. Evaluation of Haemoglobin levels between groups

	General anesthesia Txa used Group 1	General anesthesia Txa unused Group 2	Spinal anesthesia Txa used Group 3	Spinal anesthesia Txa unused Group 4	χ^2 (Kruskal-Wallis H)	p[a]
	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))		
HG0	(10.9-14.9)-(12.9±1.1 (12.9))	(10.5-13.3)-(12.08±1.02 (12))	(10.9-15.8)-(13.36±1.18 (13.4))	(10-15.6)-(13.29±1.11 (13.2))	10.355	**0.016***
HG1	(10-13.3)-(11.72±0.88 (11.9))	(9.4-12.7)-(11.01±1.14 (11))	(8.6-15.2)-(11.95±1.29 (12))	(8.9-14.3)-(10.95±1.31 (10.8))	14.405	**0.002***
HG3	(8.6-13.2)-(10.74±1.44 (10.5))	(8.8-12.8)-(10.48±1.51 (9.9))	(9.2-14.3)-(10.79±1.31 (10.3))	(8.2-13.5)-(10.16±1.37 (9.9))	5.888	**0.117**
HG5	(8.8-12.2)-(10.19±1 (10.3))	(7.7-12.2)-(9.63±1.47 (9.6))	(9.5-14.2)-(10.8±1.2 (10.5))	(7.6-13.4)-(9.9±1.25 (10))	11.250	**0.010***
HG0-HG1 p[b]	0.001*	0.004*	0.000*	0.000*		
HG0-HG3 p[b]	0.001*	0.009*	0.000*	0.000*		
HG0-HG5 p[b]	0.001*	0.018* +	0.000*	0.000*		

[a]Kruskal-Wallis Test, [b]Wilcoxon Signed Ranks Test, *p<0.05

+ The significance level for pairwise comparisons within the group was set at p<0.0167 after Bonferroni correction.

After Bonferroni correction, the decrease observed at the HG5 level compared to HG0 was not statistically significant in the General Anesthesia Txa unused (Group 2), (p=0.018; p>0.0167)

Table 5.1. Post-Hoc Table

	Post-Hoc					
	HG0		HG1		HG5	
	Dunn Testi	*p*	Dunn Testi	*p*	Dunn Testi	*p*
Group 1-Group 2	18.524	0.090	15.970	0.144	14.679	0.224
Group 1-Group 3	-10.885	0.204	-3.152	0.713	-10.789	0.195
Group 1-Group 4	-7.733	0.361	20.000	0.018*	8.018	0.329
Group 2-Group 3	-29.409	0.002*	-19.121	0.046*	-25.468	0.019*
Group 2-Group 4	-26.258	0.006*	4.030	0.671	-6.661	0.536
Group 3-Group 4	3.152	0.635	23.152	0.000*	18.807	0.003*

*$p<0.05$

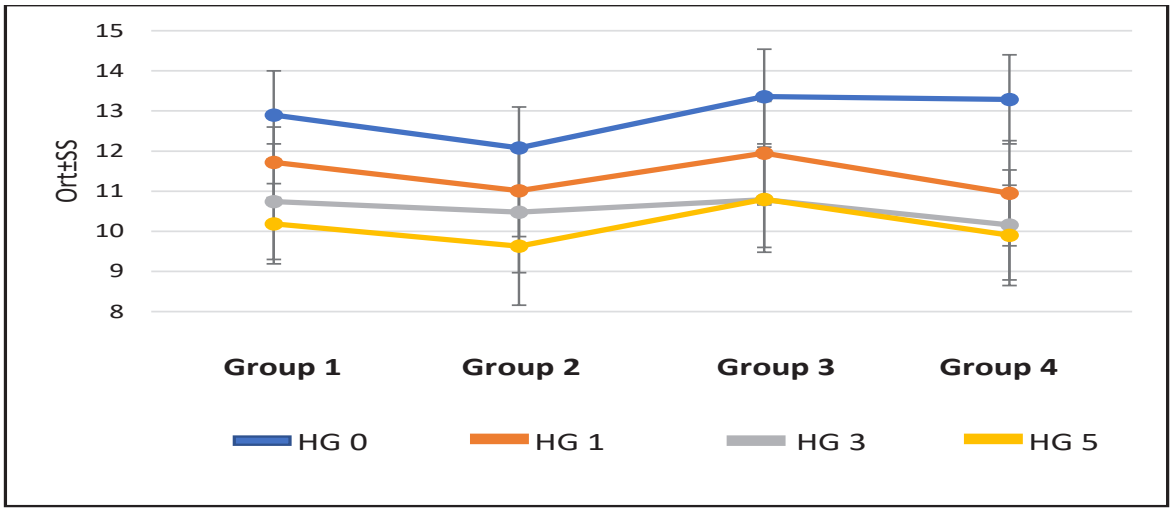


Figure 1. Comparison of haemoglobin amounts in the preoperative and postoperative periods between groups

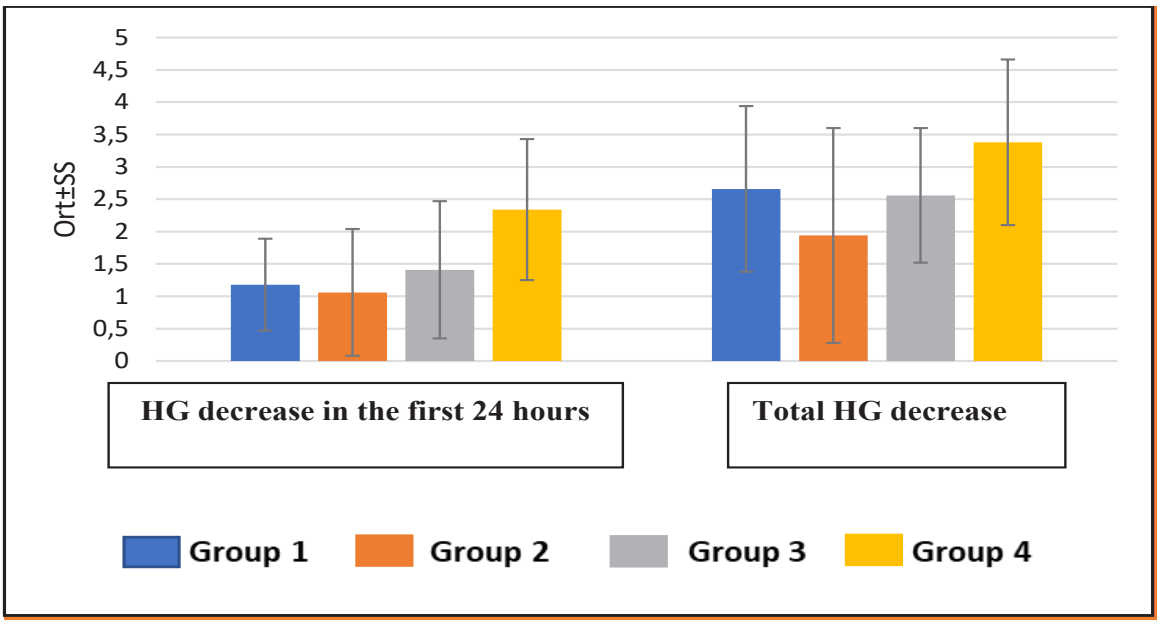


Figure 2. Comparison of the first 24 hours and total haemoglobin decrease between groups

Table 6. Evaluation of first 24 hours and total Hg decreases between groups

	General anesthesia Txa used Group 1	General anesthesia Txa unused Group 2	Spinal anesthesia Txa used Group 3	Spinal anesthesia Txa unused Group 4	X^2 (Kruskal-Wallis H)	*p* [a]
	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))		
HG decrease in the first 24 hours	(0.1-2.5)-(1.18±0.71 (1.1))	(-0.1-3.6)-(1.06±0.98 (1))	(0.1-4.8)-(1.41±1.06 (1.3))	(0.6-4.7)-(2.34±1.09 (2.3))	21.301	0.000*
Total HG decrease	(0.1-4.4)-(2.66±1.28 (2.6))	(0-5.4)-(1.94±1.66 (1.1))	(0.4-4.6)-(2.56±1.04 (2.7))	(1.1-5.7)-(3.38±1.28 (3.3))	12.182	0.007*

[a]Kruskal-Wallis Test, *p<0.05

Table 6.1. Post-Hoc table

	Post-Hoc			
	First 24-hour HG decrease		Total HG decrease	
	Dunn Test	***p***	**Dunn Test**	***p***
Group 1 - Group 2	5.242	0.632	15.967	0.144
Group 1 - Group 3	-4.909	0.567	2.436	0.776
Group 1 - Group 4	-27.889	0.001*	-13.530	0.103
Group 2 - Group 3	-10.152	0.290	-13.797	0.158
Group 2 - Group 4	-33.131	0.000*	-29.764	0.002*
Group 3 - Group 4	-22.980	0.001*	-16.234	0.014*

*$p<0.05$

Drain values differed significantly between groups (p=0.000*). Pairwise comparisons were used to identify the source of these differences. Drain values in group 4 were substantially higher compared to Groups 1-3 (p=0.000*). Conversely, in group 1, the drain values were statistically significantly lower than in Groups 2-4 (p1:0.001; p2:0.032; $p3$:0,000*). Drain values did not differ significantly across the other groups (p1:0.060; p2:0.135; p>0.05) (Table 7, Table 7.1).

There were substantial differences in blood replacement distribution rates between the groups (p=0.002). Pairwise comparisons were used to identify the source of these differences. Group 3 had significantly lower blood replacement rates compared to Group 1 (26.7%), Group 2 (45.5%), and Group 4 (25%) at a replacement level of 2 units (6.1%) (p1:0.002; p2:0.000). There were insufficiently significant variations in blood replacement distribution rates among the other groups (p1:0.128; p2:0.143; p3:0.166; p4:0.682 p>0.05) (Table 7.1).

Table 7. Evaluation of the amount of blood coming from the drain and blood replacement parameters between groups

	General anesthesia Txa used Group 1	General anesthesia Txa unused Group 2	Spinal anesthesia Txa used Group 3	Spinal anesthesia Txa unused Group 4	χ^2 (Kruskal-Wallis H)	*p*
	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))	(Min-Max)-(Median±SD (median))		
Amount of drain	(40-150)-(66±26.61 (60))	(70-200)-(106.36±40.38 (90))	(50-220)-(84.24±28.59 (80))	(85-160)- (113.75±21.86 (112.5))	44.389	[a] 0.000*
	n (%)	**n (%)**	**n (%)**	**n (%)**	χ^2	
Amount of blood replacement					16.177	[b] 0.002*
0	10 (66.7%)	3 (27.3%)	27 (81.8%)	12 (33.3%)		
1	1 (6.7%)	3 (27.3%)	4 (12.1%)	13 (36.1%)		
2	4 (26.7%)	5 (45.5%)	2 (6.1%)	9 (25%)		
3	0 (0%)	0 (0%)	0 (0%)	2 (5.6%)		

[a] Kruskal-Wallis Test, [b]Chi-Square analysis test, *p<0.05

Table 7.1. Post-Hoc table

	Post-Hoc p- value			
	Amount of drain		Amount of blood replacement	
	Dunn Test	***p***	**x^2**	***p***
Group 1 - Group 2	-36.336	0.001*	4.217	0.128
Group 1 - Group 3	-18.321	0.032*	3.727	0.143
Group 1 - Group 4	-50.478	0.000*	6.546	0.066
Group 2 - Group 3	18.015	0.060	11.867	0.002*
Group 2 - Group 4	-14.141	0.135	1.741	0.682
Group 3 - Group 4	-32.157	0.000*	16.392	0.000*

*$p<0.05$

Discussion

The results of our study showed a significant decrease in haemoglobin levels in the first twenty-four hours after surgery in the group that underwent total knee arthroplasty with spinal anaesthesia and did not receive local tranexamic acid (Group 4). There was no significant difference between the groups in haemoglobin control values on the third day. Postoperative haemoglobin values in the group that underwent total knee arthroplasty with spinal anaesthesia and local tranexamic acid was not administered (group 3) were statistically significantly higher than the other groups. Group 4, in which total knee arthroplasty was performed with spinal anaesthesia and local tranexamic acid was not administered, had significantly higher drain volumes compared to Groups 1-3. The drain volumes in group 1 were statistically significantly lower than the other groups. When the 2 units (6.1%) replacement amount was evaluated, blood replacement rates in group 3 were significantly lower than the other groups.

Total knee arthroplasty (TKA) serves as an effective solution for restoring function and alleviating pain in patients with advanced arthritis. It is estimated that approximately 700,000 cases are documented annually in the United States [10]. As the number of total knee arthroplasty procedures increases, various strategies are being explored to mitigate bleeding-related complications. Among these strategies, studying the relationship between the use of tranexamic acid and the type of anaesthesia is likely to help progress these approaches.

Studies in the literature have revealed that women have a higher incidence and that the prevalence increases with age [11-13]. In our study, 82.1% of the patients were female, which aligns with findings in the literature indicating a higher prevalence of total knee arthroplasty among women. Pierson et al. [14] reported that patients who underwent TKA had an average decrease in haemoglobin levels of 3.8 g/dL. In our study, changes in the patients' haemoglobin values on the 1st, 3rd, and 5th days were evaluated. The average haemoglobin (HG) decrease in the first 24 hours is 1.69±1.13 g/dl. The average total HG reduction was 2.81±1.33 g/dl. We examined the dynamics of haemoglobin decline and postoperative bleeding following total knee arthroplasty (TKA). The data demonstrated that a significant amount of total haemoglobin drop occurred within the first 24 hours after surgery, highlighting the importance of early postoperative blood loss. This finding is consistent with prior studies, which found a considerable drop in haemoglobin levels within the first three days after TKA [15, 16].

In our study, haemoglobin levels dropped dramatically in the first few days, and the average haemoglobin values on the fifth day were comparable to those on the third day. In our study, the haemoglobin decrease in the early period (first day) after total knee arthroplasty was highest in Group 4. In our opinion, the main explanation for this could be a lack of local tranexamic acid administration.

Another possibility is that spinal anaesthesia may have a longer-term effect on peripheral vascular dilatation. Sympathetic nervous system blockade after spinal anaesthesia application It causes hypotension by reducing venous return and systemic vascular resistance [17]. Venous dilatation is more effective than arterial dilatation in the formation of hypotension [18]. However, no significant difference in hemoglobin decline was observed between the groups by the 3rd postoperative day. Interestingly, haemoglobin values were notably higher in Group 3 by the 5th postoperative day, suggesting reduced blood loss compared to other groups in the late postoperative phase. In group 1, TKA was administered under general anaesthesia, while Group 3 had spinal anaesthesia. Tranexamic acid was administered to both groups. However, the amount of bleeding in Group 3 on the 5th postoperative day was significantly lower than in Group 1, suggesting that spinal anesthesia may have an effect on bleeding in the late period. A definitive judgement cannot be reached. However, the effect of anaesthesia type on late bleeding in total knee arthroplasty needs to be investigated with further studies. Additionally, when the preoperative haemoglobin amounts were compared with the postoperative 1st, 3rd, and 5th days in each group, the haemoglobin amounts decreased significantly. There are studies in the literature supporting that haemoglobin values decrease for more than 3 days after total knee arthroplasty [19].

Blood loss from the drain ranged between 40 and 220 ml, with a mean of 95.11±32.55 ml. The quantity of blood emanating from the drain was observed to be consistent with what is reported in the literature [20]. Our study compared the amount of bleeding from the drain. There were statistically significant variations between the groups in the drain values. The amount of bleeding from the drain in group 4 was statistically significantly higher compared to Groups 1-3. In Group 1, the amount of bleeding from the drain was statistically significantly lower than in Groups 2, 3, and 4. The literature supports that the admission of tranexamic in total knee arthroplasty reduces the amount of bleeding [7, 21-23]. The amounts of blood coming from the drain between Groups 1 and 3, where tranexamic acid was applied, and Groups 2 and 4, where tranexamic acid was not applied, are significantly different. It shows that the type of anaesthesia is also effective, as is the effect of tranexamic acid.

In one of the oldest systematic reviews, Rodgers et al. [24] study evaluating complications related to general anesthesia and regional anaesthesia found that regional anaesthesia had a lower transfusion requirement. Our study also evaluated the amount of blood transfusion between groups. The rate of 2 units of blood transfusion was significantly lower in Group 3, where spinal anaesthesia and local tranexamic acid were administered.

This significant value suggests that tranexamic acid and spinal anaesthesia have a bleeding-reducing effect. In a meta-analysis comprising 12 studies with 1189 patients, Yue et al. [25]. They found that local tranexamic acid (TXA) decreased blood loss by an average of 280.65 mL, leading to a decrease in the need for blood transfusion. In the study by Wei et al. [26], regional anaesthesia was associated with a decreased likelihood of perioperative blood transfusion compared to combined anaesthesia and general anaesthesia. However In the study of Lee et al. [27], they did not find any difference in transfusion rates between patients receiving general and spinal anaesthesia during total knee arthroplasty.

In our study, the transfusion rates in group 4, where spinal anaesthesia was administered without local tranexamic acid, were found to be comparable to the other groups where general anaesthesia was utilised. Our study's weaknesses include its limited sample size and retrospective nature. The study's strength is that it examines the effect of local tranexamic acid usage on bleeding in total knee arthroplasty against general or spinal anaesthesia.

The findings of this study hold potential significance for clinical practice. They are consistent with prior studies demonstrating the usefulness of local tranexamic acid administration in minimising bleeding and the need for blood transfusions in total knee arthroplasty. However, more research is needed to determine the effect of anaesthesia types in relation to local TXA on bleeding in total knee arthroplasty.

In conclusion, our study emphasises the importance of local tranexamic acid administration in managing blood loss during TKA, particularly in mitigating early postoperative decreases in haemoglobin and reducing the need for blood transfusions. Understanding the interplay of anaesthesia types and tranexamic acid application contributes valuable insights to optimising perioperative care in TKA procedures.

Funding: None.

Authors contributions: B.B. created the main idea and hypothesis of the study. B.B. and E.I. developed the theory and edited the materials and methods section. I.T. evaluated the data in the results section. Discussion section of the article was written by B.B. Reviewed, edited and approved by E.I. Additionally, all authors discussed the entire study and approved the final version.

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

References

1. Hart A, Khalil JA, Carli A, Huk O, Zukor D, Antoniou J. Blood transfusion in primary total hip and knee arthroplasty. Incidence, risk factors, and thirty-day complication rates. *J Bone Joint Surg Am*. 2014;96(23):1945-1951. doi:10.2106/JBJS.N.00077
2. Kato H, Nakayama T, Uruma M, et al. A retrospective observational study to assess adverse transfusion reactions of patients with and without prior transfusion history. *Vox Sang*. 2015;108(3):243-250. doi:10.1111/vox.12208
3. Bernard AC, Davenport DL, Chang PK, Vaughan TB, Zwischenberger JB. Intraoperative transfusion of 1 U to 2 U packed red blood cells is associated with increased 30-day mortality, surgical-site infection, pneumonia, and sepsis in general surgery patients. *J Am Coll Surg*. 2009;208(5):931-939. doi:10.1016/j.jamcollsurg.2008.11.019
4. Browne JA, Adib F, Brown TE, Novicoff WM. Transfusion rates are increasing following total hip arthroplasty: risk factors and outcomes. *J Arthroplasty*. 2013;28(8 Suppl):34-37. doi:10.1016/j.arth.2013.03.035
5. Banerjee S, Issa K, Kapadia BH, et al. Intraoperative nonpharmacotherapeutic blood management strategies in total knee arthroplasty. *J Knee Surg*. 2013;26(6):387-393. doi:10.1055/s-0033-1353993
6. Chareancholvanich K, Siriwattanasakul P, Narkbunnam R, Pornrattanamaneewong C. Temporary clamping of drain combined with tranexamic acid reduce blood loss after total knee arthroplasty: a prospective randomized controlled trial. *BMC Musculoskelet Disord*. 2012;13:124. Published 2012 Jul 20. doi:10.1186/1471-2474-13-124
7. Fillingham YA, Ramkumar DB, Jevsevar DS, et al. The Efficacy of Tranexamic Acid in Total Knee Arthroplasty: A Network Meta-Analysis. *J Arthroplasty*. 2018;33(10):3090-3098.e1. doi:10.1016/j.arth.2018.04.043
8. Cam N, Balkanli B, Altuntas Y, Kanar M, Ozdemir HM. The Effect of Local Tranexamic Acid Administration on Blood Loss and Transfusion in Total Knee Arthroplasty: A Retrospective Study. *Sisli Etfal Hastan Tip Bul*. 2023;57(2):245-249. Published 2023 Jun 20. doi:10.14744/SEMB.2023.42800
9. Heckmann ND, De A, Porter KR, Stambough JB. Spinal Versus General Anesthesia in Total Knee Arthroplasty: Are There Differences in Complication and Readmission Rates?. *J Arthroplasty*. 2023;38(4):673-679.e1. doi:10.1016/j.arth.2022.10.036
10. Good L, Peterson E, Lisander B. Tranexamic acid decreases external blood loss but not hidden blood loss in total knee replacement. *Br J Anaesth.* 2003;90:596-599. https://doi.org/10.1093/bja/aeg111
11. Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum*. 1987;30(8):914-918. doi:10.1002/art.1780300811
12. Driban JB, Bannuru RR, Eaton CB, et al. The incidence and characteristics of accelerated knee osteoarthritis among women: the Chingford cohort. *BMC Musculoskelet Disord*. 2020;21(1):60. Published 2020 Jan 31. doi:10.1186/s12891-020-3073-3
13. Hong JW, Noh JH, Kim DJ. The prevalence of and demographic factors associated with radiographic knee osteoarthritis in Korean adults aged ≥ 50 years: The 2010-2013 Korea National Health and Nutrition Examination Survey. *PLoS One*. 2020;15(3):e0230613. Published 2020 Mar 20. doi:10.1371/journal.pone.0230613
14. Pierson JL, Hannon TJ, Earles DR. A blood-conservation algorithm to reduce blood transfusions after total hip and knee arthroplasty. *J Bone Joint Surg Am*. 2004;86(7):1512-1518. doi:10.2106/00004623-200407000-00022
15. Kwon JB, Choi JH, Che SH, Choi WK. The natural course of hemoglobin levels after allogenic blood transfusion in total knee arthroplasty. *Medicine (Baltimore)*. 2023;102(12):e33387. doi:10.1097/MD.0000000000033387

16. Cho MR, Jun CM, Song SK, Choi WK. Natural course of hemoglobin level after total knee arthroplasty and the benefit of tranexamic acid injection in the joint. *Medicine (Baltimore)*. 2021;100(35):e27097. doi:10.1097/MD.0000000000027097
17. Hartmann B, Junger A, Klasen J, et al. The incidence and risk factors for hypotension after spinal anesthesia induction: an analysis with automated data collection. *Anesth Analg*. 2002;94(6):. doi:10.1097/00000539-200206000-00027
18. Collins VJ. Principles of Anesthesiology: General and Regional Anesthesia. 3rd ed. Lea & Febiger; 1993:1445-1497, 1498-1512.
19. Schatz C, Plötz W, Beckmann J, Bredow K, Leidl R, Buschner P. Associations of preoperative anemia and postoperative hemoglobin values with hospital costs in total knee arthroplasty (TKA). *Arch Orthop Trauma Surg*. 2023;143(11):6741-6751. doi:10.1007/s00402-023-04929-4
20. Zhang Y, Zhang JW, Wang BH. Efficacy of tranexamic acid plus drain-clamping to reduce blood loss in total knee arthroplasty: A meta-analysis. *Medicine (Baltimore)*. 2017;96(26):e7363. doi:10.1097/MD.0000000000007363
21. Bemelmans Y, Van Haaren E, Boonen B, Hendrickx R, Schotanus M. Low blood transfusion rate after implementation of tranexamic acid for fast- track hip- and knee arthroplasty. An observational study of 5205 patients. *Acta Orthop Belg*. 2021;87(1):9-16.
22. Franchini M, Mengoli C, Marietta M, et al. Safety of intravenous tranexamic acid in patients undergoing majororthopaedic surgery: a meta-analysis of randomised controlled trials. *Blood Transfus*. 2018;16(1):36-43. doi:10.2450//2017.0219-17
23. Poeran J, Rasul R, Suzuki S, et al. Tranexamic acid use and postoperative outcomes in patients undergoing total hip or knee arthroplasty in the United States: retrospective analysis of effectiveness and safety. *BMJ*. 2014;349:g4829. Published 2014 Aug 12. doi:10.1136/bmj.g4829
24. Rodgers A, Walker N, Schug S, et al. Reduction of postoperative mortality and morbidity with epidural or spinal anaesthesia: results from overview of randomised trials. *BMJ*. 2000;321(7275):1493. doi:10.1136/bmj.321.7275.1493
25. Yue C, Pei F, Yang P, Xie J, Kang P. Effect of Topical Tranexamic Acid in Reducing Bleeding and Transfusions in TKA. *Orthopedics*. 2015;38(5):315-324. doi:10.3928/01477447-20150504-06
26. Wei C, Muthiah A, Gu A, et al. Association of Anesthesia Type with Postoperative Outcome and Complications in Patients Undergoing Revision Total Knee Arthroplasty. *J Knee Surg*. 2022;35(4):345-354. doi:10.1055/s-0040-1713776
27. Lee S, Kim MK, Ahn E, Jung Y. Comparison of general and regional anesthesia on short-term complications in patients undergoing total knee arthroplasty: A retrospective study using national health insurance service-national sample cohort. *Medicine (Baltimore)*. 2023;102(8):e33032. doi:10.1097/MD.0000000000033032

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1516784

Investigation of the effect of low back pain severity on functional level, quality of life and fear-avoidance behavior in women with hip osteoarthritis

Kalça osteoartritli kadınlarda bel ağrısı şiddetinin fonksiyonel düzey, yaşam kalitesi ve korku kaçınma davranışına etkisinin incelenmesi

Sümena Hareket, Emine Aslan Telci, Nihal Büker, Nusret Ök, Hande Şenol

Posted date:17.07.2024 Acceptance date:24.11.2024

Abstract

Purpose: The objective of the this study was to investigate the effect of low back pain (LBP) severity on functional level, quality of life (QoL), and fear-avoidance behavior in patients with hip osteoarthritis (HOA).

Material and methods: A cross-sectional study conducted on a total of 43 female patients diagnosed with HOA. The patients were divided into two groups according to pain intensity; Group 1 consisted of patients with moderate-to-high intensity low back pain (VAS:3.5-10 cm; n=21), Group 2 consisted of patients with mild back pain (VAS:0.5-3.49 cm; n=22) occurred. Functional level (Lumbar Spine Mobility, Harris Hip Score, Timed Up and Go Test), quality of life (Nottingham Health Profile) and kinesiophobia (Tampa Scale of Kinesiophobia) were evaluated.

Results: When the groups were compared, it was found that the QoL, QoL-pain and emotional reactions sub-parameters scores of Group 1 were significantly higher than Group 2 (p=0.001, p=0.002, p=0.002). However, there was no statistically significant difference between the two groups in terms of functional level and kinesiophobia (p>0.05).

Conclusion: The results of the study showed that the QoL-pain and emotional reaction sub-parameters and QoL were negatively affected in patients with moderate-high severity of LBP in HOA in comparison with mild severity of LBP.

Keywords: Hip osteoarthritis, low back pain, quality of life, pain, assessment.

Hareket S, Aslan Telci E, Buker N, Ok N, Senol H. Investigation of the effect of low back pain severity on functional level, quality of life and fear-avoidance behavior in women with hip osteoarthritis. Pam Med J 2025;18:272-280.

Öz

Amaç: Bu çalışmanın amacı kalça osteoartritli (KOA) hastalarda bel ağrısı şiddetinin fonksiyonel düzey, yaşam kalitesi ve korku kaçınma davranışı üzerine etkisini incelemektir.

Gereç ve yöntem: Yapılan kesitsel çalışmaya KOA tanısı konmuş toplam 43 kadın hasta dahil edilmiştir. Hastalar ağrı şiddetine göre iki gruba ayrılmış; Grup 1 orta-yüksek şiddette bel ağrısı olan hastalardan (VAS:3,5-10 cm; n=21), Grup 2 ise hafif bel ağrısı olan hastalardan (VAS:0,5-3,49 cm; n=22) oluşturulmuştur. Fonksiyonel düzey (Lumbar Bölge Hareketliliği, Harris Kalça Skoru, Zamanlı Kalk ve Yürü Testi), yaşam kalitesi (Nottingham Sağlık Profili) ve kinezyofobi (Tampa Kinezyofobi Ölçeği) değerlendirilmiştir.

Bulgular: Gruplar karşılaştırıldığında Grup 1'in yaşam kalitesi, yaşam kalitesi-ağrı ve emosyonel reaksiyonlar alt parametre skorlarının Grup 2'ye göre anlamlı derecede yüksek olduğu görüldü (p=0,001, p=0,002, p=0,002). Ancak fonksiyonel düzey ve kinezyofobi açısından iki grup arasında istatistiksel olarak anlamlı fark yoktu (p>0,05).

Sonuç: Çalışmanın sonuçları, KOA'da orta-yüksek şiddette bel ağrısı olan hastalarda, hafif şiddette bel ağrısına kıyasla yaşam kalitesi-ağrı ve emosyonel reaksiyonlar alt parametrelerinin ve yaşam kalitesinin olumsuz etkilendiğini göstermiştir.

Anahtar kelimeler: Kalça osteoartriti, bel ağrısı, yaşam kalitesi, ağrı, değerlendirme.

Hareket S, Aslan Telci E, Büker N, Ök N, Şenol H. Kalça osteoartritli kadınlarda bel ağrısı şiddetinin fonksiyonel düzey, yaşam kalitesi ve korku kaçınma davranışına etkisinin incelenmesi. Pam Tıp Derg 2025;18:272-280.

Sümena Hareket, M.D. Institute of Health Sciences, Department of Physical Therapy and Rehabilitation, Katip Çelebi University, İzmir, Türkiye, e-mail: sumenahareket4@gmail.com (https://orcid.org/0000-0002-2072-8731) (Corresponding Author)

Emine Aslan Telci, Prof. Faculty of Physical Therapy and Rehabilitation, Department of Physical Therapy and Rehabilitation, Pamukkale University, Denizli, Türkiye, e-mail: eatelci@pau.edu.tr (https://orcid.org/0000-0003-2749-295X)

Nihal Büker, Prof. Faculty of Physical Therapy and Rehabilitation, Department of Physical Therapy and Rehabilitation, Pamukkale University, Denizli, Türkiye, e-mail: nasuk@pau.edu.tr (https://orcid.org/0000-0001-7259-7983)

Nusret Ök, Assoc. Prof. Medical Faculty, Department of Orthopedics and Traumatology, Pamukkale University, Denizli, Türkiye, e-mail: oknusret@gmail.com (https://orcid.org/0000-0003-3811-1884)

Hande Şenol, Asst. Prof. Medical Faculty, Department of Biostatistics, Denizli, Türkiye, e-mail: Hande Şenol (https://orcid.org/0000-0001-6395-7924)

Introduction

Hip osteoarthritis (HOA) is an important source of morbidity that causes pain, gait abnormalities, and functional disorders [1]. In the literature, hip osteoarthritis is reported to occur particularly after the age of 50, with a prevalence of approximately 10%, and it is predicted to increase [2-4]. The prevalence of HOA is higher in women than in men after 50 years of age [5].

Symptomatic HOA, which significantly impairs both quality of life (QoL) and daily activities, frequently coexists with lumbar spine issues, even when occurring in isolation [6]. Individuals who experience low back pain (LBP) throughout their lives often encounter a decline in their QoL due to diminished physical, mental, and social functioning [7]. The perception of pain is closely linked to fear-avoidance beliefs [8]. Fear of movement negatively impacts both pain experience and functional impairment in osteoarthritis patients [9]. Furthermore, it has been demonstrated that fear of movement at the onset of LBP serves as a mediator in the correlation between pain severity and functional decline [10].

Patients diagnosed with HOA typically exhibit an abnormal spine-hip relationship [11]. In 1983, Offierski and MacNab introduced the concept of Hip-Spine Syndrome, characterizing the co-occurrence of lumbar spine and hip disorders [12]. Offierski and MacNab proposed that hip flexion deformities lead to gradual anteversion of the pelvis, resulting in compensatory lumbar hyperlordosis and subsequent subluxation of the lumbar posterior facets, thereby eliciting LBP [12]. It is thought that there may be other factors that play a role in the mechanism of LBP secondary to HOA. Severe osteoarthritis of the hip joint, abnormal sagittal spine alignment and disordered balance may cause trudge and are associated with LBP [13].

The aim of the present study was to investigate the effect of LBP severity on functional level, QoL, and fear-avoidance behavior in women with HOA.

Material and methods

Study design and sample

This is a cross-sectional study, and approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (date: 03.08.2017, number: 60116787-020/49863). The study was performed in accordance with the rules of the Declaration of Helsinki. Informed consent forms were obtained from the participants in the study. The study was conducted with 43 female patients who were diagnosed with stage 2 and 3 HOA according to Kellgren-Lawrence Stage on their radiographs taken in the last 6 months between October 2017 and January 2018 at Pamukkale University Department of Orthopedics and Traumatology. Patients were required to have LBP along with HOA. Patients with HOA were divided into 2 study groups according to the severity of non-specific LBP as a result of the evaluation with Visual Analog Scale (VAS) (0-10 cm) [14]. Group 1 included patients with moderate-high LBP (VAS:3.5-10 cm; n=21) and Group 2 included patients with mild LBP (VAS:0.5-3.49 cm; n=22), respectively [15]. The inclusion criteria for both groups, other than having LBP, were patients over 50 years of age, being diagnosed with HOA according to the American College of Rheumatology Criteria, having at least stage 2 according to the Kellgren-Lawrence Staging on radiographs taken in the last 6 months, patients with pain problems around the groin and hip for at least 3 months, and having a hip pain severity of 3.5 cm or higher on the VAS. Patients who had undergone knee and/or hip replacement, had spinal surgery, received physiotherapy for hip, knee, or waist in the last 6 months, had a neurological diagnosis, and had secondary HOA were not included in the assessment.

Sample size

Power analysis results from the study conducted by French et al. [16] (2015) were utilized. According to the study result, they had a large effect size (d=1.45). Assuming we can achieve a lower effect size (d=0.9), a power analysis was performed before the study. Accordingly, when at least 42 participants (21 per each group) were included in the study, that

would result in 80% power with 95% confidence level (5% type 1 error rate). A total of 48 patients were enrolled during the study's duration. Exclusions from the study encompassed one patient who declined to participate, 2 patients who did not complete the assessment, and 1 patient who encountered a health issue during the evaluation. Of the 44 total patients who met criteria, only one was male.

Data collection

The patient information form

For the participants in the study, information regarding age, weight, height, body mass index, and radiological stage of HOA were recorded on a prepared form.

Pain

The duration of hip and LBP (in months) was queried for the patients participating in the study. The severity of both LBP and hip pain for the patients was assessed using the Visual Analog Scale (VAS) [14]. Additionally, participants were asked to specify the duration (in months) of their hip pain complaints.

Functional level

The functional level measurement of the patients was determined by evaluating lumbar region mobility with the Modified Schober Test [17], range of the motion (ROM) of hip [18], Harris Hip Score [19], and performance-based functional mobility measurement with the Timed Up and Go Test [20, 21].

a. The Modified Schober Test (MST)

The MST was employed to assess the active flexion range of motion in the lumbar region [22]. To conduct this assessment, the upper edge of the sacrum was identified and marked over both spina iliaca posterior superior. The marked area was determined to be 5 cm below and 10 cm above the vertical line, resulting in a 15 cm difference. The distance between the two marked points was then measured while patients were instructed to lean forward as far as possible. The increase in the distance between the marks functioned as a quantitative gauge of the normal range of motion for lumbar flexion [17].

b. The Range of Motion of Hip

The range of motion of hip flexion, extension, and abduction was measured using a standard two-arm goniometer [18].

Flexion/in supine position: The pivot point of the goniometer was positioned at the greater trochanter, while the stationary arm was held parallel to the axilla. The flexion value was recorded by following the lateral midline of the femur with the moving arm of the goniometer [23].

Extension/in prone position: The patient was asked to extend the hip while keeping the knee extended on the side being measured [23].

Abduction/in supine position: The stationary arm of the goniometer was kept parallel to the anterior superior iliac spines. The abduction value of the hip joint was recorded by tracking the anterior midline of the femur with the moving arm of the goniometer [23].

c. Harris Hip Score (HHS)

Hip function was evaluated with HHS; it consists of four subsections: pain, function, deformity, and range of motion [24, 25]. 90-100 points: excellent, 80-89 points: good, 70-79 points: medium, below 70 points: poor [26].

d. The Timed Up and Go Test (TUGT)

The TUGT was employed to determine the functional performance levels of the patients based on objective measures. The test commenced with the individual rising from a chair and concluded when the person walked a distance of 3 meters and returned to the the chair. Timing was initiated upon issuing the "go" command to the patient and concluded when the person made contact with the back of the chair [20, 21].

Quality of Life (QoL)

The Nottingham Health Profile (NHP) was used to determine QoL [27]. The NHP encompasses 38 statements that assess subjective distress across six domains: physical activity, pain, sleep, energy, social isolation, and emotional reactions [28]. Each section is scored between 0-100. In this scale, 0 represents the best health, while a score of 100 signifies the worst health [29].

Kinesiophobia

Kinesiophobia behaviors related to low back pain of the patients were evaluated with the Tampa Scale of Kinesiophobia (TSK). The scale consists of 17 items, which encompass parameters related to injury/re-injury and fear avoidance in work-related activities. It employs a 4-point Likert scoring system. In TSK, where the possible score varies between 17-68, high scores indicate that kinesiophobia is also high [30]. The scores greater than 37 indicate a high degree of kinesiophobia [31].

Statistical analysis

Statistical analyses were conducted using the 'IBM SPSS Statistics 24 software (Armonk, NY: IBM Corp.)'. The variables were provided as mean, standard deviation (SD), and percent. Whether the obtained data was suitable for normal distribution was evaluated by the Shapiro -Wilk test. When parametric test assumptions are provided, a t test in Independent Groups is used to compare differences. When the parametric test assumptions were not met, the Mann-Whitney U test was employed to compare independent group differences. The difference between categorical variables was examined by Chi-Square analysis. In all analyzes, $p<0.05$ was considered statistically significant.

Results

There was no statistically significant difference between the two groups in terms of sociodemographic and clinical data [age, height, body mass index (BMI), radiological stage of hip osteoarthritis ($p=0.099$, $p=0.921$, $p=0.466$, $p=0.391$) (Table 1). Pain severity of low back was 8.2±1.8 cm in Group 1 and 1.6±1.0 cm in Group 2. There was no statistically significant difference between the two groups in terms of hip pain severity and pain duration ($p=0.187$, $p=0.075$) (Table 1).

The results obtained from the HHS indicate that hip function was poor in the groups. There was no statistically significant difference between the groups regarding pain, function, deformity, and joint motion sub-parameters and total score of HHS, MST, and TUGT ($p=0.563$, $p=0.243$, $p=0.274$, $p=0.682$, $p=0.284$, $p=0.128$, $p=0.654$) (Table 2).

Table 1. Comparison of sociodemographic and clinical data between groups

	Group 1 (n=21) **Mean ± SD**	**Group 2 (n=22)** **Mean ± SD**	***p* (Z/t)**
Age (years)	62.6±8.0	66.6±7.4	0.099[a] t:(-1.690)
Height (cm)	157.5±0.1	157.4±0.1	0.921[a] t:(0.102)
Weight (kg)	81.0±11.2	78.9±8.1	0.486[a] t:(0.703)
BMI (kg/m²)	32.8±5.2	31.9±3.0	0.466[a] t:(0.737)
Hip Pain Severity (VAS)	9.2±1.2	8.8±1.0	0.187[b] Z:(-1.319)
Hip Pain Duration (Month)	125.1±129.8	67.3±108. 8	0.075[b] Z:(- 1.779)
Kellgren Lawrence Classification	**n (%)**	**n (%)**	***p***
Grade 2	16 (76.2)	19 (86.4)	0.391[c]
Grade 3	5 (23.8)	3 (13.6)	

BMI: Body Mass Index, VAS: Visual Analog Scale, [a]T-test in Independent Groups, [b] Mann-Whitney U Test, [c]Fisher exact test, t value is used to describe the T test of the comparison of the normal distribution variables. Z value is used to describe the Mann–Whitney U Test of the comparison of the non-normal distribution variables

Table 2. Comparison of ROM of Hip, HHS parameters, MST and TUGT values between groups

	Group 1 (n=21) Mean ± SD	Group 2 (n=22) Mean ± SD	*p* (Z/t)
ROM of Hip Flexion	82.1±13.8	84.7±13.8	0.544[a] Z:(-0.612)
Extension	4.7±3.2	4.5±2.2	0.802[b] t:(0.253)
Abduction	24.4±5.3	22.4±6.1	0.266[b] t:(1.128)
HHS Pain	12.4 ± 4.4	13.1±4.1	0.563[a] Z:(-0.578)
Function	23.2±9.2	26.1±6.6	0.243[b] t:(-1.185)
Deformity	3.6±0.5	3.5±0.5	0.274[a] Z:(-1.094)
Joint Motion	4.3±0.4	4.4±0.4	0.682[a] t:(-0.413)
Total Score	43.6±12.2	47.2±9.0	0.284[b] t:(-1.088)
MST (cm)	3.6±1.5	4.3±1.6	0.128[a] Z:(-1.551)
TUGT (Sec)	17.5±4.1	18.2±5.9	0.654 [b] t:(-0.452)

ROM: Range of motion, HHS: Harris Hip Score, MST: Modified Schober Test, TUGT: Timed Up and Go Test, SD: Standard Deviation
[a]: Mann-Whitney U Test, [b]: T-test in Independent Groups
t value is used to describe the T test of the comparison of the normal distribution variables
Z value is used to describe the Mann–Whitney U Test of the comparison of the non-normal distribution variables

Statistically, Group 1's total scores of QoL, QoL- emotional reactions, and pain sub-parameters were significantly higher than Group 2 (p=0.001, p=0.002, p=0.002).

There were no statistically significant differences in QoL-physical activity, sleep, energy level, social isolation sub-parameters between the groups (p=0.458, p=0.066, p=0.306, p=0.147). The results obtained with the TSK indicate high kinesiophobia in the groups. There was no statistically significant difference between the two groups regarding kinesiophobia (p=0.241) (Table 3).

Table 3. Comparison of NHP sub-parameters and kinesiophobia between groups

	Group 1 (n=21) Mean ± SD	Group 2 (n=22) Mean ± SD	*p* (Z/t)
NHP Physical Activity	65.8±17.8	61.8±16.3	0.458[a] t:(0.749)
Pain	94.9±9.1	79.9 ±17.5	0.002[a*] Z:(-3.142)
Sleep	64.5±26.1	47.1±33.4	0.066[b] t:(1.891)
Energy Level	62.8±8.5	61.0±0.0	0.306[a] Z:(-1.024)
Social Isolation	52.4±25.5	39.5±29.5	0.147[b] t:(1.479)
Emotional Reaction	75.9±20.6	52.9±24.3	0.002[b*] t:(3.328)
Total Score	416.1±64.2	342.5±70.3	0.001[b*] t:(3.576)
TSK	49.8±6.2	47.6±6.2	0.241[b] t:(1.191)

NHP: Nottingham Health Profile, TSK: Tampa Scale of Kinesiophobia, [a]: Mann-Whitney U Test
[b]: T-test in Independent Groups , *=p<0.05. t value is used to describe the T test of the comparison of the normal distribution variables
Z value is used to describe the Mann–Whitney U Test of the comparison of the non-normal distribution variables

Discussion

In this study, it was found that the QoL sub-parameters associated with pain and emotional reactions and the total QoL were negatively affected in patients with moderate-high severity of LBP in HOA compared to mild severity of LBP. This result showed that the severity of LBP is an important factor in QoL in patients with HOA.

The study aims to investigate the mechanisms underlying the co-occurrence of HOA and LBP, provide prognostic information to clinicians associated with this condition, and examine the impact of varying levels of low back pain in patients with HOA on health-related parameters.

The findings from the restricted number of studies examining lumbar region mobility in patients with HOA and concurrent LBP appear to be inconclusive. For instance, French et al. [16] conducted a comparison between groups of patients with HOA, with and without LBP. De Araújo et al. [32] similarly made a comparison between sailors with and without LBP. They reported no statistically significant difference in lumbar spine mobility, a result that is in line with the study. In a study by Moll and Wright [33], it was noted that the flexion values according to the Modified Schober Test ranged from 4 to 8.5 cm for women aged 55-64 and from 3.5 to 6.5 cm for women aged 65-74. These values suggest a decrease in lumbar spine mobility in the group experiencing moderate-high LBP, while it remained within normal limits for the group with mild LBP. Therefore, even though there was no statistically significant difference in lumbar spine mobility between the groups, we can infer from this distribution that an increase in the severity of LBP negatively impacts lumbar spine mobility. In substantiation of this situation, Tateuchi et al. [34] reported that hip degeneration and LBP severity negatively affect lumbar spine mobility. Latimer et al. [35] and Shirley [36] noted an association between increased pain intensity, decreased voluntary mobility, and abnormal spinal stiffness. While both study groups in this research were comprised of individuals with HOA, it is possible that lumbar spine mobility was more impacted in the group experiencing moderate-high levels of LBP severity. Furthermore, it has been noted that a decrease in mobility can occur as a result of muscle spasms in painful conditions [37]. The reasons for the lack of an influence of LBP severity on lumbar region mobility in patients with HOA in our study may be attributed to several factors. Firstly, it is possible that HOA, independently of the severity of LBP, exerts a negative impact on lumbar biomechanics. Secondly, both study groups have experienced issues related to LBP, albeit with varying degrees of severity.

In this study, it was observed that the severity of LBP did not have an impact on hip function based on the results of the HHS and hip ROM isolated from HHS in patients with HOA. Parvizi et al. [38] conducted a study involving a significant number of participants with HOA, encompassing both those with and without LBP. Similarly, Staibano et al. [39] conducted a comparison between individuals with HOA who did not experience LBP or had mild LBP and those with moderate-high LBP. In both of these studies, similar to the research, it was reported that the groups exhibited similar HHS, and it was noted that the hip function in these groups was deemed “poor.” Furthermore, Ran et al. [40] observed that improvements in hip function, as assessed by the HHS, correlated with enhancements in spine function and a reduction in LBP. In our study, hip involvement caused by osteoarthritis was similar between the groups. HOA may affect hip function more than LBP severity. Therefore, it can be asserted that hip function exhibits similarity between the groups, irrespective of the severity of LBP.

In the literature, the duration of the TUGT applied to patients with HOA varies; Arnold and Faulkner [41] (2007) recorded the duration of the TUGT as 12.8 seconds, while Ceballos Laita et al. [42] (2021) determined it as 10.5, 9.63 , and 9.5 seconds. In our study, compared to the literature, it was observed that the duration of the TUGT was higher in both groups. The presence of LBP indicates that the performance-based functional level is negatively affected in HOA, regardless of the severity of LBP. In addition, it has been found in our study that the severity of LBP had no effect on performance. Results of performance-based tests were correlated with the strength of hip abductors and knee extensors [43]. Similar HOA involvement and hip function may result in a similar loss of muscle strength in the lower extremity.

The QoL was poor in both groups; however, it was significantly worse in the group with moderate to severe LBP. In our study, the QoL score showed that moderate-high LBP negatively affected the QoL compared to mild pain. It was also noted that pain and emotional reactions related to QoL were more negatively affected in the group with moderate-high LBP. High pain level is associated with significant physical limitations and worse prognosis; these sub-parameters have a negative impact on health-related QoL [44]. Our study results confirm this situation. However, there are different results in the literature. Parvizi et al. [38] reported that the QoL of patients with and without LBP was similar in a study in which most of the participants were HOA patients. Stupar et al. [45] stated that patients with hip and knee OA with LBP had a lower QoL related to physical function, mental, and general health parameters compared to patients without LBP. In studies examining HOA and LBP together in the literature, heterogeneity is observed among patient groups. There is a need for more homogeneous studies in this regard.

The study shows that kinesiophobia is high in both groups, but there is no significant difference between the two groups. This result shows that HOA, regardless of the severity of LBP, and in addition to osteoarthritis, LBP, regardless of its severity, can cause kinesiophobia. In order to better elucidate this issue, future studies should include HOA patients with different LBP severities as well as a group without LBP, which will further clarify the situation. From a different perspective, Kopp et al. [46], in their study, the limitation and pain caused by HOA, reported that it is more strongly correlated with individual and psychological aspects, especially cognitive coping strategies such as kinesiophobia, rather than pathological and anatomical factors such as the location and severity of arthritis. This may be why kinesiophobia is mainly associated with hip pain, and anatomically, hip-related LBP severity has no effect on kinesiophobia.

In this study, the effect of LBP of different severity in HOA on health-related parameters was examined. However, people without LBP were not included in the study. Therefore, it has become challenging to make interpretations regarding certain parameters. This can be seen as a limitation of our study. We believe that future studies including individuals with HOA who do not have LBP can provide more conclusive results. At the same time, no biomechanical evaluation was made in our study. We believe that biomechanical changes caused by HOA and LBP, as well as the small sample size in the study, could potentially influence the outcomes.

In conclusion, the presence of moderate to high levels of LBP in patients with HOA revealed a significant adverse impact on various sub-parameters of QoL, particularly on quality of life in relation to pain and emotional reactions, when compared to patients with mild LBP. It is crucial for clinicians to assess the severity of LBP in patients with HOA and subsequently incorporate comprehensive evaluation and management of all aspects of QoL, including physical activity, pain, sleep, energy, social isolation, and emotional reactions, into the treatment plan.

Funding: None.

Acknowledgment: The research has been presented as an oral presentation at the 1st Congress on Education, Research, and Innovation in Physiotherapy and Rehabilitation at Katip Çelebi University.

Authors contributions: Contributions of the authors to the article E.A.T. constructed the main idea and hypothesis of the study. E.A.T., S.H. developed the theory, and E.A.T., S.H., N.B. and N.O arranged/edited the material and method section. N.B. and H.S. have done the evaluation of the data in the Results section. The discussion section of the article was written by S.H. E.A.T. reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.

Conflicts of interest: No conflict of interest was declared by the authors.

References

1. Kim C, Nevitt MC, Niu J, et al. Association of hip pain with radiographic evidence of hip osteoarthritis: diagnostic test study. *BMJ*. 2015;351:h5983. doi:10.1136/bmj.h5983
2. MacDonald KV, Sanmartin C, Langlois K, Marshall DA. Symptom onset, diagnosis and management of osteoarthritis. *Health Rep*. 2014;25:10-17.

3. Jordan JM, Helmick CG, Renner JB, et al. Prevalence of hip symptoms and radiographic and symptomatic hip osteoarthritis in African Americans and Caucasians: the Johnston County Osteoarthritis Project. *J Rheumatol*. 2009;36(4):809-815. doi:10.3899/jrheum.080677
4. Faber BG, Frysz M, Boer CG, et al. The identification of distinct protective and susceptibility mechanisms for hip osteoarthritis: findings from a genome-wide association study meta-analysis of minimum joint space width and Mendelian randomisation cluster analyses. *EBioMedicine*. 2023;95:104759. doi:10.1016/j.ebiom.2023.104759
5. Felson DT. Epidemiology of hip and knee osteoarthritis. *Epidemiol Rev*. 1988;10:1-28. doi:10.1093/oxfordjournals.epirev.a036019
6. Wang W, Sun M, Xu Z, Qiu Y, Weng W. The low back pain in patients with hip osteoarthritis: current knowledge on the diagnosis, mechanism and treatment outcome. *Ann Joint*. 2016;1:9. doi:10.21037/aoj.2016.0
7. Panahi R, Mohammadi B, Kazemi SS, Shamsi Nejad Geshti MR. Low back pain, disability and quality of life among university students. *IJMPP*. 2016;1:173-177.
8. Gatchel RJ, Neblett R, Kishino N, Ray CT. Fear-avoidance beliefs and chronic pain. *J Orthop Sports Phys Ther*. 2016;46(2):38-43. doi:10.2519/jospt.2016.0601
9. Sánchez Herán Á, Agudo Carmona D, Ferrer Peña R, et al. Postural stability in osteoarthritis of the knee and hip: analysis of association with pain catastrophizing and fear-avoidance beliefs. *PM R*. 2016;8(7):618-628. doi:10.1016/j.pmrj.2015.11.002
10. Olsson LE, Hansson E, Ekman I. Evaluation of person-centred care after hip replacement—a controlled before and after study on the effects of fear of movement and self-efficacy compared to standard care. *BMC Nurs*. 2016;15(1):53. doi:10.1186/s12912-016-0173-3
11. Rivière C, Lazic S, Dagneaux L, Van Der Straeten C, Cobb J, Muirhead Allwood S. Spine-hip relations in patients with hip osteoarthritis. *EFORT Open Rev*. 2018;3(2):39-44. doi:10.1302/2058-5241.3.170020
12. Offierski CM, MacNab I. Hip-spine syndrome. *Spine (Phila Pa 1976)*. 1983;8(3):316-321. doi:10.1097/00007632-198304000-00014
13. Ben Galim P, Ben Galim T, Rand N, et al. Hip-spine syndrome: the effect of total hip replacement surgery on low back pain in severe osteoarthritis of the hip. *Spine (Phila Pa 1976)*. 2007;32(19):2099-2102. doi:10.1097/BRS.0b013e318145a3c5
14. Dixon JS, Bird HA. Reproducibility along a 10 cm vertical visual analogue scale. *Ann Rheum Dis*. 1981;40(1):87-89. doi:10.1136/ard.40.1.87
15. Boonstra AM, Schiphorst Preuper HR, Balk GA, Stewart RE. Cut-off points for mild, moderate, and severe pain on the visual analogue scale for pain in patients with chronic musculoskeletal pain. *Pain*. 2014;155(12):2545-2550. doi:10.1136/ard.40.1.87
16. French HP, O'Donnell B, Cuddy V, O'Connell P. Clinical features of low back pain in people with hip osteoarthritis: a cross sectional study. *Physiother Pract Res*. 2015;36(1):15-22. doi:10.3233/PPR-140048
17. Robinson HS, Mengshoel AM. Assessments of lumbar flexion range of motion: intertester reliability and concurrent validity of 2 commonly used clinical tests. *Spine (Phila Pa 1976)*. 2014;39(4):270-275. doi:10.1097/BRS.0000000000000131
18. Poulsen E, Christensen HW, Penny JØ, Overgaard S, Vach W, Hartvigsen J. Reproducibility of range of motion and muscle strength measurements in patients with hip osteoarthritis - an inter-rater study. *BMC Musculoskelet Disord*. 2012;13:242. doi:10.1186/1471-2474-13-242
19. Çelik D, Can C, Aslan Y, Ceylan HH, Bilsel K, Ozdincler AR. Translation, cross-cultural adaptation, and validation of the Turkish version of the Harris Hip Score. *Hip Int*. 2014;24(5):473-479. doi:10.5301/hipint.5000146
20. Çifçili S, Ünalan PC. Yaşlılarda fonksiyonel kayıplara yaklaşım. *Türk Aile Hek Derg*. 2004;8(4):166-173.
21. Lin MR, Hwang HF, Hu MH, Wu HDI, Wang YW, Huang FC. Psychometric comparisons of the timed up and go, one-leg stand, functional reach, and Tinetti balance measures in community-dwelling older people. *J Am Geriatr Soc*. 2004;52(8):1343-1348. doi:10.1111/j.1532-5415.2004.52366.x
22. Tousignant M, Poulin L, Marchand S, Viau A, Place C. The Modified-Modified Schober Test for range of motion assessment of lumbar flexion in patients with low back pain: a study of criterion validity, intra- and inter-rater reliability and minimum metrically detectable change. *Disabil Rehabil*. 2005;27(10):553-559. doi:10.1080/09638280400018411
23. Otman AS, Köse N. Tedavi hareketlerinde temel değerlendirme prensipleri. 5. Baskı. Ankara: Meteksan Matbaacılık ve Teknik Sanayi Ticaret A.Ş.; 2013:74-76.
24. Harris WH. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation. *J Bone Joint Surg Am*. 1969;51(4):737-755.
25. Küçükdeveci AA. Functional assessment measures in osteoarthritis. *Turkish J Geriatrics*. 2011;14:37-44.
26. Elibol N. Türk toplumunda total kalça protezi uygulanan hastaların beklentilerinin incelenmesi. Yüksek lisans Tezi. Dokuz Eylül Üniversitesi Sağlık Bilimleri Enstitüsü, Fizik Tedavi ve Rehabilitasyon, İzmir; 2011.
27. Küçükdeveci AA, McKenna SP, Kutlay S, Gürsel Y, Whalley D, Arasil T. The development and psychometric assessment of the Turkish version of the Nottingham Health Profile. *Int J Rehabil Res*. 2000;23(1):31-38. doi:10.1097/00004356-200023010-00004

28. Uutela T, Kautiainen H, Hakala M. Nottingham health profile questionnaire incorporates important aspects of the patient perspective into outcome assessment in rheumatoid arthritis. *Clin Exp Rheumatol.* 2008;26(1):39-44.
29. Madenci E, Gürsoy S, Arıca E, Keven S. Primer fibromiyalji sendromlu hastalarda yaşam kalitesinin Nottingham sağlık profili ile değerlendirilmesi. *Türkiye Klinikleri*. 2003;3(1):11-14.
30. Tunca Yilmaz Ö, Yakut Y, Uygur F, Ulug N. Turkish version of the Tampa scale for kinesiophobia and its test-retest reliability. *Fizyoter Rehabil*. 2011;22(1):44-49.
31. Bränström H, Fahlström M. Kinesiophobia in patients with chronic musculoskeletal pain: differences between men and women. *J Rehabil Med*. 2008;40(5):375-380. doi:10.2340/16501977-0186
32. de Araújo LM, Dell'Antonio E, Hubert M, Ruschel C, Roesler H, Pereira SM. Trunk muscular endurance, lumbar spine mobility and hip flexibility in sailors with and without low back pain. *Fisioter Mov.* 2020;33(1):e003334. doi:10.1590/1980-5918.033.AO34
33. Moll JM, Wright V. Normal range of spinal mobility. An objective clinical study. *Ann Rheum Dis.* 1971;30(4):381-386. doi:10.1136/ard.30.4.381
34. Tateuchi H, Akiyama H, Goto K, So K, Kuroda Y, Ichihashi N. Sagittal alignment and mobility of the thoracolumbar spine are associated with radiographic progression of secondary hip osteoarthritis. *Osteoarthritis Cartilage*. 2018;26(3):397-404. doi:10.1016/j.joca.2017.12.005
35. Latimer J, Lee M, Adams R, Moran CM. An investigation of the relationship between low back pain and lumbar posteroanterior stiffness. *J Manipulative Physiol Ther*. 1996;19(9):587-591.
36. Shirley D. Muscle activity and lumbar PA stiffness. PhD thesis. University of Sydney, School of Physiotherapy, Sydney, 2002.
37. Yalgın S, Karacan İ, Çelikdelen A. Mekanik bel ağrısı şiddeti ve süresi ile kas kuvveti ilişkisinin değerlendirmesi. *Dirim Tıp Gazetesi* 2008;83(3):117-123.
38. Parvizi J, Pour AE, Hillibrand A, Goldberg G, Sharkey PF, Rothman RH. Back pain and total hip arthroplasty: a prospective natural history study. *Clin Orthop Relat Res*. 2010;468(5):1325-1330. doi:10.1007/s11999-010-1236-5
39. Staibano P, Winemaker M, Petruccelli D, de Beer J. Total joint arthroplasty and preoperative low back pain. *J Arthroplasty*. 2014;29(5):867-871. doi:10.1016/j.arth.2013.10.001
40. Ran TF, Ke S, Li J, et al. Relieved low back pain after total hip arthroplasty in patients with both hip osteoarthritis and lumbar degenerative disease. *Orthop Surg*. 2021;13(6):1882-1889. doi:10.1111/os.13135
41. Arnold CM, Faulkner RA. The history of falls and the association of the timed up and go test to falls and near-falls in older adults with hip osteoarthritis. *BMC Geriatr*. 2007;7:17. doi:10.1186/1471-2318-7-17
42. Ceballos Laita L, Jiménez Del Barrio S, Marín Zurdo J, et al. Effectiveness of dry needling therapy on pain, hip muscle strength, and physical function in patients with hip osteoarthritis: a randomized controlled trial. *Arch Phys Med Rehabil*. 2021;102(5):959-966. doi:10.1016/j.apmr.2021.01.077
43. Zeni Jr J, Abujaber S, Pozzi F, Raisis L. Relationship between strength, pain, and different measures of functional ability in patients with end-stage hip osteoarthritis. *Arthritis Care Res (Hoboken)*. 2014;66(10):1506-1512. doi:10.1002/acr.22329
44. Mutubuki EN, Beljon Y, Maas ET, et al. The longitudinal relationships between pain severity and disability versus health-related quality of life and costs among chronic low back pain patients. *Qual Life Res.* 2020;29(1):275-287. doi:10.1007/s11136-019-02302-w
45. Stupar M, Côté P, French MR, Hawker GA. The association between low back pain and osteoarthritis of the hip and knee: a population-based cohort study. *J Manipulative Physiol Ther*. 2010;33(5):349-354. doi:10.1016/j.jmpt.2010.05.008
46. Kopp B, Furlough K, Goldberg T, Ring D, Koenig K. Factors associated with pain intensity and magnitude of limitations among people with hip and knee arthritis. *J Orthop*. 2021;25:295-300. doi:10.1016/j.jor.2021.05.026

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1503792

Subclinical seizures on EEG with sleep phases and clinical significance in patients with focal epilepsy

EEG'de uyku evresinde görülen subklinik nöbetler ve fokal epilepsili hastalarda klinik önemi

Çağla Erdoğan, İsmail Hakkı Akbudak, Ebru Apaydın Doğan

Posted date:24.06.2024 Acceptance date:09.12.2024

Abstract

Purpose: Studies have observed that subclinical seizures significantly impact diagnosis, prognosis, and treatment responses. The aim of our study is to investigate the presence of these seizures, their relationship with sleep, their contribution to diagnosis, and their impact on treatment selection and prognosis in patients diagnosed with partial epilepsy.

Materials and methods: A total of 1.626 EEGs conducted in the EEG unit were examined. Among them, 626 were found to belong to patients diagnosed with partial epilepsy. 140 patients who underwent twenty-minute EEG monitoring and 28 patients who underwent twenty-four-hour EEG monitoring were included in the study.

Results: A comparative analysis was conducted between two cohorts of patients: one subjected to a twenty-minute EEG and the other undergoing a 24-hour EEG. In the presence of resistant epilepsy, the risk of SCS is increased by 3.1 times. The presence of SCS in frontal lobe epilepsies demonstrated a 2.2-fold elevation compared to temporal lobe epilepsies (1.0-5.3). A statistically significant relationship between Levetiracetam usage and seizures was observed (p=0.044). The calculated Odds Ratio for risk indicated a 1.2-fold (1-1.6) higher frequency of seizures with Levetiracetam usage. Risk analysis revealed a 1.5%-fold (1.2-1.9) higher incidence of seizures with the use of well-known drugs in favor of lacosamide-zonisamide.

Conclusion: SCSs are gaining increasing clinical significance in terms of diagnosis, prognosis, and treatment. The relationship between these periods of sleep and SCSs is important for future research with a larger number of patients and medical professionals.

Keywords: Subclinical seizures, sleep, focal epilepsy.

Erdogan C, Akbudak IH, Apaydin Dogan E. Subclinical seizures on EEG with sleep phases and clinical significance in patients with focal epilepsy. Pam Med J 2025;18:282-292.

Öz

Amaç: Çalışmalarda, subklinik nöbetlerin tanı, prognoz ve tedavi yanıtları üzerinde önemli bir etkisi olduğu gözlemlenmektedir. Çalışmamızın amacı, parsiyel epilepsi tanısı konmuş hastalarda bu nöbetlerin varlığını araştırmak, uyku ile ilişkilerini incelemek, tanıya katkılarını ve tedavi seçimi ile prognoz üzerindeki etkilerini değerlendirmektir.

Materyal ve yöntem: EEG ünitesinde yapılan toplam 1,626 EEG incelendi. Bunların içinde, parsiyel epilepsi tanısı konmuş 626 hastaya ait EEG bulundu. Çalışmaya yirmi dakika EEG monitörizasyonu yapılan 140 hasta ve yirmi dört saat EEG monitörizasyonu yapılan 28 hasta dahil edildi.

Bulgular: İki hasta grubu arasında karşılaştırmalı bir analiz yapıldı: biri yirmi dakika EEG'ye tabi tutulan diğeri 24 saat EEG monitörizasyonu yapılan. Dirençli epilepsi varlığında, SCS riski 3.1 kat artmaktadır. Frontal lob epilepsilerinde SCS varlığı, temporal lob epilepsilere kıyasla 2.2 kat daha yüksek bulundu (1,0-5,3). Levetirasetam kullanımı ile nöbetler arasında istatistiksel olarak anlamlı bir ilişki gözlemlendi (p=0,044). Risk oranı hesaplaması, Levetirasetam kullanımı ile nöbetlerin 1.2 kat (1-1,6) daha sık oluşma olasılığını gösterdi. Risk analizi, lakozamid-zonisamid gibi bilinen ilaçların kullanımı lehine nöbetlerin %1,5 kat (1,2-1,9) daha sık görüldüğünü ortaya koydu.

Sonuç: Subklinik nöbetler, tanı, prognoz ve tedavi açısından giderek artan klinik öneme sahiptir. Bu nöbetlerin uyku ile ilişkisi, daha geniş hasta ve merkezlerle yapılacak gelecek araştırmalar için önem taşımaktadır.

Anahtar kelimeler: Subklinik nöbetler, uyku, fokal epilepsi.

Erdoğan Ç, Akbudak İH, Apaydın Doğan E. EEG'de uyku evresinde görülen subklinik nöbetler ve fokal epilepsili hastalarda klinik önemi. Pam Tıp Derg 2025;18:282-292.

Çağla Erdoğan, Ph.D. Pamukkale University, Medicine Faculty, Intensive Care Department, Denizli, Türkiye, e-mail: caglaerdogan89@gmail.com (https://orcid.org/0000-0001-8772-6565) (Corresponding Author)

İsmail Hakkı Akbudak, Assoc. Prof. Pamukkale University, Medicine Faculty, Intensive Care Department, Denizli, Türkiye, e-mail: ishakbudak@gmail.com (https://orcid.org/0000-0002-3716-9243)

Ebru Apaydın Doğan, Prof. Akdeniz University, Medicine Faculty, Neurology Department, Antalya, Türkiye, e-mail: eapaydindogan@yahoo.com (https://orcid.org/0000-0002-6818-3021)

Introduction

The electroencephalography (EEG) examinations conducted on patients with epilepsy reveal various patterns related to pathologies. Sharp waves, spike waves, and spike-wave complexes are identified among these pathologies. Numerous studies exist regarding both the characteristics and clinical significance of these patterns. However, the clinical significance of subclinical seizures is not clearly defined. Subclinical seizures (SCS) are defined as electrographic seizures consisting of rhythmic ictal discharges that do not lead to objective or subjective changes in consciousness or behavior and are spread over time. SCS can be detected through intracranial or surface EEG monitoring. In many studies, data suggest that SCS originating from the same cortical area as clinical seizures can be used for diagnosis by determining the epileptogenic area [1].

There are few studies on SCS. Although the exact prevalence of SCS is unknown, a study by Velkey et al. [2] found a subclinical seizure rate of 18% in long-term EEG monitoring in children, contributing to the diagnostic process by 85%. When reviewing the literature, it is indicated that SCS can be used for surgical prognosis and predictions of seizure freedom [3]. In a study by Fernandez Torré et al. [4], the presence of SCS in patients with glioma is reported as a sign in favor of progression. Evidence also suggests that SCS may be associated with memory deficits and autonomic dysfunction. Vossel et al. [5] investigated the presence of SCS in patients followed up with a diagnosis of Alzheimer's disease. SCS was detected in 42% of the patients, and when these patients were followed up, it was observed that cognition and executive functions were lost faster in patients with SCS. 60% of the identified SCS occurred during sleep.

Jin et al. [6] conducted studies on the prevalence and characteristics of SCS. In these studies, the presence of these seizures was found to be associated with pharmacoresistant epilepsy and abnormal MRI. The prevalence was found to be 5.3%, and it was stated that SCS provided information about the localization of the epileptogenic focus in 79.4% of the patients with SCS. It was reported that 7.7% of these seizures were detected in a 20-minute EEG, 83.3% in a 24-hour EEG, and 92.8% in a 48-hour EEG. Most of these seizures were detected during sleep.

In a recent study, a significant increase in the risk of seizures was found in the presence of epileptiform discharges detected in EEG. It was suggested that subclinical and clinical seizure patterns have similar circadian rhythms and are formed by the same regulatory mechanisms. Studies have suggested that SCS can cause permanent neuronal changes and may lead to cognitive defects that could result in sequelae. For this reason, many authors believe that SCS requires treatment in the same way as clinical seizures. Anderson et al. [7] examined the circadian pattern of SCS. It was determined to be most common between 23:00 and 05:00. Investigating SCS during sleep is considered effective in understanding clinical treatment responses and selecting the most appropriate treatment time.

It is observed that SCS have a significant impact on the diagnosis, prognosis, and treatment responses. In our clinic, we plan to investigate the presence of these seizures, their relationship with sleep, their contribution to diagnosis, and their impact on treatment selection and prognosis in patients diagnosed with focal epilepsy.

Material and methods

Patients who applied to our clinic and underwent monitoring in the EEG unit within the last year were included in the study. Retrospectively, the demographic information of the patients, details of the disease diagnosis and treatment, and the presence of resistant epilepsy were scanned through the hospital information management system. Patients with focal epilepsy who underwent EEG monitoring and had pathological changes in their EEG were included in the study.

Permission was obtained from the Akdeniz University Clinical Research Ethics Committee for the study (permission date: 13/07/2017 and permission number: 70904504/258).

EEG monitoring

EEG monitoring was conducted using surface electrodes placed according to the international 10-20 system and VEEG digital systems.

Routine 20-minute EEG monitoring sessions and 24-hour EEG monitoring sessions performed on the presenting patients were considered for analysis. This study aimed to investigate the detection rate and clinical significance of subclinical seizures (SCSs) and sleep through routine EEG monitoring. For this reason, short EEG monitoring sessions were also included in the study. In statistical analyses, short-term and 24-hour EEG monitoring sessions were evaluated separately and, ultimately, together.

The presence of sleep in the conducted EEGs was assessed according to the EEG patterns defined in the literature based on the American Academy of Sleep Medicine (AASM) scoring system for sleep stages.

The presence of SCSs in EEGs and its relationship with sleep were examined. Whether SCSs were observed during wakefulness or sleep, and if observed during sleep, in which stage they occurred.

A total of 1.626 EEGs conducted in the EEG unit were examined. Ninety-six of these EEGs had a duration of twenty-four hours. Among them, 626 were found to belong to patients diagnosed with focal epilepsy. After being evaluated by two independent EEG specialists, these EEGs were scrutinised, with a joint decision that those exhibiting focal epileptiform features were included in the study. As a result, a total of 140 patients who underwent twenty-minute EEG monitoring and 28 patients who underwent twenty-four-hour EEG monitoring were included in the study.

Subclinical seizure definition

SCSs (Subclinical Seizures) are defined as paroxysmal rhythmic epileptiform EEG patterns that spread over time and localization without subjective or objective clinical findings. The absence of alpha rhythm in wakefulness, posterior reactive rhythm, and focal or generalized slowing are considered abnormal. The absence of age-appropriate sleep spindles, the presence of asymmetric sleep spindles, and continuous asynchrony are considered abnormal during sleep. Electrographic seizure is defined as rhythmic epileptiform activity lasting longer than 10 seconds, not giving rise to clinical symptoms but showing development over time and space, even causing changes in connected electrodes [3].

During video EEG monitoring, patients' subjective complaints are questioned and objectively monitored for seizures. Electrographic seizures without any clinical signs are considered subclinical seizures (SCSs).

Statistical analysis

Statistical analysis was conducted utilizing SPSS 18 Software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were applied to summarize continuous data, expressing them as the median with the range and mean with standard deviation (mean±SD). Categorical data were presented in terms of numbers and percentages. The Chi-square test was used to compare categorical variables, and a significance test for the difference between two means was employed to compare continuous variables. For variables with significant results in the Chi-square test, Odds Ratio (OR) was calculated. A significance level of $p<0.05$ was considered.

Results

Of the patients who underwent a twenty-minute EEG, 56.4% were female, and 43.6% were male. The patients who underwent a twenty-minute EEG ranged from 17 to 89 years old. Their average age was calculated as 42.5±19.9. It was determined that 22.1% of the participants have resistant epilepsy, 33.6% have the presence of sleep in their EEG, and when EEGs with sleep were examined, 60.6% of the seizures occurred in the NREM-1 stage. Additionally, 37.1% of the patients were found to have a frontal lobe epilepsy focus. The patients who underwent a twenty-minute EEG ranged from 17 to 89 years old. Their average age was calculated as 42.5±19.9. The duration of illness varies between 1 and 45 years, with an average of 8.3±10.1 years (Table 1).

Among the cohort subjected to a twenty-four-hour EEG, 67.9% were of the female gender, while 32.1% were male. Notably, 53.6% of the participants had resistant epilepsy. When examining the presence of subclinical seizures (SCS) during sleep, researchers found that 3.6% of seizures occurred in the light sleep stage (NREM-1), while 96.4% occurred during deep sleep. A predominant frontal lobe epilepsy focus was discerned in 60.7% of the patients. The age range of individuals undergoing the EEG protocol ranged from 18 to 67 years, with an average age of 33.9±12.7. Regarding the duration of illness, a variance between 1 and 39 years was noted, with an average duration of 11.21±10.5 years (Table 1).

Table 1. Distribution of demographic and clinical characteristics of patients

Parameters	20 Minutes EEG Group	24-Hour EEG Group
Gender Distribution	Female: 79 (56.4%)	Female: 19 (67.9%)
	Male: 61 (43.6%)	Male:9 (32.1%)
Age (Mean)	42.5	33.9
Refractory Epilepsy	31 (22.1%)	13 (53.6%)
Presence of Sleep in EEG	93 (66.4%)	28 (100%)
Presence of SCS in Sleep	66 (47.1%)	28 (100%)
Stages of Sleep for SCS	NREM-1: 40 (60.4%)	NREM-1: 1 (3.6%)
	NREM-2: 26 (39.4%)	NREM-2/3: 27 (96.4%)
Epileptic Focus	Frontal: 52 (37.1%)	Frontal: 17 (60.7%)
	Temporal: 60 (42.9%)	Temporal: 9 (32.1%)
		Other: 2 (7.1%)
Disease Duration (Mean)	8.3 year	11.21 year

When looking at medication use in patients who had a 20-minute EEG, it was found that 14% were not taking any medication. Notably, 40% were on monotherapy, while those employing five medications comprised a mere 2%. Upon examination of the pharmacological profiles of patients subjected to a twenty-four-hour EEG, it was observed that 18% refrained from using any medication. Additionally, 36% were noted to be utilizing a singular medication, with the highest medication usage capped at three, constituting 21% of the patient cohort (Table 2).

When examining the pharmaceutical usage among 168 patients in terms of both numerical counts and percentages, levetiracetam emerged as the predominant medication with a utilization rate of 50%. Subsequently, zonisamide followed with a utilization rate of 17.9%, oxcarbazepine with 14.9%, and lacosamide also with 14.9%. Phenytoin constituted a 3% usage rate, while phenobarbital concluded the list with a usage rate of 1.8% (Table 2).

Table 2. Data related to the treatment of patients

Parameters	Value
Number of Medications	
20 Minutes EEG group	
No medication	14%
1	49%
2	19%
3	12%
4	4%
5	2%
24 Hour EEG Group	
0	18%
1	36%
2	25%
3	21%
Medications	
Levetiracetam	84 (50%)
Zonisamide	30 (17.9%)
Oxcarbazepine	25 (14.9%)
Lacosamide	25 (14.9%)
Carbamazepine	23 (13.7%)
Valproate	22 (13%)
Lamotrigine	20 (11.9%)
Topiramate	8 (4.8%)
Phenytoin	5 (3%)
Phenobarbital	3 (1.8%)

In the cohort undergoing a twenty-minute EEG, an analysis was conducted on the relationships between the presence of SCS in sleep and gender, the number of medications used, the presence of refractory epilepsy, and the epileptic focus. A statistically significant association was identified solely between the presence of SCS and the epileptic focus (p=0.004) (Table 3).

A comparative analysis was conducted between two cohorts of patients: one subjected to a twenty-minute Electroencephalogram (EEG) and the other undergoing a 24-hour EEG. The former group is denoted as Group 1. In Group 1, 56.4% of subjects were female, contrasting with 67.9% in Group 2. No statistically significant distinction in gender distribution was discerned between the two cohorts (p=0.263). Upon comparing the two cohorts, a similar prevalence of singular drug usage was observed. Specifically, 49.3% in Group 1 and 35.7% in Group 2 reported single-drug administration. No statistically significant variance in drug utilization was identified between the groups (p=0.432) (Table 4).

Resistant epilepsy was diagnosed in 22.1% of Group 1 and 46.4% of Group 2, revealing a noteworthy statistical discrepancy (p=0.004). Notably, the incidence of resistant epilepsy was markedly higher in subjects undergoing a 24-hour EEG. In evaluating sleep stages between the two groups, it was noted that 60.6% of Group 1 SCSs manifested during Non-Rapid Eye Movement Stage 1 (NREM1), while 96.4% of Group 2 exhibited SCSs during NREM2 and NREM3 stages. A statistically significant disparity was evident between the two cohorts (p=0.0001) (Table 4).

Concerning the comparative assessment of epileptic foci, patients with frontal lobe epilepsy comprised 37.1% in Group 1 and 60.7% in Group 2, whereas those with temporal lobe epilepsy constituted 42.9% in Group 1 and 32.1% in Group 2. A statistically significant distinction was observed between the two groups (p=0.005). An OR analysis was employed to ascertain the risk in subjects exhibiting SCS and significant statistical differences. In the presence of resistant epilepsy, the risk of SCS is increased by 3.1 times (1.3-7.08). Furthermore, the presence of SCS during NREM2 and NREM3 stages exhibited a 41.5-fold increase compared to NREM1 (5.3-324.6). Lastly, the presence of SCS in frontal lobe epilepsies demonstrated a 2.2-fold elevation compared to temporal lobe epilepsies (1.0-5.3) (Table 4).

An examination was conducted on the relationship between the presence of Levetiracetam, the most-used medication in the groups, and subclinical seizures. Levetiracetam usage was identified in 56.4% of patients with subclinical seizures. A statistically significant relationship between Levetiracetam usage and seizures was observed (p=0.044). The calculated OR for risk indicated a 1.2-fold (1-1.6) higher frequency of seizures with Levetiracetam usage (Table 5).

Table 3. The relationship between the presence of SCS in sleep and other parameters in the 20-minute EEG cohort

	No SCS in Sleep		Presence of SCS in Sleep		
Parameters	**Number of patients**	**Percent of patients (%)**	**Number of patients**	**Percent of patients (%)**	***p*-value**
Gender					
Female	42	56.8	37	56.1	0.953
Male	32	43.2	29	43.9	X^2:0.007[a]
Total	74	100	66	100	
Number of Medication					
0	6	8.1	14	21.2	
1	39	52.7	30	45.5	
2	16	21.6	11	16.7	0.355
3	8	10.8	8	12.1	X^2:5.527[a]
4	4	5.4	2	3	
5	1	1.4	1	1.5	
Total	74	100	66	100	
Refractory Epilepsy					
Yes	17	23	14	21.2	0.482
No	57	77	52	78.8	X^2:0.063[a]
Total	74	100	66	100	
Epileptic Focus					
Frontal	18	24.3	34	51.5	0.004*
Temporal	38	51.4	22	33.3	X^2:11.054[a]
Others	18	24.3	10	15.2	
Total	74	100	66	100	

[a]Pearson Chi-Square Test, *:p<0.05 statistically significant

Table 4. Comparison of the demographic and clinical characteristics of patient groups undergoing twenty minutes and twenty-four hours of EEG

	Group 1		Group 2				
	Number	**Percent**	**Number**	**Percent**	***p*-value**	**OR**	**CI-Confidence Interval**
Gender							
Female	79	56.4	19	67.9	0.263	-	
Male	61	43.6	9	32.1	X^2:1.254[a]		
Number of Medication							
0	20	14.3	5	17.9			
1	69	49.3	10	35.7			
2	27	19.3	7	25	0.432		
3	16	11.4	6	21.4	X^2:4.872[a]	-	
4	6	4.3	0	0			
5	2	1.4	0	0			
Refractory Epilepsy							
Yes	31	22.1	13	46.4	0.004*		
No	109	77.9	15	53.6	X^2:7.119[a]	3.1	(1.3-7.08)
Sleep Stage							
NREM1	40	60.6	1	3.6	0.0001*		
NREM2+3	26	39.4	27	96.4	X^2:65.61[a]	41.5	(5.3-324.6)
Epileptic Focus							
Frontal	52	37.1	17	60.7			
Temporal	60	42.9	9	32.1	0.05* X^2:5.969[a]	2.2	(1.0-5.3)
Others	28	20	2	7.1			

[a]Pearson Chi-Square Test, *:p<0.05 statistically significant

Table 5. The relationship between Levetiracetam usage and subclinical seizures

Levetiracetam Usage	**Subclinical Seizures**				***p*-value**
	No		**Yes**		
	Number	**Percent**	**Number**	**Percent**	
Yes	31	41.9	53	56.4	0.044*
No	43	58.1	41	43.6	X^2:3.47[a]
Total	74	100	94	100	

[a]Pearson Chi-Square Test OR:1.2 (CI-confidence interval: 1-1.6), *:p<0.05 statistically significant

The relationship between the long-standing use of well-known anti-seizure medications and the new-generation anti-seizure treatments with seizures has been investigated in the groups. Patients using Levetiracetam, valproate, oxcarbazepine, and carbamazepine (Group 1) were compared with those using lacosamide and zonisamide (Group 2). Among patients experiencing subclinical seizures, 34% were using Group 1 drugs, while in those without subclinical seizures, this percentage was found to be 13.5%. A significant statistical difference was observed between the two groups (p=0.002). Risk analysis revealed a 1.5-fold (1.2-1.9) higher incidence of seizures with the use of Group 1 drugs (Table 6).

Table 6. The relationship between the usage of medications in Group 1 and Group 2 and seizures

	Subclinical Seizures				
	No		Yes		*p*-value
	Number	Percent	Number	Percent	
Group 1	10	13.5	32	34	0.002* X^2:9.3[a]
Group 2	64	86.5	62	66	
Total	74	100	94	100	

[a]Pearson Chi-Square Test OR: 1.5 (CI-confidence interval:1.2-1.9), *:p<0.05 statistically significant

Discussion

In the preliminary findings of our clinical study, it has been observed that sleep patterns can be detected even in the twenty-minute EEG monitoring of patients presenting to the outpatient clinic. The more frequent detection of SCSs during sleep also assists in capturing foci.

In our study, 1626 EEGs were examined. Of these, 626 belonged to patients diagnosed with focal epilepsy (38.4%). When looking at the presence of subclinical seizures in these EEGs, it was observed that a total of 168 showed epileptiform changes. This figure represents 10.3% of all EEGs, in line with other studies described below, and 26.8% of patients diagnosed with focal epilepsy. Our clinic is an advanced treatment centre that follows up on refractory epilepsy and offers surgical treatment options. Consequently, subclinical seizures are observed more frequently in our examinations.

A recent study examined the presence of subclinical seizures in stereotactic EEG. Subclinical seizures were detected in 84 out of 164 patients, indicating a high rate of around 50%. Although the surface electrodes we routinely use do not detect at such high rates, the detection percentage increases in the presence of appropriate indications and refractory epilepsy. Since our patients fit these conditions, the presence of subclinical seizures is detected at a higher rate [8].

There is limited research on the clinical and treatment-related data of SCSs, and their prevalence is not well-known. In a study by Velkey et al. [2], the observed rate of SCSs in long-term EEG monitoring in children was 18%, and their contribution to the diagnostic process was determined to be 85%. There is also a study utilizing positron emission tomography (PET) in the presence of SCSs, demonstrating the detection of the focus and pathology [9].

Jin et al. [6] have conducted studies on the prevalence and characteristics of SCSs. In these studies, the presence of these seizures has been found to be associated with pharmacoresistant epilepsy and abnormal MRI. The prevalence was determined to be 5.3%, and it has been noted that the presence of SCSs provides information on epileptogenic focus localization in 79.4% of patients. It was indicated that 7.7% of SCSs were detected in 20-minute EEG, 83.3% in 24-hour EEG, and 92.8% in 48-hour EEG. The majority of these seizures were observed during sleep.

In our study, the presence of sleep was detected in 66% of patients who underwent a twenty-minute EEG, and 47% of these individuals exhibited SCSs. In patients undergoing 24-hour EEG monitoring, all of them displayed sleep patterns, and SCSs were identified in 100% of these cases. All seizures that were observed occurred during sleep. Given that polysomnography and electrooculography were not used, differentiation of the REM phase was not considered in the assessment.

NREM2 and NREM3 stages were collectively considered as deeper sleep stages than NREM1. A significant association was found between sleep staging and SCS, with a noticeable increase in risk favoring deep sleep. Due to the small number of patients, an OR of 41 was determined. The occurrence of SCS during surface sleep in a patient undergoing 24-hour EEG monitoring may have contributed to this result. It is believed that with an increase in the number of patients, this ratio will be more normalized. The higher risk associated with deep sleep aligns with findings in other studies [10].

In the study by Farooque and Duckrow [3], SCSs in patients who underwent epilepsy surgery were considered. The results indicated that 64% of patients showing the presence of SCSs in the area where epilepsy surgery was performed had a favorable prognosis. It was suggested that SCSs could be used for predicting surgical prognosis and non-seizure follow-ups. In the same study, it was shown that SCSs in the included patients mostly originated from outside the temporal region, although in many other studies, they were predominantly thought to be temporal in origin [1].

In patients subjected to a twenty-minute EEG, a temporal focus was identified in 42% of cases, while a frontal focus was noted in 37%. Among those who underwent a 24-hour EEG, a frontal focus was detected in 60% of cases. This result, which differs from other studies, is thought to be related to patient selection. Patients with detected frontal and temporal foci had previously received these diagnoses and are being followed up accordingly.

Advanced statistical analyses revealed that the presence of SCS was 2.2 times more likely in individuals with a frontal focus compared to those with a temporal focus. The risk of experiencing SCSs during sleep was found to be elevated in patients with frontal lobe epilepsy. This heightened risk was consistently observed in both short-term and long-term EEG recordings. This observation suggests that the probability of diagnosing frontal lobe epilepsy increases when patients are induced into sleep during short-term EEG recordings.

Reducing the frequency and severity of epileptic attacks leads to a noticeable improvement in clinical outcomes. Various studies have shown that patients with SCS have decreased in memory and cognitive functions. There is evidence supporting the notion that SCSs contribute to memory impairment and autonomic dysfunction [9-13]. The treatment and elimination of SCSs have been associated with a significant improvement in cognitive functions in these cases [11, 14, 15].

In the study conducted by Vossel et al. [5] on patients diagnosed with Alzheimer's disease (AD), SCSs were identified in 42.2% of patients with AD. At the time SCSs were detected, there were no clinical symptoms present in the patients. However, follow-up observations have shown that patients with SCSs experience a faster cognitive decline process.

To assess the relationship between the treatment process of SCSs and medications, the study included data on the number and types of medications used by patients. Analysis of medication usage revealed a higher prevalence of monotherapy in patients undergoing short-term EEG. The use of more than three medications was similarly observed in this group. Upon closer examination of these patients, it was noted that all of them were in a medication transition phase. In contrast, many of the patients who underwent 24-hour EEG were potential candidates for epilepsy surgery, and their medications were more consistently regulated and monitored. As a result, a more even distribution of medication numbers was observed. While no significant difference was found between the number of medications and SCSs, the proximity to statistical significance suggests that with an increase in the number of patients, this relationship could gain significance.

Therefore, the study suggests that polytherapy may be favorable in patients with SCSs. It was observed that 50% of the patients in the study used levetiracetam. The high prevalence of levetiracetam usage was attributed to the difficulty in distinguishing between focal epilepsy and generalized epilepsy, leading to a broad spectrum of medication choices. Consequently, when examining the OR in patients using levetiracetam, a 1.2-fold increase in the risk of SCSs was observed. This finding underscores the importance of meticulous diagnosis of focal epilepsy and suggests that medication selection based on this diagnosis may be more effective in seizure control.

In recent years, new treatment options have emerged in the battle against epilepsy. Among these, the most preferred ones are lacosamide and zonisamide. The study indicates a high utilization rate of 17.9% and 14.9% for these two drugs among the patients. The effectiveness of these two drugs was compared with others. In the comparison between patients using frequently preferred drugs in neurology clinics (levetiracetam, valproate, oxcarbazepine, and carbamazepine) and those using newer-generation drugs, a significant statistical difference was observed. In patients using four

drugs, a 1.5-fold increase in the risk of SCS was detected according to the calculated OR. Increasing the number of patients could allow for a more detailed analysis of this comparison, potentially revealing more substantial differences.

The reason for categorizing patients into two groups lies in the absence of 24-hour EEG recording facilities in many centres. This underscores the need to demonstrate whether meaningful findings can be detected with short-term EEG. In most short-term EEG recordings, a sleep pattern is evident, and most seizures are detected during sleep stages. This suggests that the probability of detecting epileptiform changes in EEGs recorded with patients being induced into sleep is higher, providing us with an easier diagnostic opportunity. Even in a twenty-minute EEG recording, the presence of characteristics of NREM1 and NREM2 stages is valuable when considering the relationship between SCS and sleep.

In patients presenting with suspicion of epileptic seizures, a diagnosis of focal or generalized epilepsy should be meticulously established, and appropriate medication selection should be made. The study indicates that broad-spectrum anti-seizure treatment options are less effective compared to newer-generation medications, with a particular increase in risk noted for levetiracetam.

Regarding the limitations of our study, it is thought that the small number of patients who underwent 24-hour EEG may cause the statistical risk ratio to be overestimated. Studies with larger sample sizes need to be planned for more consistent responses. It was observed that many of the patients who underwent 24-hour EEG were followed up because they were diagnosed with resistant epilepsy or were planned for surgery. Due to this situation, a significant difference was observed in the number of medications between the two groups.

Sleep serves as one of our tools in examining both physiological and pathological events. It is utilized to reveal underlying pathologies that may not be apparent. SCSs are gaining increasing clinical significance in terms of diagnosis, prognosis, and treatment. The relationship between these two aspects holds importance for future research with a larger number of patients and centres.

Funding: None.

Authors contributions: C.E., E.A.D. have constructed the main idea and hypothesis of the study. C.E., E.A.D. developed the theory and arranged the material and method section. C.E., E.A.D. have done the evaluation of the data in the Results section. The Discussion section of the article was written by C.E. C.E., E.A.D., İ.H.A. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Zangaladze A, Nei M, Liporace JD, Sperling MR. Characteristics and clinical significance of subclinical seizures. *Epilepsia*. 2008;49(12):2016-2021. doi:10.1111/j.1528-1167.2008.01672.x
2. Velkey A, Siegler Z, Janszky J, Duray B, Fogarasi A. Clinical value of subclinical seizures in children with focal epilepsy. *Epilepsy Res*. 2011;95(1-2):82-85. doi:10.1016/j.eplepsyres.2011.03.001
3. Farooque P, Duckrow R. Subclinical seizures during intracranial EEG recording: are they clinically significant?. *Epilepsy Res*. 2014;108(10):1790-1796. doi:10.1016/j.eplepsyres.2014.09.020
4. Fernández Torre JL, Hernández Hernández M, Martino J, Hinojo C. Subclinical focal seizures as a sign of progression in gliomas. *Epileptic Disord*. 2014;16(4):546-553. doi:10.1684/epd.2014.0701
5. Vossel KA, Ranasinghe KG, Beagle AJ, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol*. 2016;80(6):858-870. doi:10.1002/ana.24794
6. Jin B, Wang S, Yang L, et al. Prevalence and predictors of subclinical seizures during scalp video-EEG monitoring in patients with epilepsy. *Int J Neurosci*. 2017;127(8):651-658. doi:10.1080/00207454.2016.1220946
7. Anderson CT, Tcheng TK, Sun FT, Morrell MJ. Day-Night Patterns of Epileptiform Activity in 65 Patients With Long-Term Ambulatory Electrocorticography. *J Clin Neurophysiol.* 2015;32(5):406-412. doi:10.1097/WNP.0000000000000183
8. Cox BC, Khattak JF, Starnes K, et al. Subclinical seizures on stereotactic EEG: characteristics and prognostic value. *Seizure*. 2022;101:96-102. doi:10.1016/j.seizure.2022.07.015

9. la Fougère C, Rominger A, Förster S, Geisler J, Bartenstein P. PET and SPECT in epilepsy: a critical review. *Epilepsy Behav*. 2009;15(1):50-55. doi:10.1016/j.yebeh.2009.02.025
10. Frauscher B, Gotman J. Sleep, oscillations, interictal discharges, and seizures in human focal epilepsy. *Neurobiol Dis*. 2019;127:545-553. doi:10.1016/j.nbd.2019.04.007
11. Brotherstone R, McLellan A. Parasympathetic alteration during sub-clinical seizures. *Seizure*. 2012;21(5):391-398. doi:10.1016/j.seizure.2012.03.011
12. Akman CI, Montenegro MA, Jacob S, et al. Subclinical seizures in children diagnosed with localization-related epilepsy: clinical and EEG characteristics. *Epilepsy Behav*. 2009;16(1):86-98. doi:10.1016/j.yebeh.2009.06.026
13. Adjei P, Surges R, Scott CA, Kallis C, Shorvon S, Walker MC. Do subclinical electrographic seizure patterns affect heart rate and its variability?. *Epilepsy Res*. 2009;87(2-3):281-285. doi:10.1016/j.eplepsyres.2009.08.011
14. Puteikis K, Wolf P, Mameniškienė R. Longer-term verbal and visual memory patterns in patients with temporal lobe and genetic generalized epilepsies. *Epilepsia Open*. 2023;8(4):1279-1287. doi:10.1002/epi4.12779
15. Kanazawa K, Matsumoto R, Shimotake A, et al. Persistent frequent subclinical seizures and memory impairment after clinical remission in smoldering limbic encephalitis. *Epileptic Disord*. 2014;16(3):312-317. doi:10.1684/epd.2014.0664

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1554747

Syphilis coinfection in individuals living with HIV: tertiary university hospital data

HIV ile yaşayan bireylerde sifiliz eş enfeksiyonu: üçüncü basamak üniversite hastanesi verileri

Işıl Deniz Alıravcı, Sevinç Yenice Aktaş, Selçuk Kaya

Posted date:24.09.2024 Acceptance date:16.12.2024

Abstract

Purpose: This study aimed to examine the coinfection of syphilis in HIV-positive individuals and the epidemiological and clinical characteristics of these patients.

Material and methods: Our study was conducted in Çanakkale Onsekiz Mart University, between 13.02.2018 and 13.02.2024, in 26 patients with clinically and laboratory-confirmed syphilis co-infection among 142 HIV/AIDS patients who were followed up and confirmed in the infectious diseases and clinical microbiology outpatient clinic.

Results: A total of 142 HIV positive and 26 (18.3%) syphilis coinfected patients between the ages of 17-77 were included in the study. The mean ages of the infected and syphilis coinfected patients were 39.1 and 41.07, respectively. Six (23%) of the coinfected patients were married, the remaining 14 (53.8%) were single or divorced, five had multiple partners; 20 (77%) were heterosexual, four (15%) were men who have sex with men (MSM), and two (8%) were bisexual. Simultaneous HIV/syphilis positivity was detected in 14 (54%) of the coinfected patients at the time of diagnosis, while 7 (27%) were detected during the HIV treatment follow-up period, and 5 (19%) were detected before HIV diagnosis. Of the patients with coinfection, five (19.2%) had primary syphilis, 10 (38.5%) had latent syphilis, and 11 (42.3%) had secondary syphilis at the time of diagnosis. Twenty-two (85%) of the patients had been diagnosed and treated for syphilis only once, while the remaining (15%) had received two or more treatments.

Conclusions: The prevalence of syphilis coinfection and reinfection in patients was 18.3% and 15%, respectively. In cases of coinfection, male gender, alcohol and drug consumption, being MSM, irregular lifestyles, and sexual habits were found to be effective. It is important to conduct screening for syphilis and other sexually transmitted diseases (STDs) in HIV-infected patients. Providing psychiatric support to these patients in organizing their life plans is also very important for preventing HIV transmission and syphilis coinfection.

Keywords: Syphilis, HIV, coinfection.

Aliravcı ID, Yenice Aktas S, Kaya S. Syphilis coinfection in individuals living with HIV: tertiary university hospital data. Pam Med J 2025;18:294-302.

Öz

Amaç: Bu çalışmada HIV ile infekte bireylerde sifilizin koenfeksiyonunun varlığı ve bu hastaların epidemiyolojik ve klinik özelliklerinin incelenmesini amaçladık.

Gereç ve yöntem: Çalışmamız 13.02.2018-13.02.2024 tarihleri arasında Çanakkale Onsekiz Mart Üniversitesi Hastanesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji polikliniğinde takip edilen ve tanısı doğrulanan 142 HIV/AIDS hastası arasından klinik ve laboratuvar olarak doğrulanmış sifiliz koenfeksiyonu olan 26 hastada gerçekleştirildi.

Bulgular: Çalışmaya yaşları 17-77 arasında değişen 142 HIV pozitif ve 26 (%18,3) sifiliz koenfekte hasta dahil edildi. Enfekte ve sifiliz koenfekte hastaların yaş ortalamaları sırasıyla 39,1 ve 41,07 idi. Koenfekte hastaların altısı (%23) evliyken, kalan 14'ü (%53,8) bekar veya boşanmış, beşi multipartnerli; 20'si (%77) heteroseksüel, dördü (%15) erkeklerle seks yapan erkekti (men who have sex with men – MSM), ikisi (%8) biseksüeldi. Koenfekte hastaların 14'ünde (%54) eş zamanlı HIV/sifiliz pozitifliği tanı anında tespit edilirken, 7'si (%27) HIV tedavi-takip sürecinde ve 5'i (%19) HIV tanısı öncesinde tanı almıştı. Koenfekte hastaların beşinde (%19,2) tanı anında primer, 10'unda (%38,5) latent ve 11'inde (%42,3) tanı anında sekonder sifiliz vardı. Hastaların 22'sine (%85) sadece bir kez sifiliz tanısı konulmuş ve tedavi edilmişken, kalan (%15) hastalar iki veya daha fazla tedavi almıştı.

Işıl Deniz Alıravcı, Asst. Prof. Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye, e-mail: dr_isildeniz@hotmail.com (https://orcid.org/0000-0002-4740-1579) (Corresponding Author)

Sevinç Yenice Aktaş, Asst. Prof. Faculty of Medicine, Department of Medical Microbiology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye, e-mail: sevincyenice@gmail.com (https://orcid.org/0000-0003-0877-9162)

Selçuk Kaya, Prof. Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye, e-mail: kayaselcuk1973@gmail.com (https://orcid.org/0000-0003-4607-2870)

Sonuç: Hastalarda sifiliz koenfeksiyonu ve reenfeksiyon prevalansı sırasıyla 18,3% ve 15% idi. Koenfekte hastalarda erkek cinsiyet, alkol ve uyuşturucu tüketimi, erkeklerle seks yapan erkek olmak (MSM), düzensiz yaşam tarzı ve cinsel alışkanlıkların etkili olduğu bulundu. HIV enfeksiyonlu hastalarda sifiliz ve diğer cinsel yolla bulaşan hastalıklar (CYBH) için tarama yapmak önemlidir. Bu hastalara yaşam planlarını düzenlemeleri konusunda psikiyatrik destek sağlamak HIV bulaşmasını ve sifiliz koenfeksiyonunu önlemek için çok önemlidir.

Anahtar kelimeler: Sifiliz, HIV, koenfeksiyon.

Alıravcı ID, Yenice Aktaş S, Kaya S. HIV ile yaşayan bireylerde sifiliz eş enfeksiyonu: üçüncü basamak üniversite hastanesi verileri. Pam Tıp Derg 2025;18:294-302.

Introduction

Sexually transmitted diseases, such as HIV and syphilis, have shown an increasing trend worldwide. The most common method of transmission is heterosexual sexual intercourse. In addition, transmission routes, such as homosexual/bisexual sexual intercourse, intravenous drug use, infected blood transfusion, sharp object injuries, and transmission from mother to baby, are also known. Although HIV-positive patients receive antiretroviral therapy, the continuation of risky behaviors leads to an increase in other sexually transmitted diseases [1].

Syphilis is a sexually transmitted systemic infectious disease caused by a bacterium from the spirochete family, *Treponema pallidum,* and is a major public health problem worldwide [2]. There has been an increase in syphilis cases in Türkiye in recent years, with 3533 cases detected as of December 31, 2022 [3].

Syphilis and HIV are both sexually transmitted, and co-infection often occurs in men who have sex with men (MSM). Individuals who have recently been diagnosed with HIV are at higher risk of developing syphilis due to overlapping risk behaviors, such as having unprotected sexual activity. HIV infection has various effects on the onset, diagnosis, disease progression, and treatment of syphilis. While HIV infection negatively affects the prognosis of syphilis, genital ulcers observed in the course of syphilis may also increase the risk of HIV transmission [4].

HIV, anti-HIV therapies, and psychosocial stressors in HIV disease lead to psychiatric morbidity like depression throughout the disease course. Depressive symptoms increase the progress of the disease [5]. As a result, patients postpone applying to a health institution.

Especially among MSM, there is a high rate of HIV/syphilis co-infection due to the common transmission routes of HIV and syphilis and the increased infectiousness of one in the presence of the other [6]. Therefore, detecting the seroprevalence and risk factors of syphilis in HIV-infected individuals will draw a road map in preventing the spread of these infections. Considering the common transmission routes and increasing importance of Syphilis and HIV, our study aimed to investigate the presence of syphilis coinfection in individuals with HIV infection and analyze the risk factors, prognostic features, and clinical and laboratory data of these patients.

Material and methods

Our study was carried out in patients coinfected with HIV and Syphilis, whose diagnosis was confirmed and followed up by the Çanakkale Onsekiz Mart University Faculty of Medicine, Infectious Diseases and Clinical Microbiology Department, between 13.02.2018 and 13.02.2024. Retrospective data, including demographic and clinical/laboratory information, were collected from medical records obtained from the hospital's doctor and laboratory information system, including age, sex, HIV transmission route, coinfection with hepatitis B virus (HBV), and coinfection with hepatitis C virus (HCV).

HIV screening in serum samples was performed by ELISA (Architect-i2000-ABBOTT-USA), and recurrent reactive samples were confirmed by immunoblotting in the Turkish Public Health Institution Reference Laboratory. In the Western blot / "line immunoassay" test, in accordance with the recommendations of the CDC and the World Health Organization [7], at least two envelope protein bands (sgp120/gp41 or sgp105, gp36) or at least one envelope

protein band (sgp120/gp41 or sgp105, gp36) with the presence of a p24 antigen band was accepted as a confirmatory criterion for the diagnosis. Detection of bands other than those specified was considered indeterminate, and absence of a band was considered negative [8].

For serological syphilis diagnosis, the reverse algorithm was followed between 13.02.2018 and 13.02.2024, and all serum samples were tested as a non-treponemal test using VDRL (Trepolipin, Tulip Diagnostic, India) and RPR (Carbogen, Tulip Diagnostic, India), and as a treponemal test, TPHA (Spinreact, Girona, Spain). For both tests, the manufacturer's recommendations were followed. Serum titers detected at 1/80 and above in the TPHA test were considered positive.

Primary syphilis: Cases characterized by the presence of one or more ulcers (chancres) with positive serological tests. Secondary syphilis is characterized by characteristic lesions (localized or mucocutaneous) accompanied by generalized lymphadenopathy, often without the presence of a primary chancre, with positive serological tests. Latent syphilis: Cases detected with reactive non-treponemal and treponemal tests without any clinical signs or symptoms or with a fourfold increase in non-treponemal test titers compared to previously reported non-treponemal tests, or with a significant increase in titers according to the latest non-treponemal tests in the presence of a history of syphilis treatment. Latent syphilis is categorized into early latent (less than one year), late latent (more than one year), and indeterminate, based on the duration of infection. The patients were staged according to these definitions.

The study was approved by the Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University Medical School by protocol number 2024-07-06 and date: 24.07.2024.

Since our study was conducted retrospectively based on patient anamnesis files and hospital information system physician notes, informed consent was not obtained from the patients. There had been no face-to-face contact with the patients during the study.

Results

One hundred forty-two patients diagnosed with HIV were tested for syphilis, and 26 (18.3%) of them were coinfected. The average age of HIV and coinfected patients was found to be 39.1, ranging from 17 to 77 years old and 41.07, ranging from 23 to 61 years. 90.1% of HIV patients (n=128) and 100% (n=26) of coinfected patients were male (Table 1). Patients who tested negative for TPHA were considered to have never encountered syphilis during their lifetime. Positive results detected at a dilution of 1/80 or higher in the TPHA test were considered statistically significant. All HIV-syphilis coinfected patients were TPHA-positive. In TPHA positive patients, 61.5% (n=16) of them were RPR, and 76.9% (n=20) of them were VDRL positive (Figure 1). The findings obtained in the study include the demographic and clinical characteristics of the coinfected patients shown in Table 2.

Table 1. Distribution and characteristics of all patients

	Total number of patients (n)	Mean Age	Gender
HIV-Positive	142	39.1 (17-77)	Male:128 (90.1%) Female:14 (9.9%)
HIV+ Syphilis	26	41.07 (23-61)	Male:26

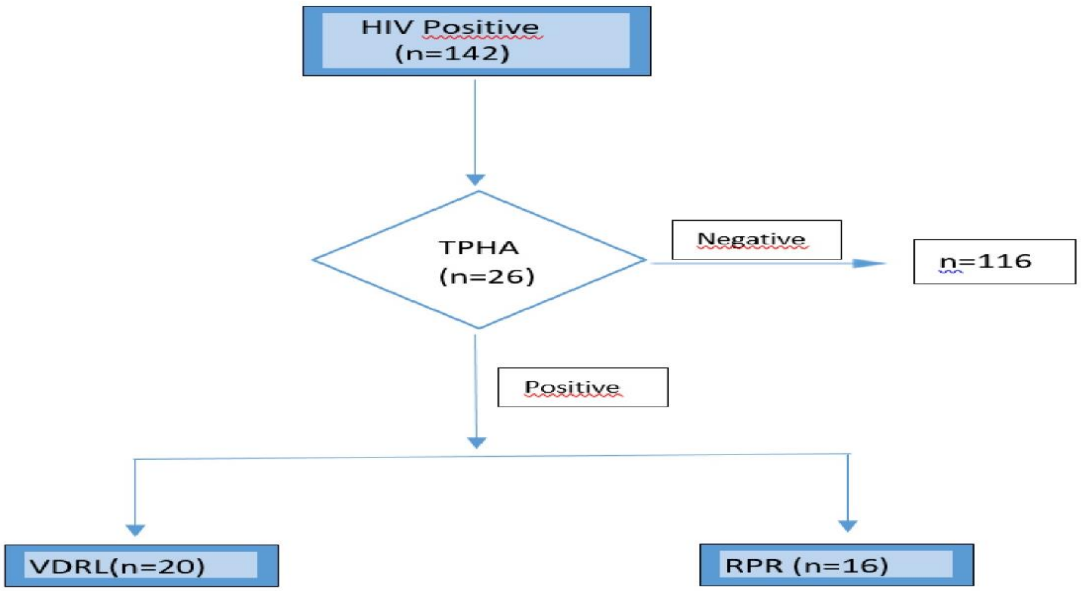


Figure 1. Syphilis serological test results of all patients

Table 2. Distribution and characteristics of HIV/Syphilis coinfected patients

	Total number of patients (n)	Percent (%)
Gender/ Male	26	100
MSM	4	15
Bisexual	2	8
Married	6	23
Multiple partners	5	19
Alcohol consumption	17	74
Drug Addicition	5	19
No-Comorbid diseases	18	69
Urethritis	5	19
Depression	5	19
Hypertension	4	15
Diabetes mellitus	3	11
Hepatitis C	2	8
Hepatitis B	2	8
Non-Hodgkin lymphoma	1	4
Extra pulmoner tuberculosis	1	4
Asthma	1	4
Prisoner	2	8
Refugee	1	4
Sex worker	1	4

While all primary and secondary syphilis cases were VDRL positive, in latent cases positivity was 5 (45.4%) at the time of diagnosis. When the VDRL dynamics of these cases before and after treatment were examined, a decrease and disappearance of the VDRL titer was observed after treatment. In all cases, the VDRL was negative for at least 12th month after treatment. When HIV-syphilis co-infected patients were screened for HBV and HCV, HBsAg positivity was detected in two patients, isolated anti-Hbc IgG positivity was detected in one patient, and two patients were anti-HCV positive.

While 18 (69.2%) patients had no comorbidities, the remaining 8 (31.8%) had additional conditions such as diabetes, hypertension, depression, asthma, and hepatitis B, and C. Among the patients, 17 (60.7%) had a history of alcohol consumption, 5 (19.2%) had a history of drug addiction, and 1 had received immunosuppressive agents due to non-Hodgkin lymphoma. Six (23%) of the coinfected patients were married, while the remaining 14 (53.8%) were single or divorced; 20 (77%) were heterosexual, four (15%) were men who have sex with men (MSM), and two (8%) were bisexual. Regarding the transmission routes of syphilis, one patient had a history of dental procedures in prison, two had a common history of using personal care items, and one had a history of hospitalization in Tanzania, two had an IV drug history, while the majority (n=20, 77%) had a history of unprotected sexual intercourse. As a matter of fact, when the demographic data of the patients were examined, it was seen that one was a sex worker, two were prisoners, five were drug addicts, one was an immigrant, three had a history of working abroad, and all patients were male.

While 14 (54%) of the co-infected patients were simultaneously HIV/syphilis positive, seven (27%) were found to be syphilis positive during the HIV treatment follow-up period, and five (19%) were positive to be syphilis positive before HIV diagnosis (Figure 2).

Among those with coinfection, 5 (19.2%) had primary syphilis, 11 (42.3%) secondary syphilis, and 10 (38.5%) latent syphilis (Figure 3). Genital chancre was the common finding in primary syphilis cases, whereas rash and lymphadenopathy were the most common findings in secondary syphilis. One patient had an ocular syphilis infection. While 24 patients (88.8%) were treated with 2.4 million units of benzathine penicillin G once a week for 3 weeks, one patient received doxycycline due to penicillin allergy, and another received ceftriaxone therapy due to ocular syphilis for 14 days.

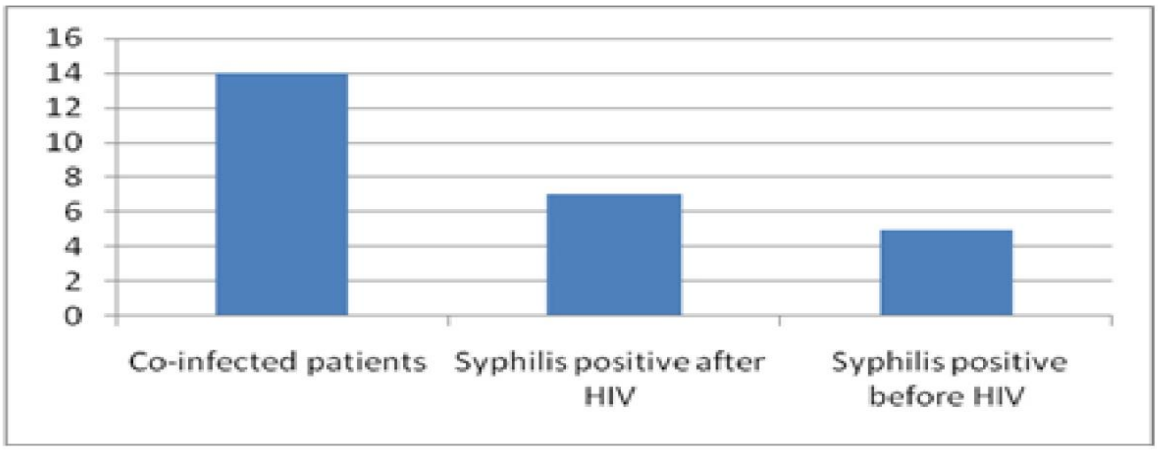


Figure 2. Separation of patients according to the order of coinfection

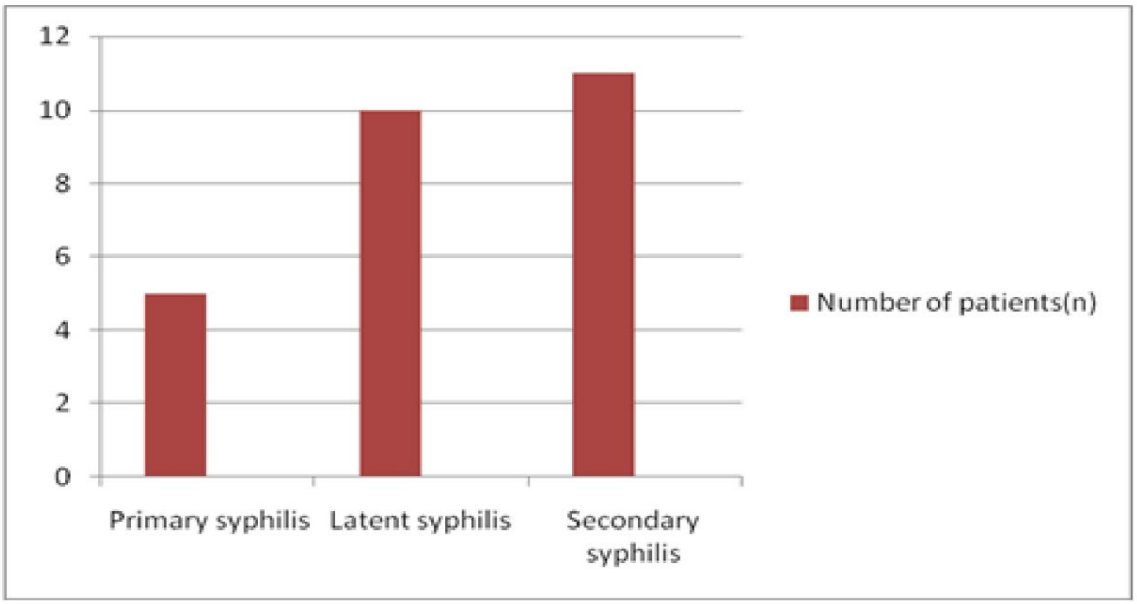


Figure 3. Clinical staging of syphilis in coinfected patients

Twenty-two (85%) patients had received syphilis diagnosis and treatment only once, while the remaining (15%) had received two or more treatments. When the partners were questioned whether they had syphilis, it was seen that one partner of four (15%) had syphilis as known. HIV-positive patients received antiretroviral treatments between 2 and 149 months (average, 64 months). While twenty-four (92%) patients had been receiving HIV treatment for more than a year, two patients were diagnosed in the last 2 months. Thirteen of the patients received bictegravir, emtricitabine, and tenofovir alafenamide; four patients received abacavir, lamivudine, dolutegravir; 4 patients received lamivudine, dolutegravir; 3 patients received dolutegravir, emtricitabine, and tenofovir disoproxil; and two patients received elvitegravir, emtricitabine, and tenofovir alafenamide combination therapy. After at least 12 months of follow-up, in the last examination of patients receiving regular antiretroviral treatments, HIV RNA positivity was detected in four (22.2%).

While none of the co-infected patients had a CD4 count below 200 during syphilis infection, the control CD4 count after treatment was above 400 and only four patients had a mean CD4 count of 202.

Discussion

In a multicenter study conducted in our country, the HIV/syphilis coinfection rate was found to be 8% [9]; this rate was found to be between 1-25% in the literature review [10]. In 2022 Türkiye statistics, most patients diagnosed with syphilis were male (81.5%) and between the ages of 25-29 [11]. Müderris et al. [12] stated that all patients except one were male among HIV/syphilis coinfected patients, and more than 90% of them were over 25 years of age. Consistent with the literature, in our study, HIV-syphilis comorbidity was found to be 18% in HIV patients, and all patients were male (100%) between the ages of 23-61 (mean: 41.07).

The coexistence of HIV infection and syphilis has been increasing worldwide in recent years, especially among MSM [13]. Fan et al. [14] stated in his meta-analysis published by 2021 that 40.1% of HIV/Syphilis co-infected patients were receiving ART and 42.5% of them were MSM. In our study, all patients were receiving ART, and five patients had multiple partners and four were MSM and two were bisexual.

Most patients diagnosed with HIV between 1985 and 08 November 2023 were men (81.4%) and Turkish citizens (83.1%) [15]. In our study, only one patient was an immigrant, and the rest were Turkish citizens (96%). Although no

mortality was observed in our patients during an average of 6 years of follow-up, it is taken into consideration that this rate may change in longer-term follow-up.

Co-transmission of *T. pallidum* and HIV may be due to the risky behavior of patients, such as unprotected intercourse and multiple partnerships. Therefore, in addition to applying a syphilis screening test to patients newly diagnosed with HIV infection, syphilis screening tests should be repeated during routine follow-up of patients with risky behavior or suspicious clinical findings [16, 17]. In our study, six patients were married, and all patients had a history of risky sexual behavior. Seven patients (27%) caught syphilis during HIV treatment and follow-up.

Sarıgül et al. [17] stated that 25% of 384 HIV-positive patients had HIV/syphilis coinfection, and 94% of these patients had positive RPR and TPHA tests. In our study, 77% of patients had VDRL and TPHA, and 61% of patients had RPR and TPHA positivity, respectively.

Remera et al. [18] stated that HIV and hepatitis B virus coinfection was found in 0·5%, whereas HIV and hepatitis C coinfection was 0·1%, and no coinfection for any three viruses was observed. In our study, there were 2 separate patients with hepatitis B (7.7%) and C (7.7%) coinfected, and 5 (19%) of them had urethritis. Our high rate might be due to the low total number of patients. We believe that the reason for our high rate is that Türkiye is in the medium-endemicity region in terms of hepatitis.

Recently, a significant increase in the number of primary and secondary syphilis cases has been reported worldwide. According to CDC data, approximately half of homosexual men with primary and secondary syphilis are infected with HIV. Studies have shown that the symptoms of secondary syphilis appear more frequently during the same period as chancres in patients with HIV infection [19]. In our study, five (19.2%) had primary syphilis, 10 (38.5%) had latent syphilis, and 11 (42.3%) had secondary syphilis. While the symptoms of syphilis are very diverse, the untreated disease passes into the latent phase over time, and the symptoms disappear, making it difficult to combat the disease and seeming to be a significant problem. Although the secondary syphilis patient group was the most frequently admitted patient group in our study (42.3%), the high number of patients in the latent phase (38.5%) attracted our attention.

The most common manifestation of syphilis in the eye is uveitis, which can occur at any stage of infection. Although most patients recover well with penicillin treatment, some may develop visual loss due to macular edema and endarteritis, causing retinal ischemia [20, 21]. After penicillin treatment, 67% of patients experienced improved vision, with a 92% of rate reduced inflammatory signs. However, despite treatment, relapse of ocular diseases may still occur [9, 22]. In this study, only one patient developed ocular syphilis during the second syphilis stage, and no vision loss was observed during the post-treatment follow-up.

In HIV/syphilis co-infection, HIV is more likely to fail syphilis treatment, and co-infection leads to profound neurocognitive impairment [23]. In our study, no neurological findings other than depression (n=5,19%) were observed. In addition, in the only case of ocular syphilis, there was no neurological involvement. In our study, no treatment failure was observed for syphilis.

Fan et al. [14] stated that the syphilis reinfection rate was 7% in 7 years. In our study, four patients (15%) received syphilis treatment multiple times. The higher reinfection rate in our study than that reported in the literature was associated with the small number of patients.

The main limitation of the study was that patients tended to give hesitant answers when questioned about sexual history in outpatient clinics.

In our study, syphilis coinfection and reinfection rates in HIV patients were found to be 18% and 15%, respectively. We observed that our patients applied to health institutions, especially in the late stages of syphilis, or received their diagnosis late. This situation is thought to be due to reasons such as lack of education and sexual stigma. Considering the importance of early diagnosis in terms of prognosis and public health, planning educational activities aimed at increasing the level of sexual knowledge in our country and increasing the number and functionality of Voluntary Counseling and Testing Centers (VTCs) reveals the importance of approaches.

Even though 46% of the patients were coinfected with HIV or syphilis after the first diagnosis and the fact that approximately 1/3 of the patients diagnosed with syphilis were in the latent stage, as well as the 19% presence of concurrent urethritis, made us think that the patients continued their unprotected sexual habits and they did not avoid risky contact.

In addition, the development of mood disorders, such as depression, in this group of patients, who are mostly unmarried (n=20.77%) and do not live a regular life, also contributes to the course of the disease. Considering the increase in morbidity and mortality that may be caused by these disorders, as a natural consequence of the disease by both the patient and the physician normalized, ignored, or overlooked during the disease process, the patient's cognitive and psychosocial status and mental complaints should be routinely questioned independent of the infection. However, under outpatient clinic conditions, our patients may not accurately express information about their psychosocial status or sexual preferences. Considering the large number and diversity of outpatient clinic patients in our country, examining this patient group in separate clinic rooms and on different days reserved for HIV patients will yield more accurate results in terms of correctly identifying transmission routes and analyzing psychosocial and sexual problems that may adversely affect the course of the disease.

Screening for syphilis in HIV-infected patients, planning training for sexually transmitted diseases with treatment, providing psychological support, and advising patients to organize their life plans are very important to prevent syphilis and HIV transmission.

Main Points: Our analysis about HIV and syphilis coinfected patients who had received a secondary sexually transmitted disease (STD) diagnosis after receiving their first STD diagnosis made us think that the patients continued their unprotected sexual habits and they did not avoid risky contact. We also found that all coinfected patients in our study were male.

Not being married and having multi-partner sexual life or an irregular work schedule contributes to the course of the disease.

The main reason why these patients applied to healthcare institutions late or received their diagnosis late is thought to be due to reasons such as lack of education and sexual stigma.

Screening for syphilis in HIV-infected patients, planning training for sexually transmitted diseases with treatment, providing psychological support, and advising patients to organize their life plans are very important to prevent syphilis and HIV transmission.

Funding: None.

Author contributions: Concept – I.D.A.; Design – I.D.A.; Supervision – S.K.; Resources – I.D.A., S.Y.A.; Materials – I.D.A., S.Y.A.; Data Collection and/or Processing – I.D.A., S.Y.A.; Analysis and/or Interpretation – I.D.A.; Literature Search – I.D.A.; Writing – I.D.A., S.Y.A.; Critical Review – S.K.

Conflict of interest: The authors have no conflict of interest to declare.

References

1. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet*. 2014;384(9939):241-248. doi:10.1016/S0140-6736(14)60604-8
2. Hook EW 3rd. Syphilis [published correction appears in Lancet. 2019 Mar 9;393(10175):986. doi:10.1016/S0140-6736(19)30483-0]. *Lancet*. 2017;389(10078):1550-1557. doi:10.1016/S0140-6736(16)32411-4
3. Sfiliz vaka dağılımı. Available at: https://hsgm.saglik.gov.tr/depo/birimler/bulasici-hastaliklar-ve-erken-uyari db/Dokumanlar/Istatistikler/sifiliz_istatistikleri.pdf. Accessed February 13, 2024
4. Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. *Lancet Infect Dis*. 2004;4(7):456-466. doi:10.1016/S1473-3099(04)01061-8
5. Castellon SA, Hinkin CH, Wood S, Yarema KT. Apathy, depression, and cognitive performance in HIV-1 infection. *J Neuropsychiatry Clin Neurosci*. 1998;10(3):320-329. doi:10.1176/jnp.10.3.320
6. Centers for Disease Control and Prevention (CDC). Notes from the field: repeat syphilis infection and HIV coinfection among men who have sex with men--Baltimore, Maryland, 2010-2011. *MMWR Morb Mortal Wkly Rep*. 2013;62(32):649-650.
7. World Health Organization (2016). WHO guidelines for the treatment of Treponema pallidum (syphilis). World Health Organization. https://iris.who.int/handle/10665/249572. Accessed February 13, 2024

8. Dinç HM, Özbey D, Sirekbasan S, et al. Seroepidemiologic Evaluation of HIV Scanning and Verification Datas During The Period of 2015-2018. ANKEM. 2019;33(3):89-94. doi:10.5222/ankem.2019.089
9. Sarigül F, Sayan M, İnan D, et al. Current status of HIV/AIDS-syphilis co-infections: a retrospective multicentre study. *Cent Eur J Public Health*. 2019;27(3):223-228. doi:10.21101/cejph.a5467
10. 10.Can Bilek H, Deveci A, Aksakal Tanyel E. Seroprevalence of hepatitis A virus, hepatitis B virus, hepatitis C virus, and syphilis among human immunodeficiency virus-infected people at a university hospital, Turkey. *Archives of Medical Science*. 2020. doi:10.5114/aoms.2020.97889
11. Sifiliz İstatistik. (2024) Available: https://hsgm.saglik.gov.tr/tr/bulasici-hastaliklar. Accessed February 13, 2024
12. Müderris T, Peker BO, Gülvardar Baran N, Aksoy Gökmen A, Kaya S, Yurtsever SG. HIV pozitif hastalarda sifiliz seroprevalansı. Flora. 2022;27(1):21-7. doi:10.5578/flora.20228585
13. Said MA, German D, Flynn C, *et al.* Uptake of Testing for HIV and Syphilis Among Men Who Have Sex with Men in Baltimore, Maryland: 2004–2011. *AIDS Behav*. 2015;19:2036-2043. doi:10.1007/s10461-015-1106-y
14. Fan L, Yu A, Zhang D, et al. Consequences of HIV/Syphilis Co-Infection on HIV Viral Load and Immune Response to Antiretroviral Therapy. *Infection and Drug Resistance.* 2021;14:2851-2862. doi:10.2147/IDR.S320648
15. HIV-AIDS Statistics (2023) T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü; Available: https://hsgm.saglik.gov.tr/tr/bulasici-hastaliklar/hiv-aids/hivaids-liste/hiv-aids-istatislik.html. Accessed February 13, 2024
16. Schmidt R, Carson PJ, Jansen RJ. Resurgence of Syphilis in the United States: An Assessment of Contributing Factors. *Infect Dis (Auckl)*. 2019;12:1178633719883282. Published 2019 Oct 16. doi:10.1177/1178633719883282
17. Sarıgül F, Üser Ü, Öztoprak N. Seroprevalence and risk factors in HIV/AIDS patients coinfected with syphilis. *Klimik*. 2019;32:161-164. doi:10.5152/kd.2019.35
18. Remera E, Tuyishime E, Kayitesi C, et al. HIV and hepatitis B, C co-infection and correlates of HIV infection among men who have sex with men in Rwanda, 2021: a respondent-driven sampling, cross-sectional study. *BMC Infect Dis*. 2024;24(1):347. Published 2024 Mar 23. doi:10.1186/s12879-024-09206-2
19. Sayan M, Sargin F, Inan D, et al. HIV-1 Transmitted Drug Resistance Mutations in Newly Diagnosed Antiretroviral-Naive Patients in Turkey. *AIDS Res Hum Retroviruses*. 2016;32(1):26-31. doi:10.1089/AID.2015.0110
20. Tsan GL, Claiborne RT. Ocular syphilis. *Clin Exp Optom*. 2021;104(7):756-759. doi:10.1080/08164622.2021.1906848
21. Harford DA, Green Sanderson K, Stephenson KAJ, et al. *BMJ Case Rep*. 2021;14. doi:10.1136/bcr-2020241403
22. Ghanem KG, Erbelding EJ, Wiener ZS, Rompalo AM. Serological response to syphilis treatment in HIV-positive and HIV-negative patients attending sexually transmitted diseases clinics. *Sex Transm Infect*. 2007;83(2):97-101. doi:10.1136/sti.2006.021402
23. Marra CM, Deutsch R, Collier AC, et al. Neurocognitive impairment in HIV-infected individuals with previous syphilis. *Int J STD AIDS*. 2013;24(5):351-355. doi:10.1177/0956462412472827

Research Article
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1592341

The effect of thymoquinone on cell proliferation, 8-hydroxy-2' -deoxyguanosine level and expression changes of DNA repair and oxidative stress-related genes in MCF-7 breast cancer cells

Timokinonun MCF-7 meme kanseri hücrelerinde hücre proliferasyonu, 8-hidroksi-2' –deoksiguanozin seviyesi ile DNA tamiri ve oksidatif stres ilişkili genlerin ekspresyon değişimlerine etkisi

Mücahit Seçme, Sümeyya Deniz Aybek, Gonca Gülbay, Yavuz Dodurga

Posted date:27.11.2024 Acceptance date:17.01.2025

Abstract

Purpose: The aim of this study was to determine the effects of thymoquinone on cell proliferation, 8-hydroxy-2' -deoxyguanosine level and expression changes of oxidative stress and DNA repair-related genes in MCF-7 breast cancer cells.

Material methods: Cell proliferation in MCF-7 cells after thymoquinone exposure was determined by MTT assay. 8-hydroxy-2' -deoxyguanosine protein concentration was measured by ELISA assay. Total RNA isolation from control and thymoquinone treated cell was performed by Trizol and cDNA was synthesized. mRNA expression changes of *OGG1, NEIL-1, CRCC1 SOD2, CAT* and *NRF-2* were also determined in RT-PCR using SYBER Green method.

Results: In this study, the IC50 dose of thymoquinone in MCF-7 cells was determined as 7.867 µM at 24th hour. It was investigated that thymoquinone inhibited 8-hydroxy-2' -deoxyguanosine level in breast cancer cells according to RT-PCR results, thymoquinone increased *XRRC1* expression 4.71-fold and catalase expression 6.68-fold in breast cancer cells.

Conclusion: In conclusion, TQ inhibits MCF-7 cell proliferation under in vitro conditions. It also alters the expression of genes associated with DNA repair and oxidative stress and acts through this oxidative stress mechanism. This study contributes to the existing literature and provides preliminary data for more comprehensive studies.

Keywords: Thymoquinone, MCF-7 cells, breast cancer, DNA repair genes, oxidative stress.

Secme M, Aybek SD, Gulbay G, Dodurga Y. The effect of thymoquinone on cell proliferation, 8-hydroxy-2' -deoxyguanosine level and expression changes of DNA repair and oxidative stress-related genes in MCF-7 breast cancer cells. Pam Med J 2025;18:304-312.

Öz

Amaç: Bu çalışmanın amacı, timokinonun MCF-7 meme kanseri hücrelerinde hücre proliferasyonu, 8-hidroksi-2' -deoksiguanozin düzeyi ve oksidatif stres ve DNA onarımı ile ilişkili genlerin ekspresyon değişiklikleri üzerindeki etkilerini belirlemektir.

Gereç ve yöntem: Timokinon maruziyetinden sonra MCF-7 hücrelerinde hücre proliferasyonu MTT testi ile belirlendi. 8-hidroksi-2' -deoksiguanozin protein konsantrasyonu ELISA testi ile ölçüldü. Kontrol ve timokinon uygulanan hücrelerden Trizol ile total RNA izolasyonu yapıldı ve cDNA sentezlendi. *OGG1, NEIL-1, CRCC1 SOD2, CAT* ve *NRF-2*'nin mRNA ekspresyon değişiklikleri de SYBER Green yöntemi ile RT-PCR'da belirlendi.

Bulgular: Bu çalışmada, MCF-7 hücrelerinde timokinonun IC50 dozu 24. saatte 7,867 µM olarak belirlenmiştir. Timokinonun meme kanseri hücrelerinde 8-hidroksi-2' -deoksiguanozin seviyesini inhibe ettiği araştırılmıştır. Ayrıca RT-PCR sonuçlarına göre, timokinon meme kanseri hücrelerinde *XRRC1* ekspresyonunu 4,71 kat ve katalaz ekspresyonunu 6,68 kat artırmaktadır.

Sonuç: Sonuç olarak, TQ in vitro koşullar altında MCF-7 hücre proliferasyonunu inhibe eder. Ayrıca DNA onarımı ve oksidatif stres ile ilişkili genlerin ekspresyonunu değiştirir ve bu oksidatif stres mekanizması yoluyla etki eder. Bu çalışma mevcut literatüre katkıda bulunmakta ve daha kapsamlı çalışmalar için ön veri sağlamaktadır.

Mücahit Seçme, Assoc. Prof. Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Türkiye, e-mail: mehtersecme@gmail.com (https://orcid.org/0000-0002-2084-760X) (Corresponding Author)

Sümeyya Deniz Aybek, Asst. Prof. Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Türkiye, e-mail: sumeyyadenizcelik@gmail.com (https://orcid.org/0000-0001-9957-5485)

Gonca Gülbay, Assoc. Prof. Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Türkiye, e-mail: gonca.gulbay44@gmail.com (https://orcid.org/0000-0001-5201-6352)

Yavuz Dodurga, Prof. Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Türkiye, e-mail: yavuzdodurga@gmail.com (https://orcid.org/0000-0002-4936-5954)

Anahtar kelimeler: Timokinon, MCF-7 hücreleri, meme kanseri, DNA onarım genleri, oksidatif stres.

Seçme M, Aybek SD, Gülbay G, Dodurga Y. Timokinonun MCF-7 meme kanseri hücrelerinde hücre proliferasyonu, 8-hidroksi-2' –deoksiguanozin seviyesi ile DNA tamiri ve oksidatif stres ilişkili genlerin ekspresyon değişimlerine etkisi. Pam Tıp Derg 2025;18:304-312.

Introduction

Cancer is a pathological condition characterised by the excessive and uncontrolled growth of cells that have acquired abnormal properties and have a highly complex molecular genetic structure. These abnormal cells have malignant properties, including the ability to invade and metastasise [1, 2]. The process of carcinogenesis is a gradual integration of biological events in which oncogenic mutations in a normal cell affect cellular mechanisms such as survival, differentiation, growth, and signal transduction. This leads to the acquisition of new capabilities by the cells and provides a clonal advantage [3, 4].

Breast cancer is the second leading cause of cancer-related mortality in women, following lung cancer. In terms of incidence, breast cancer accounts for 32% of all cancer cases and 15% of cancer-related deaths [5]. Breast cancer detected at an early stage can be treated with treatments such as surgery and radiotherapy. However, metastatic breast cancer is the leading cause of death [6]. Although the development of resistance, toxicity in non-target organs, and side effects are limitations of chemotherapy and other cancer treatment strategies, studies are underway to develop potential cancer drugs and treatment strategies that overcome these limitations [7-10].

Natural and bioactive compounds isolated from plants have been used for many years in the treatment of many diseases due to their strong pharmacological properties, low side effects, low toxicity, natural abundance, and cost-effectiveness. Scientific studies have also highlighted the discovery of the strong anticancer potential of these bioactive molecules [11-14]. *Nigella sativa* is a dicotyledonous plant of the Ranunculaceae family. It has been used for many years as a sweetener, appetizer, spice or food in many regions, particularly in Asia, Europe, and Africa. The main bioactive compound of the widely used medicinal plant *Nigella sativa* is thymoquinone (TQ) [15-17]. Both cell culture and animal model studies have demonstrated that TQ exhibits anti-cancer, anti-microbial, anti-parasitic, anti-inflammatory, anti-diabetic, and antioxidant properties. [6, 18]. TQ has been shown to have antiproliferative activity in several types of cancer, including larynx, colon, breast, endometrium, pancreatic, and lung cancer. Its effects are mediated through cell cycle inhibition, induction of apoptosis, and regulation of gene expressions involved in various signalling pathways [17-22]. Recent studies have reported that the combination of TQ with nanoparticles or nanoparticle-based functionalised nanoformulations enhances its bioavailability and helps to make it more effective in cancer treatment. These properties are believed to make thymoquinone nanoparticles an attractive potential agent for cancer therapy [22, 23]. Although the antiproliferative effects of thymoquinone in breast cancer have been demonstrated, studies to elucidate its mechanisms of action are still ongoing. In this study, we aimed to determine the molecular biological mechanism underlying the antiproliferative effects of thymoquinone in MCF-7 cells by examining changes in the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and the mRNA expression of genes related to oxidative stress and DNA repair.

Materials and methods

Cell culture

The MCF-7 breast cancer cells were cultured in-Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) enriched with 10%. Fetal bovine serum (FBS; HyClone, USA) and 1% penicillin (100 U/mL)/streptomycin (0.1 mg/mL; Sigma Aldrich, USA) under standard conditions of 37°C in a humidified incubator with 5% CO2. Subculturing was performed in T-flasks two to three times weekly, with cells being harvested for assays once they reached approximately 90% confluency.

MTT assay

The antiproliferative effect of TQ was analysed by MTT assay (GoldBio, USA) according to kit the protocol. The MCF-7 cells

(1.0×10^4 cells/well) were cultured in 96-well plates in media for 24 hours. Cells were treated with increasing concentrations of 12.5, 25, and 50 µM of Thymoquinone (Sigma Aldrich, USA) for 24 h. 10 µL of MTT stock solution (5 mg/mL) was added to each well, and the plates were incubated at 37°C for 3 h. After incubation, the media were carefully removed by aspiration. Dimethyl sulfoxide (DMSO, 100 µL/well; Sigma Aldrich, USA) was used to solubilize the formazan crystals, and the plates were incubated at 37°C for 15 minutes. Finally, the absorbance was read at 570 nm with a microplate reader (Biotek-EPOCH2). The half-maximum inhibitory concentration (IC_{50}) of TQ was determined via non-linear regression by using GraphPad Prism (version 9.4.1). The percentage of MCF-7 cell viability was determined using the following formula:

Cell Viability (%) = [(Treatment group OD570 - Blank well OD570) / (Untreated group OD570 - Blank well OD570)] ×100%.

All experiments were performed using IC50 dose of TQ obtained from cell proliferation assays for MCF-7 cells.

8-hydroxy-2′-deoxyguanosine (8-OHdG) ELISA assay

The protein level of 8-OHdG in MCF-7 cells was investigated by an enzyme-linked immunosorbent assay (BT LAB, catalog no: EA0048Hu, China ELISA) kit according to the kit protocol. Control and dose group MCF-7 cells were homogenized in lysis buffer including protease inhibitors cocktail and centrifuged at 12.000 g for 15 min at 4°C. The supernatant was harvested and utilized for measuring 8-OHdG levels. The concentration of 8-OHdG was determined by comparing the sample readings to a pre-established standard curve and was expressed in ng/mL.

Gene expression assay

MCF-7 cells were treated with TQ at IC_{50} dose detected through MTT assay or media only (control). Total RNA was isolated using TRIzol (Hibrizol, Hibrigen, Türkiye), following the protocol provided by the manufacturer. NanoDrop was used to measure the amount and quality of RNA (BioSpec-nano, Shimadzu, Japan). The cDNA for each sample was then synthesized using the OneScript Plus cDNA Synthesis Kit (Abm, Cat No: G236, Canada), following the manufacturer's protocol. Relative RNA levels were detected using the Rotor Gene 6000 Real-time PCR Thermocycler (Corbett, USA) with NucleoGene qPCR SYBER-Green Master Mix (NucleoGene, Türkiye). The primer sequences for amplification of human *NEIL1, OGG1*, *XRCC1, NRF2, SOD2,* and *CAT* are presented in Table 1. The cycle conditions were as follows: 95°C for 15 min, 95°C for 15 s, and 60°C for 1 min, for a total of 40 cycles. The expression fold-change for each target was calculated by the $2^{-\Delta\Delta CT}$ method using *ACTB* as a normalization control.

Table 1. Reverse and Forward sequences of the primers

Gene symbol	Gene name	Primers
NEIL1	Nei like DNA glycosylase 1	Forward:GACAGAGGCAAGTGGCAAAGCA Reverse:GCCTCATTCACAAACTGGCTGG
OGG1	8-oxoguanine DNA glycosylase	Forward: GGCTCAACTGTATCACCACTGG Reverse:GGCGATGTTGTTGTTGGAGGAAC
XRCC1	X-ray repair cross complementing 1	Forward: CGGATGAGAACACGGACAGTGA Reverse: GAAGGCTGTGACGTATCGGATG
SOD2	Superoxide dismutase 2	Forward: CTGGACAAACCTCAGCCCTAAC ReverseAACCTGAGCCTTGGACACCAAC
NRF2	NFE2 like bZIP transcription factor 2	Forward:CACATCCAGTCAGAAACCAGTGG Reverse: GGAATGTCTGCGCCAAAAGCTG
CAT	Catalase	Forward: GTGCGGAGATTCAACACTGCCA Reverse: CGGCAATGTTCTCACACAGACG
ACTB	Actin beta	Forward: CACCATTGGCAATGAGCGGT Reverse: AGGTCTTTGCGGATGTCCAC

Statistical analyses

The Real-time PCR data were quantified using the ΔΔCT method, with analysis facilitated by the Gene Globe RT-PCR Analysis RT2 Profile PCR Array Data Analysis tool (Qiagen). Statistical evaluations were conducted using GraphPad Prism 9.4.1 software. All results are presented as mean ± standard deviation (S.D.) derived from at least three independent biological replicates. Mean comparisons were performed using either an Unpaired t-test or one-way analysis of variance (ANOVA), with Dunnett's test applied for post hoc analysis.

Results

Antiproliferative effect of thymoquinone treatment on MCF-7 cells

The MCF-7 cell viability after TQ treatment (12.5, 25, and 50 µM) for 24 hours was assessed by the MTT assay. The cell viability results and IC_{50} values are presented in Figure 1. As depicted in Figure 1A, 12.5, 25, and 50 µM of TQ significantly inhibited cell viability in MCF-7 cells, with an IC50 value of 7.867 µM (95% CI: 4.64-11.74 µM) (Figure 1B).

The effect of thymoquinone treatment on 8-hydroxy-2′ deoxyguanosine level

Treatment with TQ significantly decreased 8OHdG level in MCF-7 cells from 0.245±0.04 to 0.056±0.01 ng/mL compared with untreated control samples (Figure 2; p=0.0016).

The effect of TQ treatment on gene expression

To determine the effect of TQ on to influence the expressions of genes associated with DNA repair and oxidative stress response, MCF-7 cells were treated with TQ at the IC_{50} dose, for 24 h. Gene expressions were analyzed using RT-qPCR, and all expression levels are normalized to *ACTB* expression levels. Figure 3 and Table 2 showed that treatment of MCF-7 with TQ at the IC50 dose significantly increased the mRNA expression of *XRCC1* by 4.71-fold in comparison to untreated cells. Similarly, MCF-7 treated with TQ at the IC_{50} dose enhanced *CAT* mRNA expression by 6.68-fold in comparison to untreated cells. MCF-7 treated with TQ at the IC_{50} dose increased the expressions of *NEIL1, OGG1*, and *NRF2*, while decreased the mRNA expression level of *SOD2*, although not quite significantly (p>0.05). Fold changes in genes where no significant changes were observed were determined to be 5.72 for *NEIL-1,* 7.36 for *OGG1*, 1.43 for *NRF-2* and 0.70 for SOD2.

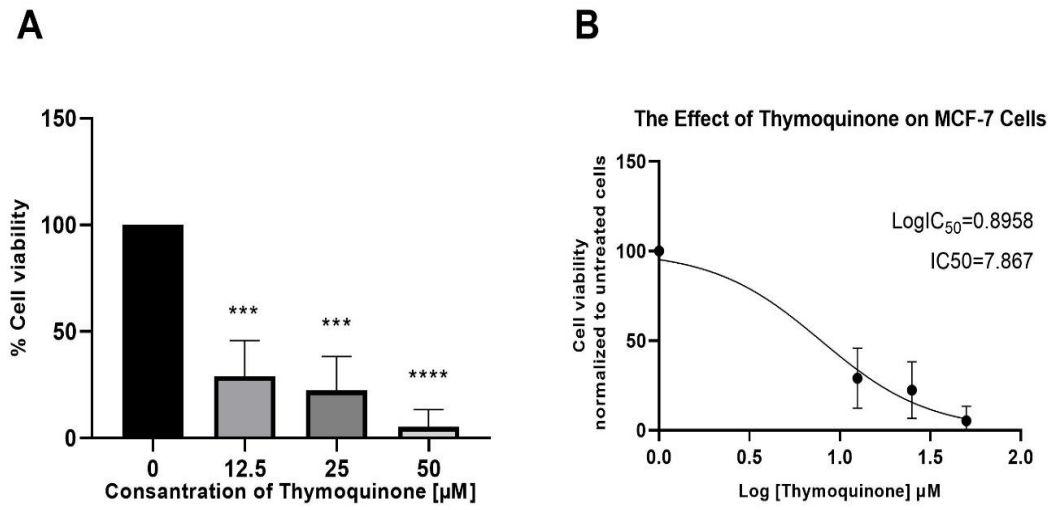


Figure 1. In vitro antiproliferative effect of TQ on MCF-7 cells at 24 h post-stimulation. (A) The effect of TQ on cell viability. The comparison of each dose group with the control was statistically significant (p values are shown on the graph; F=35.15), and (B) Dose-response curve of cell viability with respect to treatment with different concentrations of TQ

The results are reported as cell viability percentage (%) normalized to untreated MCF-7 cells. Comparisons between means were performed using one-way ANOVA, and Dunnett's test was utilized for post hoc analysis (ns p>0.05, * p≤0.05, ** p≤0.01, *** p≤0.001, and **** p≤0.0001)

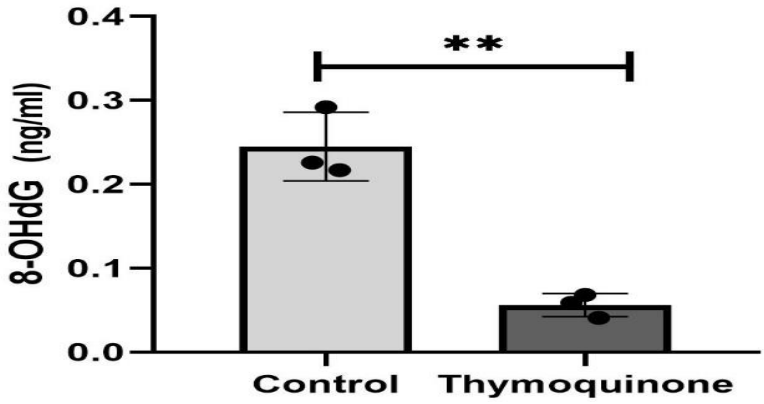


Figure 2. Effect of TQ on the content of 8-OHdG in MCF-7 cells. MCF-7 cells were treated with TQ at IC50 dose

The data are presented as the mean ± S.D. from at least three independent biological replicates. Comparisons between means were performed using Unpaired t-test (ns $p>0.05$, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, and **** $p\leq0.0001$)

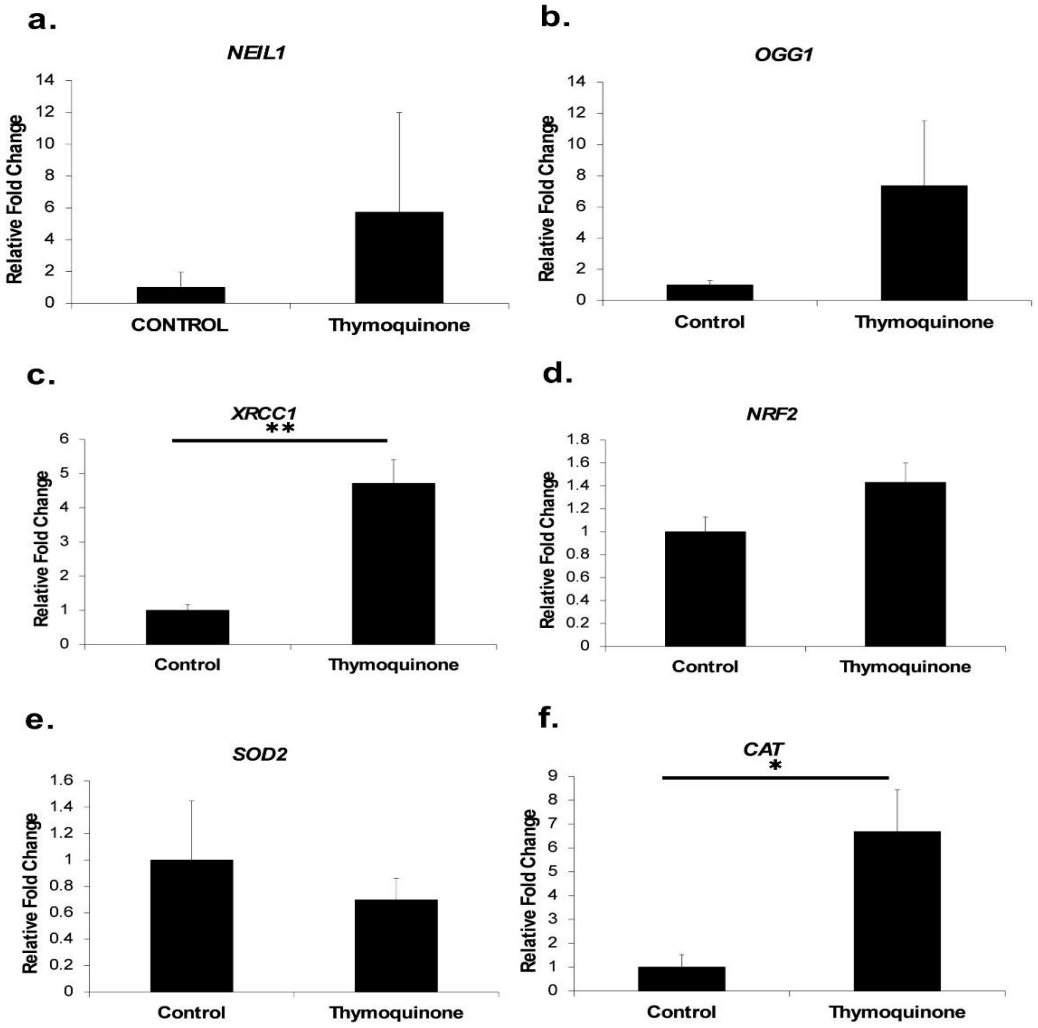


Figure 3. The effect of TQ treatment on the expression levels of genes related to DNA repair and oxidative stress response in breast cancer cells. XRCC1 and CAT show significantly different expression in MCF-7 compared to untreated cells. Data are expressed as fold change

Table 2. Gene expressions including fold-change and *P* value of genes associated with DNA repair and oxidative stress response, MCF-7 cells were treated with TQ

Gene symbol	Gene name	Fold-change	*p* value
NEIL1	Nei like DNA glycosylase 1	5.72	0.31
OGG1	8-oxoguanine DNA glycosylase	7.36	0.10
XRCC1	X-ray repair cross complementing 1	4.71	0.005**
SOD2	Superoxide dismutase 2	0.70	0.38
NRF2	NFE2 like bZIP transcription factor 2	1.43	0.087
CAT	Catalase	6.68	0.023*

*represents a significant difference ($p \leq 0.05$), ** represents a significant different ($p \leq 0.01$)

Discussion

Natural compounds isolated from plants, fungi, and other organisms have been widely used from ancient times to the present as pharmaceutical agents for various human diseases, especially cancer [12-14]. One of the most important reasons for their use is their strong antioxidant potential, which allows them to exert effects by modulating oxidative stress [12, 24]. One of the most popular phyto-natural products studied for its antineoplastic activities is TQ, a bioactive compound from Nigella sativa, which has been shown to exhibit anticancer activities by inhibiting cell proliferation, migration, angiogenesis, and invasion in cancer cells. [15, 16]. Cancer types in which the effects and anti-cancer activity of TQ have been demonstrated include lung, breast, colon, leukaemia, and cervical cancer [17-24].

In this study, by determining the dose-dependent antiproliferative activity of TQ in the range of 12.5-50 μM, it was found that TQ decreased cell viability depending on the increasing dose. The IC_{50} value was determined to be 7.867 μM in MCF-7 cells at the end of the 24th hour. One study found that TQ exhibited cytotoxic activity in T47D and MCF-7 cells under in vitro conditions. It was observed that TQ showed a sudden cytotoxic effect at concentrations higher than 100 μM in T47D cells, while TQ showed a cytotoxic effect in MCF-7 cells. The IC_{50} values were 80.1±9.8, 32.7±1.1 and 64.9±14.5 for 24, 48, and 72 h, respectively [25]. In another study using the MTS assay, the cytotoxic effect of TQ exposure in the dose range of 25-300 μM on breast cancer cells was demonstrated, and the IC_{50} value was reported to be 25 μM. In addition to the antiproliferative effect of TQ, it was also reported that TQ induced apoptosis in breast cancer cells and exhibited anticancer activity through cell cycle arrest [26].

Cancer is a multifaceted disease that involves alterations in the regulation of gene expression [3, 9]. Several studies have indicated that TQ possesses therapeutic properties and lowers the risk of cancer by modifying genetic pathways [9, 25, 26]. A recent study reported that TQ suppressed VEGF-mediated angiogenesis and stimulated apoptosis in breast cancer [27]. Another study on the molecular biological mechanism of action of TQ in triple-negative breast cancer reported that TQ arrests the cell cycle in the G1/S phase via cyclin E, cyclin D1 and p27, suppresses histone deacetylase (HDAC) activity, induces apoptosis by upregulating Bax and downregulating Bcl-2, and affects cell survival by increasing PTEN expression [28].The protective role of TQ in breast tumour xenograft mouse models and breast cancer cells has been demonstrated through molecular mechanisms mediated by induction of p38 phosphorylation, reactive oxygen species (ROS) production, decrease in Ki67, increase in apoptotic cell density, and increase in catalase, glutathione, and superoxide dismutase concentrations [29].

We investigated the changes in the expressions of the DNA repair-related genes *OGG1, NEIL-1,* and *XRCC1* and the oxidative stress-related genes NRF2, SOD2, and CAT. According to the real-time PCR results we obtained, the DNA repair-related genes showed the following fold increases: *OGG1* 7.36-fold, *NEIL-1* 5.72-fold, and *XRCC1* 4.71-fold. Among these increases, the upregulation of *XRCC1* was found to be statistically significant. The results demonstrate that TQ significantly

increases the expression of DNA repair genes. In addition, in our study, the changes in the protein concentration of the important oxidative stress marker 8-OHdG were detected using the ELISA method. After TQ exposure, the 8-OHdG level in breast cancer cells showed a significant decrease. In addition, in this study, it was observed that NRF-2 and catalase expressions increased as a result of TQ exposure. Catalase gene expression increase was found to be statistically significant. SOD expression was partially downregulated after TQ exposure.

8-OHdG generated as a result of E2-induced oxidative stress is regarded as a cellular indicator of oxidative stress. 8-OHdG is generated as a result of oxidative stress triggered by E2 and is regarded as a cellular indicator of oxidative DNA damage [30, 31]. Decreased levels of OGG1 have been shown to have a significant effect on tumour progression and growth, promoting cancer development [30-33]. The mechanisms of action of substances with antioxidant potential are known to suppress oxidative stress by acting as free radical scavengers through the induction of NRF2-dependent antioxidant enzymes. NRF2 is a transcription factor sensitive to redox changes, playing a crucial role in shielding cells from oxidative harm [31, 34]. It has been suggested that NRF2 may be an important distinguishing feature in cancer development stages associated with breast cancer growth and cell invasion [35]. Mutations or oxidative damage have been implicated in the pathogenesis of several diseases, particularly cancer. Base excision repair (BER) deficiency contributes to genomic instability and promotes carcinogenesis. NEIL-1 also fulfils important repair processes in the DNA repair mechanism [36]. In a study on TQ and its mechanisms of action in cancer treatment, TQ was found to induce apoptosis through ROS production in primary effusion lymphoma [37]. It has recently been reported that the combined application of TQ with piperine reduced oxidative damage in liver tissue and reduced malondialdehyde (MDA) and nitric oxide (NO) levels and induced glutathione peroxidase, SOD, and catalase [38]. Evaluating the data obtained in this study, TQ was shown to reduce proliferation in MCF-7 breast cancer cells. In addition, it is thought that TQ attempts to reduce the increased oxidative damage in breast cancer by upregulating the expression of DNA repair genes. Limitations of this study include the use of a single cell line, the lack of confirmation of gene expression changes at the protein level, and the fact that other pathways were not included in the study. Another limitation of this study is that the cytotoxic effect of TQ on normal, non-cancerous cells was not demonstrated using healthy breast cells. The fact that DNA repair genes were also examined in this study as a contribution to similar studies in the literature is an important originality that distinguishes this study from other studies.

In conclusion, this study shows that thymoquinone has significant effects on cell proliferation, 8-OHdG levels, and expression of genes associated with DNA repair and oxidative stress in MCF-7 breast cancer cells. TQ treatment resulted in a decrease in cell proliferation, demonstrating its potential as an anticancer agent. TQ also modulated the expression of key genes involved in DNA repair and oxidative stress responses, further supporting its role in disrupting cellular homeostasis in cancer cells. These findings highlight the potential of TQ to target oxidative stress and DNA repair mechanisms that may contribute to its anticancer effects. A comprehensive understanding of the cellular mechanisms underlying the anti-cancer effects of TQ would further contribute to the therapeutic application of this extensively studied molecule and support strategies for the development of new therapeutic agents. In conclusion, this study will contribute to studies to better understand the molecular mechanisms of action of TQ and the molecular pathways through which it acts and to evaluate its pharmacological potential in the treatment of breast cancer.

Funding: This study was not funded by any organisation.

Author contributions: M.S., S.D.A., G.G. contributed to the conceptualization and design. Y.D. supervised the study. All authors conducted all experiments and wrote the first draft of the manuscript. M.S., S.D.A., G.G. collected, analyzed and interpreted the data. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

Conflict of interest: The authors have no conflicts of interest.

References

1. Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of cancer. *Mol Cancer Res*. 2023;21(11):1142-1147. doi:10.1158/1541-7786.MCR-23-0411
2. Khan SU, Fatima K, Malik F, Kalkavan H, Wani A. Cancer metastasis: molecular mechanisms and clinical perspectives. *Pharmacol Ther*. 2023;250:108522. doi:10.1016/j.pharmthera.2023.108522
3. Park JH, Pyun WY, Park HW. Cancer metabolism: phenotype, signaling and therapeutic targets. *Cells*. 2020;9(10):2308. doi:10.3390/cells9102308
4. Zhang S, Xiao X, Yi Y, et al. Tumor initiation and early tumorigenesis: molecular mechanisms and interventional targets. *Signal Transduct Target Ther*. 2024;9(1):149. doi:10.1038/s41392-024-01848-7
5. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin*. 2024;74(1):12-49. doi:10.3322/caac.21820
6. Maroufi NF, Ashouri N, Mortezania Z, et al. The potential therapeutic effects of melatonin on breast cancer: an invasion and metastasis inhibitor. *Pathol Res Pract*. 2020;216(10):153226. doi:10.1016/j.prp.2020.153226
7. Moo TA, Sanford R, Dang C, Morrow M. Overview of breast cancer therapy. *PET Clin*. 2018;13(3):339-354. doi:10.1016/j.cpet.2018.02.006
8. Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduct Target Ther*. 2018;3:7. doi:10.1038/s41392-017-0004-3
9. Shabani H, Karami MH, Kolour J, et al. Anticancer activity of thymoquinone against breast cancer cells: mechanisms of action and delivery approaches. *Biomed Pharmacother*. 2023;165:114972. doi:10.1016/j.biopha.2023.114972
10. Liu B, Zhou H, Tan L, Siu KTH, Guan XY. Exploring treatment options in cancer: tumor treatment strategies. *Signal Transduct Target Ther*. 2024;9(1):175. doi:10.1038/s41392-024-01856-7
11. Nahata A. Anticancer agents: a review of relevant information on important herbal drugs. *Int J Clin Pharmacol Toxicol*. 2017;6(2):250-255. doi:10.19070/2167-910x-1700042
12. Blowman K, Magalhães M, Lemos M, Cabral C, Pires IM. Anticancer properties of essential oils and other natural products. *Evid Based Complement Alternat Med*. 2018;2018:3149362. doi:10.1155/2018/3149362
13. Secme M, Dodurga Y. Usnic acid inhibits cell proliferation and downregulates lncRNA UCA1 expression in Ishikawa endometrial cancer cells. *Nat Prod Biotechnol*. 2021;1(1):28-37.
14. Yuan M, Zhang G, Bai W, Han X, Li C, Bian S. The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects. *Oxid Med Cell Longev*. 2022;2022:1429869. doi:10.1155/2022/1429869
15. Dajani EZ, Shahwan TG, Dajani NE. Overview of the preclinical pharmacological properties of Nigella sativa (black seeds): a complementary drug with historical and clinical significance. *J Physiol Pharmacol*. 2016;67(6):801-817.
16. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. *J Pharmacol Phytother*. 2017;20(3):179-193. doi:10.3831/KPI.2017.20.021
17. Alshaibi HF, Aldarmahi NA, Alkhattabi NA, Alsufiani HM, Tarbiah NI. Studying the anticancer effects of thymoquinone on breast cancer cells through natural killer cell activity. *Biomed Res Int*. 2022;2022:9218640. doi:10.1155/2022/9218640
18. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: a review. *J Ethnopharmacol*. 2016;190:372-386. doi:10.1016/j.jep.2016.06.061
19. Rooney S, Ryan MF. Effects of alpha-hederin and thymoquinone, constituents of Nigella sativa, on human cancer cell lines. *Anticancer Res*. 2005;25(3B):2199-2204.
20. Imran M, Rauf A, Khan IA, et al. Thymoquinone: a novel strategy to combat cancer: a review. *Biomed Pharmacother*. 2018;106:390-402. doi:10.1016/j.biopha.2018.06.159
21. Zhao ZX, Li S, Liu LX. Thymoquinone affects hypoxia-inducible factor-1α expression in pancreatic cancer cells via HSP90 and PI3K/AKT/mTOR pathways. *World J Gastroenterol*. 2024;30(21):2793-2816. doi:10.3748/wjg.v30.i21.2793
22. Gulbay G, Secme M, Ilhan H. Exploring the potential of thymoquinone-stabilized selenium nanoparticles: in HEC1B endometrial cancer cells revealing enhanced anticancer efficacy. *ACS Omega*. 2023;8(42):39822-39829. doi:10.1021/acsomega.3c06028
23. Sohrabi B, Qadbeigi M, Sabouni F, Hamta A. Thymoquinone nanoparticle induces apoptosis and cell migration retardation through modulating of SUMOylation process genes in breast cancer cell line. *Iran J Biotechnol*. 2024;22(1):e3676. doi:10.30498/ijb.2024.390400.3676
24. Almajali B, Al Jamal HAN, Taib WRW, et al. Thymoquinone, as a novel therapeutic candidate of cancers. *Pharmaceutics*. 2021;14(4):369. doi:10.3390/ph14040369

25. Bashmail HA, Alamoudi AA, Noorwali A, et al. Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities. *Sci Rep*. 2018;8(1):11674. doi:10.1038/s41598-018-30046-z
26. Motaghed M, Al Hassan FM, Hamid SS. Cellular responses with thymoquinone treatment in human breast cancer cell line MCF-7. *Phcog Res*. 2013;5(3):200-206. doi:10.4103/0974-8490.112428
27. Talib WH. Regressions of breast carcinoma syngraft following treatment with piperine in combination with thymoquinone. *Sci Pharm*. 2017;85(3):27. doi:10.3390/scipharm85030027
28. Barkat MA, Harshita, Ahmad J, Khan MA, Beg S, Ahmad FJ. Insights into the targeting potential of thymoquinone for therapeutic intervention against triple-negative breast cancer. *Curr Drug Targets*. 2018;19(1):70-80. doi:10.2174/1389450118666170612095959
29. Woo CC, Hsu A, Kumar AP, Sethi G, Tan KH. Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: the role of p38 MAPK and ROS. *PLoS One*. 2013;8(10):e75356. doi:10.1371/journal.pone.0075356
30. Boiteux S, Radicella JP. The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. *Arch Biochem Biophys*. 2000;377(1):1-8. doi:10.1006/abbi.2000.1773
31. Singh B, Chatterjee A, Ronghe AM, Bhat NK, Bhat HK. Antioxidant-mediated up-regulation of OGG1 via NRF2 induction is associated with inhibition of oxidative DNA damage in estrogen-induced breast cancer. *BMC Cancer*. 2013;13:253. doi:10.1186/1471-2407-13-253
32. Cooke MS, Olinski R, Evans MD. Does measurement of oxidative damage to DNA have clinical significance? *Clin Chim Acta*. 2006;365(1-2):30-49. doi:10.1016/j.cca.2005.09.009
33. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. *Mutat Res*. 2004;567(1):1-61. doi:10.1016/j.mrrev.2003.11.001
34. Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A. Nrf2, the master regulator of anti-oxidative responses. *Int J Mol Sci*. 2017;18(12):2772. doi:10.3390/ijms18122772
35. Kumar H, Kumar RM, Bhattacharjee D, Somanna P, Jain V. Role of Nrf2 signaling cascade in breast cancer: strategies and treatment. *Front Pharmacol*. 2022;13:720076. doi:10.3389/fphar.2022.720076
36. Xue W, Liu Y, Xin N, et al. Nei endonuclease VIII-like1 (NEIL1) inhibits apoptosis of human colorectal cancer cells. *Biomed Res Int*. 2020;2020:5053975. doi:10.1155/2020/5053975
37. Hussain AR, Ahmed M, Ahmed S, et al. Thymoquinone suppresses growth and induces apoptosis via generation of reactive oxygen species in primary effusion lymphoma. *Free Radic Biol Med*. 2011;50(8):978-987. doi:10.1016/j.freeradbiomed.2010.12.034
38. Abdel Daim MM, Sayed AA, Abdeen A, et al. Piperine enhances the antioxidant and anti-inflammatory activities of thymoquinone against microcystin-LR-induced hepatotoxicity and neurotoxicity in mice. *Oxid Med Cell Longev*. 2019;2019:1309175. doi:10.1155/2019/1309175

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1633888

Sports participation and ankle injury prevalence among medical school students

Tıp fakültesi öğrencileri arasında spor katılımı ve ayak bileği yaralanmalarının yaygınlığı

Mehmet Yücens, Hediye Aktaş, Rümeysa Ümmügülsüm Sandalcı, Ayça Polat

Posted date:05.02.2025 Acceptance date:15.02.2025

Abstract

Purpose: The aim of this study is to evaluate the prevalence of ankle injuries and the tendency of medical students to participate in sports, and the effect of medical school preparation and study process on participation in sports.

Material and methods: A cross-sectional descriptive study was conducted among medical students at Pamukkale University Faculty of Medicine. Data were collected using an online questionnaire covering aspects of sports participation before and after medical school, types and frequency of sports played, and history of ankle injuries. Statistical analysis was performed to examine changes in sports participation and injury prevalence.

Results: A total of 253 students took part in the study. Of these, 135 (53.4%) participated in sports before medical school, with volleyball (28%), football (17%) and basketball (17%) being the most common. Ankle injuries were particularly common in football (50%), basketball (33%) and volleyball (21%). After starting medical school, 48 of the students reduced or stopped their sports activities, citing academic workload as the main reason. However, 46 students started sports activities espacially fitness.

Conclusion: The study highlights a significant decline in sports participation during the university entrance exam among medical students due to academic pressure, with a notable prevalence of ankle injuries in high-impact sports. During medical school education, there is an increasing tendency to engage in fitness-style sports with a lower risk of injury.

Keywords: Sports participation, medical students, ankle injuries, physical activity.

Yucens M, Aktas H, Sandalci RU, Polat A. Sports participation and ankle injury prevalence among medical school students. Pam Med J 2025;18:314-319.

Öz

Amaç: Bu çalışma, tıp fakültesi öğrencilerinin spora katılım eğilimleri ve ayak bileği yaralanmalarının yaygınlığını ve tıp fakültesi hazırlanma ve okuma sürecinin spora katılıma etkisini değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Pamukkale Üniversitesi Tıp Fakültesi öğrencileri arasında kesitsel tanımlayıcı bir çalışma yapılmıştır. Veriler, tıp fakültesinden önce ve sonra spora katılım, yapılan spor türleri ve sıklığı ile ayak bileği yaralanması öyküsünü kapsayan çevrimiçi bir anket kullanılarak toplanmıştır. Spora katılım ve yaralanma prevalansındaki değişiklikleri incelemek için istatistiksel analiz yapılmıştır.

Bulgular: Çalışmaya toplam 253 öğrenci katıldı. Bunlardan 135'i (%53,4) tıp fakültesine başlamadan önce herhangi bir spor dalında aktivite yapmıştır. Öğrencilerin yaptığı en yaygın sporlar voleybol (%28), futbol (%17) ve basketbol (%17) idi. Ayak bileği yaralanmaları özellikle futbol (%50), basketbol (%33) ve voleybolda (%21) yaygındı. Tıp fakültesine başladıktan sonra, öğrencilerin 48'i spor faaliyetlerini azaltmış veya durdurmuş, bunun ana nedeni olarak da akademik iş yükünü göstermiştir. Ancak, 46 öğrenci özellikle fitness olmak üzere tıp fakültesine başladıktan sonra spor aktivitelerine başlamıştır.

Sonuç: Bu çalışma, tıp öğrencileri arasında üniversite giriş sınavı sırasında akademik baskı nedeniyle spora katılımda önemli bir düşüş olduğunu ve yüksek etkili sporlarda ayak bileği yaralanmalarının yaygınlığını vurgulamaktadır. Tıp fakültesi eğitimi sırasında, yaralanma riski daha düşük olan fitness tarzı sporlara katılım eğilimi artmaktadır.

Anahtar kelimeler: Spor katılımı, tıp fakültesi öğrencileri, ayak bileği yaralanmaları, fiziksel aktivite.

Yücens M, Aktaş H, Sandalcı RÜ, Polat A. Tıp fakültesi öğrencileri arasında spor katılımı ve ayak bileği yaralanmalarının yaygınlığı. Pam Tıp Derg 2025;18:314-319.

Mehmet Yücens, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Orthopaedics and Traumatology, Denizli, Türkiye, e-mail: aflyucens@yahoo.com (https://orcid.org/0000-0001-6924-6613) (Corresponding Author)

Hediye Aktaş, MA Student, Pamukkale University Faculty of Medicine, Student, Denizli, Türkiye, e-mail: hediyeaktas649@gmail.com (https://orcid.org/0009-0002-0584-9583)

Rümeysa Ümmügülsüm Sandalcı, MA Student, Pamukkale University Faculty of Medicine, Student, Denizli, Türkiye, e-mail: sandalcirumeysa@gmail.com (https://orcid.org/0009-0000-6562-7240)

Ayça Polat, MA Student, Pamukkale University Faculty of Medicine, Student, Denizli, Türkiye, e-mail: aycapolat26@gmail.com (https://orcid.org/0009-0004-5728-1496)

Introduction

Participation in sports is an important component of overall health. It contributes to physical fitness, injury prevention, and mental well-being. Previous research has shown that participating in sports has many benefits. These include improved cardiovascular function, musculoskeletal strength, and cognitive performance [1]. There are many benefits to taking part in sports. These include improvements in physical and mental health, self-esteem, the ability to work in a team, and a reduction in cigarette smoking. Despite these benefits, the risk of injury must also be considered. In the United States, there are an estimated 4.5 million sports- and recreation-related injuries among children and young adults each year [2]. However, the transition from secondary school to medical school often leads to significant changes in lifestyle, including a decrease in physical activity due to increased academic demands [3].

Studies have shown that time constraints, academic workload and stress management challenges are the main reasons for a decrease in physical activity among medical students [4]. Studies focusing on high school and college athletes have shown that injuries, especially ankle injuries, are prevalent in those participating in sports such as basketball, football and volleyball [5]. Injury rates among high school and college athletes remain significant, with ankle sprains among the most commonly reported injuries, according to the first decade of web-based sports injury surveillance [6].

Furthermore, an analysis of epidemiological trends in collegiate athletics indicates that the incidence of foot and ankle injuries varies according to sport, frequency of participation, and history of previous injury [7]. Understanding these trends is critical to developing injury prevention strategies tailored to medical students. Medical students may have limited opportunities for structured rehabilitation due to academic commitments [8]. In addition, the importance of early intervention and rehabilitation programmes to reduce long-term disability has been highlighted in studies of lateral ligament complex ankle sprains and lower limb injuries in athletes [9].

This study aims to assess the impact of medical education on trends in sports participation and the prevalence of ankle injuries among medical students. Through the study of these factors, educational institutions can develop tailored interventions to promote physical activity whilst minimising the risk of injury [10].

Materials and methods

This cross-sectional descriptive study was conducted with the approval of Pamukkale University Non-Interventional Clinical Research Ethics Committee. The study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (date: 05.03.2020 and number:60116787-020/19227).

The study sample included students of Pamukkale University Faculty of Medicine in different academic years: Year 1 (78 students, 30.8%), Year 2 (118 students, 46.6%), Year 3 (38 students, 15%), Year 4 (9 students, 3.6%), Year 5 (7 students, 2.8%), and Year 6 (3 students, 1.2%).

Data was collected using an online questionnaire via Google Forms. The survey consisted of 26 questions covering different aspects of sports participation and injury, including

- Sports participation before and after starting medical school.

- Type and frequency of sports played.

- History of ankle injuries during sports participation.

- Incidence of ankle injuries during medical training.

The questionnaire was distributed online on 21 May 2024, and responses were collected until 4 June 2024. The collected data were analysed using statistical and graphical methods.

Results

A total of 253 students participated in the survey. Of these, 135 students (53.4%) were involved in sports before starting medical school. Of these, 59 (43%) were female and 76 (57%) were male. The most common sports were volleyball (28%), football (17%), basketball

(17%), fitness (13%), tennis (8%), swimming (8%), and contact sports (7%).

During the preparation period for the university entrance examination, 101 out of 135 students (74%) stopped playing sports, mainly because of the academic workload. Of these, 60 students (59%) resumed sport after starting medical school, while 41 (40%) continued to do no sport due to academic intensity and lack of motivation. In addition, 34 students continued to play sports during exam preparation, of whom 27 (79%) continued to play sports during medical school, while 7 (20%) stopped due to academic workload.

After entering medical school, 87 students who had previously participated in sports continued to do so. The distribution of sports among them was as follows: 31 students (35%) participated in fitness, 18 (20%) in football, 17 (19%) in volleyball, 6 (6%) in tennis, 6 (6%) in running, and 3 (3%) in basketball. In terms of frequency, 4 students (4%) reported doing sports once a month, 27 (31%) once a week, 45 (51%) three times a week, and 11 (12%) daily.

Of the 118 students who did not exercise before medical school, 46 (38%) started exercising after enrolling in medical school. The most common sports chosen were fitness (25 students, 54%), pilates (6 students, 13%), football (4 students, 8%), running (3 students, 6%), volleyball (2 students, 4%), tennis (2 students, 4%), and swimming (2 students, 4%). In terms of frequency of exercise, 4 students (7%) reported exercising once a month, 14 (26%) once a week, 32 (61%) three times a week, and 2 (3%) daily.

In total, 133 (52%) of the 253 students surveyed continued or started playing sports during medical school. The most common sports were fitness (56 students, 42%), football (22 students, 16%), volleyball (19 students, 14%), running (9 students, 6%), and tennis (8 students, 6%). In terms of frequency, 8 students (6%) exercised once a month, 41 (30%) once a week, 77 (57%) three times a week, and 13 (9%) daily. Total participation in sport before medical school, during university entrance exams, and during medical school, as shown in Figure 1 and Table 1. Ankle injury rates varied between the sports listed in Table 2.

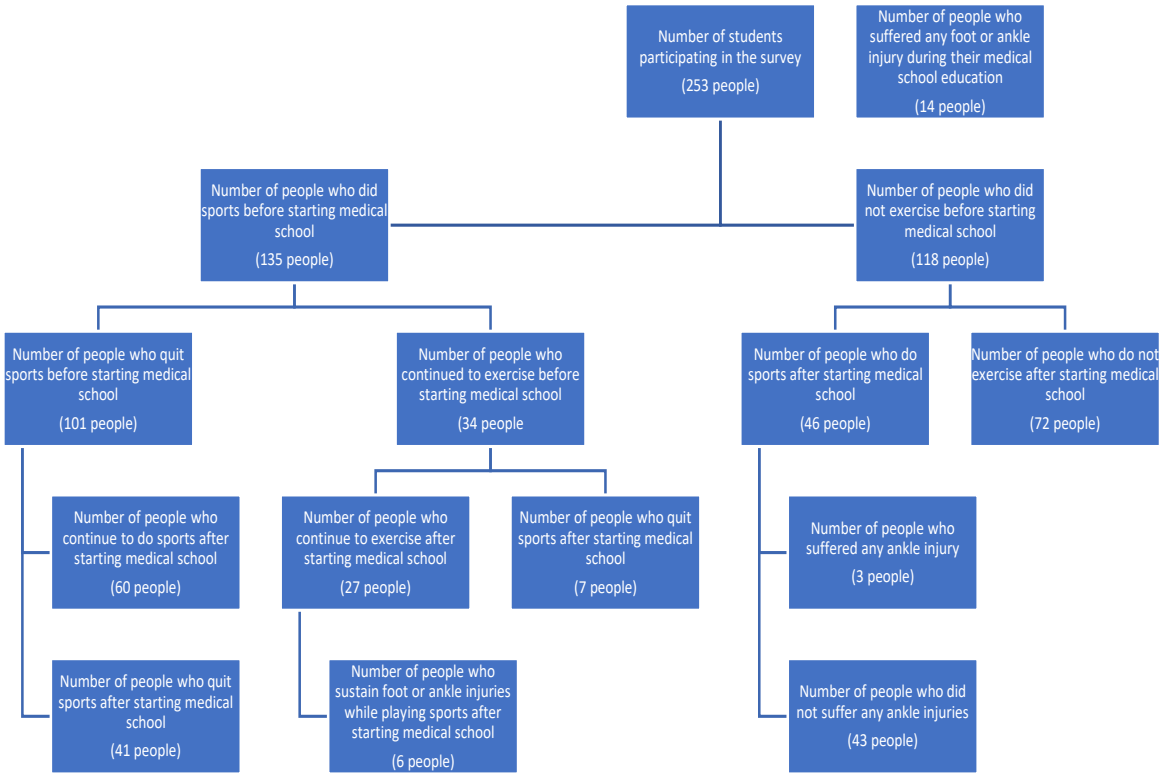


Figure 1. Participation in sports before medical school, during university entrance exams and during medical school

Table 1. Participation in sports before starting medical school and during medical school

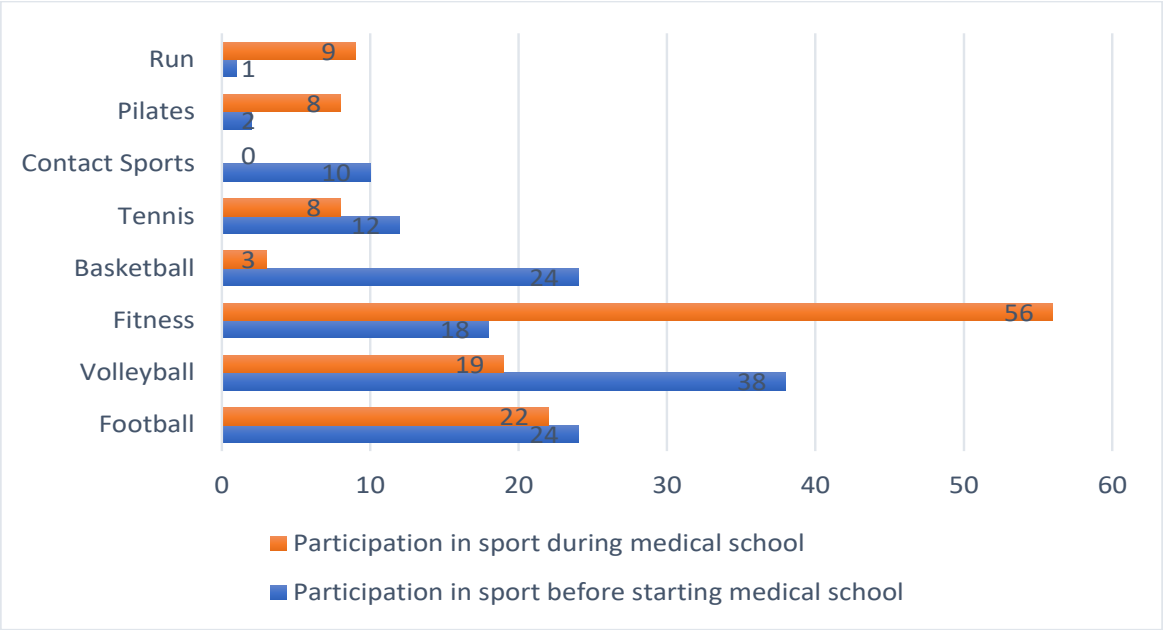


Table 2. Ankle injury rates varied between the sports

	Minor Injury (Healing within 1-15 days)		Moderate Injury (Healing within 15 days-2 months)		Serious Injury (Healing over 2 months)		Injury rate in people doing sports as a percentage	
	Before starting medical school	After starting medical school	Before starting medical school	After starting medical school	Before starting medical school	After starting medical school	Before starting medical school	After starting medical school
Football	9 people	3 people	3 people	1 person	-	-	50%	22%
Basketball	6 people	-	1 person	-	1 person	-	33%	-
Volleyball	5 people	2 people	2 people	1 person	1 person	-	21%	11%
Tennis	2 people	-	1 person	-	-	-	25%	-
Contact sports	1 person	-	1 person	-	-	-	20%	-
Pilates	-	1 person	-	-	-	-	-	16%

Discussion

The results of this study are consistent with previous research indicating that academic responsibilities significantly reduce student sport participation [2]. A study by Kerr et al. [11] of the first decade of web-based sports injury surveillance and a survey of sports participation and sports injuries in Calgary area high schools highlight a decline in student sports participation due to increasing academic pressures. Similarly, our study found that 74% of students who participated in sports prior to medical school discontinued their activities during the pre-medical school period. And 40% of them never took up sports again. However, relevant associations between academic performance and VO2 max, diastolic blood pressure, and respiratory insomnia were found by Redondo Flórez et al. [12]. They emphasise the importance of implementing different programmes to improve these factors, especially physical activity and sleep habits, to improve academic performance [12].

According to this study, volleyball was the most common sport before starting medical school (28%), whereas fitness was the most common sport during medical school (42%). This can be interpreted as students moving away from contact sports to reduce the risk of injury, to stay fit, and to increase the importance of looking good. The pressure to look good is an issue for students regardless of their course, according to Sundgot Borgen et al. [13]. It is also thought that the fact that fitness sport can be done individually, does not require a team, does not require an instructor, and is easily accessible may have contributed to the increase in participation.

In this study, the incidence of foot and ankle injuries among students before they start medical school is 23%, with football and basketball having the highest rates of ankle injuries. Ankle injuries are a common problem among student athletes, particularly in high-impact sports. Research suggests that ankle sprains are among the most common injuries in sports such as basketball, football, and volleyball, including the epidemiology of lateral ligament complex ankle sprains in National Collegiate Athletic Association sports and the incidence and epidemiology of foot and ankle injuries in elite collegiate athletes [6]. Hunt et al. [5] examined the incidence and epidemiology of foot and ankle injuries in elite college athletes and found that 27% of injuries to the foot and ankle occurred in college athletes.

Furthermore, the importance of early intervention and rehabilitation programmes is highlighted by the characteristics of athletic training services for patients with ankle sprains sustained during high school athletics and the epidemiology of lower leg soft tissue injuries in high school athletes [10]. In this study, 38% of students who sustained an ankle injury continued to participate after treatment, highlighting the need for appropriate protocols to help a facilitate safe return to play.

In view of the demanding nature of medical education, our findings suggest that universities could incorporate structured programmes of physical activity that fit into the schedules of students.

Funding: No funding.

Authors contributions: M.Y. have constructed/constructed the main idea and hypothesis of the study. M.Y., H.A., R.S. developed the theory and arranged/edited the material and method section. H.A., R.S., A.P., have done the evaluation of the data in the results section. Discussion section of the article was written by M.Y., H.A., R.S and A.P., M.Y. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Pizzarro J, Chiang B, Malyavko A, et al. Epidemiology of Sports Injuries Among High School Athletes in the United States: Data From 2015 to 2019. *Orthop J Sports Med*. 2024;12(5):23259671241252637. Published 2024 May 22. doi:10.1177/23259671241252637
2. Spitnale MJ, Mathews CG 3rd, Barnes AJ, Thier ZT, Jackson JB 3rd. Epidemiology of Lower Leg Soft Tissue Injuries in High School Athletes. *Foot Ankle Orthop*. 2022;7(1):24730114211057886. Published 2022 Jan 11. doi:10.1177/24730114211057886
3. Clifton DR, Hertel J, Onate JA, et al. The First Decade of Web-Based Sports Injury Surveillance: Descriptive Epidemiology of Injuries in US High School Girls' Basketball (2005-2006 Through 2013-2014) and National Collegiate Athletic Association Women's Basketball (2004-2005 Through 2013-2014). *J Athl Train*. 2018;53(11):1037-1048. doi:10.4085/1062-6050-150-17
4. Emery CA, Meeuwisse WH, McAllister JR. Survey of sport participation and sport injury in Calgary and area high schools. *Clin J Sport Med*. 2006;16(1):20-26. doi:10.1097/01.jsm.0000184638.72075.b7
5. Hunt KJ, Hurwit D, Robell K, Gatewood C, Botser IB, Matheson G. Incidence and Epidemiology of Foot and Ankle Injuries in Elite Collegiate Athletes. *Am J Sports Med*. 2017;45(2):426-433. doi:10.1177/0363546516666815
6. Roos KG, Kerr ZY, Mauntel TC, Djoko A, Dompier TP, Wikstrom EA. The Epidemiology of Lateral Ligament Complex Ankle Sprains in National Collegiate Athletic Association Sports. *Am J Sports Med*. 2017;45(1):201-209. doi:10.1177/0363546516660980
7. Harmon KG, Drezner J, Gammons M, et al. American Medical Society for Sports Medicine position statement: concussion in sport. *Clin J Sport Med*. 2013;23(1):1-18. doi:10.1097/JSM.0b013e31827f5f93

8. Manoel LS, Xixirry MG, Soeira TP, Saad MC, Riberto M. Identification of Ankle Injury Risk Factors in Professional Soccer Players Through a Preseason Functional Assessment. *Orthop J Sports Med*. 2020;8(6):2325967120928434. Published 2020 Jun 24. doi:10.1177/2325967120928434

9. Gulbrandsen M, Hartigan DE, Patel KA, Makovicka JL, Tummala SV, Chhabra A. Ten-year epidemiology of ankle ınjuries in men's and women's collegiate soccer players. *J Athl Train*. 2019;54(8):881-888. doi:10.4085/1062-6050-144-18

10. Simon JE, Wikstrom EA, Grooms DR, Docherty CL, Dompier TP, Kerr ZY. Athletic training service characteristics for patients with ankle sprains sustained during high school athletics. *J Athl Train*. 2019;54(6):676-683. doi:10.4085/1062-6050-449-16

11. Kerr ZY, Gregory AJ, Wosmek J, et al. The first decade of web-based sports injury surveillance: Epidemiology of injuries in high school girls' volleyball and collegiate women's volleyball. *J Ath Tra.* 2018;53(10):926-937. doi:10.4085/1062-6050-162-17

12. Redondo Flórez L, Ramos Campo DJ, Clemente Suárez VJ. Relationship between Physical Fitness and Academic Performance in University Students. *Int J Environ Res Public Health*. 2022;19(22):14750. Published 2022 Nov 10. doi:10.3390/ijerph192214750

13. Sundgot Borgen C, Sundgot Borgen J, Bratland Sanda S, et al. Body appreciation and body appearance pressure in Norwegian university students comparing exercise science students and other students. *BMC Public Health*. 2021;21(1):532. Published 2021 Mar 19. doi:10.1186/s12889-021-10550-0

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1561951

Dysphonia prevalence and its relationship with risk factors among teachers

Öğretmenlerde disfoni prevelansı ve risk faktörleri ile ilişkisi

Funda Tümkaya, Cüneyt Orhan Kara

Posted date:05.10.2024 Acceptance date:03.03.2025

Abstract

Purpose: The aim of this study was to determine the relationship between the prevalence of and risk factors for dysphonia among teachers.

Materials and methods: According to the questionnaire results of 167 teachers, 87 teachers who described dysphonia were included in the patient group and 80 teachers who did not describe dysphonia were included in the control group. For both groups, statistical evaluations were performed using non-parametric statistical methods.

Results: 87 (52.1%) of 167 teachers included in the study had the complaint of dysphonia. The presence of laryngopharyngeal reflux and allergic diseases caused increase in the risk of dysphonia. The risk of dysphonia was also increased with increasing stage of education that the teachers were responsible for. Laryngeal endoscopy revealed no organic lesion in 65.5% of the patients, whereas showed laryngopharyngeal reflux findings in 26.4%, vocal cord nodule in 6.8% and vocal cord polyp in 1.1%.

Conclusion: This study showed that dysphonia is a common disorder among teachers and while evaluating these subjects the presence of laryngopharyngeal reflux and allergic diseases should primarily be considered.

Keywords: Dysphonia, risk factors, laryngopharyngeal reflux, allergic diseases.

Tumkaya F, Kara CO. Dysphonia prevalence and its relationship with risk factors among teachers. Pam Med J 2025;18:320-328.

Öz

Amaç: Öğretmenlerde disfoni prevelansını ve disfoni yapan risk faktörleri arasındaki ilişkiyi tesbit etmek.

Gereç ve yöntem: Araştırmaya alınan 167 öğretmenin anket sonuçlarına göre disfoni tarifleyen 87 öğretmen hasta grubu, disfoni tariflemeyen 80 öğretmen anketi ise kontrol grubu olarak alındı. Her iki grup için non parametrik istatistiksel yöntemler kullanılarak değerlendirmeler yapıldı.

Bulgular: Çalışmaya alınan 167 öğretmenin 87'sinde (%52,1) disfoni şikayeti saptandı. Larengofarengeal reflü varlığının ve alerjik hastalık varlığının disfoni riskini arttırdığı belirlendi. Öğretmenlerin sorumlu olduğu eğitim-öğretim kademesinin arttıkça disfoni riskinin de arttığı görüldü. Larenks endoskopisinin %65,5'inde organik bir lezyon saptanmazken, %26,4'ünde larengofarengeal reflü bulguları, %6,8'de vokal kord nodülü ve %1,1'de vokal kord polibi olduğu görüldü.

Sonuç: Çalışmamız, öğretmenlerdeki ses kalite bozukluğunun yaygın görülen fiziksel bir rahatsızlık olduğunu ve bu kişilerin değerlendirilirken larengofarengeal reflü ve alerjik hastalık varlığının öncelikle sorgulanması gerektiğini düşündürmektedir.

Anahtar kelimeler: Disfoni, risk faktörleri, larengofarengeal reflü, alerjik hastalıklar.

Tümkaya F, Kara CO. Öğretmenlerde disfoni prevelansı ve risk faktörleri ile ilişkisi. Pam Tıp Derg 2025;18:320-328.

Funda Tümkaya, Asst. Prof. Pamukkale University, School of Medicine, Department of Otolaryngology, Head-Neck Surgery, Denizli, Türkiye, e-mail: fundatumkaya@gmail.com (https://orcid.org/0000-0002-3213-8106) (Corresponding Author)

Cüneyt Orhan Kara, Prof. Pamukkale University, School of Medicine, Department of Otolaryngology, Head-Neck Surgery, Denizli, Türkiye, e-mail: cokara@yahoo.com (https://orcid.org/0000-0003-2219-4283)

Introduction

Voice complaints are known to be more frequent in those who perform their profession with their voice compared with the general population [1]. Teachers, among professional voice users, are in a high-risk group due to a high incidence of voice problems, and the teachers who present with various voice problems to ear, nose and throat clinics are frequently encountered [2-7]. The most common voice symptoms among teachers are hoarseness, vocal fatigue, voice coarsening and bifurcation, throat clearing requirement, fading out at the end of a sentence, throat soreness, dryness and sensitivity, difficulty in hearing, and insufficiency of breath while speaking [2, 5-11].

The voice of the teachers has been found to be exposed to different risk factors. These are grouped under four main headings: Voice burden, physical risk factors, psychosocial risk factors, and environmental risk factors [12-14]. Different situations that increase voice complaints are of a different importance. Studies that address all the risk factors about the increase and emergence of voice complaints have revealed that physical and psycho-emotional factors are more important than voice burden and environmental risk factors. Knowing the effects of risk factors and their combinations is closely related to the diagnosis, treatment, prevention, and economic loss [15].

The hypotheses of this study are that the frequency of dysphonia will increase in teachers and that this frequency is increased by laryngopharyngeal reflux, allergic rhinitis, allergic asthma, hearing loss, hypothyroidism, previous head and neck surgery, chemical inhaler exposure, and smoking and alcohol use. According to this hypothesis, the purpose of this study was to determine the relationship between the prevalence of and risk factors for dysphonia and to identify pathologies that cause dysphonia among teachers who are the occupational risk group.

Materials and methods

The study was designed prospectively. In order for the data to be generalizable, data were collected between September 2006 and February 2007 from 3 different socioeconomically known lower, middle, and upper regions of Denizli. This study was conducted by filling out survey forms of 176 teachers in Denizli. The survey form questioned teachers' socio-demographic characteristics, voice complaints, and potential risk factors (The survey form Annex-1).

Of 167 teachers who completed the survey, 87 with a variety of voice complaints underwent endoscopic laryngeal examination. 9 teachers who completed the survey but were unable to attend the later stages of the study for various reasons were excluded. A hearing test was performed on those who had a complaint of hearing loss.

Statistical analysis

For both groups, statistical evaluations were performed using non-parametric statistical methods. The differences between the two groups in terms of categorical variables, such as gender distribution, working hours, presence of upper respiratory tract infections associated with voice symptoms, hearing loss and diagnostic symptoms of reflux disease, accompanying allergic diseases, and smoking status were investigated by using the Chi-square (χ^2) test. As the level of class that teachers are responsible for was increased, the evaluation of voice symptoms was performed using the χ^2 test for trend. When calculating the average age between the two independent groups, an independent t-test was used. Frequency analysis was conducted for the diagnostic distribution of the group with voice symptoms that underwent examination.

Approval for the study was obtained from Pamukkale University Non-Invasive Clinical Research Ethics Committee in 2006. This research is based on a master's thesis.

Results

The study group included 105 female and 62 male teachers between the ages of 22 and 56. The four most common voice symptoms were questioned. The group of teachers who answered ''yes'' to even one of these questions was evaluated as having dysphonia. Table 1 shows comparisons of sociodemographic and occupational characteristics properties of dysphonia and non-dysphonia groups. There was no statistically significant difference between the groups with and without dysphonia in terms of gender and average age.

ANNEX - 1

THE SURVEY FORM

1) **Do you have a complaint about your voice, for example hoarseness or talking fatigue?**

Yes 0 No 0

2) **Do you have tickle the throat, dry cough, or suffering from a feeling of obsession?**

Yes 0 No 0

3) **Do you suffer from stomach contents or brackish, sour water coming in your mouth?**

Yes 0 No 0

4) **Do you have a systemic disease?**

Yes (Thyroid diseases 0, Other ….) No 0

5) **Have you had a recent upper respiratory tract infection?**

Yes 0 No 0

6) **Do you have a hearing loss?**

Yes 0 No 0

7) **Did you have a surgical operation or an accident?**

Yes (Tonsilectomy 0 / Adenoidectomy 0 / Both are 0 / No 0
Neck mass removal 0 / Other 0….)

8) **Do you have an allergic illness has been determined?**

Yes (Asthma 0 / Allergic rhinitis 0) No 0

9) **Do you use cigarettes and alcohol?**

Yes (cigarettes 0 alcohol 0 Both are 0) No 0

10) **Have you been exposed to an irritating agent in your breath**?

Yes 0 No 0

11) **Have you consulted a doctor because of hoarseness?**

Yes 0 No 0

Table 1. The comparisons of sociodemographic and occupational characteristics properties of dysphonia and non-dysphonia groups

	Dysphonia (+) S: 87	Dysphonia (-) S: 80	Total S: 167	Test Statistic	*p*
Age	40.91±7.29	38.7±8.26	39.85±7.82	t=1.844	0.067
Gender					
Woman	58 (66.7(%)	47 (58.8(%)	105 (62.9(%)	x^2=1.119	0.290
Male	29 (33.3(%)	33 (41.2(%)	62 (37.1%)		
Education Stage					
Kindergarten teachers	16 (18.4%)	30 (37.5%)	46 (27.5%)		
Primary school (classroom) teachers	34 (39.1%)	24 (30.0%)	58 (34.7%)	Trend χ2 7.626	0.022*
Specialist teachers	37 (42.5%)	26 (32.5%)	63 (37.7%)		
Weekly Working-Hours					
Less than 30 hours per week	25 (28.7%)	20 (25%)	45 (26.9%)	x^2=0.295	0.587
More than 30 hours per week	62 (71.3%)	60 (75%)	122 (73.1%)		

*: *p*<0.05, t:independent samples t test, x^2: Chi square test, Mean±Standard deviation values were shown for age

All teachers in the study group were divided into three main groups according to the education level that they were responsible for. The first group consisted of kindergarten teachers responsible for children aged 4 to 6 years. The second group consisted of classroom teachers responsible for the first five years of primary school. The third group consisted of specialist teachers responsible for the last three years of primary school. In particular. In general, the age of the students that the teachers were responsible for increased gradually from the first group to the third group. When the three groups were compared in terms of a history of dysphonia symptoms, a significant difference was found between them (X^2=7.626, df=2, *p*=0.022). Pairwise comparisons revealed that the significant differences were between the first group and the second group (X^2=5.840, df=1, *p*=0.016), as well as between the first group and the third group (X^2=6.104, df=1, *p*=0.013).

For all the teachers icluded in the study, active working hours per week were recorded. Frequency analysis revealed a range of weekly working hours between 10 and 54 hours with 44.3% (74 teachers) working 30 hours per week. The median weekly working hours were 30, while the mean value was 31.29±7.58 hours. Weekly working hours were divided into two groups based on a threshold of 30 weekly working hours, and the effect of working hours on voice symptoms was investigated. No significant difference was found between the two groups in terms of weekly active working hours (Table 1).

Table 2 shows the comparisons of dysphonia and non-dysphonia groups with regard to allergic diseases and laryngopharyngeal reflux. In this study, laryngeal endoscopic findings of all patients with dysphonia and LPR (laryngopharyngeal reflux) symptoms were consistent with the findings of reflux disease. For this reason, all of the patients with LPR symptoms were considered to have the diagnosis of LPR. The concurrent presence of laryngopharyngeal reflux symptoms in the group with dysphonia was statistically significant. In this study, allergic diseases, including allergic rhinitis and allergic asthma, were questioned together with dysphonia. Of 87 subjects with dysphonia, 7 (8.05%) had allergic rhinitis, 3 (3.44%) had allergic asthma, and 2 (2.29%) had both allergic asthma and rhinitis. In other words, a total of 12 (13.8%) subjects had diagnoses related to allergic diseases. 2 (2.5%) of the 80 subjects without dysphonia had allergic rhinitis. Frequency analysis was used for these assessments. Comparisons between the two groups were performed by the “Chi-square test’’. The presence of allergic disease reached statistical significance in the group with dysphonia.

Table 2. The comparisons of dysphonia and non-dysphonia groups with regard to allergic diseaese and laryngopharyngeal reflux

	Dysphonia (+) S: 87	Dysphonia (-) S: 80	Total S: 167	Test Statistic	*p*
Allergic diseases (-)	75 (86.2%)	78 (97.5%)	153 (91.6%)	χ^2 =6.920	0.009*
Allergic diseases (+)	12 (13.8%)	2 (2.5%)	14 (8.4%)		
Laryngopharyngeal reflux (-)	39 (44.8%)	69 (86.2%)	108 (64.7%)	χ^2 =31.298	0.001*
Laryngopharyngeal reflux (+)	48 (55.2%)	11 (13.8%)	59 (35.3%)		

*: $p<0.05$, $x^{2:}$ Chi square test

In this study, the relationship between dysphonia and past history of recent upper respiratory tract infection was questioned. 32 (36.8%) of 87 subjects with dysphonia and 26 (32.5%) of 80 subjects without dysphonia had positive results. This difference was not statistically significant, and no relationship was found between dysphonia and past history of recent upper respiratory tract infection (p=0.562).

In this study, the evaluation addressed the relationship between dysphonia and smoking status. 19 (21.8%) of 87 subjects with dysphonia were smokers, while 24 (30%) of subjects without dysphonia were non-smokers. Statistical analysis of the results revealed no significant relationship between voice symptoms and smoking status (p=0.228).

Hypothyroidism, history of surgical operation and trauma that may cause dysphonia, alcohol use, and exposure to chemical inhalants, which were questioned in the questionnaire form, could not be evaluated since no effect on voice symptoms was found.

All the subjects with voice symptoms underwent laryngeal examination with a 90-degree rigid endoscope. The frequency analysis revealed normal laryngeal examination in 65 (65.5%) subjects, supporting findings of laryngopharyngeal reflux in 23 (26.4%) subjects, vocal cord nodules in 6 (6.8%) subjects, and vocal cord polyp in 1 (1.1%) subject.

Discussion

The frequency of voice complaints is known to be higher in teachers than in the general population [1, 3, 5, 12]. Although specific calculations depend on the definition of voice problems and examination approaches, 3 to 9% of the general population was found to have voice anomalies [16]. This prevalence is higher in teachers, and 87 (52.1%) of 167 teachers in our study had at least one existing voice symptom. This result was considered as close to the rate of 47.5%, which was obtained from the query of hoarseness, the most common voice symptom, in Smith et al.'s [2] survey study and to the rate of 42%, which was the rate of at least one symptom per week and/or day in Smolander et al.'s [10] study on 76 teachers. A phoniatric and stroboscopic study [17] conducted in Poland on 425 female teachers working full-time indicated deterioration in voice quality in 37.4% of teachers. In another study [18] conducted in the Netherlands on 214 female teacher candidates who were still students, the rate of at least one existing voice symptom was 56%. In our study, we could not evaluate the subjects according to their symptom numbers. Hence, our results may appear to be higher than the results of other studies that reported more than one symptom. However, our results are compatible with the results of different survey studies in the literature on the prevalence of voice symptoms among teachers.

The mean age of the teachers with voice symptoms in our study was 40.9 years, whereas the mean age of the teachers without voice complaints was 38.7 years. There was no statistically significant difference between the two groups. When considered that the age of teachers is correlated with the seniority in their job [19], this result suggests that working time in the teaching profession has no effect on voice symptoms. In a study of Roy et al. [20] on American teachers about voice problems, they divided teachers with voice symptoms between the ages of 20 and 60 years into 5 different groups, each containing 10 subjects. In this study, in which the mean age was 43.2±11.6

years, there was no significant relationship between prevalence of voice symptoms and age range. Again, the results of the study of de Jong et al. [13] on Dutch teachers about voice problems and epidemiologic data showed a median age of 38 (21 to 62) years in teachers with voice burden and 41 (21 to 64) years in teachers without voice burden. The difference was not significant. As a result, the results of Roy et al. [20] and de Jong et al. [13] were compatible with our results. Yet, there is an increasing use of voice among teachers in the practice of their profession compared to other occupational voice users [21]. Their voice gradually decreases in advancing course hours while performing their duties, and reducing the use of voice due to voice problems in their profession is often not practical and possible [12]. At this point, different studies in which students can participate with their feedback about their teachers should perhaps be planned. However, it can be said that teachers indeed develop voice preventive techniques either consciously or unconsciously over the years or reduce energy they expend in the first years of their profession at the expense of reducing their productivity.

There was no statistically significant difference between groups in terms of gender. Although the present assessment in which gender is not a risk factor for voice symptoms has been supported by some studies [10], the publications reporting that female gender is a risk factor for voice symptoms are in the majority [2, 3, 8, 13, 15, 20]. The factors that make the female gender a risk factor are that they use their voices worse than their male colleagues and that they are affected more from the voice problems that arise [8]. In addition, an important point that should not be ignored is that the structure of the male vocal cords is different than that of female vocal cords. In a study of normal human vocal cords in America, it has been found that the amount of hyaluronic acid in the central part of the lamina propria layer of vocal cords is greater in amount in males than in females in particular [22]. Given that hyaluronic acid is a key ingredient for creating resistance against the compression of the lamina propria, as well as its role in proliferation, regeneration, and tissue repair, it is not surprising to see in many studies that female gender constitutes a risk group for voice symptoms [22].

All teachers in the study were divided into three main groups according to the stage of education that they were responsible for. In general, the age of the students that the teachers were responsible for was increased gradually from the first group to the third group (Table 1). The first group was compared with the other two groups; the history of voice symptoms increased from the first group to the other groups. Results were statistically significant. This shows that specialist teachers and classroom teachers are at more risk than kindergarten teachers. In the literature, the stage of education has not been considered as a risk factor in large-scale studies based on the stage of education among teachers. In Russell et al.'s study [3], the school types have been classified as kindergarten, primary, secondary, and area schools (schools located in rural areas in which the students between the ages of 5 and 12 years are trained), and no relationship was found between school types and voice problems of the teachers. However, our results are seen to be different than the result of Russell et al.'s study [3]. It is not difficult to understand that teachers' voice complaints are increased with the increasing age range of the students. Kindergarten teachers educate the same class every day. This also allows better recognition of the group of students under responsibility and time allocation for themselves for voice rest later in the day according to the curriculum. However, primary school and specialist teachers who have to enter into a separate class for each course are expected to give the same performance up to the last hours. In addition, participation of these teachers in courses about prolonged voice use due to the Secondary Schools Student Placement Examination in Turkey increases the voice burden they are exposed to. As shown in our study, increasing stage of education and the specialization lead to an increase in voice symptoms.

The concurrent presence of laryngopharyngeal reflux symptoms in the group with voice symptoms reached statistical significance. The risk analysis showed that LPR caused a 4.7-fold increase in voice symptoms. In the literature, several publications indicating that voice disorders are the most common symptoms of reflux at the laryngeal level are in agreement with our results [23, 24]. In Koufman et al.'s [25] study conducted on 113 patients with

laryngeal reflux and voice disorders, the patients underwent dual-probe 24-hour esophageal pH monitoring, and findings were consistent with laryngopharyngeal reflux in 78 (69%) patients. 69 (88%) of these 78 patients complained about hoarseness, which indicates that this symptom is the most common laryngeal and voice symptom. Years ago, Morrison and Rammage [26] defined gastroesophageal reflux (the term laryngopharyngeal reflux became common at that time) as one of the four internal factors that affect phonation processes. Again, in Perez Fernandez et al.'s [27] study on a teacher group who had vocal cord nodules and was healthy, reflux has been shown as one of the four factors associated with personal factors in the pathologic group. In our study, the subjects with laryngopharyngeal reflux symptoms underwent concurrent direct laryngoscopic examination, and the results were consistent with reflux laryngitis.

Mucosal problems, which were found to be effective on both the general population and study groups among the teachers, were not effective in our study. Perhaps conducting future studies by separating the term ‘’upper respiratory tract infection” into sub-groups may give us more meaningful results. Because in a study conducted by Roy et al. [28] on the general population, it was determined that frequent colds and sinus infections are an increased risk for chronic voice disorders, in which chronic postnasal drips constitute the major cause.

The presence of allergic disease reached statistical significance in the group with voice symptoms. In the literature, in Gotaas and Starr’s [29] study, which is very similar to our study and results, 22 teachers with the complaint of voice fatigue and 17 teachers without complaints were compared, and it was concluded that allergy causes the complaint of voice fatigue via mucosal edema and inflammation. In the study of Roy et al. [20] on voice disorders in the general population, the rate of voice disorders was 41.84% in patients with respiratory tract allergies, whereas this rate was 27.23% in patients without respiratory tract allergies. The difference was considered significant. Given that allergies are a risk factor for voice symptoms even in the general population, it is anyway inevitable to think that allergies are a factor that enhances the complaints of teachers at risk of occupational voice disorders.

Harmful effects of smoking are evident. However, the high rate of smoking in society and the groups being compared, the emergence of the effects of smoking occurs in the long term, and studying multiple factors in the studies has influenced not only the results of our study, but also the results of many other important studies in the literature [11, 20]. For this reason, no significant correlation was found between smoking and dysphonia.

All the subjects with voice symptoms underwent laryngeal examination with a 90-degree rigid endoscope, which revealed normal laryngeal examination in 65 (65.5%) subjects, reflux laryngitis in 23 (26.4%) subjects, vocal cord nodules in 6 (6.8%) subjects, and vocal cord polyp in 1 (1.1%) subject. A comparison of our results with similar studies in the literature revealed differences in methods and approaches.

In Ma and Yiu’s [30] study, the survey forms about voice complaints were distributed to two different groups, including 40 subjects with dysphonia and 40 healthy individuals. Then, the group of dysphonia was subtyped according to the laryngeal pathology. 40 individuals with dysphonia; 12 (30%) had vocal cord nodules, 3 (7.5%) had vocal cord polyps, 9 (22.5%) had chronic laryngitis, 6 (15%) had thickened vocal cords, 3 (7.5%) had vocal cord paralysis, and 7 (17.5%) had different laryngeal pathologies. Here, the most common organic cause of dysphonia was identified to be vocal cord nodules. The rates seem to be higher according to our results. Because dysphonia cases in Ma and Yiu’s [30] study were selected from volunteers from Hong Kong hospitals who had applied for healing.

In a larger study conducted by Preciado et al. [31], the prevalence of voice disorders was found to be 57%. Of this ratio, 20.3% were diagnosed as organic lesions (14% nodules, 2% polyps, 1.4% submucous accumulation, 1.2% Reinke edema, 0.4% sulcus vocalis, 0.6% scar, 0.02% leukoplakia, and 0.02% vocal cord paralysis), whereas 8.1% was chronic laryngitis (3.9% smoking, 2.8% non-specific, 2.5% reflux laryngitis), and 29% was functional impairment. In this study, a questionnaire, vocal cord examination, acoustic analysis, and video-stroboscopy equipment were used as a diagnostic tool. The results show that

the most common cause of voice disorders is functional dysphonia. To diagnose the functional dysphonia, a physiological evaluation with an acoustic analysis and video stroboscopy, as well as the survey studies and examination [32]. In our study, the dysphonia cases with normal vocal cord examination have not been evaluated in terms of functional dysphonia. For this reason, the rate of the cases considered as healthy was higher in our study.

We have applied laryngoscopic examination for at least one existing voice symptom in our study. But this application was insufficient for the diagnosis of functional voice disorders, as stroboscopic examination was not performed. This constitutes the limitation of our study. In our study, the most important etiologic factor has been reflux laryngitis. According to Koufmann [23], who is an author on this subject, laryngopharyngeal reflux has been shown to be the underlying cause or etiological cofactor in two-thirds of patients with laryngeal or voice disorders. On the other hand, the diagnosis of reflux laryngitis has been ignored in studies about the voice symptoms of teacher groups [7, 11, 30, 31].

In conclusion, our study suggests that one of the two teachers has a symptom of dysphonia, and the presence of laryngopharyngeal reflux and allergic diseases must primarily be questioned.

Funding: None.

Authors contributions: F.T. and C.O.K. have constructed the main idea and hypothesis of the study. F.T. and C.O.K. developed the theory and arranged/edited the material and method section. F.T. and C.O.K. have done the evaluation of the data in the Results section. Discussion section of the article was written by F.T. and C.O.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Williams NR. Occupational groups at risk of voice disorders: a review of the literature [published correction appears in Occup Med (Lond). 2012 Oct;62(7):588]. *Occup Med (Lond)*. 2003;53(7):456-460. doi:10.1093/occmed/kqg113
2. Smith E, Gray SD, Dove H, Kirchner L, Heras H. Frequency and effects of teachers' voice problems. *J Voice*. 1997;11(1):81-87. doi:10.1016/s0892-1997(97)80027-6
3. Russell A, Oates J, Greenwood KM. Prevalence of voice problems in teachers. *J Voice*. 1998;12(4):467-479. doi:10.1016/s0892-1997(98)80056-8
4. Urrutikoetxea A, Ispizua A, Matellanes F. Pathologie vocale chez les professeurs: une étude vidéo-laryngo-stroboscopique de 1.046 professeurs [Vocal pathology in teachers: a videolaryngostroboscopic study in 1046 teachers]. *Rev Laryngol Otol Rhinol (Bord)*. 1995;116(4):255-262.
5. Roy N, Merrill RM, Thibeault S, Gray SD, Smith EM. Voice disorders in teachers and the general population: effects on work performance, attendance, and future career choices. *J Speech Lang Hear Res*. 2004;47(3):542-551. doi:10.1044/1092-4388(2004/042)
6. Sapir S, Keidar A, Mathers Schmidt B. Vocal attrition in teachers: survey findings. *Eur J Disord Commun*. 1993;28(2):177-185.doi:10.3109/13682829309041465
7. Sala E, Laine A, Simberg S, Pentti J, Suonpää J. The prevalence of voice disorders among day care center teachers compared with nurses: a questionnaire and clinical study. *J Voice*. 2001;15(3):413-423. doi:10.1016/S0892-1997(01)00042-X
8. Smith E, Kirchner HL, Taylor M, Hoffman H, Lemke JH. Voice problems among teachers: differences by gender and teaching characteristics. *J Voice*. 1998;12(3):328-334. doi:10.1016/s0892-1997(98)80022-2
9. Sliwińska Kowalska M, Fiszer M, Niebudek Bogusz E, Kotyło P, Rzadzińska A. Ocena stanu narzadu głosu u studentów szkoły pedagogicznej [Evaluation of voice quality in students from teaching colleges]. *Med Pr*. 2000;51(6):573-580.
10. Smolander S, Huttunen K. Voice problems experienced by Finnish comprehensive school teachers and realization of occupational health care. *Logoped Phoniatr Vocol*. 2006;31(4):166-171. doi:10.1080/14015430600576097
11. Simberg S, Laine A, Sala E, Rönnemaa AM. Prevalence of voice disorders among future teachers. *J Voice*. 2000;14(2):231-235. doi:10.1016/s0892-1997(00)80030-2
12. Thomas G. The Voice of Student Teachers and Teachers. Yayınlanmamış Yüksek Lisans Tezi. Radboud University Nijmegen Medical Centre, Nijmegen, 2005.
13. de Jong FI, Kooijman PG, Thomas G, Huinck WJ, Graamans K, Schutte HK. Epidemiology of voice problems in Dutch teachers. *Folia Phoniatr Logop*. 2006;58(3):186-198. doi:10.1159/000091732

14. Kooijman PG, de Jong FI, Oudes MJ, Huinck W, van Acht H, Graamans K. Muscular tension and body posture in relation to voice handicap and voice quality in teachers with persistent voice complaints. *Folia Phoniatr Logop*. 2005;57(3):134-147. doi:10.1159/000084134
15. Kooijman PG, de Jong FI, Thomas G, et al. Risk factors for voice problems in teachers. *Folia Phoniatr Logop*. 2006;58(3):159-174. doi:10.1159/000091730
16. Ramig LO, Verdolini K. Treatment efficacy: voice disorders. *J Speech Lang Hear Res*. 1998;41(1):S101-S116. doi:10.1044/jslhr.4101.s101
17. Sliwinska Kowalska M, Niebudek Bogusz E, Fiszer M, et al. The prevalence and risk factors for occupational voice disorders in teachers. *Folia Phoniatr Logop*. 2006;58(2):85-101. doi:10.1159/000089610
18. Meulenbroek LF, de Jong FI. Voice quality in relation to voice complaints and vocal fold condition during the screening of female student teachers. *J Voice*. 2011;25(4):462-466. doi:10.1016/j.jvoice.2010.01.003
19. Pahn J, Pahn E. Vorschlag zur Einschätzung des Schweregrades von Stimmstörungen [Assessment of the degree of severity of voice disorders]. *Folia Phoniatr (Basel)*. 1975;27(06):472-479.
20. Roy N, Merrill RM, Gray SD, Smith EM. Voice disorders in the general population: prevalence, risk factors, and occupational impact. *Laryngoscope*. 2005;115(11):1988-1995. doi:10.1097/01.mlg.0000179174.32345.41
21. Mattiske JA, Oates JM, Greenwood KM. Vocal problems among teachers: a review of prevalence, causes, prevention, and treatment. *J Voice*. 1998;12(4):489-499. doi:10.1016/s0892-1997(98)80058-1
22. Hammond TH, Zhou R, Hammond EH, Pawlak A, Gray SD. The intermediate layer: a morphologic study of the elastin and hyaluronic acid constituents of normal human vocal folds. *J Voice*. 1997;11(1):59-66. doi:10.1016/s0892-1997(97)80024-0
23. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope*. 1991;101(4 Pt 2 Suppl 53):1-78. doi:10.1002/lary.1991.101.s53.1
24. Ulualp SO, Toohill RJ, Shaker R. Pharyngeal acid reflux in patients with single and multiple otolaryngologic disorders. *Otolaryngol Head Neck Surg*. 1999;121(6):725-730. doi:10.1053/hn.1999.v121.a98010
25. Koufman JA, Amin MR, Panetti M. Prevalence of reflux in 113 consecutive patients with laryngeal and voice disorders [published correction appears in Otolaryngol Head Neck Surg 2001 Jan;124(1):104]. *Otolaryngol Head Neck Surg*. 2000;123(4):385-388. doi:10.1067/mhn.2000.109935
26. Morrison MD, Rammage LA. Muscle misuse voice disorders: description and classification. *Acta Otolaryngol*. 1993;113(3):428-434. doi:10.3109/00016489309135839
27. Pérez Fernández CA, Preciado López J. Nódulos de cuerdas vocales. Factores de riesgo en los docentes. Estudio de casos y controles [Vocal fold nodules. Risk factors in teachers. A case control study design]. *Acta Otorrinolaringol Esp*. 2003;54(4):253-260. doi:10.1016/s0001-6519(03)78412-x
28. Roy N, Merrill RM, Gray SD, Smith EM. Voice disorders in the general population: prevalence, risk factors, and occupational impact. *Laryngoscope*. 2005;115(11):1988-1995. doi:10.1097/01.mlg.0000179174.32345.41
29. Gotaas C, Starr CD. Vocal fatigue among teachers. *Folia Phoniatr (Basel)*. 1993;45(3):120-129. doi:10.1159/000266237
30. Ma EP, Yiu EM. Voice activity and participation profile: assessing the impact of voice disorders on daily activities. *J Speech Lang Hear Res*. 2001;44(3):511-524. doi:10.1044/1092-4388(2001/040)
31. Preciado J, Pérez C, Calzada M, Preciado P. Incidencia y prevalencia de los trastornos de la voz en el personal docente de La Rioja. Estudio clínico: cuestionario, examen de la función vocal, análisis acústico y vídeolaringoestroboscopia [Prevalence and incidence studies of voice disorders among teaching staff of La Rioja, Spain. Clinical study: questionnaire, function vocal examination, acoustic analysis and videolaryngostroboscopy]. *Acta Otorrinolaringol Esp*. 2005;56(5):202-210. doi:10.1016/s0001-6519(05)78601-5
32. Morrison MD, Nichol H, Rammage LA. Diagnostic criteria in functional dysphonia. *Laryngoscope*. 1986;96(1):1-8. doi:10.1288/00005537-198601000-00001

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1583891

Combined and alone apoptotic effects of Ankaferd hemostat and silver nanoparticles on chronic myeloid leukemia (CML) cell line K562

Ankaferd kanama durdurucu ve gümüş nanopartiküllerin kronik miyeloid lösemi (KML) hücre hattı K562 üzerindeki kombine ve tek başına apoptotik etkileri

Başak Ünver Koluman, Mücahit Seçme, Yavuz Dodurga

Posted date:12.11.2024 Acceptance date:13.03.2025

Abstract

Purpose: This study aimed to evaluate the effects of Ankaferd hemostat (ABS; Ankaferd Blood Stopper®) and silver nanoparticles (AgNPs), alone or in combination, on human chronic myeloid leukemia (CML) cells.

Materials and methods: The cytotoxicity of ABS and AgNPs on K562 CML cells was assessed using the XTT assay, measuring cell viability over time and across different doses. The half maximal inhibitory concentration (IC_{50}) was determined at 72 hours. Apoptosis-related gene expression was analyzed by Real-Time PCR, and oxidative stress was assessed by total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

Results: AgNPs reduced cell viability at higher doses, with the IC_{50} for 40 nm AgNPs being 107.8854 ppm at 72 hours. ABS reduced cell viability by 75% even at maximum dose. Significant changes (p<0.05) were observed in bcl-2, caspase-8 and CDK4 in the AgNPs group. In the ABS group, bcl-2, and CDK4 expressions were significantly elevated. The combined treatment increased caspase-8 and caspase-9 expressions, promoting apoptosis. No significant differences were found in TAS-TOS, but all groups showed higher oxidant activity compared to the control, with the combination group exhibiting the highest antioxidant effect.

Conclusion: ABS, a herbal treatment with minimal side effects, and AgNPs, a promising therapeutic agent, both showed potential in inhibiting tumor cells. Their combination enhanced apoptotic effects, warranting further investigation.

Keywords: Chronic myeloid leukemia, Ankaferd hemostat, silver nanoparticles, apoptosis, oxidative stress.

Unver Koluman B, Secme M, Dodurga Y. Combined and alone apoptotic effects of Ankaferd hemostat and silver nanoparticles on chronic myeloid leukemia (CML) cell line K562. Pam Med J 2025;18:330-338.

Öz

Amaç: Bu çalışma, Ankaferd hemostat (ABS; Ankaferd Blood Stopper®) ve gümüş nanopartiküllerinin (AgNP'ler), tek başına veya kombinasyon halinde, insan kronik miyeloid lösemi (KML) hücreleri üzerindeki etkilerini değerlendirmeyi amaçlamıştır.

Gereç ve yöntem: ABS ve AgNP'lerin K562 KML hücreleri üzerindeki sitotoksik etkisi, XTT testi ile değerlendirilmiş ve farklı dozlar ve zaman dilimlerinde hücre canlılığı ölçülmüştür. Yarı maksimal inhibitör konsantrasyonu (IC_{50}) değeri 72 saat sonunda belirlenmiştir. Apoptoz ile ilişkili gen ekspresyonları Real-Time PCR ile analiz edilmiş, oksidatif stres ise total antioksidan durumu (TAS), total oksidan durumu (TOS) ve oksidatif stres indeksi (OSI) kullanılarak değerlendirilmiştir.

Bulgular: AgNP'ler, doz arttıkça hücre canlılığını düşürmüş ve 40 nm AgNP'ler için IC_{50} değeri 72 saat sonunda 107.8854 ppm olarak bulunmuştur. ABS, maksimum dozda bile hücre canlılığını %75 oranında düşürmüştür. AgNP grubunda bcl-2, kaspaz-8 ve CDK4 ekspresyonlarında anlamlı değişiklikler (p<0,05) gözlemlenmiştir. ABS grubunda ise bcl-2 ve CDK4 ekspresyonları anlamlı şekilde artmıştır. Kombine tedavi, kaspaz-8 ve kaspaz-9 ekspresyonlarını artırarak apoptozu teşvik etmiştir. TAS-TOS arasında anlamlı bir fark bulunmamış, ancak tüm gruplarda kontrol grubuna kıyasla daha yüksek oksidan aktivite gözlemlenmiş, kombinasyon grubunda ise en yüksek antioksidan etki saptanmıştır.

Sonuç: Yan etkileri minimal olan bir bitkisel tedavi olan ABS ve umut verici bir tedavi ajanı olan AgNP'ler, tümör hücrelerini inhibe etme potansiyeli göstermiştir. Kombinasyonları apoptoz üzerindeki etkilerini artırmış ve daha fazla araştırma yapılmasını gerektirmiştir.

Anahtar kelimeler: Kronik miyeloid lösemi, Ankaferd hemostat, gümüş nanopartiküller, apoptoz, oksidatif stress.

Başak Ünver Koluman, Asst. Prof. Department of Hematology, Asst. Prof. Pamukkale University, Faculty of Medicine, Denizli, Türkiye, e-mail: basakunver@yahoo.com (https://orcid.org/0000-0003-1106-5021) (Corresponding Author)

Mücahit Seçme, Assoc. Prof. Department of Medical Biology, Assoc. Prof. Ordu University, Faculty of Medicine, Ordu, Türkiye, e-mail: mehtersecme@gmailcom (https://orcid.org/0000-0002-2084-760X)

Yavuz Dodurga, Prof. Department of Medical Biology, Prof. Pamukkale University, Faculty of Medicine, Denizli, Türkiye, e-mail: yavuzdodurga@gmail.com (https://orcid.org/0000-0002-4936-5954)

Ünver Koluman B, Seçme M, Dodurga Y. Ankaferd kanama durdurucu ve gümüş nanopartiküllerin kronik miyeloid lösemi (KML) hücre hattı K562 üzerindeki kombine ve tek başına apoptotik etkileri. Pam Tıp Derg 2025;18:330-338.

Introduction

Chronic myeloid leukemia (CML) is a disease characterized by aberrant clonal growth of myeloid precursor cells. The annual global incidence rate of CML is 0.87/100 000, and it can reach 1.52 in people over the age of 70. The average age of diagnosis is 56 [1-3]. CML is caused by the Philadelphia chromosome, which causes the bcr-abl fusion gene and its end result, the bcr-abl protein. In the early 2000s, the initiation of a tyrosine kinase inhibitor (TKI) by targeting this protein dramatically changed the treatment of CML.

Prior to TKI, allogeneic hematopoietic stem cell transplantation was attempted as soon as a suitable donor was found for the patients. Imatinib, dasatinib, nilotinib, and bosutinib are some of the most common treatment choices now available. Allogeneic hematopoietic stem cell transplantation is now only used on a very limited basis [4]. CML has been transformed from a fatal disease to a chronic disease because of TKI. However, due to the prevalence of TKI resistance, treatment-free remission and deep molecular response are difficult to achieve.

TKI resistance can be dependent on bcr-abl or independent of bcr-abl. Dose escalation or switching to another TKI is preferred in bcr-abl-dependent resistance. Nevertheless, this approach has limitations, particularly when higher doses are not tolerated. On the other hand, bcr-abl independent resistance is more complex and difficult to manage. Cancer cells exhibit negative effect adaptation behaviors in addition to pharmacogenetics in bcr-abl-independent resistance and its effects on patients. These cells are attempting to survive, which causes changes in the expression of a variety of proteins (such as bcl-2 and MCL1, MDR1, transcription factor STAT5, and imatinib uptake transporter OCT1). These cancer stem cells are strong, resistant, and incurable; they form tumors, renew themselves, and frequently cause disease progression and relapse [5].

Ankaferd hemostat (ABS; Ankaferd Blood Stopper®) is an herbal mixture utilized for many years in Anatolia for hemostasis. ABS contains Glycyrrhiza glabra, Alpinia officinalis, Thymus vulgaris, Vitis vinifera, and Urtica dioica, among others [6]. It also contains anti-inflammatory, antioxidative, anti-infective, and lastly, antineoplastic properties in addition to hemostasis. ABS inhibits cell proliferation by influencing cell metabolism and cell cycle mechanisms. KPNA2, SND1, and PARK7 are potential cancer therapy targets. It also increases the expression of the tumor suppressor proteins RPL5 and UCHL1. RPL5 causes apoptosis by directly activating the p53 apoptotic pathway [6, 7]. ABS toxicology studies have also revealed that it is a very safe agent [8].

Silver nanoparticles (AgNPs) are structures ranging in size from 1 to 100 nm. They have low electrical and thermal resistance and have been used in a variety of consumables, electronics, and healthcare applications [9]. It possesses potent antimicrobial properties [10]. Recent research on lymphoma, breast cancer cells, and liver cancer cells has also revealed that these molecules have anti-tumor properties [11].

CML is one of the most well-controlled cancers, yet it remains an important issue for research due to resistance development, complications induced by patients' extra co-morbidities, and TKI side effects. Additionally, although ABS and AgNPs have shown promising antitumor effects in various cancer models, there is still a notable gap in research on their combined effects on chronic CML cells. This study hypothesizes that the combination of ABS and AgNPs will synergistically enhance apoptosis in CML cells, providing a more effective therapeutic approach than either agent alone. By investigating these effects on the K562 CML cell line, we aim to assess the potential of ABS and AgNPs to induce apoptosis via both extrinsic and intrinsic pathways, which may offer an additional therapeutic mechanism compared to traditional treatments. This study's findings could establish a basis for new adjunctive strategies in CML treatment, addressing drug resistance and enhancing antitumor efficacy through a multi-mechanistic approach.

Materials and methods

Cell culture

In this study, K562 human CML cells were cultured in RPMI 1640 (Gibco™) enriched with 10% heat-inactivated fetal bovine serum (FBS; Capricorn Scientific), 20 units/mL penicillin and 20 µg/mL streptomycin, 1 mM sodium pyruvate, and 0.1 mM amino acid solution (Biological Industries) and cultured at 37°C in 5% CO_2.

AgNPs (Sigma-Aldrich, 40 nm) were applied to the K562 cells with concentrations including 5, 10, 25, 50, 100, and 200 ppm, and Ankaferd hemostat (ABS; Ankaferd Blood Stopper®, Istanbul, Türkiye) was decided to apply to the K562 cells with various concentrations including 2.5, 5, 10, 25, 50, and 100 ppm to evaluate the antiproliferative effect at 24, 48, and 72 hours.

XTT Assay

The antiproliferative activities of AgNPs (40 nm), ABS, and their combination on K562 cells were evaluated using the XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. The test was conducted on 1×10^4 cells per well in 96-well plates, the provided instructions (Cell Proliferation Kit; Biological Industries Cat No: 20-300-1000). Following the completion of the dosing procedures, the XTT combination was administered corresponding to the recommended dose and time. The synthesis of farmazon was assessed spectrophotometrically (using a Multiskan GO microplate spectrophotometer, Termo) and chromatically at 450 nm (reference wavelength: 630 nm). The viability percentage is calculated by dividing the absorption of the experimental well by the absorption of the control well, and then multiplying the ratio by 100.

The AAT Bioquest online web page was utilized to calculate the IC_{50} dose of AgNPs (40 nm) and ABS on K562 cells (https://www.aatbio.com/tools/ic50-calculator). The IC_{50} dosage was utilized as the dose group in the remaining molecular tests.

RT- PCR assay

Trizol (Invirogen, USA) was applied to obtain total RNA from cells, in accordance with the directions provided by the manufacturer. The A.B.T. synthesis kit with RNase Inh. was used to synthesize cDNA (ABT, Türkiye). RT-PCR was performed to measure changes in mRNA expression of bcl-2, caspase-3, bax, caspase-9, CDK4, caspase-8, CDK6, URG4, p21, BID, and CCND1 (Applied Biosystem, StepOne Plus). Beta-actin was utilized to normalize the results. The identical primer sequences as in Dodurga et al. [12] 2015 and Alur et al. [13] 2016 were applied. Real-time RT-PCR was utilized for PCR testing with the SYBR Green qPCR Master Mix ABTTM 2X qPCR SYBR-Green MasterMix (Türkiye) methodology.

TAS- TOS and OSI measurement

Rel Assay kits (Rel Assay Kit Diagnostics, Gaziantep, Türkiye) were applied to assess the total antioxidant status (TAS) and total oxidant status (TOS) in control cells as well as cells treated with AgNPs, ABS, and their IC_{50} value combinations. TAS and TOS values were obtained by a microplate reader (Biotek). The oxidative stress index (OSI) was calculated by converting the mmol unit of TAS to the µmol unit of TOS. The oxidative stress index was determined by the OSI= TOS / TAS* 1/10 technique.

Statistical analysis

The statistical analysis was carried out using SPSS 23.0 (IBM SPSS Statistics 23 software (Armonk, NY: IBM Corp.)). Continuous variables were defined by mean ± standard deviation. One-way ANOVA test was used for comparisons of multi-group parameters (TOS (µmol H_2O_2 equiv./L) and TAS (mMOL Trolox Equiv./L). The $2^{-\Delta\Delta CT}$ method in the RT² ProfilerTM PCR Array Data Analysis program was used to quantify RT-PCR data. Data were expressed as mean + standard deviation and for group comparisons. Independent samples t test was used with cRT² ProfilerTM PCR Array Data Analysis program. Statistical significance was determined as $p\leq0.05$.

Results

The results of this study provide a comprehensive assessment of the effects of ABS and AgNPs on K562 CML cells, demonstrating distinct responses across cell viability, gene expression, and oxidative stress. The XTT assay showed that AgNPs, both alone and in combination with ABS, significantly reduced cell viability in a dose- and time-dependent manner,

achieving an IC_{50} of 107.8854 ppm at 72 hours, whereas ABS alone had a comparatively limited effect. Gene expression analysis via RT-PCR further revealed that combination treatment increased apoptotic markers, particularly in caspase-8 and caspase-9 expressions. The antioxidant activity was most pronounced in the combined treatment. These findings collectively underscore the apoptotic and oxidative impacts of ABS and AgNPs on CML cells, with combination therapy showing potential for more substantial therapeutic effects. The results obtained from this study are given below in detail.

XTT assay

The viability of K562 cells was evaluated by the XTT assay following treatment with ABS, AgNPs (40 nm), and their combination. K562 cell viability decreased in a time- and dose-dependent way. At the 24- and 48-hour marks, the vitality of the cells treated with AgNPs decreased somewhat as the dose rose; however, at the 72-hour mark, a more significant effect was observed. The IC_{50} dose of AgNPs (40 nm) was found to be 107.8854 ppm at 72 hours in the K562 cell line (Figure 1a, Figure 2). The influence of ABS on K562 cell viability is illustrated in Figure 1b. A comparable reduction in cell viability was not found with increasing ABS dosage. Even at the highest dose rate, ABS could only reduce cell viability up to 75% within 72 hours.

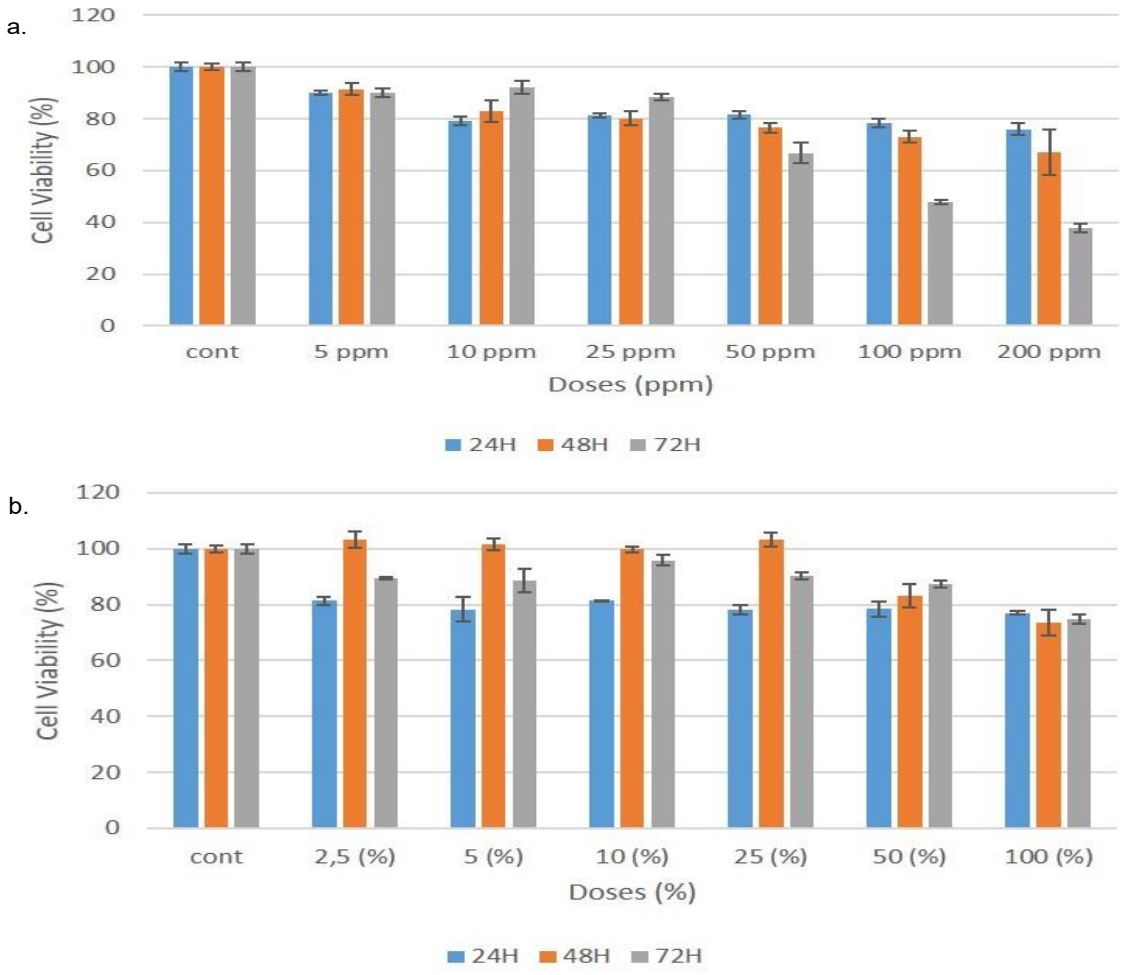


Figure 1. Cell viability of groups

a. K562 cells were treated with AgNPs at different concentrations and time intervals and their viability was assessed by XTT assay. Data shows the average results of three independent experiments. IC_{50} doses of AgNPs in K562 cells were detected as 107.8854 ppm at the 72 hours

b. K562 cells were treated with ABS at different concentrations and time intervals and their viability was assessed by XTT assay. Data shows the average results of three independent experiments

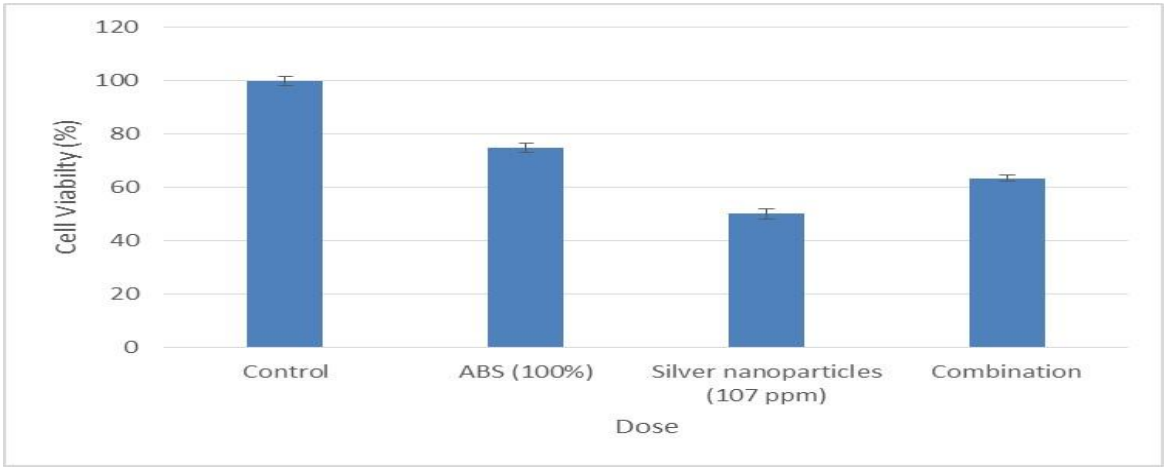


Figure 2. Cell viability of control and dose groups including IC_{50} value of AgNPs, ABS (100%) and their combination in K562 cells

Real Time-PCR

Following total RNA removal from the cells, cDNA synthesis was carried out. RT-PCR was used to examine the gene expression of bcl-2, caspase-8, caspase-9, bax, caspase-3, CDK6, CDK4, URG4, p21, BID, and CCND1. The SYBR Green qPCR Master Mix technique was followed. RT- PCR was utilized to assess mRNA expression alterations in genes implicated in the triggering of apoptosis and cell cycle arrest. In comparison to the control group, the expression of key genes related to apoptosis, such as caspase-8 and caspase-9, increased in the combination group. Significant alterations (p<0.05) were seen in bcl-2, caspase-8, and CDK4 expressions in the AgNPs group. Statistically significant (p<0.05) increases in bcl-2 and CDK4 expressions were found in the ABS group. In the combined group, the increase in both caspase-8 and caspase-9 expressions was found to be statistically significant in inducing apoptosis. Table 1 summarizes all gene phase changes and associated p-values (Table 1).

Table 1. Fold regulation and p values in dose groups with comparing to control group

		Group 1*		Group 2*		Group 3*	
		Fold Regulation	*p*-value	Fold Regulation	*p*-value	Fold Regulation	*p*-value
1	**Beta-actin**	1.00	non	1.00	non	1.00	non
2	**Caspase-3**	-10.96	0.343256	9.44	0.555935	14.21	0.353469
3	**Caspase-8**	4.14	0.313246	28.27	0.005590	12.06	0.004980
4	**Caspase-9**	1.88	0.213277	1.39	0.537163	5.89	0.011216
5	**bax**	2.89	0.201103	2.56	0.100986	1.87	0.083722
6	**bcl-2**	8.81	0.012183	5.48	0.006919	37.03	0.019985
7	**CDK4**	5.64	0.003648	2.14	0.019540	7.18	0.000171
8	**CDK6**	9.39	0.050577	20.24	0.306986	451.47	0.003172
9	**CCND1**	4.55	0.272031	5.89	0.000166	6.27	0.015260
10	**URG4**	1.74	0.048724	1.78	0.178441	2.09	0.320371
11	**p21**	2.20	0.055106	1.55	0.026678	1.49	0.374144
12	**BID**	1.80	0.410042	1.16	0.935613	9.65	0.081735

*Group 1: ABS, Group 2: AgNPs, Group 3: Combination

TAS-TOS and OSI determination

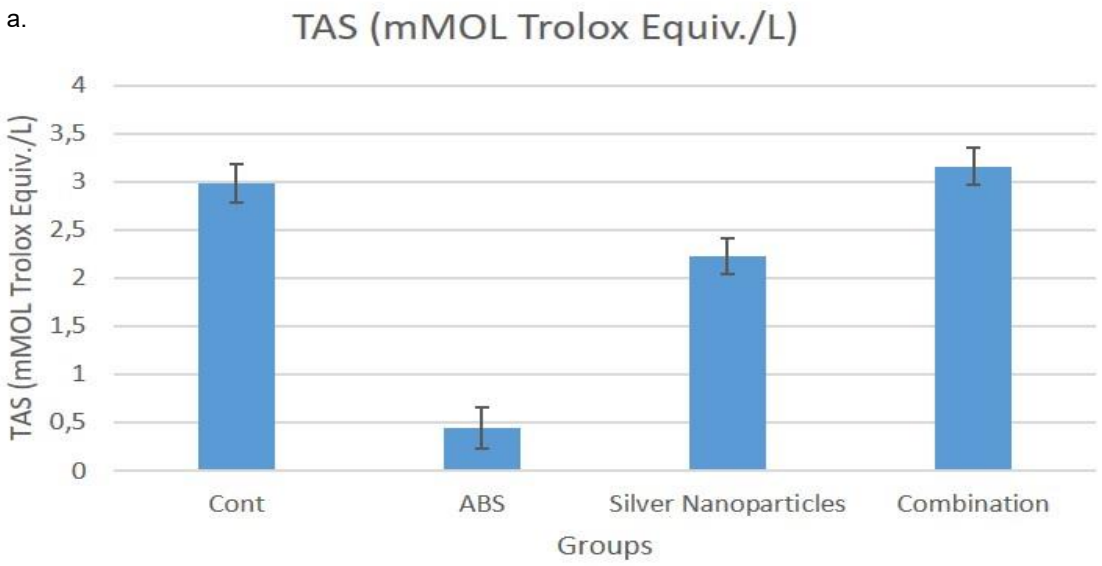
Table 2 shows that there was no statistically significant difference (p>0.05) in the TAS-TOS between the groups. ABS, AgNPs, and their combination showed increased total oxidant activity than the control group (p>0.05), despite the lack of a statistically significant difference (Table 2). After assessing each group's total antioxidant capacity, we discovered that, while there was no statistically significant difference, the combination group had the greatest antioxidant impact (Table 2). OSI values are shown in the Figures 3a-c.

Table 2. Comparison of TAS and TOS values

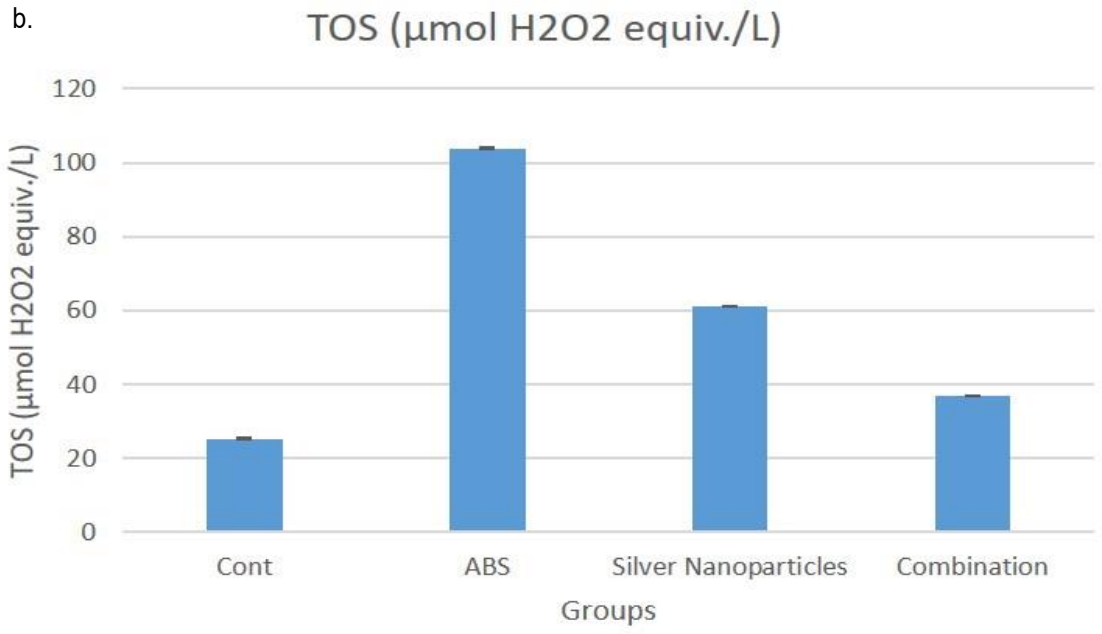
	Control	Group1*	Group 2*	Group 3*	*p*-value
TAS	2.97±0.08	0.43±0.09	2.22±0.15	3.15±0.04	0.417 (F=0.325)
TOS	25.31±0.11	103.8±0.22	61.12±0.16	36.97±0.13	0.561 (F=0.733)

*Group 1: ABS, Group 2: AgNPs, Group 3: Combination

a.



b.



c.

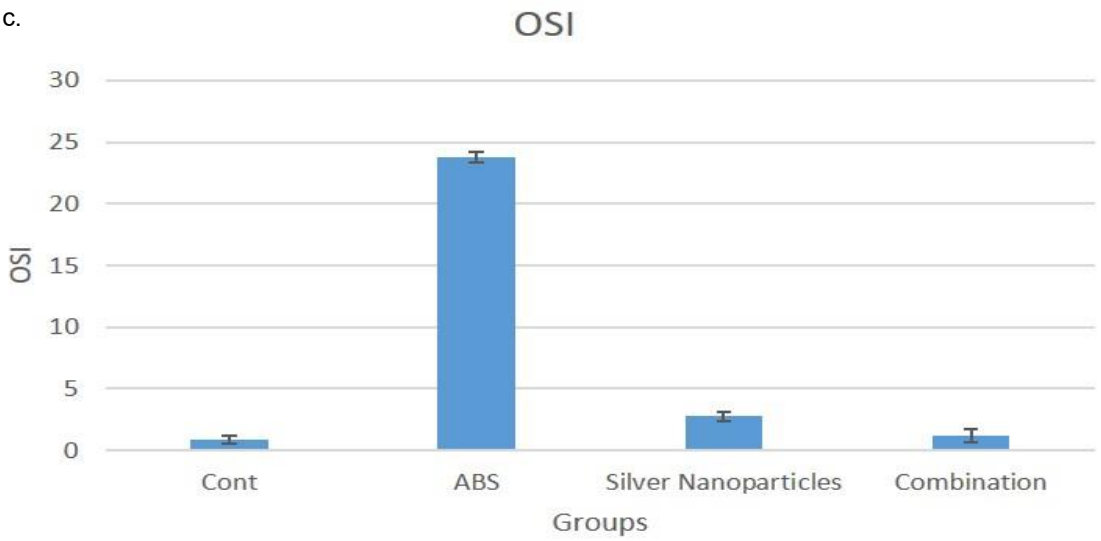


Figure 3. Total antioxidant (a), oxidant (b) and oxidative stress index (c) in K562 cells treated with AgNPs, ABS and their combination

Discussion

This study demonstrates that the combined treatment of ABS and AgNPs significantly enhances apoptotic activity in K562 CML cells. Key findings include a dose- and time-dependent decrease in cell viability with AgNPs alone, reaching optimal efficacy at 72 hours, while the combined treatment markedly increased expression of apoptotic markers, both caspase-8 and caspase-9. These results indicate that the ABS-AgNP combination amplifies apoptotic responses beyond the effects of either agent alone, which could offer a multi-faceted approach for targeting CML cells. This combined effect highlights the potential of ABS and AgNPs as adjunctive agents in CML therapy, addressing limitations of current treatments and potentially overcoming challenges related to drug resistance.

Apoptosis is a physiological condition required for a cell's normal growth and development. It is also necessary for a stable internal environment. The mitochondrial pathway is divided into segments, such as the death receptor pathway. Caspase-mediated apoptosis occurs because of mitochondrial cytochrome c release. When the mitochondrial permeability transition pores open in response to an external stimulus, the mitochondrial membrane potential decreases and apoptosis is induced [11, 14, 15]. In our study, we noticed a statistically significant increase in caspase 8 activities in the AgNPs group. This suggests that the inhibitory effect of these nanoparticles on malignant cells in CML in our study occurs via apoptosis. The increase in caspase-9 expression was also shown to be statistically significant in inducing apoptosis in the combined group. One of the expressions of genes implicated in inducing cell cycle arrest, known as Cyclin D1, increased in the AgNPs group.

Mitochondria produce reactive oxygen radicals and provide energy to cells. These radicals are a type of molecule that causes apoptosis. AgNPs have been shown in some studies to cause oxidative damage to tumor cells by causing the production of reactive oxygen radicals. This impact was not only detected in our study, but it was also observed more frequently in ABS [11, 16, 17].

CML is one of the most prevalent hematological cancers. TKI in CML affects not only bcr-abl1, but also a wide range of other targets. This situation has a slew of unintended consequences. Furthermore, despite its mildness, it interferes with humoral and cellular immunity. Side effects make life difficult for both the doctor and the patient, especially in patients with co-morbidities and the elderly [18]. Immunity issues are addressed, particularly in pediatric patients with CML. Concerns are also raised by the fact that these children will need

to use TKIs for an extended period of time. These children, for example, have immunization programs, which must be carefully managed due to the use of TKIs. In these two difficult patient groups, research is being conducted on various treatment options. Future investigations into the potential use of ABS and/or AgNPs in the treatment of these adult or pediatric patients may be guided by our findings.

Numerous cellular processes, including angiogenesis, apoptosis, cell cycle regulation, inflammation, signal synthesis, metabolism, and immunological pathways, have been shown to be subject to the pleiotropic effects of ABS [19, 20]. ABS has been demonstrated to prevent the B-CLL cell line from transforming into an aggressive, blastic lymphoid form [21]. Mumcuoglu et al. [19] investigated the effects of ABS in two different human leukemia cell lines, K562 and Jurkat cells, and discovered that it induced apoptosis by regulating the expression of PAR1 and EPCR (Endothelial cell protein C receptor) in these cells. ABS has been shown to have a dose- and time-dependent effect on EPCR expression in both cell lines, causing cycles of decreasing and increasing EPCR expression. However, in high-dose ABS application, the effect of decreased EPCR expression was observed to be statistically more stable and continuous in Jurkat cells compared to K562 cells. Given the results of this study in the literature, we investigated the effects of ABS and AgNPs on the CML cell line. Tumor cell viability decreased as the dose and period of therapy increased. ABS and/or AgNPs may be able to help these patients, as demonstrated in our study, because of their good efficacy and low side-effect profile.

We can see that AgNPs have hosted some cancer studies in recent years. In one study, AgNPs were modified with folic acid to actively and selectively recognize tumor cells, and it was demonstrated that these particles inhibit lymphoma cells by increasing the apoptosis of stem cells in vitro and in vivo, with no significant side effects [11]. Guo et al. [22] found that AgNPs may enter K562 cells, a CML cell line, and reside in endosomes. In our study, the K562 cell line was also selected. According to Guo et al. [22], the effect of AgNPs on these cells could be cytotoxicity and apoptosis via reactive oxygen radicals. These effects have been shown to be reversible with vitamin C, an antioxidant. Another study found that AgNPs increase the intracellular accumulation of daunorubicin, a common chemotherapeutic agent, in K562 leukemia cells, activating the killing effect of these cells [23].

While this study provides valuable insights, several limitations should be addressed in future research. The scope of gene analysis was limited to key apoptotic markers, and additional gene targets and pathways could be explored to better understand the molecular mechanisms at play. Additionally, potential off-target effects of AgNPs, especially in a clinical setting, require further investigation. The lack of in vivo data restricts the ability to fully translate these findings to therapeutic applications. Future studies should include in vivo experiments to evaluate the safety and efficacy of the ABS-AgNP combination in a living organism, alongside pathway-specific analyses to confirm and expand these initial findings. Exploring the pharmacokinetics, biodistribution, and long-term effects of these agents in vivo would be essential for assessing their therapeutic potential in CML treatment.

In conclusion, due to the development of drug resistance in CML, additional co-morbidities in patients, and TKI side effects, research into different treatment options is still ongoing. We tested two different options on CML cell lines, ABS, a herbal medicine with very low potential for side effects, and AgNPs, which is a very effective and promising agent in this regard, and found that they inhibited tumor cells. Furthermore, it has been demonstrated that when these two agents are used together, the apoptotic anti-tumor effects increase. This study, we believe, is a groundbreaking study for future research.

Funding: None.

Authors contributions: B.U.K. and Y.D. constructed the hypothesis of the study. B.U.K. and M.S. arranged the material and method section. M.S., B.U.K. and Y.D. evaluated the data of the Results section. Discussion section of the article was written by B.U.K. Also B.U.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Eden RE, Coviello JM. Chronic Myelogenous Leukemia. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531459/. Accessed January 16, 2023
2. Hoffmann VS, Baccarani M, Hasford J, et al. The EUTOS population-based registry: incidence and clinical characteristics of 2904 CML patients in 20 European Countries. *Leukemia.* 2015;29(6):1336-1343. doi:10.1038/leu.2015.73
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin.* 2017;67(1):7-30. doi:10.3322/caac.21387
4. Snyder D. CML Chapter. *Cancer Treat Res.* 2021;181:97-114. doi:10.1007/978-3-030-78311-2_6
5. Schoepf AM, Gust R. Novel strategies to eradicate resistant cells in chronic myeloid leukemia. *Future Med Chem*. 2020;12(23):2089-2092. doi:10.4155/fmc-2020-0278
6. Çiftçiler R, Haznedaroglu İC. Ankaferd hemostat: from molecules to medicine. *Turk J Med Sci.* 2020;50(SI-2):1739-1750. doi:10.3906/sag-1908-161
7. Koçak E, Çelebier M, Haznedaroglu IC, Altınöz S. Analysis of the Antiproliferative Effect of Ankaferd Hemostat on Caco-2 Colon Cancer Cells via LC/MS Shotgun Proteomics Approach. *Biomed Res Int*. 2019;2019:5268031. doi:10.1155/2019/5268031
8. Koluman A, Akar N, Malkan UY, Haznedaroglu IC. Qualitative/Chemical Analyses of Ankaferd Hemostat and Its Antioxidant Content in Synthetic Gastric Fluids. *Biomed Res Int.* 2016;2016:8957820. doi:10.1155/2016/8957820
9. Islam MA, Jacob MV, Antunes E. A critical review on silver nanoparticles: From synthesis and applications to its mitigation through low-cost adsorption by biochar. *J Environ Manage*. 2021;281:111918. doi:10.1016/j.jenvman.2020.111918
10. Bruna T, Maldonado Bravo F, Jara P, Caro N. Silver Nanoparticles and Their Antibacterial Applications. *Int J Mol Sci*. 2021;22(13):7202. doi:10.3390/ijms22137202
11. Zhao Q, Sun XY, Wu B, et al. Construction of biomim etic silver nanoparticles in the treatment of lymphoma. *Mater Sci Eng C Mater Biol Appl*. 2021;119:111648. doi:10.1016/j.msec.2020.111648
12. Dodurga Y, Seçme M, Eroğlu C, et al. Investigation of the effects of a sulfite molecule on human neuroblastoma cells via a novel oncogene URG4/URGCP. *Life Sci.* 2015;143;27-34. doi:10.1016/j.lfs.2015.10.005
13. Alur İ, Dodurga Y, Seçme M, et al. Anti-tumor effects of bemiparin in HepG2 and MIA PaCa-2 cells. *Gene*. 2016;585(2):241-246. doi:10.1016/j.gene.2016.03.044
14. Bukeirat M, Sarkar SN, Hu H, Quintana DD, Simpkins JW, Ren X. MiR-34a regulates blood-brain barrier permeability and mitochondrial function by targeting cytochrome c. *J Cereb Blood Flow Metab*. 2016;36(2):387-392. doi:10.1177/0271678X15606147
15. Pérez MJ, Ponce DP, Aranguiz A, Behrens MI, Quintanilla RA. Mitochondrial permeability transition pore contributes to mitochondrial dysfunction in fibroblasts of patients with sporadic Alzheimer's disease. *Redox Biol*. 2018;19:290-300. doi:10.1016/j.redox.2018.09.001
16. Wu H, Lin J, Liu P, et al. Reactive oxygen species acts as executor in radiation enhancement and autophagy inducing by AgNPs. *Biomaterials*. 2016;101:1-9. doi:10.1016/j.biomaterials.2016.05.031
17. Nayak D, Kumari M, Rajachandar S, Ashe S, Thathapudi NC, Nayak B. Biofilm Impeding AgNPs Target Skin Carcinoma by Inducing Mitochondrial Membrane Depolarization Mediated through ROS Production. *ACS Appl Mater Interfaces*. 2016;8(42):28538-28553. doi:10.1021/acsami.6b11391
18. Albayrak M, Celebi H, Albayrak A, et al. Serious skin reaction associated with imatinib in a patient with chronic myeloid leukemia. *Eurasian J Med.* 2011;43(3):192-195. doi:10.5152/eajm.2011.42
19. Mumcuoglu M, Akin DF, Ezer U, Akar N. Ankaferd Blood Stopper induces apoptosis and regulates PAR1 and EPCR expression in human leukemia cells. *Egypt J Med Hum Gen*. 2015;16(1):19-27. doi:10.1016/j.ejmhg.2014.10.001
20. Malkan UY, Haznedaroglu IC. Antineoplastic Effects of Ankaferd Hemostat. *Biomed Res Int.* 2022;2022:2665903. doi:10.1155/2022/2665903
21. Akalın İ, Okur FV, Haznedaroğlu İC, et al. Acute in Vitro effects of ABS (Ankaferd Hemostat) on the Lymphoid Neoplastic Cells (B-CLL and RAJI Tumor Cell Lines). *UHOD*. 2014;24(4):253-259. doi:10.4999/uhod.13026
22. Guo D, Zhao Y, Zhang Y, et al. The cellular uptake and cytotoxic effect of silver nanoparticles on chronic myeloid leukemia cells. *J Biomed Nanotechnol*. 2014;10(4):669-678. doi:10.1166/jbn.2014.1625
23. Zhou Y, Wang X. Study on synergistic effect of new functionalized Ag nanoparticles for intracellular drug uptake in cancer cells. *Nano Biomed Eng*. 2010;2(4):208-213. doi:10.5101/nbe.v2i4.p208-213

Research Article
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1625516

Effect of probiotics on kidney tissue in an experimental diabetes model

Deneysel diyabet modelinde probiyotiklerin böbrek dokusuna etkisi

Hülya Çetin, İlknur Lafcı, Semih Tan, Bilgin Kıray Vural, Saim Özdamar

Posted date:27.01.2025 Acceptance date:18.03.2025

Abstract

Purpose: Diabetes, a major public health issue, is an endocrine and metabolic disease that causes damage to various tissues, including the kidneys. Probiotics are thought to play a beneficial role in the prevention and treatment of diseases when used in sufficient amounts. This study aims to investigate the effects of probiotic supplementation on kidney tissue damage induced by diabetes.

Materials and methods: A total of 34 rats were divided into five groups. The control group (K, n=5) received PBS, while the probiotic-only group (Pm, n=5) was given probiotics (0.6 mg/kg). In the diabetes group (Dm, n=8), diabetes was induced using streptozotocin (50 mg/kg). The PmD group (n=8) received probiotic supplementation before diabetes induction, and probiotic administration continued after diabetes was induced. In contrast, the DmP group (n=8) first underwent diabetes induction, followed by probiotic supplementation. Kidney tissues were examined histopathologically and immunohistochemically, with Bcl-2 and alpha-SMA antibody expressions evaluated.

Results: No histopathological alterations were observed in the control group. In the Dm group, moderate-to-mild nephrotoxicity was detected, while the probiotic-supplemented diabetes groups exhibited mild nephrotoxicity. Bcl-2 expression was decreased in the Dm group but was found to be higher in the DmP and PmD groups. Conversely, alpha-SMA expression was elevated in the Dm group, whereas it was lower in the DmP and PmD groups.

Conclusion: Histopathological and immunohistochemical analyses indicate that probiotics exert a protective effect against diabetes-induced kidney damage.

Keywords: Diabetes, probiotic, kidney, alpha-SMA, Bcl-2.

Cetin H, Lafci I, Tan S, Vural BK, Ozdamar S. Effect of probiotics on kidney tissue in an experimental diabetes model. Pam Med J 2025;18:340-355.

Öz

Amaç: Diyabet, önemli bir halk sağlığı sorunu olan endokrin ve metabolik bir hastalıktır. Tüm dokularda olduğu gibi böbrek dokusunda da hasara yol açmaktadır. Probiyotiklerin yeterli miktarda kullanılması, hastalıkların önlenmesi ve tedavisinde faydalı olabileceği düşünülmektedir. Bu çalışmada, diyabetin böbrek dokusunda oluşturduğu hasara karşı probiyotik takviyesinin etkisini araştırmayı amaçladık.

Gereç ve yöntem: Toplam 34 sıçan beş gruba ayrıldı. Kontrol grubuna (K, n=5) PBS, yalnızca probiyotik alan gruba (Pm, n=5) ise probiyotik (0,6 mg/kg) uygulandı. Diyabet grubu (Dm, n=8) streptozotosin (50 mg/kg) ile diyabet oluşturuldu. PmD grubuna (n=8) diyabet oluşturulmadan önce probiyotik takviyesi uygulandı ve diyabet oluştuktan sonra da probiyotik uygulamasına devam edildi. DmP grubunda (n=8) ise önce diyabet oluşturuldu, ardından probiyotik takviyesi uygulandı. Böbrek dokuları histopatolojik ve immünohistokimyasal olarak incelendi; Bcl-2 ve alfa-SMA antikor ekspresyonları değerlendirildi.

Bulgular: Kontrol grubunda histopatolojik değişiklikler gözlenmezken, Dm grubunda orta-hafif nefrotoksisite saptandı. Probiyotik takviyesi alan diyabet gruplarında ise hafif nefrotoksisite gözlendi. Dm grubunda Bcl-2 ekspresyonu azalmış olup, DmP ve PmD gruplarında ise daha yüksek seviyelerde olduğu tespit edilmiştir. Alfa-SMA ekspresyonu ise Dm grubunda artarken, DmP ve PmD gruplarında daha düşük seviyelerde olduğu gözlenmiştir.

Sonuç: Histopatolojik ve immünohistokimyasal değerlendirmeler, probiyotiklerin diyabetin neden olduğu böbrek hasarına karşı koruyucu bir etki gösterdiğini ortaya koymaktadır.

Anahtar kelimeler: Diyabet, probiyotik, böbrek, alfa-SMA, Bcl-2.

Hülya Çetin, Prof. Pamukkale University Faculty of Medicine, Department of Histology and Embriology, Denizli, Türkiye, e-mail: hcsorkun@pau.edu.tr (https://orcid.org/0000-0001-8731-0631)

İlknur Lafcı, PhD Student, Pamukkale University Faculty of Medicine, Department of Histology and Embriology, Denizli, Türkiye, e-mail: ilknurlafci93@gmail.com (https://orcid.org/0009-0000-2135-6418) (Corresponding Author)

Semih Tan, Asst. Prof. Ordu University Faculty of Medicine, Department of Histology and Embriology, Ordu, Türkiye, e-mail: semihtan@ordu.edu.tr (https://orcid.org/0000-0002-5609-9594)

Bilgin Kıray Vural, Assoc. Prof. Pamukkale University, Vocational School of Health Services, Department of Medical Services and Techniques, Denizli, Türkiye, e-mail: bvural@pau.edu.tr (https://orcid.org/0000-0002-2136-8192)

Saim Özdamar, Prof. Pamukkale University Faculty of Medicine, Department of Histology and Embriology, Denizli, Türkiye, e-mail: sözdamar@pau.edu.tr (https://orcid.org/0000-0003-4440-5360)

Çetin H, Lafcı İ, Tan S, Vural BK, Özdamar S. Deneysel diyabet modelinde probiyotiklerin böbrek dokusuna etkisi. Pam Tıp Derg 2025;18:340-355.

Introduction

Diabetes mellitus (diabetes) is a chronic metabolic disease characterized by insulin deficiency and the associated pathological consequences [1]. Globally, while significant reductions in mortality from non-communicable diseases such as chronic respiratory diseases, cardiovascular diseases, and cancer were observed between 2000 and 2019, diabetes-related mortality increased by 3% during the same period [2]. Approximately 10-20% of patients with diabetes succumb to renal failure, and it has been reported that 50% of patients undergoing dialysis have diabetes [3].

Kidneys are important organs that maintain the acid-base balance of the body by filtering the blood from metabolic wastes [3]. In histopathological evaluation of the tissue in diabetes-induced renal damage, podocyte damage, cell shedding and apoptosis are among the primary pathological findings. Damage to podocytes exacerbates proteinuria in diabetes [4].

Diabetes adversely affects multiple organs, including the heart, blood vessels, kidneys, eyes, and nerves. One of the most well-documented renal complications of diabetes is diabetic nephropathy. Experimental diabetes models have demonstrated that diabetic nephropathy impairs kidney function and leads to histological damage in the glomerular and tubular structures [5].

In addition to conventional pharmacological treatments, medical nutrition therapy plays a key role in diabetes management. Patients who adhere to medical nutrition therapy achieve better blood glucose control and a reduced risk of complications [6]. In addition, the occurrence of gastrointestinal dysfunction, which shows symptoms such as obesity, delayed gastric emptying, diabetic gastroparesis, diarrhea and constipation, is considered a contributing factor in the pathogenesis of type 2 diabetes [7-9]. This suggests that the risk of developing type 2 diabetes may be influenced by factors related to the gut microbiota [10]. Probiotics, defined as live microorganisms that confer health benefits when administered in sufficient amounts, are key components of the gut microbiota. Evidence from animal studies suggests that probiotics may modulate glucose metabolism and enhance insulin sensitivity [11, 12]. Recently, research on the therapeutic potential of probiotics, particularly their antibacterial and anti-inflammatory properties, has gained momentum. The concept that modulating the gut microbiota is crucial for overall health has become increasingly recognized [13].

In this study, we aimed to investigate the effects of probiotic supplementation on the kidneys in a streptozotocin (STZ)-induced diabetic rat model by analyzing biochemical markers and assessing Bcl-2 and alpha-SMA expression via immunohistochemical methods.

Materials and methods

Permission for the study was obtained from Pamukkale University Animal Experiments Ethics Committee with the decision numbered and dated PAUHDEK-2021/07, 24.08.2021.

Experimental study design

The effect size obtained in the reference study [31] was found to be strong (F=0.786). Assuming that an effect size at this level could be obtained, as a result of the power analysis performed for 5 groups, it was calculated that 90% power could be obtained at 95% confidence level when at least 35 rats (at least 7 rats for each group) were included in the study. Since the control and probiotic-only groups were the control group, it was deemed appropriate to use 5 rats each in these groups and 8 animals each in the diabetes groups since animal loss due to diabetes could be seen.

In the study, 10-week-old Wistar rats weighing between 250-300 g, were used (n=34). The animals were kept in specially designed cages under the supervision of a veterinarian and maintained in a temperature-controlled environment with 50%±5% humidity under laboratory conditions simulating a 12-hour day-night cycle. The rats were given standard rat

pellet food and tap water ad libitum. The rats were randomly assigned to five groups: control group (group K, n=5), probiotic group (group Pm, n=5), diabetes group (group Dm, n=8), probiotic + diabetes group (group PmD, n=8) and diabetes + probiotic group (group DmP, n=8). VSL#3 was used as probiotic. VSL#3 contains 3 genera, 7 species and 8 strains. The species included 4 Lactobacilli, 3 Bifidobacteria and 1 Streptococcus strains (Table 1).

Table 1. Probiotic microorganisms contained in VSL#3

Streptococcus thermophilus BT01
Lactobacillus plantarum BP06
Lactobacillus acidophilus BA05
Lactobacillus helveticus BD08
Lactobacillus paracasei BP07
Bifidobacterium animalis subsp. lactis BL03
Bifidobacterium animalis subsp. lactis BL04
Bifidobacterium breve BB02

During the experiment, 200 µl PBS (phosphate buffered saline solution) was given to group K by gavage 5 days a week for 4 weeks. After a five-week break, the same procedure was repeated for an additional four weeks, starting from week 10. In Group Pm, 0.6 mg/kg VSL#3 probiotic was dissolved in 400 µl PBS and administered by gavage for each rat 5 days a week for the first 4 weeks [14]. Probiotic (reminder dose) was administered twice a week for the next 5 weeks. Starting from the 10th week, probiotics were given 5 times a week for 4 weeks. To induce diabetes, a single dose of streptozotocin (STZ, 50 mg/kg) was administered. Seventy-two hours later, blood samples were collected from the tail veins of STZ-treated rats, and blood glucose levels were measured using a commercial glucometer (Accu-Chek Performa) [9]. Diabetes was induced in group Dm rats by STZ administration at the 10th week of the study. Group PmD rats were administered probiotic (0.6 mg/kg VSL#3) 5 times a week for the first 4 weeks and twice a week for the following 5 weeks and diabetes was induced in the 10th week. After diabetes was induced, probiotic administration was continued 5 times a week for 4 weeks. No treatment was applied to group DmP for the first 9 weeks. Diabetes was induced in the 10th week of our experiment and probiotic solution was administered by gavage (0.6 mg/kg VSL#3) for 4 weeks starting from the 10th week. Body weights of the rats were measured weekly. After the completion of the 13th week of the experiment, blood glucose levels of the rats were measured by glucometer from blood samples taken from the lateral tail vein. One day later, the rats were anaesthetized with 90 mg/kg ketamine + 10 mg/kg xylazine intraperitoneally. For biochemical analysis, intracardiac blood samples were collected and centrifuged in yellow-capped biochemistry tubes. Urea, Blood Urea Nitrogen (BUN) and creatinine levels were determined from serum by immunoassay method (Beckman coulter Access 2).

Animal care, injection and gavage applications, sampling procedures were carried out at Pamukkale University Experimental Animals Research Unit (DEHAB).

Histopathological analysis

The kidneys excised from rats were weighed and fixed in a 10% formalin solution. For histopathological examination, the tissues were processed and embedded in paraffin. Subsequently, 5-micron thick sections were obtained using a microtome and stained histologically with hematoxylin-eosin (HE) and PAS stains (Histomed BS-0046, Lot: 092016-001).

Immunohistochemical analysis

Bcl-2 antibody [(Elabscience E-AB-64067 host: Rabbit) (1:100)], an anti-apoptotic marker and alpha-SMA antibody [(Elabscience E-AB-34268 host: Rabbit) (1:100)], a marker of myofibroblasts, were used for immunohistochemical analysis. Secondary antibody (Thermo-Scientific cat no: 37624) and DAB chromogen kit (Thermo Scientific) were then used. Nuclear staining was performed using hematoxylin. Both histochemically and immunohistochemically stained sections were examined and photographed with Olympus BX51 and Olympus DP72 brand/model devices. The immunoreactivity intensity of the sections was evaluated in 10 different areas at x40 magnification with Image-J program and the mean values were calculated for each group.

Histopathological evaluation of kidney damage

Sections taken from the kidney tissues of the groups were coded and evaluated in a blinded manner by two independent histologists. The semiquantitative evaluation of renal tissues was performed based on previously published criteria and graded according to the severity of tissue damage (Table 2) [15].

Table 2. Criteria for histopathological evaluation of kidneys [15]

Value	Glomerular damage	Acute tubular necrosis	Tubulointerstitial inflammatory infiltrates
0	None	None	None
1	less than 25% of glomeruli exhibit non-specific damage characteristics	less than 25 per cent of all renal parenchymal tubules	leukocytes confined within the interstitium
2	25-50% of glomeruli exhibit non-specific damage characteristics	25-50% of all renal parenchymal tubules	leukocytes infiltrating interstitium and tubular epithelial cells
3	50-75% of glomeruli exhibit non-specific damage characteristics	50-75% of all renal parenchymal tubules	
4	more than 75% of glomeruli exhibit non-specific damage	more than 75% of all renal parenchymal tubules	

(A) no nephrotoxicity: 0-1, (B) mild nephrotoxicity: 2-4, (C) moderate nephrotoxicity: 5-7, (D) severe nephrotoxicity: 8-10

Statistical analysis

Data were analysed with SPSS 23.0 software package. Continuous variables were given as mean ± standard deviation, median (minimum-maximum values). Shapiro-Wilk test was used to determine the suitability of the data for normal distribution. One-way analysis of variance (ANOVA) with Tukey's post hoc test was applied for comparisons among independent groups when parametric test assumptions were met. When parametric assumptions were not satisfied, the Kruskal–Wallis test was employed, followed by the Mann–Whitney U test with Bonferroni correction for post hoc comparisons. A *p*-value of <0.05 was considered statistically significant.

Results

Weight gain was observed in all experimental groups from the beginning of the study. In the tenth week, a decrease in body weight was noted in the diabetes groups. Although not statistically significant, the average weight loss in the DmP group was lower than in the other diabetes (Dm and PmD) induced groups. When the mean body weights were compared, no statistically significant difference was found between the groups (Table 3, Figure 1).

Although the difference between the mean kidney weights among the groups was not statistically significant, it was noteworthy that the mean kidney weight of Group Pm was higher than in the other groups (Table 4).

Table 3. Weight measurement results of rats (g)

Groups	Week 1	Week 2	Week 3	Week 4	Week 10	Week 11	Week 12	Week 13
K	289.4±19.42	298.4±22.17	314.2±25.78	312.8±34	371.6±55.8	378± 56.67	380±48.89	379±46.45
Pm	294.6±25.54	306.2±26.15	317.8±27.79	321.8±30.7	357.4±43.65	366.2±45.16	368±47.14	366.6±44.8
Dm	290.38±19.65	308.88±21.86	321.88±24.4	335.25±21.91	396.75±26.75	388.75±31.22	366.75±29.08	336.5±31.1
DmP	293.75±24.36	309±27.23	329±30.47	341±33.82	391.13±47.32	388.5±45.3	384.63±48.71	377±51.8
PmD	293.5±34.71	308.25±36.99	322±43.26	327.63±40.81	394.5±51.23	383±50.94	367.75±55.43	333.25±51.8
p	0.996 F= 0.047	0.965 F= 0.141	0.94 F= 0.193	0.597 F= 0.702	0.513 F= 0.837	0.911 F= 0.244	0.919 F=0.23	0.188 F=1.653

$p<0.05$ was considered significant, F=One-Way Anova Test

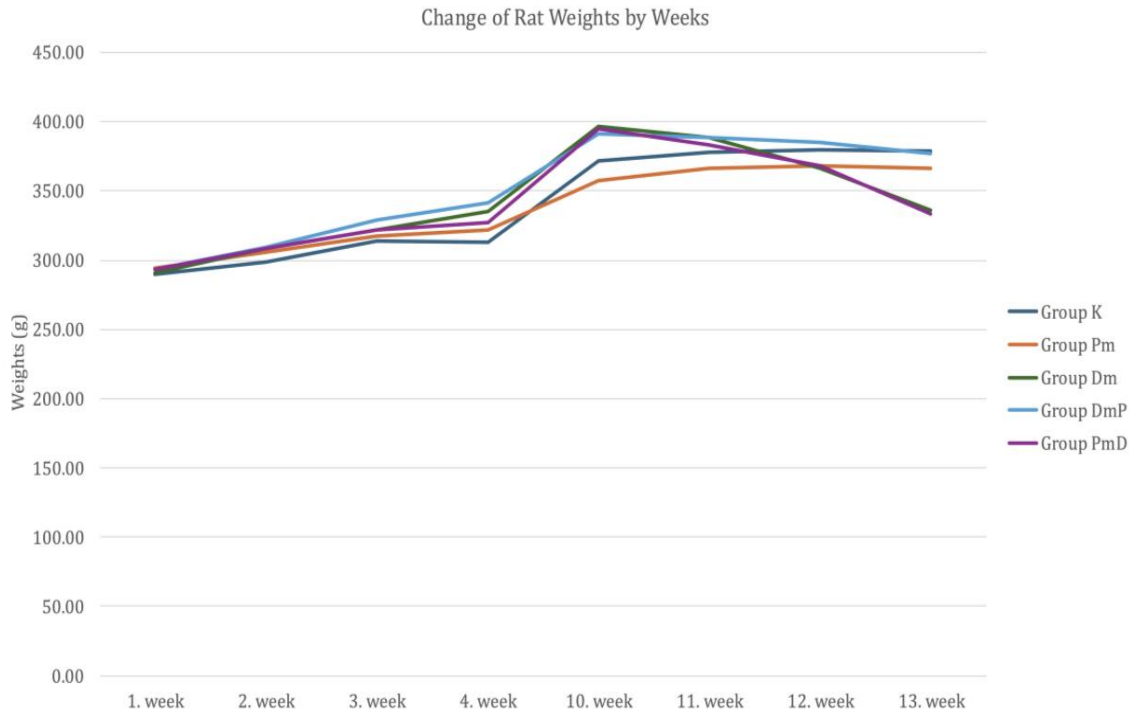


Figure 1. Weekly weight changes of rats according to groups are shown

Table 4. Mean kidney weights of the groups (g)

	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
Right Kidney Weight	1.9±0.38	2.4±0.54	1.96±0.63	1.61±0.33	1.89±0.2	0.063 F=2.511
Left Kidney Weight	1.78±0.33	2.13±0.45	1.67±0.38	2.2±0.5	1.88±0.29	0.083 F=2.294

p<0.05 was considered significant, F=One-Way Anova Test

Blood glucose values measured before sacrification were compared among the groups. The blood glucose levels in the Dm, DmP, and PmD groups were higher than those in the K and Pm groups (Table 5, Figure 2).

The blood glucose levels in the DmP and PmD groups were lower than those in the Dm group. Among the diabetes-induced groups, the PmD group, which received probiotics prior to diabetes induction, exhibited the lowest blood glucose levels. However, the differences in blood glucose levels among these three groups were not statistically significant (Table 5, Figure 2).

Table 5. Blood glucose value measurement results of the groups (mg/dL)

	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
72 hours after STZ induction	109.2±4.55	116.2±2.49	607.13±94.16 k*p*. p*p*	397.13±67.99 k*p*. p*p*	489.5±140.13 k*p*. p*p*	0.0001* kw=25.912
Before sacrification	109.8±5.97	121.2±8.23	544.13±126.87 k*p*. p*p*	454.75±110.41 k*p*. p*p*	431.13±59.55 k*p*. p*p*	0.0001* kw=22.728

k*p*=(*p*<0.05) Statistically significant with the control group (Group K), p*p*=(*p*<0.05) Statistically significant with the probiotic group (Group Pm)

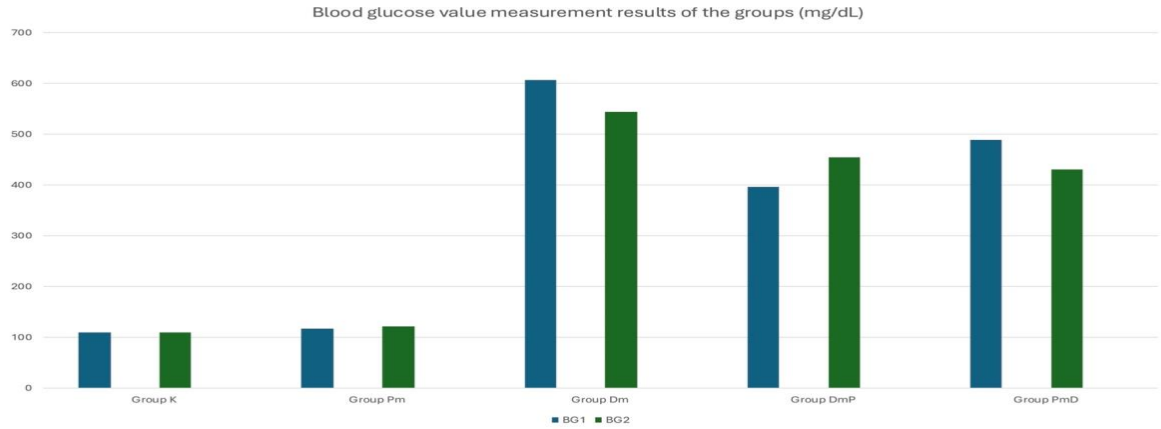


Figure 2. Blood glucose measurement results of the groups (BG1=blood glucose measurement result 72 hours after STZ induction, BG2=blood glucose measurement result just before sacrification)

The differences in mean serum urea, creatinine and BUN levels between the groups were statistically significant. The mean urea concentration in the DM group was significantly higher than groups K, P and DMP. Additionally, the mean urea concentration in the PmD group was significantly higher than in group K. Although not statistically significant, the mean urea concentration in the PmD group was lower than in the DM group. The mean serum BUN concentration in the Dm group differed significantly from those in groups K and P. Although, the mean BUN concentrations in the diabetic groups receiving probiotic supplements (groups DmP and PmD) were lower than in the Dm group, these differences were not statistically significant. When the mean creatinine concentration was compared between the groups, the difference between group Pm and group PmD was statistically significant (Table 6).

Tubular necrosis was observed in the kidney of one rat in the K group. No histopathological changes were detected in the kidney tissue of the remaining rats in the K group (Figure 3 A, F). In the P group, tubular necrosis was observed in two rats, with glomerular damage in one and tubulointerstitial inflammation in another; however, no nephrotoxicity was identified in these rats (Figure 3 B, G). Since both tubular necrosis and tubulointerstitial inflammation were observed in only one rat in the P group, mild nephrotoxicity was determined as a result of scoring (Table 7).

Two of the rats in DM group exhibited moderate nephrotoxicity, while the remaining 6 rats showed mild nephrotoxicity. As a result of scoring, no nephrotoxicity was observed in one rat in the DmP group, moderate nephrotoxicity was identified in one rat and mild nephrotoxicity was observed in the remaining 6 rats. In the PmD group, mild nephrotoxicity was observed in seven rats, with no nephrotoxicity detected in the remaining rat (Table 7).

Upon examination of the kidney sections of group Dm were examined, areas of inflammation, impaired glomerular membrane structures, enlarged Bowman's space, vacuolisation and tubular dilatation, tubular epithelial shedding and brushy edge loss were identified (Figure 3 C, H). In the kidney sections of group DmP, it was found that glomerular structures were preserved, but some tubules were damaged and pignotic nuclei were formed (Figure 3 D, I). In the kidney sections of group PmD, although glomerular and tubule structures were more smooth, tubular dilatation areas and pignotic nuclei were still observed (Figure 3 E, J). When the thickness of Bowman's capsule basement membrane was evaluated semiquantitatively in the kidney sections of the diabetic groups, an increase in thickness was noted. In the diabetic groups (DmP, PmD) given probiotic supplementation, it was observed that the basement membrane thickening was less than in group Dm (Figure 3 H-J).

Table 6. Mean serum creatinine. urea and BUN values of the groups (mg/dL)

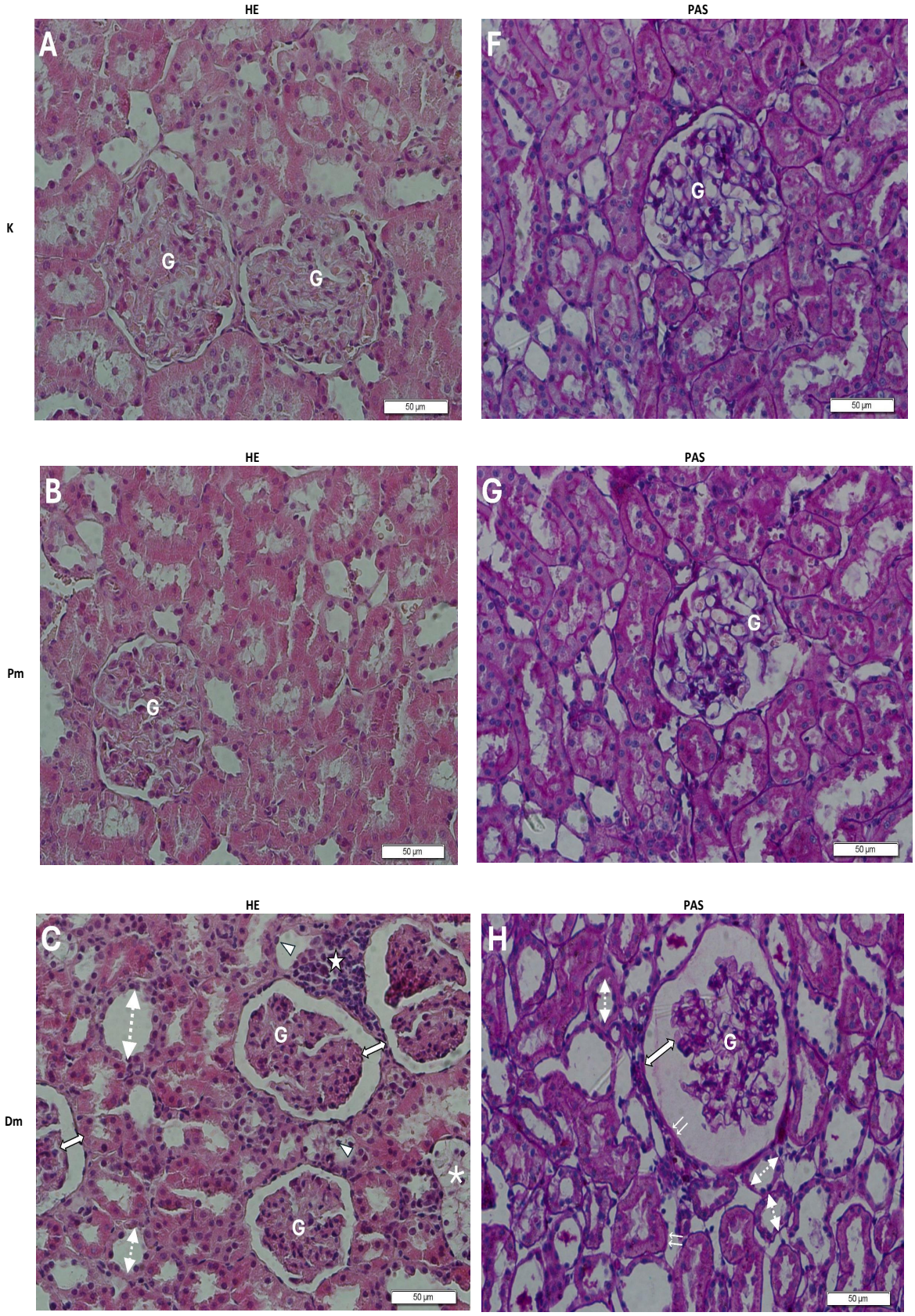
	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
Urea	47.2±5.17	54±16.81	94.63±16.74 k*p*. p*p*	71.13±1.73 d*p*	77.88±5.99 k*p*	0.0001* kw=23.631
BUN	22.06±2.38	25.26±7.8	44.15±7.93 k*p*. p*p*	33.74±1.94	34.8±2.6	0.0001* kw =22.381
Creatinine	0.36±0.03	0.34±0.03	0.38±0.05	0.39±0.06	0.44±0.07 p*p*	0.028* kw=10.87

k*p*=(*p*<0.05) Statistically significant with the control group (Group K), p*p*=(*p*<0.05) Statistically significant with the probiotic group (Group Pm) d*p*=(*p*<0.05) Statistically significant with diabetes group (Group Dm)

Table 7. Histopathological evaluation of kidneys

Animal No	Glomerular Damage	Tubular Necrosis	Tubulointerstitial Inflammation	Total Score	Scoring Scale
K1	0	0	0	0	A
K2	0	1	0	1	A
K3	0	0	0	0	A
K4	0	0	0	0	A
K5	0	0	0	0	A
Pm1	0	1	0	1	A
Pm2	0	1	0	1	A
Pm3	1	0	0	1	A
Pm4	0	0	1	1	A
Pm5	0	1	1	2	B
Dm1	1	2	1	4	B
Dm2	2	1	1	4	B
Dm3	2	2	1	5	C
Dm4	1	2	1	4	B
Dm5	2	1	1	4	B
Dm6	2	1	1	4	B
Dm7	2	2	0	4	B
Dm8	2	2	1	5	C
DmP1	1	1	0	2	B
DmP2	1	1	0	2	B
DmP3	0	1	1	2	B
DmP4	2	2	1	5	C
DmP5	0	1	1	2	B
DmP6	2	0	1	3	B
DmP7	1	1	0	2	B
DmP8	0	0	1	1	A
PmD1	0	1	1	2	B
PmD2	0	1	1	2	B
PmD3	1	1	0	2	B
PmD4	1	0	1	2	B
PmD5	0	1	0	1	A
PmD6	1	0	1	2	B
PmD7	0	1	1	2	B
PmD8	1	1	0	2	B

(A) no nephrotoxicity: 0-1. (B) mild nephrotoxicity: 2-4. (C) moderate nephrotoxicity: 5-7. (D) severe nephrotoxicity: 8-10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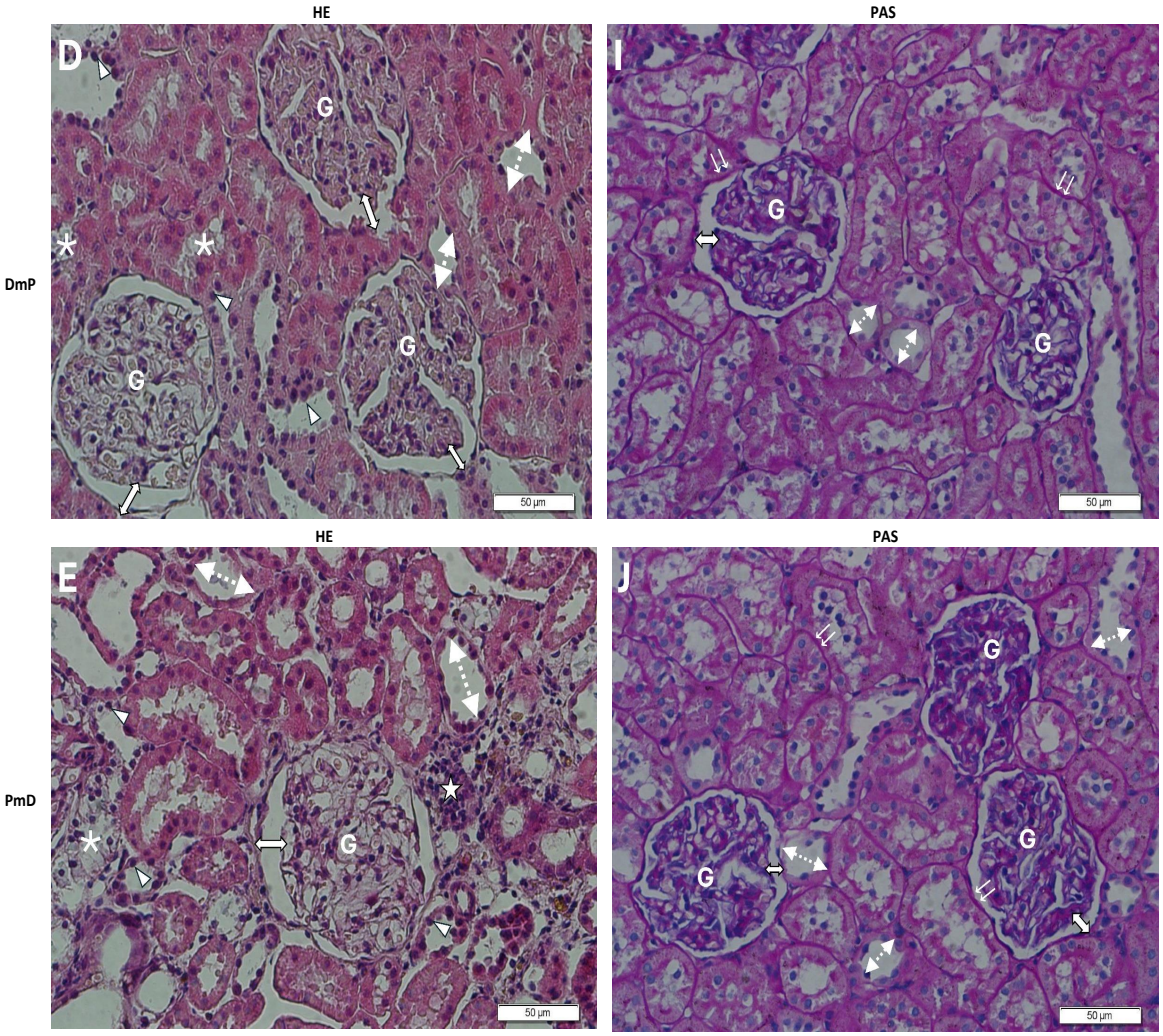
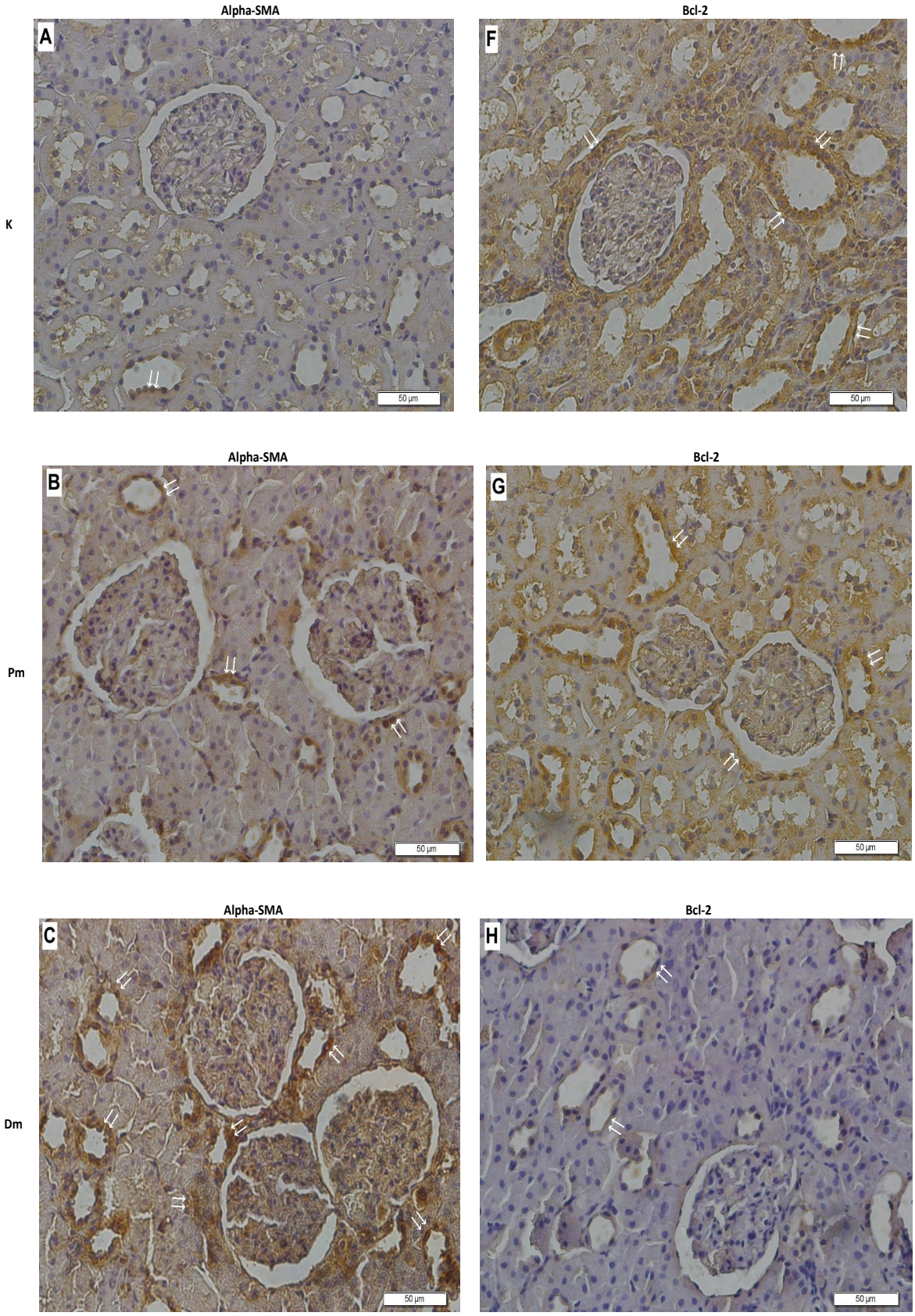


Figure 3. Glomerular structures (G) and tubules appear normal in tissues of K(A, F) and Pm(B, G) groups. Infiltration areas (star), pignotic nuclei (triangle), tubular dilatation areas (dashed two-way arrow), vacuolisation of tubule cells (asterix sign) and enlargement of Bowman's space (two-way arrow) are prominent in the renal tissue of the DM(C, H) group. In DmP(D, I) and PmD(E, J) groups, kidney tissue was less damaged. Thickening of the Bowman capsule and tubule basement membrane (double arrow) [HE staining (A-E); PAS staining (F-J) 40X]

When the kidney sections of the experimental groups were treated with Bcl-2 antibody, an anti-apoptotic marker, a 2.7% decrease in Bcl-2 expression was observed in the kidney sections of the Dm group. It was determined that Bcl-2 expression of DmP (6.2%) and PmD (8.6%), which were among the groups receiving probiotic supplements, increased compared to group Dm and showed an effect against diabetes. It was concluded that Bcl-2 expression percentages of group K and group Pm (respectively 14.3%,10.5%) were higher than the groups in which diabetes was induced (Figure 4). However, when the expression percentages were compared across the groups, it was found that it was not statistically significant (p=0.075)

Immunohistochemical evaluation of the kidney sections from our experimental groups, using alpha-SMA as a marker for myofibroblasts, revealed lower alpha-SMA expression in the kidney sections of group K (3.9%) and group Pm (8.2%) compared to group DM (13.3%). Among the diabetes groups receiving probiotic supplements, DmP (8%) and PmD (10.3%) exhibited lower alpha-SMA expression compared to group DM (Figure 4). However, no statistically significant differences were observed in the expression percentages between the groups (p=0.285).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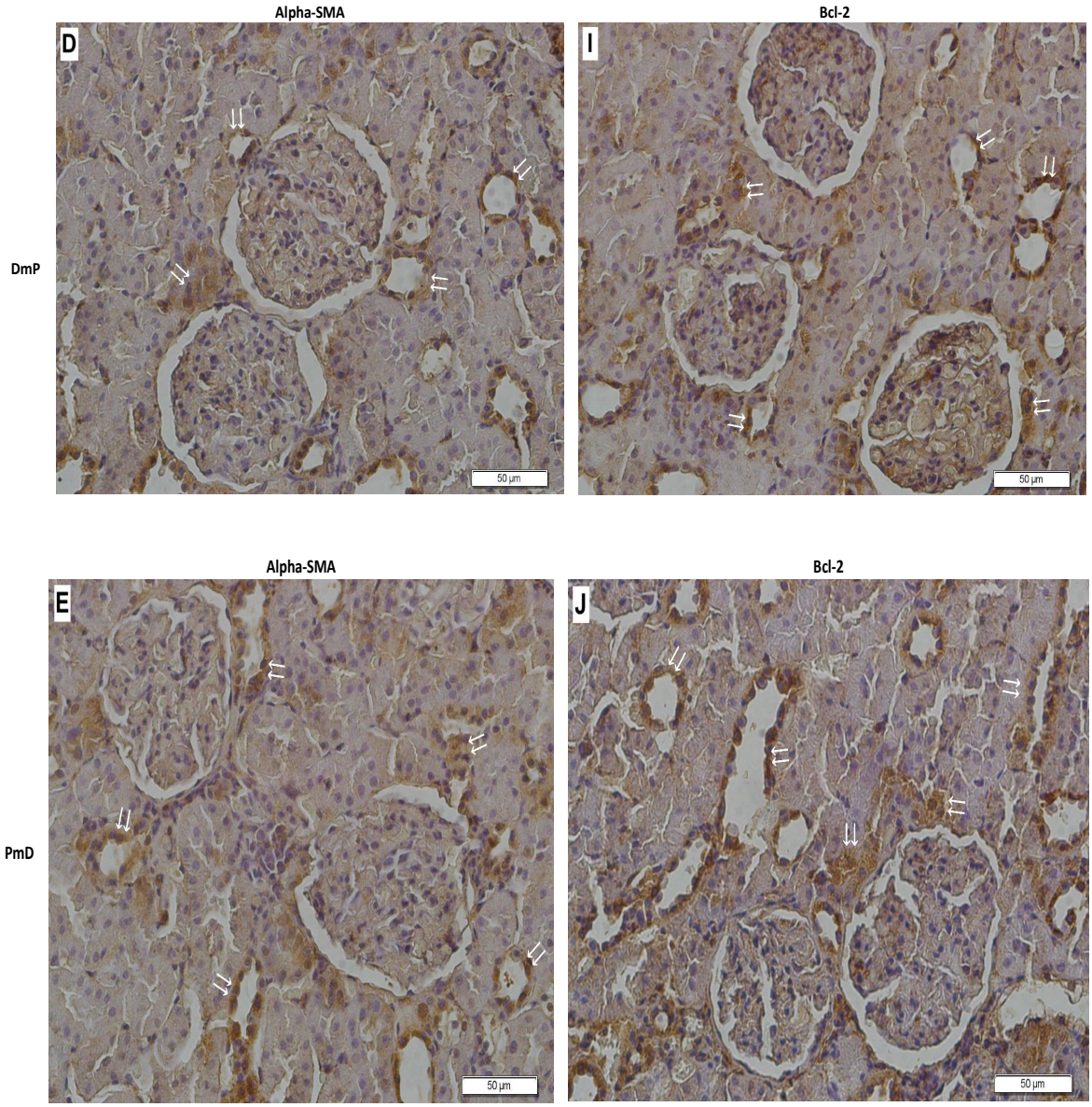

Figure 4. Immunohistochemical alpha-SMA expression was highest in DM group (C) and lowest in K (A) and Pm groups (B). Alpha-SMA expression was decreased in DmP (D) and PmD groups (E) compared to Dm group. Bcl-2 expression is highest in K (F) and P groups (G) and lowest in DM group (H). Bcl-2 expression was increased in DmP (I) and PmD groups (J) compared to Dm group (40x)

Discussion

Probiotics are known to regulate the intestinal microbiota, thus preventing or reducing inflammation by enhancing immune function. Additionally, probiotics are suggested to be effective in lowering blood sugar levels by reducing insulin resistance. It is emphasized in studies that probiotics may be an effective method in the prevention or treatment of Type 2 diabetes [16, 17]. Research has demonstrated that, in diabetes, the levels of *Lactobacillus* and *Bifidobacteria* in the intestinal microbiota decrease, while *Enterococci*, which are associated with increased insulin resistance, rise [18].

One study found that supplementation with *Lactobacillus plantarum* reduced food intake and blood glucose levels in a Type 2 diabetes model in mice [19]. Another study reported that *Lactobacillus gasseri*, derived from human

breast milk, did not result in weight loss in mice with Type 2 diabetes [20]. Additionally, some studies have found that certain bacterial strains cause weight gain, while others lead to weight loss [21]. VSL#3 probiotic supplementation has been shown to increase total fat and visceral fat [22].

The studies show that the use of probiotics containing a combination of several strains is more effective than the use of single-strain probiotics. For example, a 12-week study demonstrated that body weight decreased in individuals as a result of the use of probiotic supplements containing different strains [23]. Jones et al. [22] reported that VSL#3 probiotic supplementation caused an increase in total adiposity and trunk adiposity in subjects. In our study, the mean body weight of group Pm, which was given only probiotic, increased less compared to the control group. This result is in parallel with various studies [21, 23].

In our study, when the average weights of the groups Dm, DmP and PmD were compare during the 4-week diabetes process, weight reductions were determined despite no statistically significant differences were found. No specific effect of probiotic supplementation on weight gain or loss was determined. However, it was observed that the average body weight of the DmP group was higher than that of the Dm and PmD groups, though this difference was not statistically significant. Based on this result, we suggest that probiotic supplementation may reduce the rate of weight loss typically observed in diabetic patients.

It was reported that kidney weights increased in the diabetic mouse model in which probiotic supplement obtained from camel milk was given [24]. In a study, when the damage caused by cisplatin in kidney tissue was evaluated in rats given probiotic supplementation at different doses, it was concluded that the ratio of kidneys to body weight was close to the control in the group given low dose probiotic supplementation, but this ratio was higher in the group receiving high dose probiotic supplementation [25]. In our study, it was observed that the kidney weights of group Pm given probiotic supplementation were higher, although not significantly. However, probiotic supplementation did not have a significant effect on kidney weight changes in the diabetic groups.

It is thought that probiotics may regulate glycemic control and inflammatory response of probiotics [26]. In different studies conducted in humans, it has been reported that probiotic supplementation decreases fasting blood glucose and insulin resistance [16, 27-30]. In animal models of diabetes, it was also reported that blood glucose level decreased in the groups given probiotic supplementation, with a more pronounced effect observed in those receiving higher doses of probiotics [19, 31, 32]. In our study, there was no statistical significance when the blood glucose levels of the diabetes groups were compared. However, blood glucose levels in the diabetic groups receiving probiotic supplementation were lower than those in the diabetes group, though the difference was not statistically significant. This shows that probiotic supplementation may reduce blood glucose level in experimental diabetes model.

Diabetes in mice is known to cause an increase in serum creatinine values [33]. It was found that creatinine and urea concentrations increased in rats with diabetes, but creatinine and urea values decreased significantly with probiotic food supplementation, approaching control levels [34]. Another study reported no difference in creatinine values between the diabetes group and the group induced with diabetes and supplemented with kefir; however, a decrease in serum urea concentration was observed [35]. In our study, no statistically significant increase in mean serum creatinine concentrations was observed when comparing the control group to the diabetes-induced groups. However, the creatinine value in the PmD group was significantly higher than that of the Pm group. The group with the highest mean serum urea concentration was DM. The mean serum urea concentration of group DmP and PmD, to which we gave probiotic supplementation, was lower than group Dm. Notably, the serum urea level in the DmP group was statistically lower than that in the DM group.

The mean BUN concentration was higher in the groups in which diabetes was induced. The BUN level in the Dm group was statistically higher than in both the K and Pm groups. Although there was no statistically significant difference when the mean BUN concentrations of the diabetic groups were evaluated, we found that the BUN values of the probiotic

supplemented groups DmP and PmD were lower compared to group Dm. These findings suggest that probiotic supplementation in diabetes may have a positive effect on serum BUN, urea and creatinine levels.

Various studies have demonstrated that histopathological findings such as glomerular membrane thickening, a marked increase in Bowman's interval, tubular atrophy, tubular dilatation, tubular epithelial vacuolization, epithelial shedding, brush-like edge loss, inflammation areas and necrosis-like damage are present in experimental diabetes models [31-33, 36, 37]. In our study, tubular atrophy and dilatation, vacuolization of cells, pycnotic nuclei, epithelial shedding, thickening of the basement membrane, increase in Bowman interval and areas of inflammation were observed in diabetic kidney tissues. When evaluated semi-quantitatively, an increase in the thickness of the basement membrane of Bowman's capsule was observed in the kidneys of the diabetic groups. However, it was observed that basement membrane thickening was less in the diabetic groups given probiotic supplements. In addition, histopathological findings caused by diabetes were found to be less in the groups given probiotic supplementation. There are studies indicating that probiotic supplementation [31] and kefir supplementation [35] have ameliorative effects against diabetes damage. However, histopathological findings were found to persist in the kidney tissue of diabetic rats given probiotic yoghurt supplementation for 7 days [32].

There are studies reporting that Bcl-2 expression, which is a regulator of apoptosis, decreased in diabetic kidneys [25, 38, 39] and increased with probiotic supplementation [25]. In this study, it was observed that Bcl-2 expression decreased in diabetic kidneys and Bcl-2 expression increased in the diabetic groups (DmP and PmD) given probiotic supplementation as a result of the evaluations made with the Image-J program compared to the diabetes group. Bcl-2 expression was observed to be higher especially in distal tubules.

It was found that alpha-SMA expression in kidney tissues increased with the progression of diabetic nephropathy [40]. Additionally, a study reported that the expression of alpha-SMA in renal arteriole walls decreased in rats given probiotic supplementation for 5 weeks against renal damage caused by hyperuricemia induced by oxonic acid [41]. In our study, it was found that alpha-SMA expression increased with diabetes in kidney tissue. In parallel with other studies [24, 41], it was observed that alpha-SMA expression decreased in diabetic groups receiving probiotic supplementation.

In conclusion, when DM, DMP and PmD groups were compared with K and Pm groups, kidney damage occurred in diabetic groups. It was observed that this damage decreased in diabetic groups receiving probiotics. In this respect, it was determined that probiotic supplementation may have both protective and therapeutic aspects. Nevertheless, we think that more detailed and long-term studies on this subject will be important in clarifying the issue.

Limitations of the study: More experimental data and molecular research are needed for the data to be applicable.

Financial support: This study was supported by Pamukkale University Scientific Research Projects Coordination Office (BAP Project no: 2021SABE018).

Authors contributions: Conceptualization: H.C., S.O., Literature Review: H.C., I.L., S.O., S.T., Design: H.C., S.T., Data Collection: H.C., I.L., S.T., Analysis and Interpretation: H.C., S.O., I.L., B.K.V., Manuscript Writing: H.C, I.L, Critical Review: H.C., S.O.

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

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2013;36 Suppl 1(Suppl 1):S67-S74. doi:10.2337/dc13-S067
2. World Health Organization Available at: https://www.who.int/data/gho/publications/world-health-statistics. Accessed 25.06.2024
3. Me TA. Hastalıklarda beslenme tedavisi. *İstanbul: Hatipoğlu Matbaacılık*; 2013;1:277-304.
4. Kanchan G, Dagle P, Pushpalata C, Joshi Y, Kadam V. A review on some nephroprotective medicinal plants. *International Journal of Pharmaceutical Sciences and Research*. 2012;2012(8):2451. doi:10.13040/ijpsr.0975-8232.3(8).2451-54

5. Lu L, Zhong Z, Gu J, Nan K, Zhu M, Miao C. ets1 associates with KMT5A to participate in high glucose-mediated EndMT via upregulation of PFN2 expression in diabetic nephropathy. *Mol Med*. 2021;27(1):74. Published 2021 Jul 8. doi:10.1186/s10020-021-00339-7
6. American Diabetes Association. Diabetes and employment. *Diabetes Care*. 2009;32 Suppl 1(Suppl 1):S80-S84. doi:10.2337/dc09-S080
7. Horowitz M, Harding PE, Maddox AF, et al. Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1989;32(3):151-159. doi:10.1007/BF00265086
8. Azpiroz F, Malagelada C. Diabetic neuropathy in the gut: pathogenesis and diagnosis. *Diabetologia*. 2016;59(3):404-408. doi:10.1007/s00125-015-3831-1
9. Wu SL. Staging of type 2 diabetes mellitus. *Genet Mol Res*. 2015;14(1):2118-2121. Published 2015 Mar 20. doi:10.4238/2015.March.20.22
10. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490(7418):55-60. doi:10.1038/nature11450
11. Kobyliak N, Conte C, Cammarota G, et al. Probiotics in prevention and treatment of obesity: a critical view. *Nutr Metab (Lond)*. 2016;13:14. Published 2016 Feb 20. doi:10.1186/s12986-016-0067-0
12. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury?. *Kidney Int*. 1999;56(5):1627-1637. doi:10.1046/j.1523-1755.1999.00721.x
13. Shi R, Ye J, Fan H, et al. *Lactobacillus plantarum LLY-606* supplementation ameliorates hyperuricemia *via* modulating intestinal homeostasis and relieving inflammation. *Food Funct*. 2023;14(12):5663-5677. Published 2023 Jun 19. doi:10.1039/d2fo03411e
14. Sang LX, Chang B, Wang BY, Liu WX, Jiang M. Live and heat-killed probiotic: effects on chronic experimental colitis induced by dextran sulfate sodium (DSS) in rats. *Int J Clin Exp Med*. 2015;8(11):20072-20078. Published 2015 Nov 15.
15. Al Shabanah OA, Aleisa AM, Al Yahya AA, et al. Increased urinary losses of carnitine and decreased intramitochondrial coenzyme A in gentamicin-induced acute renal failure in rats. *Nephrol Dial Transplant*. 2010;25(1):69-76. doi:10.1093/ndt/gfp457
16. Ruan Y, Sun J, He J, Chen F, Chen R, Chen H. Effect of Probiotics on Glycemic Control: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *PLoS One*. 2015;10(7):e0132121. Published 2015 Jul 10. doi:10.1371/journal.pone.0132121
17. Kamarlı H. Pre- Probiyotikler ve Diyabet. Bes Diy Derg [Internet]. 2019;47:92-101. Erişim adresi: https://beslenmevediyetdergisi.org/index.php/bdd/article/view/1320. Erişim tarihi 22 Ocak 2025
18. Cai X, Wang L, Wang X, Liu S. Effect of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients. *Biomed. Res*. 2017;28:9371-9375.
19. Li X, Wang N, Yin B, et al. Effects of Lactobacillus plantarum CCFM0236 on hyperglycaemia and insulin resistance in high-fat and streptozotocin-induced type 2 diabetic mice. *J Appl Microbiol*. 2016;121(6):1727-1736. doi:10.1111/jam.13276
20. Yun SI, Park HO, Kang JH. Effect of Lactobacillus gasseri BNR17 on blood glucose levels and body weight in a mouse model of type 2 diabetes. *J Appl Microbiol*. 2009;107(5):1681-1686. doi:10.1111/j.1365-2672.2009.04350.x
21. Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D. Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. *Microb Pathog*. 2012;53(2):100-108. doi:10.1016/j.micpath.2012.05.007
22. Jones RB, Alderete TL, Martin AA, et al. Probiotic supplementation increases obesity with no detectable effects on liver fat or gut microbiota in obese Hispanic adolescents: a 16-week, randomized, placebo-controlled trial. *Pediatr Obes*. 2018;13(11):705-714. doi:10.1111/ijpo.12273
23. Sudha MR, Ahire JJ, Jayanthi N, Tripathi A, Nanal S. Effect of multi-strain probiotic (UB0316) in weight management in overweight/obese adults: a 12-week double blind, randomised, placebo-controlled study. *Benef Microbes*. 2019;10(8):855-866. doi:10.3920/BM2019.0052
24. Lu L, Zhong Z, Gu J, Nan K, Zhu M, Miao C. ets1 associates with KMT5A to participate in high glucose-mediated EndMT via upregulation of PFN2 expression in diabetic nephropathy. *Mol Med*. 2021;27(1):74. Published 2021 Jul 8. doi:10.1186/s10020-021-00339-7
25. Sengul E, Gelen SU, Yıldırım S, Çelebi F, Çınar A. Probiotic bacteria attenuates cisplatin-induced nephrotoxicity through modulation of oxidative stress, inflammation and apoptosis in rats. *Asian Pacific Journal of Tropical Biomedicine*. 2019;9(3):116-122.
26. Miraghajani M, Zaghian N, Mirlohi M, Feizi A, Ghiasvand R. The Impact of Probiotic Soy Milk Consumption on Oxidative Stress Among Type 2 Diabetic Kidney Disease Patients: A Randomized Controlled Clinical Trial. *J Ren Nutr*. 2017;27(5):317-324. doi:10.1053/j.jrn.2017.04.004
27. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition*. 2012;28(5):539-543. doi:10.1016/j.nut.2011.08.013

28. Ostadrahimi A, Taghizadeh A, Mobasseri M, et al. Effect of probiotic fermented milk (kefir) on glycemic control and lipid profile in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Iran J Public Health*. 2015;44(2):228-237.

29. Kobyliak N, Falalyeyeva T, Mykhalchyshyn G, Kyriienko D, Komissarenko I. Effect of alive probiotic on insulin resistance in type 2 diabetes patients: Randomized clinical trial. *Diabetes Metab Syndr*. 2018;12(5):617-624. doi:10.1016/j.dsx.2018.04.015

30. Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M, Yousefinejad A. The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo controlled trial. *Diabetes Metab Syndr*. 2019;13(1):175-182. doi:10.1016/j.dsx.2018.08.008

31. Wang Y, Dilidaxi D, Wu Y, Sailike J, Sun X, Nabi XH. Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice. *Biomed Pharmacother*. 2020;125:109914. doi:10.1016/j.biopha.2020.109914

32. Khalil NA, Eltahan NR, Elaktash HM, Aly S, Sarbini SR. Prospective evaluation of probiotic and prebiotic supplementation on diabetic health associated with gut microbiota. *Food Bioscience*. 2021;42:101149.

33. Glastras SJ, Chen H, Teh R, et al. Mouse Models of Diabetes, Obesity and Related Kidney Disease. *PLoS One*. 2016;11(8):e0162131. Published 2016 Aug 31. doi:10.1371/journal.pone.0162131

34. Negm El Dein A, Ezzat A, Aly HF, Awad G, Farid M. Lactobacillus-fermented yogurt exerts hypoglycemic, hypocholesterolemic, and anti-inflammatory activities in STZ-induced diabetic Wistar rats. *Nutr Res*. 2022;108:22-32. doi:10.1016/j.nutres.2022.10.003

35. Punaro GR, Maciel FR, Rodrigues AM, et al. Kefir administration reduced progression of renal injury in STZ-diabetic rats by lowering oxidative stress. *Nitric Oxide*. 2014;37:53-60. doi:10.1016/j.niox.2013.12.012

36. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury?. *Kidney Int*. 1999;56(5):1627-1637. doi:10.1046/j.1523-1755.1999.00721.x

37. Vardı N, Iraz M, Öztürk F, Uçar M, Gül M, Eşrefoğlu M, Otlu A. Deneysel Diyabetin Sıçan Böbreklerinde Meydana Getirdiği Histolojik Değişiklikler Üzerine Melatoninin İyileştirici Etkileri. *J Turgut Ozal Med Cent.* Haziran 2005;12(3):145-152.

38. Kikuchi Y, Nonoguchi H, Machida K, Wakamatsu S, Koga H, Tomita K. Regulation of the apoptosis-related genes, Bax and Bcl-2, in the early stage of diabetes mellitus. *Nephrology*. 2002;7(6):294-302.

39. Li A, Yi B, Han H, et al. Vitamin D-VDR (vitamin D receptor) regulates defective autophagy in renal tubular epithelial cell in streptozotocin-induced diabetic mice via the AMPK pathway. *Autophagy*. 2022;18(4):877-890. doi:10.1080/15548627.2021.1962681

40. Manaer T, Yu L, Nabi XH, Dilidaxi D, Liu L, Sailike J. The beneficial effects of the composite probiotics from camel milk on glucose and lipid metabolism, liver and renal function and gut microbiota in db/db mice. *BMC Complement Med Ther*. 2021;21(1):127. Published 2021 Apr 22. doi:10.1186/s12906-021-03303-4

41. García Arroyo FE, Gonzaga G, Muñoz Jiménez I, et al. Probiotic supplements prevented oxonic acid-induced hyperuricemia and renal damage. *PLoS One*. 2018;13(8):e0202901. Published 2018 Aug 24. doi:10.1371/journal.pone.0202901

Research Article
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1528303

Application of machine learning techniques for survival prediction in pediatric malignant non-seminomatous germ cell testicular tumors: a SEER database study

Pediatrik malign non-seminamatöz germ hücreli testis tümörlerinde sağkalım tahmini için makine öğrenme tekniklerinin uygulanması: bir SEER veritabanı çalışması

İncinur Genişol, Batuhan Bakırarar

Posted date:05.08.2024 Acceptance date:03.10.2024

Abstract

Purpose: Childhood testicular cancers constitute 1-2% of all childhood tumors. According to the Surveillance, Epidemiology, and End Results (SEER) database, based on data from 2013 to 2019, the 5-year survival rate is 95.2%. The second most common type of testicular tumor is malignant non-seminomatous germ cell tumor. In recent years, various statistical techniques and extensive databases have been used to obtain information on disease prognosis and survival. In this study, we aimed to develop software using artificial intelligence and machine learning techniques to accurately predict the overall survival of patients with malignant nonseminomatous germ cell testicular tumors.

Materials and methods: Our study included data from 788 patients aged 0-18 diagnosed with malignant nonseminomatous germ cell testicular cancer between January 1975 and December 2019. The main hypothesis of the study was to provide overall survival (OS) in years from the date of diagnosis to the date of death or the last follow-up date for surviving patients. In addition to survival analysis, we also analyzed patient age at diagnosis, race, laterality, year of diagnosis, tumor histological type, T stage, N stage, M stage, tumor size, mortality, and follow-up duration.

Results: The OS was found to be 41.29±0.43 years. The median survival time was 43.21±0.62 years for patients <15 and 40.34±0.52 years for patients aged ≥15. We developed software that enabled the provision of patient-specific survival in addition to OS for all patients.

Conclusion: Recently, artificial intelligence techniques such as machine learning, have shown remarkable advancements compared to other statistical methods. As a result, in this study, we found that the survival rate in pediatric NSCGT was higher if the tumor was diagnosed after 2000, was less than 2 cm in size, and had a T1M0N0 stage yolk sac tumor. We created a 10-year survival prediction model with the results and thought that this model would contribute to the advancement of artificial intelligence studies in prognosis, recurrence and survival analysis.

Keywords: Non-seminomatous germ cell tumors, tescicular tumors, SEER, machine learning, survival.

Genisol I, Bakirarar B. Application of machine learning techniques for survival prediction in pediatric malignant non-seminomatous germ cell testicular tumors: a SEER database study. Pam Med J 2025;18:358-367.

Öz

Amaç: Çocukluk çağı testis kanserleri, tüm çocukluk çağı tümörlerinin %1-2'sini oluşturur. 2013-2019 yılları arasındaki verilere dayanan Surveillance, Epidemiology, and End Results (SEER) veritabanına göre, 5 yıllık sağkalım oranı %95,2 olarak görülmüştür. İkinci en yaygın testis tümörü türü ise malign non-seminomatöz germ hücreli tümördür. Son yıllarda, hastalığın prognozu ve sağkalımı hakkında bilgi elde etmek için çeşitli istatistiksel teknikler ve geniş veritabanları kullanılmaktadır. Bu çalışmada, malign non-seminomatous germ hücreli testis tümörü olan hastaların genel sağkalımını doğru bir şekilde tahmin etmek için yapay zeka ve makine öğrenimi tekniklerini kullanarak yazılım geliştirmeyi amaçladık.

Gereç ve yöntem: Çalışmamız, Ocak 1975 ile Aralık 2019 tarihleri arasında malign non-seminomatous germ hücreli testis kanseri tanısı konmuş 0-18 yaş arası 788 hastanın verilerini içermektedir. Çalışmanın temel hipotezi, tanı tarihinden ölüm tarihi veya sağ kalan hastalar için son takip tarihi itibarıyla genel sağkalımı yıllar olarak gösterebilmekti. Sağkalım yanı sıra, tanı anındaki hasta yaşı, ırk, tümörün yerleşim yeri, tanı yılı, tümör histolojik tipi, T evresi, N evresi, M evresi, tümör büyüklüğü, mortalite ve takip süresi gibi faktörleri de analiz ettik.

Bulgular: Genel sağkalım 41,29±0,43 yıl olarak bulundu. Medyan sağkalım süresi, 15 yaşından küçük hastalar için 43,21±0,62 yıl ve 15 yaş ve üzeri hastalar için 40,34±0,52 yıl olarak bulundu. Tüm hastalar için genel sağkalıma ek olarak, hastaya özgü sağkalım sağlayan bir yazılım geliştirdik.

Sonuç: Son zamanlarda, makine öğrenimi gibi yapay zeka teknikleri, diğer istatistiksel yöntemlere kıyasla dikkate değer ilerlemeler göstermiştir.

İncinur Genişol, M.D. Pediatric Surgeon, Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, İzmir, Türkiye, e-mail: incinurgenisol@msn.com (https://orcid.org/0000-0003-3843-5942) (Corresponding Author)

Batuhan Bakırarar, Assoc. Prof. Ankara University Faculty of Medicine Department of Biostatistics, Ankara, Türkiye, e-mail: batuhan_bakirarar@hotmail.com (https://orcid.org/0000-0002-5662-8193)

Sonuç olarak bu çalışmamızda pediatrik NSCGT'de tümörün 2000 yılından sonra teşhis edilmesi, boyutunun 2 cm. den küçük olması, T1M0N0 evre yolk sak tümör olmasının hayatta kalma oranının daha yüksek olduğunu saptadık. Sonuçlarla 10 yıllık sağ kalma tahmin modeli oluşturduk ve bu modelin prognoz, nüks ve hayatta kalma analizinde yapay zeka çalışmalarının ilerlemesine katkı sağlayacagını düşündük.

Anahtar kelimeler: Non-seminomatöz germ hücreli tümör, testis tümörü, SEER, machine learning, sağkalım.

Genişol I, Bakırarar B. Pediatrik malign non-seminamatöz germ hücreli testis tümörlerinde sağkalım tahmini için makine öğrenme tekniklerinin uygulanması: bir SEER veritabanı çalışması. Pam Tıp Derg 2025;18:358-367.

Introduction

Testicular cancer has an incidence of 5.5/100,000 in males under the age of 19 years according to the Surveillance, Epidemiology, and End Results (SEER) database from 2015 to 2019 in the United States [1]. The prevalence of testicular tumors in childhood is estimated at 1-2% [2]. Testicular cancer accounts for 0.5% of all new cancer cases in the U.S. and ranks 25th in cancer incidence. According to the American Cancer Society's 2022 data, 9,190 new cases of testicular cancer were diagnosed in America. Testicular cancer accounted for 0.5% of all new cancer cases in the same year. Additionally, 470 men died from testicular cancer in 2022 [3]. According to the Global Cancer Observatory (GCO) 2020 global cancer data, 6,446 new cases were reported in males under the age of 19 years. Additionally, the 5-year overall survival (OS) rate was reported to be 95.2%, based on data from 2013 to 2019 [3, 4]. The American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system is currently used to determine the prognosis of cancer patients. We believe that developing alternative techniques to the TNM staging system could be beneficial for the management and follow-up of complex diseases such as cancer. Knowing the survival outcomes of malignant diseases such as cancer, can assist clinicians in making appropriate treatment decisions and reducing patient anxiety regarding treatment choices [5].

The second most common malignant testicular tumor among testicular cancers is malignant nonseminomatous germ cell tumor (NSGCT) [3]. In this study, we aimed to develop an artificial intelligence program using machine learning techniques to predict the prognosis and survival of children with NSGCTs using the extensive SEER database. The program aims to assist clinicians in making predictions and decisions more easily.

Material and methods

Data collection and study population

These data, published by the National Cancer Institute, are a compilation of testicular cancer data from 18 population-based SEER cancer registries in the United States. The SEER database contains data from approximately 47.9% of the United States population [6]. The SEER program is used to summarize data from patients' medical records, and it is estimated to capture over 95% of all cancer cases in the surveillance areas. The duration of follow-up was determined in months using the date of diagnosis and whichever of the following events occurred first: 1) date of death, 2) date when the patient was last confirmed to be alive, or 3) the designated cutoff date of December 2019, which was employed in our analysis.

Because patient data were obtained from the SEER database with permission and did not include any personal patient information, the requirement for ethical approval was waived.

The SEER website is an American system that collects tumor data from patients without including their names. It is an open-access database that does not require ethical approval. However, researchers are provided with login credentials to access the data. Access permission has been obtained under the name of the author, Batuhan Bakırarar. We are providing the password and the printout via the email address as an attachment.

A total of 788 patients with malignant NSGCT, aged 0-18 years, diagnosed between January 1975 and December 2019, were included in the study using the ICD O3 site codes C62.1 and C62.9. Patients with in situ tumors were excluded from the study. Only patients who underwent surgery and had first and solitary malignant tumors were included in the study. Only patients

who were diagnosed by histopathological examination at the hospital were included in the study, whereas those diagnosed postmortem or through imaging techniques were excluded. Tumors at all locations of the testes (descending, undescending, and unknown) were included in this study. Patients with "unknown" or "missing" causes of death recorded in the database were excluded. Patients with bilateral testicular tumors or unknown tumor involvement were also excluded from the study. Because of the high amount of missing data in the variables related to surgical management, radiation therapy, and chemotherapy techniques, these variables were not included in the study.

The main hypothesis of the study was overall survival (OS), defined as the years from the date of diagnosis until the date of death or last follow-up with surviving patients (censored observations). In addition to survival, the variables selected for analysis included age at diagnosis, race/ethnicity, laterality, year of diagnosis, histological behavior, T, N, and M stages, and tumor size. The patients were divided into two groups based on whether they were diagnosed before or after 2000, with approximately half of the patients in each group. Testicular malignancies are most commonly observed between 15 and 40 years of age [7] and the incidence of extracranial germ cell tumors increases after 15 years of age [2] Therefore, we divided the patients into two groups: those aged <15 years and those aged ≥15 years.

Statistical analysis

The data analysis was performed using SPSS Statistics version 11.5 and R version 4.0.2. Descriptive statistics such as mean±standard deviation and median (minimum-maximum), were used for quantitative variables, while the number of patients (percentage) was used for qualitative variables. To examine the relationship between two qualitative variables, the chi-square test and Fisher's exact test were used. Survival analyses of qualitative variables were conducted using the Kaplan-Meier method, and significant differences between groups were determined using the log-rank test. Statistical significance was set at $p<0.05$. The classification methods used in this study include logistic regression, multilayer perceptron, support vector machine, bagging, and decision tables. The dataset was assessed using 10-fold cross-validation. The accuracy, F1 score, Matthew's Correlation Coefficient (MCC), Precision-Recall Curve (PRC Area), and ROC Area were employed as performance metrics for data mining. The RWeka package in R was used for analysis.

Results

Table 1 presents the demographic data and other descriptive patient and tumor characteristics of 788 patients aged 0-18 from the SEER database. In this study, 396 (50.3%) patients were diagnosed with testicular cancer before 2000, and 392 (49.7%) patients were diagnosed after 2000. Among the patients included in the study, 22.6% were < 15 years of age and 77.4% were ≥15 years of age. Among the patients, 89.0% were White, 2.2% were Black, and 8.8% had other ethnic backgrounds. Of these patients, 48.7% had tumors on the left side and 51.3% had tumors on the right side. Table 1 presents the tumor histologic behavior groups and the TNM stages. Tumor size, mortality status, and follow-up duration are provided in Table 1.

Table 2 presents the comparisons between mortality and other variables. The mortality rate was 3.4% among children younger than 15 years and 8.7% among children aged 15 years and older ($p=0.018$). Among children diagnosed before 2000, 11.1% died, whereas among those diagnosed in or after 2000, 3.8% died ($p<0.001$). The highest mortality rate was observed in children with choriocarcinoma as the histological behavior type, whereas the lowest mortality rate was observed in children with yolk sac tumors ($p<0.001$). In total, 2.4%, 2.7%, and 14.3% of children with stage T1, T2, and T3 died, respectively ($p=0.047$). The highest mortality rate was observed in children with stage N3 disease, whereas the lowest mortality rate was observed in children with stage N0 disease ($p=0.001$). The mortality rate was significantly higher in children at the M1 stage than in those at the M0 stage ($p<0.001$). The mortality rate increased as tumor size increased ($p<0.001$).

Table 1. Descriptive statistics

Variables		
Age (years), n (%)	<15	178 (22.6)
	≥15	670 (77.4)
Race, n (%)	White	697 (89.0)
	Black	17 (2.2)
	Other	69 (8.8)
Year of Diagnosis, n (%)	<2000	396 (50.3)
	≥2000	392 (49.7)
Laterality, n (%)	Left	384 (48.7)
	Right	404 (51.3)
Histologic Behavior, n (%)	Embryonal Carcinoma	169 (21.4)
	Yolk Sac Tumor	115 (14.6)
	Teratoma	65 (8.3)
	Teratocarcinoma	131 (16.7)
	Mixed Germ Cell Tumor	299 (37.9)
	Choriocarcinoma	9 (1.1)
T Stage, n (%)	T1	166 (55.3)
	T2	113 (37.7)
	T3	21 (7.0)
N Stage, n (%)	N0	221 (72.9)
	N1	38 (12.5)
	N2	28 (9.3)
	N3	16 (5.3)
M stage, n (%)	M0	256 (83.9)
	M1	49 (16.1)
Tumor Size (cm), n (%)	≤2	136 (22.7)
	2-5	299 (49.8)
	>5	165 (27.5)
Mortality, n (%)	Alive	729 (92.5)
	Dead	59 (7.5)
Follow-up Time (year)	Mean±SD	18.43±12.49
	Median (Min.-Max.)	17.50 (0.08-44.75)

SD: Standard Deviation, Min: Minimum, Max: Maximum

Table 2. The relationship between qualitative variables and mortality

Variables		Mortality				*p* value	Test value
		Alive		Dead			
		n	%	n	%		
Age (years)	<15	172	96.6	6	3.4	0.018[a]	5.625
	≥15	557	91.3	53	8.7		
Race	White	642	92.1	55	7.9	0.549[a]	1.199
	Black	16	94.1	1	5.9		
	Other	66	95.7	3	4.3		
Year of Diagnosis	<2000	352	88.9	44	11.1	<0.001[a]	15.092
	≥2000	377	96.2	15	3.8		
Laterality	Left	353	91.9	31	8.1	0.543[a]	0.371
	Right	376	93.1	28	6.9		
Histologic Behavior	Embryonal Carcinoma	159	94.1	10	5.9	<0.001[a]	88.185
	Yolk Sac Tumor	109	94.8	6	5.2		
	Teratoma	59	90.8	6	9.2		
	Teratocarcinoma	122	93.1	9	6.9		
	Mixed Germ Cell Tumor	279	93.3	20	6.7		
	Choriocarcinoma	1	11.1	8	88.9		
T Stage	T1	162	97.6	4	2.4	0.047[b]	-
	T2	110	97.3	3	2.7		
	T3	18	85.7	3	14.3		
N Stage	N0	217	98.2	4	1.8	0.001[b]	-
	N1	35	92.1	3	7.9		
	N2	27	96.4	1	3.6		
	N3	12	75.0	4	25.0		
M Stage	M0	254	99.2	2	0.8	<0.001[b]	-
	M1	39	79.6	10	20.4		
Tumor Size (cm)	≤2	133	97.8	3	2.2	<0.001[a]	20.381
	2-5	291	97.3	8	2.7		
	>5	146	88.5	19	11.5		

a: Chi-square test, b: Fisher-exact test

Table 3 presents the survival analysis results. The mean overall survival (OS) for the patients was 41.29±0.43 years. In addition, age, histologic behavior, T, N, and M stages, and tumor size were significant risk factors (p<0.05). The mean survival time was found to be 43.21±0.62 years for patients aged <15 and 40.34±0.52 years for patients aged ≥15 (p=0.017). The highest mean survival time was found in patients with histologic behavior type yolk sac tumors, whereas the lowest mean survival time was observed in patients with histologic behavior type choriocarcinoma (p<0.001).

The mean survival times for patients with T1, T2, and T3 stages were found to be 15.54±0.19, 15.54±0.22, and 12.00±0.98 years, respectively (p=0.009). When evaluated according to the N stage, the lowest mean survival was observed in patients with N3 stage, whereas in terms of M stage, the lowest mean survival was observed in patients with M1 stage (p<0.001 and p<0.001, respectively). The mean survival for children with tumor size <2 cm was found to be 36.1±0.47 years, while this duration was 35.17±0.35 years for children with tumor size between 2-5 cm and 32.45±0.92 years for children with tumor size >5 cm (p<0.001) (Table 3).

Table 3. Kaplan-Meier analysis results for factors affecting mortality

Variables		Survival					*p* value	Test value
		1 year (%)	5 year (%)	10 year (%)	Survival Time			
					Mean±SE	Median±SE		
Overall Survival (years)		96.9	93.5	93.2	41.29±0.43	-	-	-
Age (years)	<15	98.8	97.0	96.3	43.21±0.62	-	0.017*	5.664[a]
	≥15	97.2	92.5	92.3	40.34±0.52	-		
Race	White	96.8	93.3	92.9	40.72±0.47	-	0.569	1.128[a]
	Black	94.1	94.1	94.1	42.15±2.52	-		
Laterality	Other	98.6	95.3	95.3	40.39±1.05	-	0.498	0.459[a]
	Left	96.0	93.0	92.3	40.12±0.63	-		
	Right	97.7	94.0	94.0	41.55±0.58	-		
Histologic Behavior	Embryonal Carcinoma	97.0	95.7	95.7	41.46±0.80	-	<0.001*	243.187[a]
	Yolk Sac Tumor	98.2	95.4	95.4	41.54±0.91	-		
	Teratoma	95.4	92.0	92.0	40.27±1.76	-		
	Teratocarcinoma	96.9	93.1	93.1	40.44±0.93	-		
	Mixed Germ Cell Tumor	99.0	94.6	93.7	39.31±0.70	-		
	Choriocarcinoma	22.2	11.1	11.1	3.77±2.95	0.58±0.12		
T Stage	T1	99.3	98.0	97.1	15.54±0.19	-	0.009*	9.415[a]
	T2	-	97.8	97.8	15.54±0.22	-		
	T3	89.7	84.4	84.4	12.00±0.98	-		
N Stage	N0	99.1	98.0	98.0	15.46±0.14	-	<0.001*	27.812[a]
	N1	97.1	94.0	94.0	14.90±0.61	-		
	N2	96.3	96.3	96.3	14.96±0.53	-		
	N3	93.3	76.4	61.1	10.03±1.64	-		
M Stage	M0	-	99.6	99.6	15.80±0.08	-	<0.001*	41.253[a]
	M1	89.4	80.1	77.4	12.81±0.87	-		
Tumor Size (cm)	<2	98.5	97.7	97.7	36.10±0.47	-	<0.001*	19.293[a]
	2-5	99.3	97.8	97.4	35.17±0.35	-		
	>5	96.9	91.0	90.1	32.45±0.92	-		

SE: Standard error, a: Log Rank test

The gain ratio attribute (GAR) method was used for variable selection to determine variable importance. This method examines the importance of variables and their contributions to the dataset. Variables deemed insignificant using the gain ratio method and considered to lack clinical relevance were excluded from the analysis. As a result, eight variables (seven independent and one dependent) were retained in the dataset. These variables included histological behavior, M stage, age, tumor size, N stage, race, T stage, and status. The percentage of variable importance with respect to the dependent variable, mortality, is shown in Figure 1.

The performance metrics for the machine learning methods used in the prediction model are presented in Table 4. After evaluating the performance metrics, we determined that logistic regression provided the best predictive model. After logistic regression, the following methods were used: support vector machine, bagging, decision table, and multilayer perceptron.

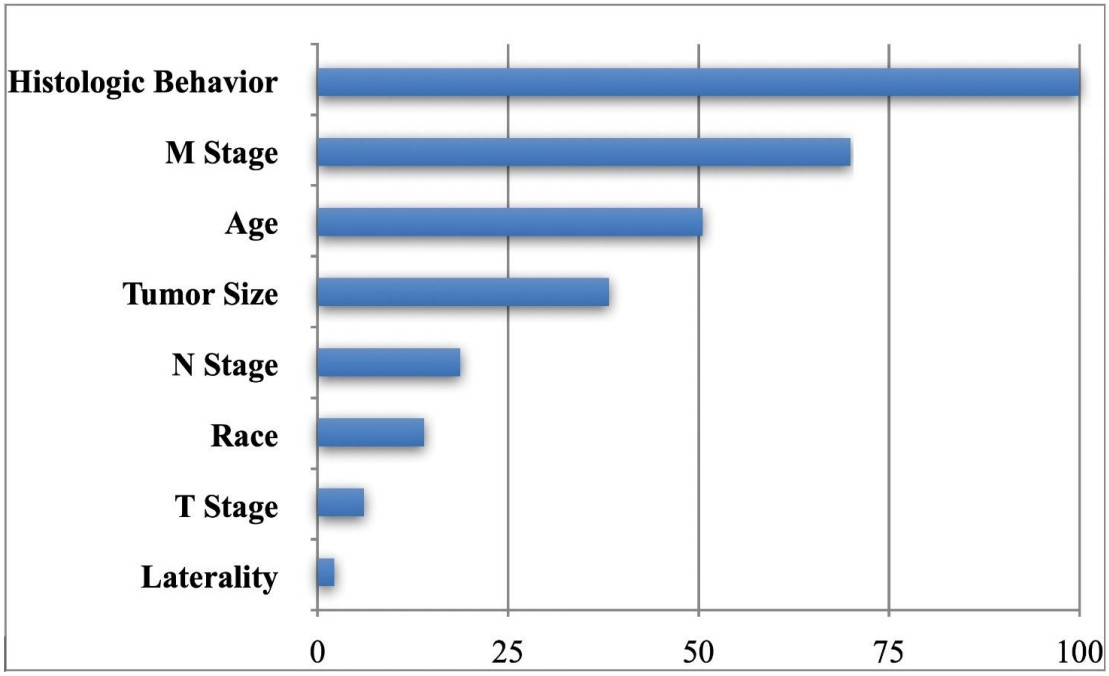

Figure 1. Variable importance for mortality

Table 4. Using machine learning methods for mortality prediction

Methods	Performance Criteria				
	Accuracy	F-measure	MCC	PRC Area	ROC Area
Logistic Regression	0.934	0.911	0.332	0.892	0.647
Multilayer Perceptron	0.926	0.906	0.243	0.755	0.919
Support Vector Machine	0.934	0.911	0.332	0.878	0.567
Bagging	0.934	0.911	0.332	0.862	0.524
Decision Table	0.931	0.907	0.287	0.851	0.420

MCC: Matthew's Correlation Coefficient, PRC Area: Precision-Recall Curve Area, ROC Area: Receiver Operating Characteristic Curve Area

Based on the best-performing machine learning method from Table 4, logistic regression, a decision support system software was developed. The variables included in the decision support system were determined based on various important factors, including histological behavior, M stage, age, tumor size, N stage, race, and T stage. The outputs of the decision support system that calculates survival based on these variables are shown in Figure 2. An overall view of the decision support system is shown in Figure 2(a), a screenshot of the survival prediction for a sample patient input is presented in Figure 2(b), and a screenshot of the mortality prediction for a sample patient input is displayed in Figure 2(c).

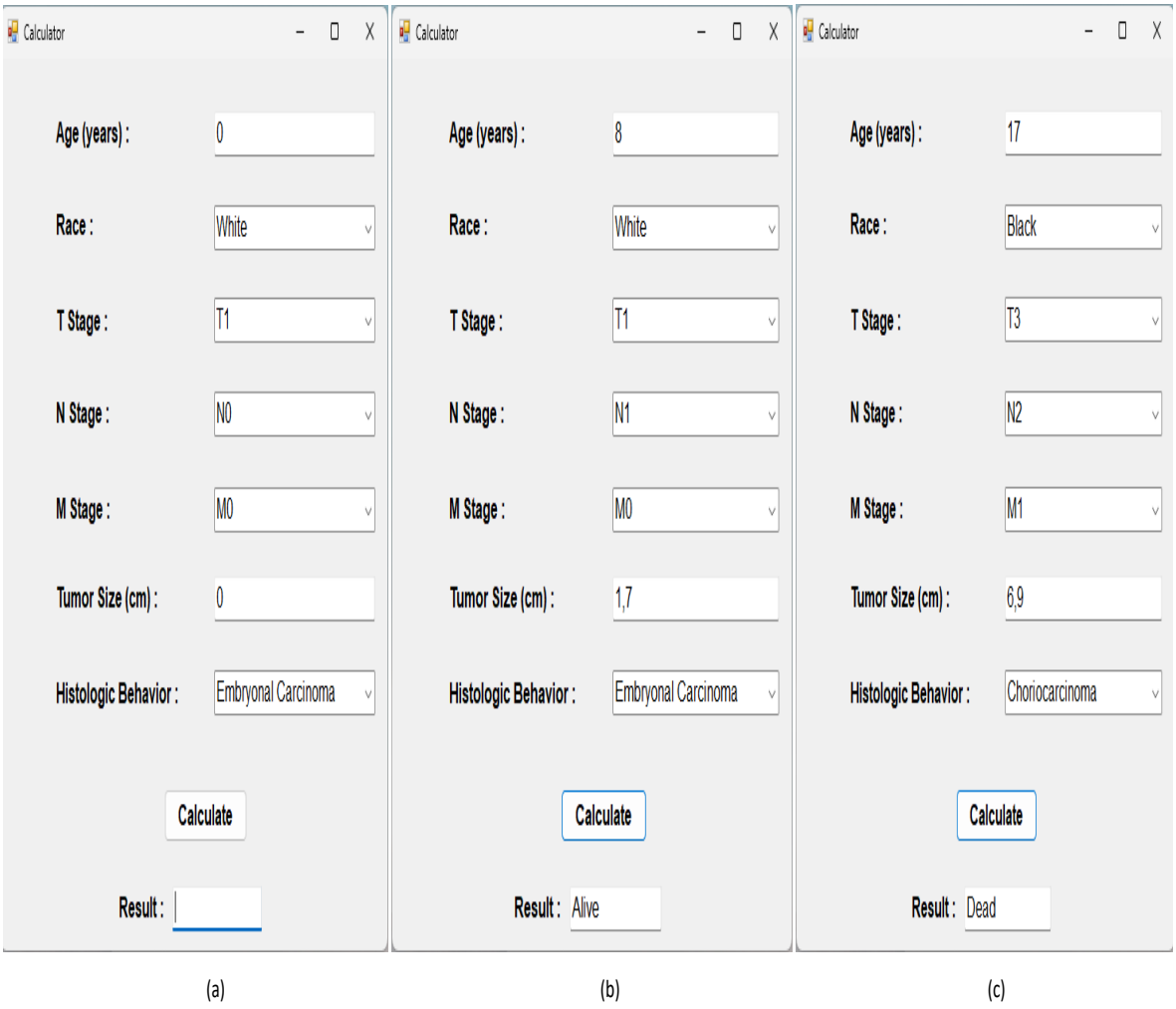

Figure 2. Output screenshots of the decision support system

(Link to download the application: https://drive.google.com/file/d/125nYPDH5b2sMovHBNjOxe3EeJwQKxd3u/view?usp=sharing)

Discussion

In this study, we used a machine learning technique to determine the survival rates of a nonseminomatous germ cell subgroup of testicular cancers, which are rare in pediatric patients. We developed a 10-year survival prediction model for pediatric patients with NSGCT. This model is presented as a software solution at the end of the study. We initially examined the demographic data of cases available in the SEER database. Only pediatric patients were included in this study. Therefore, our study is a novel and comprehensive study that specifically included a pediatric age group (0-18 years). Studies have consistently shown that the age group of 15-39 years represents the adolescent and young adult population and is most commonly affected by testicular cancer [8, 9]. Similarly, our study supports these findings, as patients aged 15 years accounted for 77.4% of the study population.

Various methods are available to predict the survival and prognosis of cancer patients [10-13]. In the study conducted by Srivastava et al. [11], for survival analysis of non-seminomatous mediastinal and testicular germ cell cancer, the researchers utilized the Cox proportional hazard model and Kaplan-Meier curves based on the SEER dataset. According to the study, the 5-year cancer-specific survival rates were 88.23% for individuals aged <19 years, 82.22% for those aged 19-30, and 74.13% for those aged >30 [11]. In our study, we conducted a survival analysis of patients aged <15 and ≥15 years. The results indicated that the 1-year, 5-year, and 10-year survival rates for patients aged < 15 years were 98.8%, 97.0%, and 96.3%, respectively. For patients aged 15 years,

the corresponding survival rates were 97.2%, 92.5%, and 92.3%, respectively. Moreover, the 1-year, 5-year, and 10-year cancer-specific survival rates for all cases were 96.9%, 93.5%, and 93.2%, respectively. The higher observed survival rates may be attributed to the focus of our study on testicular germ cell cancer. In a study by Wu et al. [14] that utilized the SEER database, the 3-year and 5-year survival rates for mediastinal malignant germ cell cancer were 63.1% and 61.2%, respectively.

There was no significant difference in tumor laterality among patients. In our literature review, we were unable to find specific data regarding the laterality of NSGCTs in pediatric patients. However, a study conducted by Berney et al. [15] reported that malignant NSGCTs were more frequently observed on the right side in individuals aged 60 years.

In our study, malignant NSGCTs were observed more frequently in Caucasians. However, no significant difference was observed in mortality between White and Black individuals. In a study conducted by Li et al. [16] on adult patients, a higher mortality rate was observed among Black individuals. This study suggests that the underlying reasons for this disparity could be attributed to factors such as hormones, dietary habits, cryptorchidism, and familial risk factors. However, the exact cause for this difference remains unclear [16].

According to the data from the SEER database, we identified mixed germ cell tumors as the most common subtype of malignant NSGCTs. According to the literature, yolk sac tumors are the most common subtype of NSGCT in children under 2 years of age (approximately 10-44% of cases) [17, 18]. Mixed germ cell tumors are the most frequently observed subtype in adolescents. We believe that the reason for the most frequent occurrence of mixed germ cell tumors in this study was that 77.4% of tumors were observed in adolescents. In our study, the highest mortality rate was observed in cases with the histological behavior of choriocarcinoma, whereas the lowest mortality rate was observed in cases of yolk sac tumors. Curto et al. [19] reported a 100% survival rate in yolk sac tumors. Our findings are consistent with those in the literature.

In our study, we observed disease-related deaths in 11.1% of patients diagnosed before 2000, whereas the rate decreased to 3% in patients diagnosed in 2000 and thereafter. We believe that advancements in medical technology as well as improvements in radiotherapy and chemotherapy may have contributed to this decrease. However, because we did not have access to a database regarding chemotherapy administered before or after surgery, we could not make a definitive conclusion regarding its impact on the observed outcomes.

In our study, the average lifespan for children with tumor sizes smaller than 2 cm was found to be 36.10±0.47 years, while it was 35.17±0.35 years for tumor sizes between 2-5 cm and 32.45±0.92 years for tumor sizes larger than 5 cm. In the study conducted by Song et al. [20], a correlation between larger radiological dimensions of tumors and malignancy was observed. Similarly, our study supports the finding that as the tumor size increases, the mortality risk also increases.

In conclusion, this study evaluated the factors influencing survival in pediatric NSCGT, which is a rare urological cancer. We examined the relationship between survival and factors such as patient age, race, histological tumor type, TNM stage, year of diagnosis, and tumor size. We found that if the tumor was diagnosed after 2000, had a size smaller than 2 cm, was diagnosed before the age of 15 years, and was a yolk sac tumor with T1N0M0 staging, the survival rate would be higher. We believe that our 10-year survival prediction model and the software created for this model will contribute to the advancement of artificial intelligence studies in prognosis, recurrence, and survival analysis.

Funding: None.

Author contributions: İ.G. and B.B. constructed the main idea and hypothesis of the study. İ.G. and B.B. developed the theory and arranged/edited the material and method section. İ.G. has done the evaluation of the data in the results section. Discussion section of the article was written by İ.G., İ.G. and B.B. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. SEER*Explorer Application. Available at: https://seer.cancer.gov/statistics-network/explorer/application.html?site=67&data_type=1&graph_type=3&compareBy=race&chk_race_1=1&rate_type=2&hdn_sex=2&advopt_precision=1&advopt_show_ci=on&hdn_view=1#resultsRegion1. Accessed April 2, 2023
2. Ahmed HU, Arya M, Muneer A, Mushtaq I, Sebire NJ. Testicular and paratesticular tumours in the prepubertal population. *Lancet Oncol.* 2010;11(5):476-483. doi:10.1016/S1470-2045(10)70012-7
3. Facts About Testicular Cancer | Testicular Cancer Statistics. Available at: https://www.cancer.org/cancer/testicular-cancer/about/key-statistics.html. Accessed March 26, 2023
4. Cancer today. Available at: http://gco.iarc.fr/today/home. Accessed April 2, 2023
5. Turabian J. Prognosis-based medicine-The importance of psychosocial factors: Conceptualization from a case of acute pericarditis. *Trends Gen Pract.* 2018;1(1):1-5. doi:10.15761/TGP.1000101
6. SEER Incidence Database - SEER Data & Software. SEER. Available at: https://seer.cancer.gov/data/index.html. Accessed April 17, 2023
7. McGlynn KA, Trabert B. Adolescent and adult risk factors for testicular cancer. *Nat Rev Urol.* 2012;9(6):339-349. doi:10.1038/nrurol.2012.61
8. Cancer statistics for adolescents and young adults, 2020 - Miller - 2020 - CA: A Cancer Journal for Clinicians - Wiley Online Library. Available at: https://acsjournals.onlinelibrary.wiley.com/doi/10.3322/caac.21637. Accessed April 20, 2023.
9. Key Statistics for Childhood Cancers. Available at: https://www.cancer.org/cancer/cancer-in-children/key-statistics.html. Accessed April 20, 2023
10. Statistical Methods in the Cancer Surveillance Research Program (SRP).
11. Srivastava A, Patel HV, Koehne E, et al. Survival of nonseminomatous germ cell tumors in pediatric patients and young adults - A stage group stratified analysis. *Urol Oncol.* 2022;40(4):169.e1-169.e12. doi:10.1016/j.urolonc.2021.12.012
12. Mao W, Wu J, Kong Q, Li J, Xu B, Chen M. Development and validation of prognostic nomogram for germ cell testicular cancer patients. *Aging.* 2020;12(21):22095-22111. doi:10.18632/aging.104063
13. Li M, Wang J, Li J, et al. Develop and validate nomogram to predict cancer-specific survival for patients with testicular yolk sac tumors. Front Public Health. 2022;10. Available at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1038502. Accessed April 10, 2023.
14. Wu P, Yang Y, Yu Z, Zhao L, Feng S. Clinical Features and Survival Outcomes in Children and Adolescents With Malignant Mediastinal Germ Cell Tumors Based on Surveillance, Epidemiology, and End Results Database Analysis. *J Surg Res.* 2023;288:362-371. doi:10.1016/j.jss.2023.03.018
15. Berney DM, Warren AY, Verma M, et al. Malignant germ cell tumours in the elderly: a histopathological review of 50 cases in men aged 60 years or over. *Mod Pathol Off J U S Can Acad Pathol Inc.* 2008;21(1):54-59. doi:10.1038/modpathol.3800978
16. Li Y, Lu Q, Wang Y, Ma S. Racial differences in testicular cancer in the United States: descriptive epidemiology. *BMC Cancer.* 2020;20(1):284. doi:10.1186/s12885-020-06789-2
17. Caballero Mora FJ, Muñoz Calvo MT, García Ros M, et al. [Testicular and paratesticular tumors during childhood and adolescence]. *An Pediatr Barc Spain 2003.* 2013;78(1):6-13. doi:10.1016/j.anpedi.2012.05.018
18. Geng R, Zheng Z, Lin Y, et al. Clinical characteristics and prognostic factors of male yolk sac tumor: a Surveillance, Epidemiology, and End Results program study. *World J Urol.* 2021;39(4):1211-1217. doi:10.1007/s00345-020-03311-y
19. Curto ML, Lumia F, Alaggio R, et al. Malignant germ cell tumors in childhood: Results of the first Italian cooperative study “TCG 91”. *Med Pediatr Oncol.* 2003;41(5):417-425. doi:10.1002/mpo.10324
20. Song G, Xiong GY, Fan Y, et al. The role of tumor size, ultrasonographic findings, and serum tumor markers in predicting the likelihood of malignant testicular histology. *Asian J Androl.* 2019;21(2):196-200. doi:10.4103/aja.aja_119_18

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1543017

Bibliometric analysis of the top 500 most cited articles in cancer epidemiology

Kanser epidemiyolojisinde en çok atıf alan ilk 500 makalenin bibliyometrik analizi

Yasemin Adalı

Posted date:03.09.2024 Acceptance date:11.11.2024

Abstract

Purpose: The objective of this work is to provide insights for future research by creating a comprehensive bibliometric analysis of the literature on "cancer epidemiology".

Material and methods: Article data were collected from the Web of Science Core Collection (WoSCC) and advanced search was performed using the following keywords: “cancer*” and “epidemi*” between 2010-2024. The results were then refined according to the publication type of Science Citation Index Expanded and Emerging Sources Citation Index, which only consist of original English articles in the WOS database. A summary of the data analysis results examined along with open-source R package Bibliometrix and VOSviewer (Version 16.20) programs.

Results: The 500 most cited documents published in 180 different sources in the time period 2010-2024 showed a negative annual growth rate of 18.97%, indicating a decrease in publication output. An average of 70.1 co-authors per document was observed, and 60.8% of the collaborative efforts in these articles were found to be foreign co-authorship. The most frequently appearing keywords were found to be "epidemiology" with a total link strength of 625, followed by "cancer" with a total link strength of 372. According to the bibliometric analysis result, the article by Ferlay, J. was found to have more than 72.000 citations.

Conclusion: This bibliometric analysis has yielded quantitative data on the expansion of the cancer epidemiology discipline, the level of scientific excellence, and the identification of keywords, so facilitating the determination of the most accomplished topics.

Keywords: Bibliometric analysis, cancer epidemiology, bibliometrix, VOSviewer.

Adali Y. Bibliometric analysis of the top 500 most cited articles in cancer epidemiology. Pam Med J 2025;18:370-382.

Öz

Amaç: Bu çalışmanın amacı, “kanser epidemiyolojisi” konusundaki literatürün kapsamlı bir bibliyometrik analizini oluşturarak gelecekteki araştırmalar için öngörüler sunmaktır.

Gereç ve yöntem: Makale verileri Web of Science Core Collection'dan (WoSCC) toplandı ve 2010-2024 yılları arasında "cancer*" ve "epidemi*" anahtar sözcükleri kullanılarak gelişmiş arama yapıldı. Sonuçlar daha sonra yalnızca WOS veri tabanındaki orijinal İngilizce makalelerden oluşan Science Citation Index Expanded ve Emerging Sources Citation Index yayın türüne göre rafine edildi. Veri analizi sonuçlarının bir özeti, açık kaynaklı R paketi Bibliometrix ve VOSviewer (Sürüm 16.20) programlarıyla birlikte araştırıldı.

Bulgular: 2010-2024 zaman aralığında 180 farklı kaynakta yayınlanan en çok atıf alan 500 belge, yıllık %18,97'lik bir negatif yönde büyüme oranı gösterdi ve bu da yayın çıktısında bir düşüş olduğunu gözlemlenmiştir. Belge başına ortalama 70,1 ortak yazar olduğu gözlenmiş ve bu makalelerdeki iş birliği çabaların %60,8'ini yabancı ortak yazarlık oluşturduğu bulunmuştur. En çok görünen anahtar kelimeler, toplam bağlantı gücü 625 olan "epidemiyoloji” olarak bulundu, daha sonra 372 toplam bağlantı gücüne sahip olan "kanser" olduğu gözlenmiştir. Bibiliometrik analiz sonucuna göre Ferlay, J. makalesi 72.000'den fazla atıf aldığı tespit edilmiştir.

Sonuç: Bu bibliyometrik analiz, kanser epidemiyolojisi disiplininin genişlemesi, bilimsel mükemmellik düzeyi ve anahtar kelimelerin belirlenmesi hakkında nicel veriler sağlamış ve böylece en başarılı konuların belirlenmesini kolaylaştırmıştır.

Anahtar kelimeler: Bibliyometrik analiz, kanser epidemiyolojisi, bibliometrix, VOSviewer.

Adalı Y. Kanser epidemiyolojisinde en çok atıf alan ilk 500 makalenin bibliyometrik analizi. Pam Tıp Derg 2025;18:370-382.

Yasemin Adalı, Asst. Prof. Pamukkale University, Faculty of Medicine, Department of Biophysics, Denizli, Türkiye; Centre for Public Health, Queen’s University Belfast, School of Medicine, Dentistry and Biomedical Sciences, Belfast, United Kingdom - Visiting Scholar, e-mail: yadali@pau.edu.tr (https://orcid.org/0000-0002-6314-4816) (Corresponding Author)

Introduction

Cancer epidemiology offers methods and approaches to comprehend cancer issues in all societies, ranging from local to worldwide scales. An in-depth and accurate comprehension of cancer epidemiology yields crucial insights into the possible etiology of these illnesses and population patterns, enabling the formulation of prompt and suitable healthcare interventions targeted at devising efficacious policies for screening, diagnosis, and prevention [1]. With the ongoing development of cancer epidemiology, there is a strong focus on enhancing research methodology. Since the 1950s, there have been advancements in research design, such as improved case-control studies, which enable more rigorous selection of case and control populations and the use of better statistical analytic tools [2]. These developments are expected to enhance the dependability of discoveries about the causes and risk elements of cancer.

In recent decades, a significant number of diligent researchers and institutions worldwide have established themselves in the subject of cancer epidemiology [3]. As a result of this growing interest, there has been a rise in scientific investigations, the entire extent of which has not yet been quantified in the field. A comprehensive bibliometric research study is underway to evaluate the quantity and progression of scientific output across different nations. Bibliometrics is the study of the scientific output of a scientist, research unit, institution, or country [4]. It involves evaluating the historical progress of a discipline, determining its significance in the scientific community, and identifying potential areas for future research [4].

However, no bibliometric analysis of publications on “cancer epidemiology” has been published till now. Therefore, this article aims to provide a bibliometric analysis report on the scholarly output in the subject of cancer epidemiology across different countries.

Materials and methods

This search covers articles published between 2010 and 2024 and was conducted on July 14, 2024. Article data was collected from the Web of Science Core Collection (WoSCC), and advanced search was performed using the following keywords: “cancer*” and “epidemi*”. Our choice was largely influenced by WoSCC’s exceptional capacity to offer thorough and in-depth descriptions of highly cited and popular papers, a capability that is unmatched in other databases. Accurately identifying and evaluating the most influential literature in the field of cancer epidemiology research depends on this feature [5].

The results were then refined according to the publication type of Science Citation Index Expanded and Emerging Sources Citation Index, which only consist of original English articles in the WoSCC database. The search was adjusted to include articles published between 2010 and 2024. As a result of these inclusion criteria, 195.265 were reached. Top 500 most cited all bibliographic data was exported from the WoSCC database and interpreted using analytical bibliometric methods.

A summary of the data analysis results is provided along with visualizations. The open-source R package Bibliometric was used to run bibliometric analyses and create data matrices for publication trends, journal rankings, authorship analysis, most prolific countries, author collaboration patterns, trend subjects, and most cited papers [6]. The VOSviewer (Version 16.20) program was also used to display bibliometric connections of data for co-authorship, co-occurrence keywords. Two standard weight attributes are applied which are defined as “Total link strength attribute” [7]. Additionally, Microsoft Office Excel 2019 was used to perform quantitative analysis of the publication.

Bibliometrix analysis, ethics committee approval is not required.

Results

Overview of obtained bibliographic data

The research output in the field of “cancer epidemiology” is depicted in Table 1, which presents a summary of bibliometric data from 2010 to 2024. The 500 documents published over this time span across 180 different sources showed a minus 18.97% annual growth rate, indicating a decrease in publication volume.

With an astounding average of 2.148 citations per document and an overall reference count of 43.687, the average document is 7.23 years old. Out of the 19.951 authors in the dataset, only 12 authored single documents. The dataset also contains 2.246 Keywords Plus and 604 author's keywords. The average number of co-authors per document is 70.1, and international co-authorship accounts for 60.8% of the collaborative efforts in these publications. The variety of document types, which include 340 articles, 145 reviews, and a few other formats, reflects the wide range of output in the field of research. Overall, this data demonstrates the complexity and richness of scholarly publications in the designated field over the specified time period.

Table 1. Summary of bibliographic statistics (n=500)

Description	Results
Authors	
Authors	19951
Authors of single-authored docs	12
Authors Collaboration	
Single-authored docs	12
Co-Authors per Doc	70.1
International co-authorships %	60.8
Document Contents	
Keywords Plus (ID)	2246
Author's Keywords (DE)	604
Document Types	
Article	340
Article; Book chapter	2
Article; Proceedings paper	3
Correction	1
Editorial material	6
Letter	1
Review	145
Review; Book chapter	2
Main Information about Data	
Timespan	2010:2024
Sources (Journals, Books, etc)	180
Documents	500
Annual Growth Rate %	-18.97
Document Average Age	7.23
Average citations per doc	2148
References	43687

Scientific output and average annual article citations

Figure 1 displays the yearly scientific output for the top 500 most cited articles related with "cancer epidemiology", along with the average number of citations per year. From 2010 to 2019, there is a noticeable overall increase in scientific production, indicating a rising inclination and allocation of resources towards research during this time frame. Nevertheless, there is a significant decrease commencing in 2020. The peak of production is achieved approximately in 2019. The subsequent significant decline can be ascribed to a multitude of factors, including global occurrences like the COVID-19 pandemic and changes in research emphases.

Figure B exhibits substantial year-to-year fluctuations, in contrast to the relatively consistent upward trend observed in Figure A. This implies that the influence of research, as quantified by citations, is more unpredictable and affected by factors beyond the sheer quantity of publications. There is a significant increase in citations around 2021, followed by a decrease. This could suggest a profoundly influential research article or a collection of studies published during that year that received substantial recognition from the scientific community.

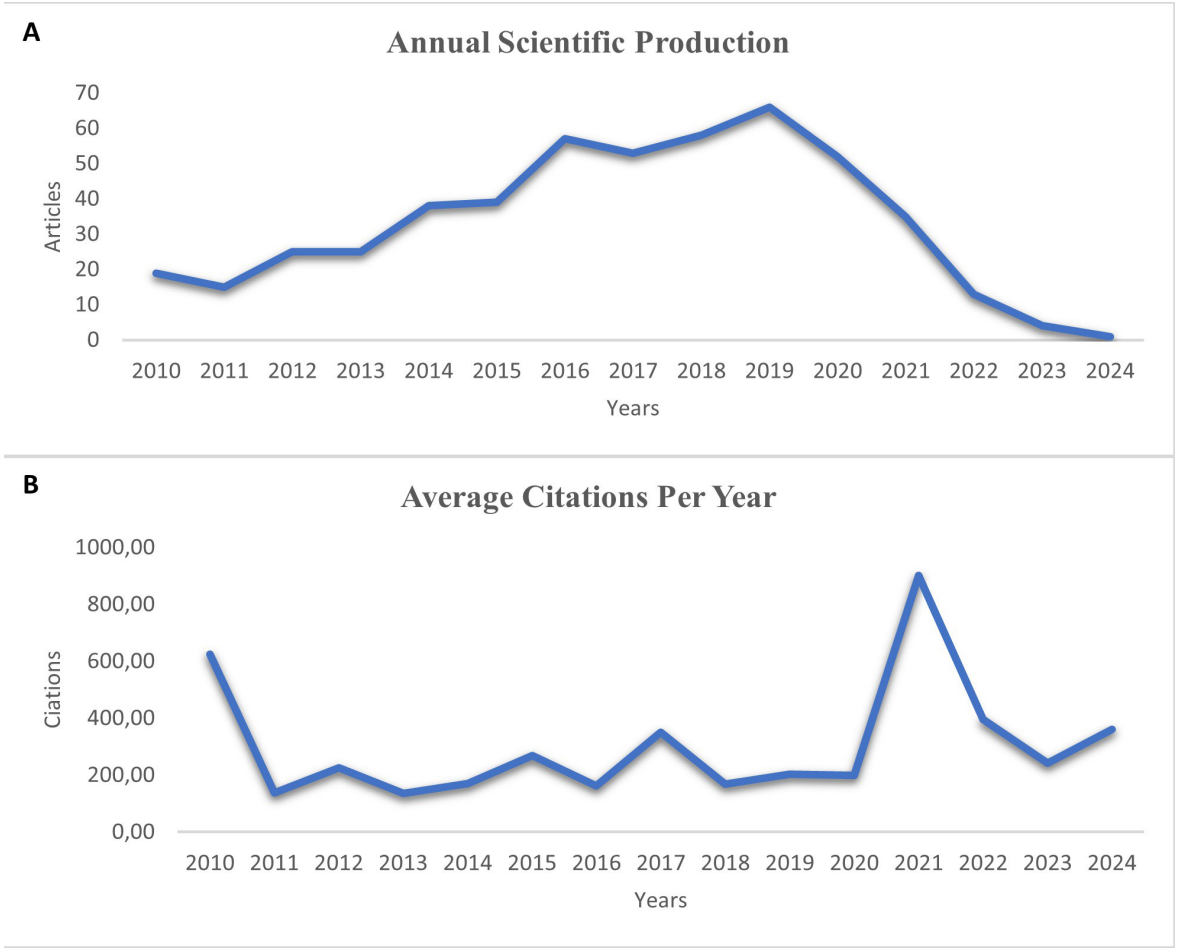

Figure 1. Annual scientific production over the years for the most cited 500 articles (A) and average citation per year (B)

Most relevant sources

Figure 2 displays the most relevant sources from which the first 500 most cited articles in the field of cancer epidemiology are published. This table displays a hierarchy of scientific journals, organized according to the frequency of citations in the field of cancer epidemiology. The substantial number of citations received by prestigious journals such as *The Lancet*, *Cancer Journal for Clinicians* and *New England Journal of Medicine* serves as a strong indication that cancer epidemiology research holds significant influence within the wider medical community.

Most relevant countries

The distribution of publications on cancer epidemiology research across 15 countries is shown in Figure 3, which makes a distinction between publications that are country-specific and those that involve multiple countries. In both categories, the United States is at the top, closely followed by nations in Western Europe like Germany, France, and the United Kingdom. There is a clear emphasis on cancer epidemiology research in these areas. The proportion of multiple-country publications is higher in Germany and Switzerland, suggesting that these countries place a strong emphasis on international collaboration in their research.

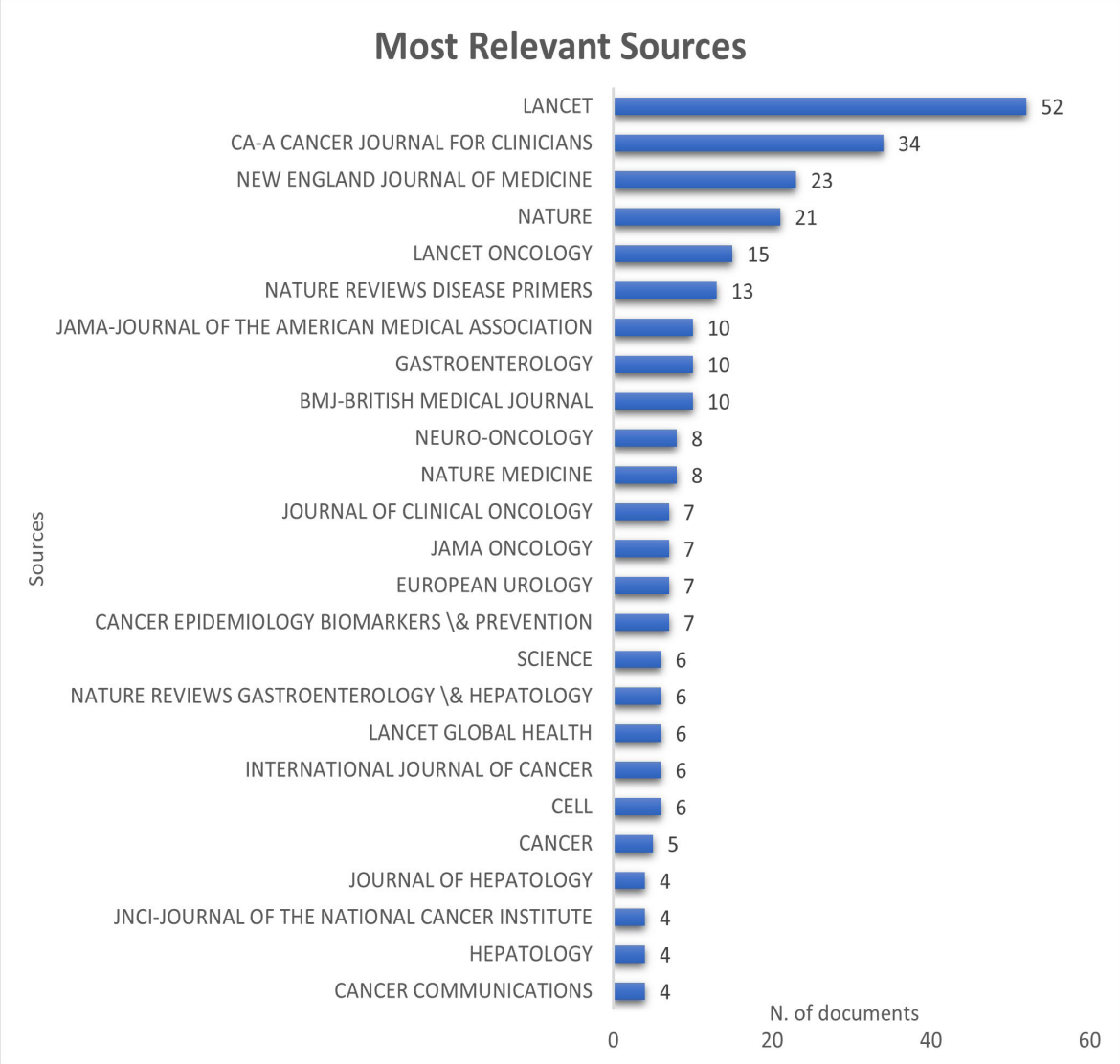

Figure 2. The most relevant sources of the 500 most cited articles in the field of cancer epidemiology

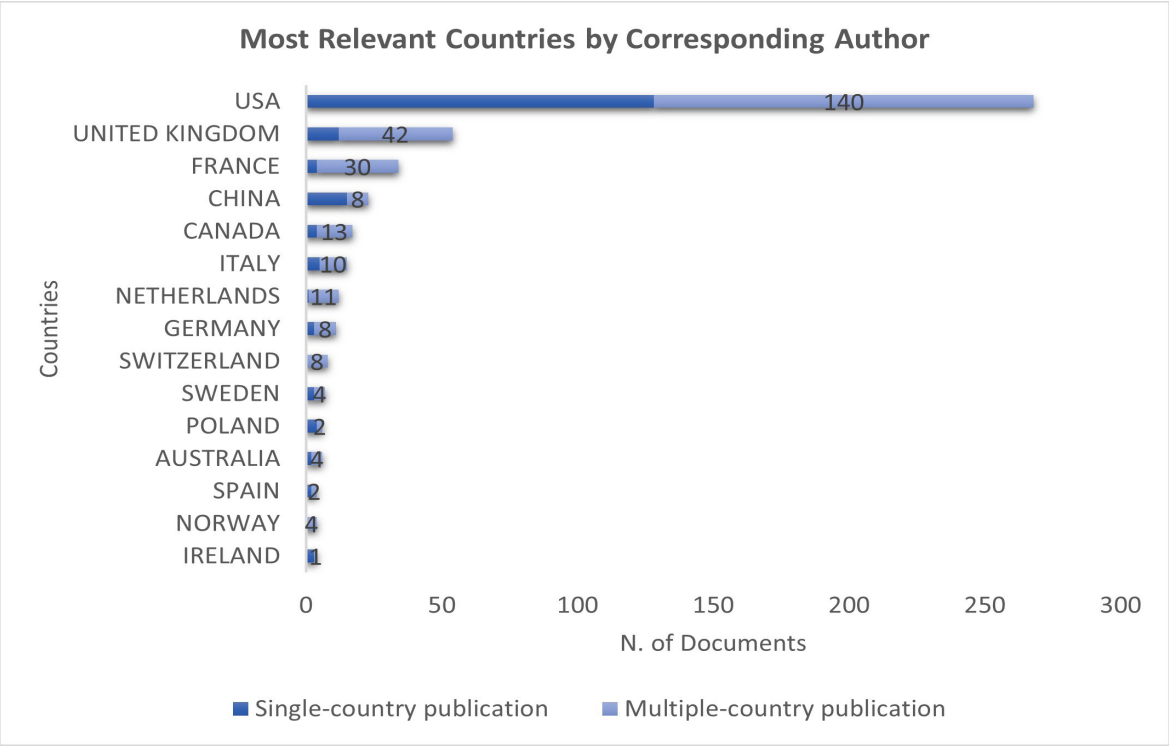

Figure 3. Most relevant countries by corresponding author

Thematic map

A thematic map based on density and centrality was created and is presented in Figure 4. The results were derived from a semi-automatic algorithm that examined the titles of all analyzed references, along with additional relevant keywords to identify more complex variations.

The upper right quadrant displays topics characterized by high density and centrality; these topics, such as “United States”, “risk factors”, and “body mass index”, could be further developed and studied due to their importance for future research. Also, the upper left quadrant displays rapidly evolving but underrepresented topics characterized by high density and low centrality, such as “glioma”, “cancer incidence”, “high grade”, “quality of life”, “open label”, and “chemotherapy”.

The lower left quadrant covers topics that have been used with low centrality and density but show a decreasing trend; this area includes “landscape”, “somatic mutation”, and “signatures”. Additionally, the lower right quadrant covers key topics characterized by high centrality and low density; These topics are important for research as general themes, and it was observed that they include “risk”, “mortality”, “epidemiology”, “genome-wide association” and “classification”.

Bibliometric analysis of co-occurrence keywords and WordCloud

The WordCloud provided in Figure 5 provided a visual representation of the most frequent terms found in the top 500 most cited articles on cancer epidemiology. This WordCloud highlights key concepts and areas of research that have been the focus of extensive research in this field (Figure 5). Prominent terms include specific types of cancer (e.g., breast cancer, prostate cancer), risk factors (e.g., smoking, obesity, genetic factors), and epidemiological concepts (e.g., incidence, prevalence, mortality). Keywords that appeared in the WOS core database more than 15 times and were supplied by the paper’s authors were included. The most frequently occurring keywords were “epidemiology,” which had a total link strength of 625, and “cancer,” which had a total link strength of 372 (Figure 6).

Bibliometric analysis of co-authorship and most cited authors

A total of 19951 authors have contributed to the “cancer epidemiology” publication for the 500 most cited articles. Figure 7 displays the co-authorship map of authors, which shows the authors who collaborate in the field of cancer epidemiology. The number of publications is indicated by the size of the circles, while different colors correspond to different clusters. The authors’ strength of connection is indicated by the thickness of the lines.

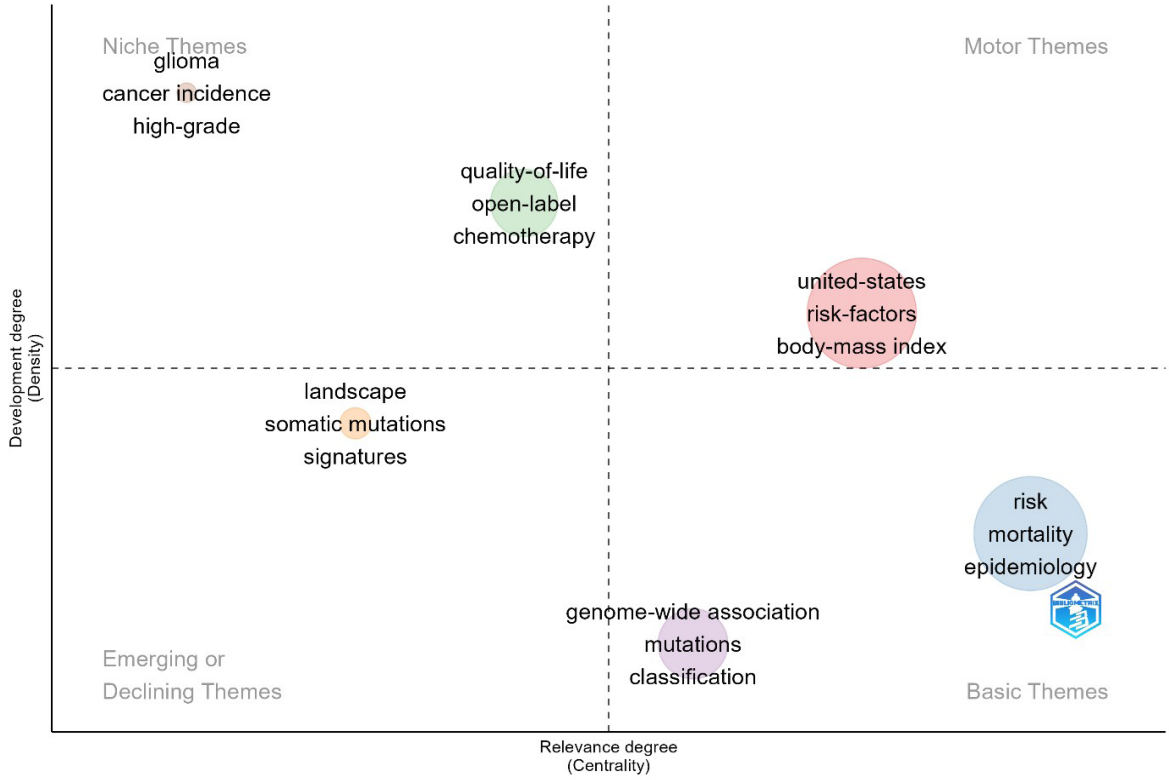

Figure 4. Thematic map based on density and centrality, divided into four topological regions

Figure 5. WordCloud

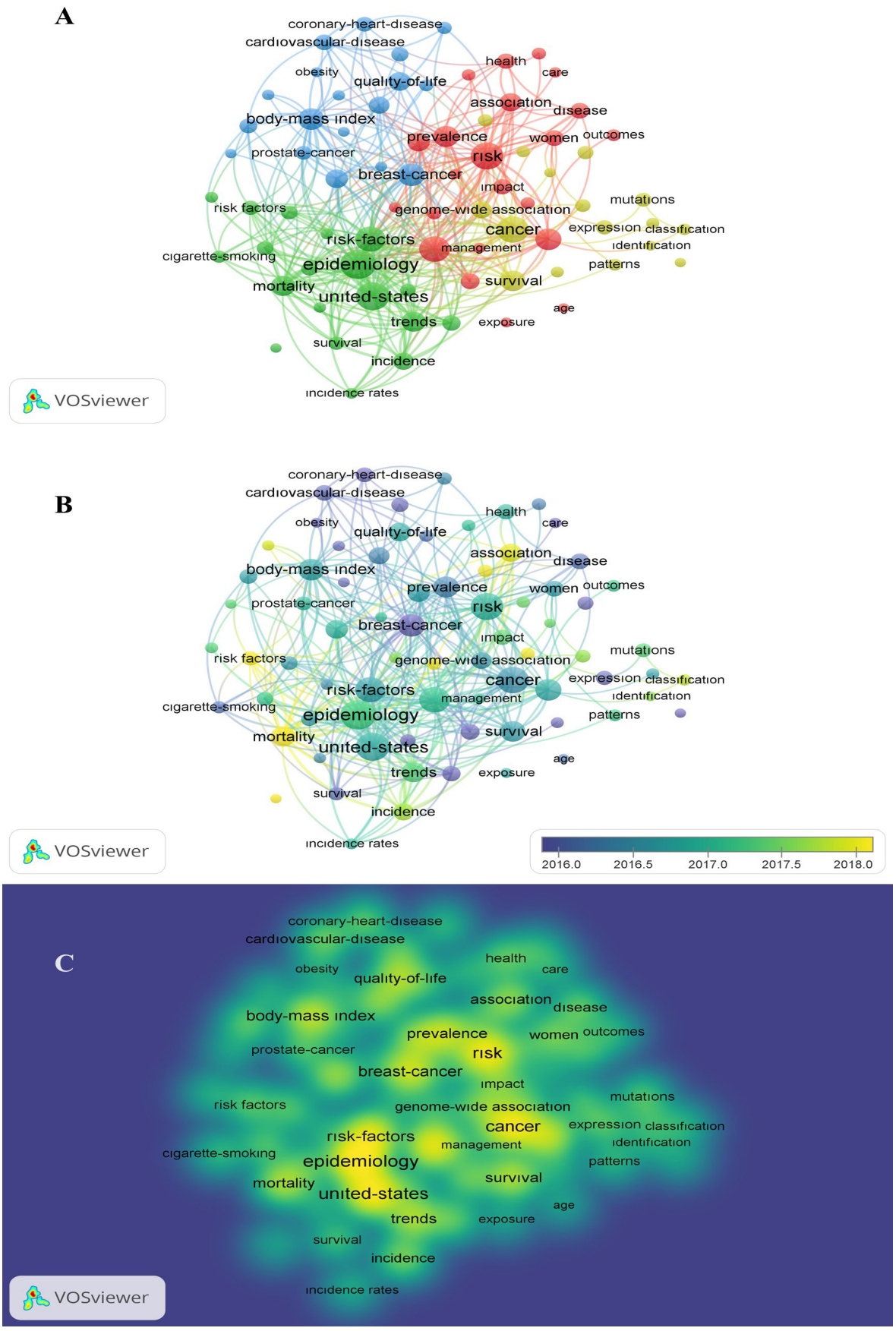

Figure 6. Co-occurrence keywords. (A) network visualization, (B) overlay visualization (C) density visualization

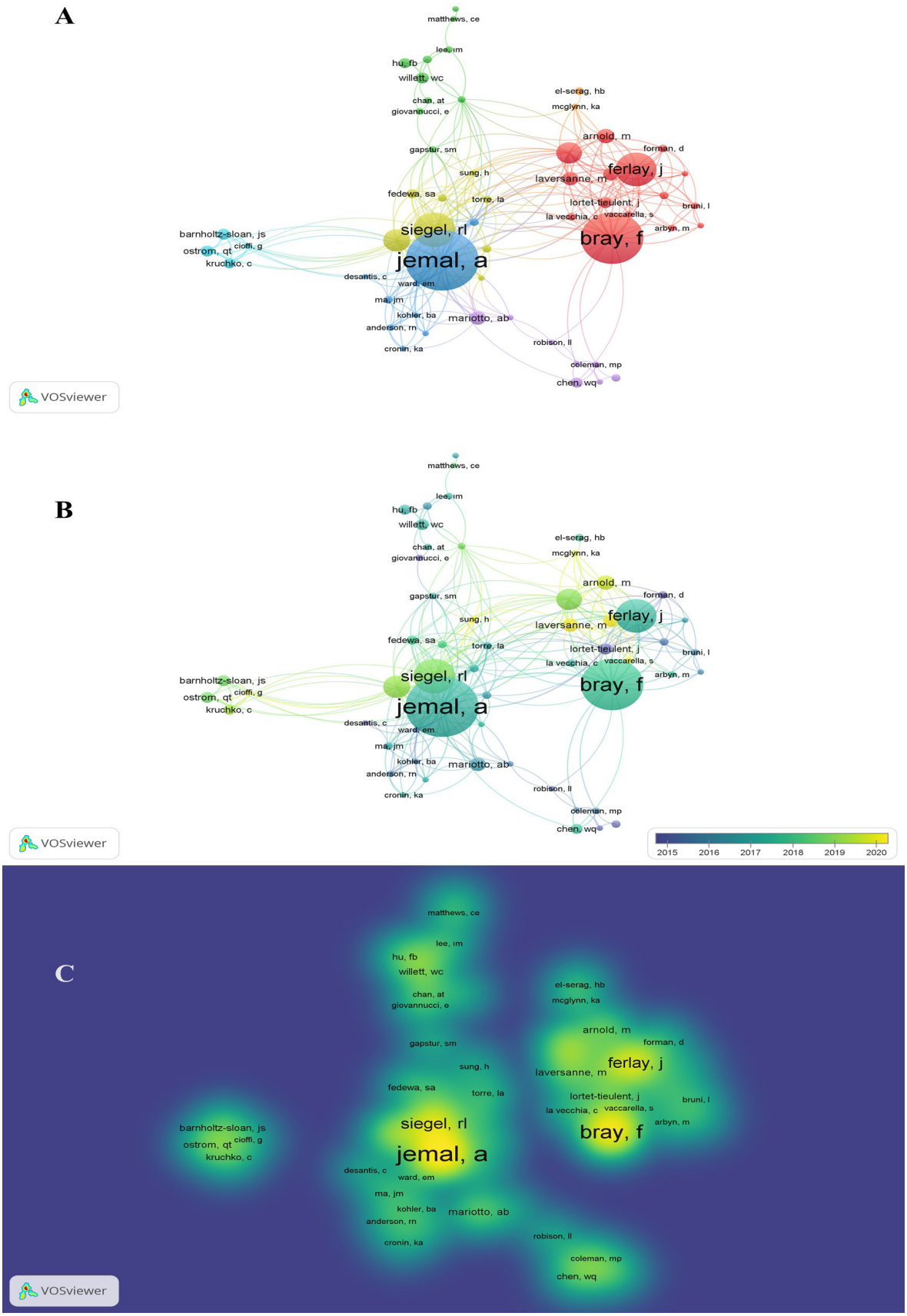

Figure 7. Co-occurrence authorship (A) network visualization, (B) overlay visualization (C) density visualization

Moher et al. [8] received more than 122.000 citations for his articles on “cancer epidemiology” with a total link strength of 5. Siegel et al. [9] (total strength link 123) received more than 72.000 citations for his research on cancer incidence and global estimates of cancer prevalence, making him the second and third most frequently referenced author (Table 2). In both 2017 and 2021, Siegel et al. [9, 10] were the authors whose research on cancer statistics received the highest number of citations. These authors, who are associated with prestigious institutions in their respective fields, have made substantial contributions to the comprehension of cancer epidemiology and statistical patterns and have influenced the discussions in cancer research. Their research, which has been published in prestigious academic journals, has been extensively embraced by researchers across the globe.

Table 2. Most cited first 15 authors

Paper / Ref	Title of articles	DOI	TC	TC per Year
Moher D, 2010 / [8]	Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement	10.1016/j.ijsu.2010.02.007	122456	8164
Sıegel Rl, 2017 / [9]	Cancer statistics, 2017	10.3322/caac.21387	72934	9117
Sıegel Rl, 2021 / [10]	Cancer statistics, 2021	10.3322/caac.21654	72934	18234
Sung H, 2021 / [11]	Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries	10.3322/caac.21660	31327	7832
Ferlay J, 2015 / [12]	Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012	10.1002/ijc.29210	21741	2174
Sıegel R, 2014 / [13]	Cancer statistics, 2014	10.3322/caac.21208	16474	1498
Torre La, 2015 / [14]	Global cancer statistics, 2012	10.3322/caac.21262	14847	1485
Chen W, 2016 / [15]	Cancer statistics in China, 2015	10.3322/caac.21338	14400	1600
Ferlay J, 2010 / [16]	Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008	10.1002/ijc.25516	12826	855
Bolyen E, 2019 / [17]	Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2	10.1038/s41587-019-0209-9	11178	1863
Koboldt Dc, 2012 / [18]	Comprehensive molecular portraits of human breast tumours	10.1038/nature11412	9002	692
Schulz Kf, 2010 / [19]	CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials	10.1136/bmj.c332	8827	588
Altshuler Dm, 2015 / [20]	A global reference for human genetic variation	10.1038/nature15393	8459	846
Sıegel Rl, 2022 / [21]	Cancer statistics, 2022	10.3322/caac.21708	8279	2760
Sıegel Rl, 2020 / [22]	Cancer statistics, 2020	10.3322/caac.21590	8120	1624

Discussion

A bibliometric analysis of the 500 most frequently referenced papers on cancer epidemiology showed a compound annual growth rate of minus 18.97%, which suggests that the number of documents has decreased over time.

The mean number of citations per paper, which is 2148, suggests that cancer epidemiology research is being extensively referenced. In addition, the substantial average number of co-authors per document (70.1) and the notable proportion of foreign co-authorship (60.8%) suggest a cooperative research setting. In general, the findings of the bibliometric study indicate a decline in the quantity of publications. However, there exists research of exceptional quality that is extensively referenced and necessitates rigorous collaboration among scholars.

Bibliometric examination of annual scientific output yields significant insights into the dynamics of scientific production in the field of cancer epidemiology. Understanding these trends enables researchers and policy makers to make well-informed decisions to progress the field and enhance cancer prevention and treatment.

The analysis of the top 500 cited articles in cancer epidemiology indicates that the most often referenced sources are general medical journals such as *The Lancet*, *New England Journal of Medicine*, *Nature*, and *JAMA*. This implies that innovative research in cancer epidemiology frequently receives publication in these influential, interdisciplinary journals. In addition to the prevalence of general medical journals, there is a notable presence of cancer-specific journals such as *Lancet Oncology*, *Journal of Clinical Oncology*, and *Cancer Epidemiology, Biomarkers & Prevention*. These specialized publications are essential for the dissemination of research that is specifically focused on cancer epidemiology. The presence of such variation implies that cancer epidemiology is a wide-ranging discipline that encompasses research on numerous aspects of the disease. The prevalence of general medical publications indicates that research in cancer epidemiology has substantial consequences for wider medical practice and public health objectives.

This bibliometric analysis demonstrates that cancer epidemiology research is a global endeavor, and the collaborative nature of research, as evidenced by articles published by multiple countries, highlights the importance of international partnerships in addressing this complex disease. The United States was the clear leader in English written publication, with a significantly higher number of publications than other countries. This suggests that the United States is a major center for cancer epidemiology research. European countries, including the United Kingdom, France, Germany, and Italy, are significant contributors to the field. While the United States and European countries dominate, other countries such as Canada, Australia, and China also have a significant presence, indicating a growing global interest in cancer epidemiology.

Important study fields in cancer epidemiology encompass the discovery of environmental and genetic variables that increase the risk of cancer, progress in research techniques, and the assessment of screening and prevention approaches [23]. The field also highlights the influence of socio-economic position on the occurrence and death rates of cancer, exposing significant variations in cancer treatment and results among various global locations [23]. Although there have been notable improvements, there are still difficulties that need to be overcome, especially in dealing with the changeable elements that lead to the occurrence of cancer. Ongoing research endeavors seek to augment comprehension of these variables, advocate for healthy behaviors, and diminish the overall prevalence of cancer, especially among populations at greater risk [24]. The advancements in cancer epidemiology will have a pivotal impact on the development of efficient public health interventions and policies targeted at preventing and managing cancer.

The thematic analysis results showed that “risk”, “mortality”, “genome-wide association”, “classification”, and “epidemiology” are important for research, and the future study trend may shift in this direction. The most studied and intensive topics were “risk factor”, “body mass index” and the “United States”. This may be due to the fact that cancer epidemiology studies are mostly conducted on cancer cohorts in the United States, and they mostly examine risk factors through obesity.

There were various restrictions on our study. Initially, our analysis of articles was restricted to citation counts, which hindered our ability to examine more recent developments in our field. Second, there might be a restriction associated with using the WOS Journal Citation Report. It has previously been questioned if citation indices are a reliable indicator of the importance and relevance of a piece of work [25]. Technical constraints may have resulted in the unintentional omission of specific articles from our analysis. Nonetheless, our study exhibits numerous strengths. No prior bibliometric analysis has been performed on cancer epidemiology. My current analysis encompassed only the 500 most cited articles in the field, which may be inadequate for a thorough examination of trends in this subspeciality. Furthermore, articles published in languages other than English were omitted, potentially constituting a limitation.

In conclusion, this bibliometric analysis represents the first effort to examine the distribution and development of the cancer epidemiology field worldwide. It has been observed that there is currently an additional need for not only a formal definition but also a functional definition that can precisely define the boundaries of the discipline. This analysis will enable both clinicians and researchers to readily determine the popularity of articles on cancer epidemiology and the predominant topics that receive more citations. Lastly, the findings of this bibliometric analysis will serve as a reference for research investigating cancer epidemiology.

Financial support: There are no financial supports.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Mattiuzzi C, Lippi G. Current cancer epidemiology. *J Epidemiol Glob Health*. 2019;9(4):217-222. doi:10.2991/jegh.k.191008.001
2. Kocic B, Filipovic S, Petrovic B, Nikolic M. Case-control design as investigative approach to assessing cancer etiology: development and future perspectives - PubMed. *BUON*. 2012;17(3):428-435. Accessed August 26, 2024. https://pubmed.ncbi.nlm.nih.gov/23033277/
3. Piña Sánchez P, Chávez González A, Ruiz Tachiquín M, et al. Cancer Biology, Epidemiology, and Treatment in the 21st Century: Current Status and Future Challenges From a Biomedical Perspective. *Cancer Control*. 2021;28. doi:10.1177/10732748211038735
4. Ohlan R, Ohlan A. A comprehensive bibliometric analysis and visualization of smart home research. *Technol Forecast Soc Change*. 2022;184:121975. doi:10.1016/J.TECHFORE.2022.121975
5. Alryalat SAS, Malkawi LW, Momani SM. Comparing bibliometric analysis using pubmed, scopus, and web of science databases. *Journal of Visualized Experiments*. 2019;2019(152). doi:10.3791/58494
6. Aria M, Cuccurullo C. bibliometrix: An R-tool for comprehensive science mapping analysis. *J Informetr*. 2017;11(4):959-975. doi:10.1016/j.joi.2017.08.007
7. Stephan P, Veugelers R, Wang J. Reviewers are blinkered by bibliometrics. *Nature 2017 544:7651*. 2017;544(7651):411-412. doi:10.1038/544411a
8. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *International Journal of Surgery*. 2010;8(5):336-341. doi:10.1016/j.ijsu.2010.02.007
9. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30. doi:10.3322/caac.21387
10. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7-33. doi:10.3322/caac.21654
11. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660
12. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359-E386. doi:10.1002/ijc.29210
13. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64(1):9-29. doi:10.3322/caac.21208
14. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108. doi:10.3322/caac.21262
15. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115-132. doi:10.3322/caac.21338
16. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127(12):2893-2917. doi:10.1002/ijc.25516

17. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37(8):852-857. doi:10.1038/s41587-019-0209-9

18. Koboldt DC, Fulton RS, McLellan MD, et al. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70. doi:10.1038/nature11412

19. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *The BMJ*. 2010;340(7748):698-702. doi:10.1136/BMJ.C332

10. Altshuler DM, Durbin RM, Bentley DR, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74. doi:10.1038/nature15393

21. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33. doi:10.3322/caac.21708

22. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30. doi:10.3322/caac.21590

23. Arem H, Loftfield E. Cancer Epidemiology: A Survey of Modifiable Risk Factors for Prevention and Survivorship. *Am J Lifestyle Med*. 2018;12(3):200. doi:10.1177/1559827617700600

24. Maresso KC, Basen Engquist K, Hawk E. Cancer Epidemiology, Prevention, and Survivorship. *Perioperative Care of the Cancer Patient*. Published online January 1, 2023:3-14. doi:10.1016/B978-0-323-69584-8.00001-3

25. Schoonbaert D, Roelants G. Citation analysis for measuring the value of scientific publications: Quality assessment tool or comedy of errors? *Tropical Medicine and International Health*. 1996;1(6):739-752. doi:10.1111/j.1365-3156.1996.tb00106.x

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1552581

Evaluation of C-reactive protein and procalcitonin as a mortality indicator in febrile neutropenic patients

Febril nötropenik hastalarda C-reaktif protein ve prokalsitoninin mortalite göstergesi olarak değerlendirilmesi

Hasan Öksüzoğlu, Duygu Mert, Gülşen İskender, Nagihan Ulu Demirci, Mustafa Ertek

Posted date:01.10.2024 Acceptance date:27.11.2024

Abstract

Purpose: Febrile neutropenia (FN) is a common side effect of chemotherapy in cancer patients, leading to complications, increased healthcare costs, and mortality. Microbiological agents can be identified in 30-50% of FN cases. Therefore, there is a need for specific, highly effective, and rapid markers to indicate infection. Various biomarkers are currently under investigation and in clinical use. This study aims to evaluate their effectiveness in the early detection of infection and mortality by comparing quantitative C-reactive protein (CRP) and procalcitonin levels at the onset of FN and during treatment.

Material and methods: This study is a retrospective case-control study. It included 572 patients with febrile neutropenia (FN) who were followed up in the Hematology Clinic, Bone Marrow Transplantation Unit, and Medical Oncology Clinic between September 3, 2018, and May 25, 2022. A total of 748 FN episodes were recorded in these patients. Data were retrieved from the hospital information management system and documented using a pre-prepared form. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 26.0 software.

Results: Of the febrile neutropenia (FN) patients included in the study, 118 experienced mortality, whereas 630 survived. The mean age was 51.6 years in the mortality group and 50.5 years in the non-mortality group. Females constituted 47.9% of the cohort, while males accounted for 52.1%. Hematological malignancies were present in 67.2% of all patients. Bacteremia was identified in 36.5% of FN episodes. Although E. coli was the most frequently isolated microorganism, P. aeruginosa and A. baumannii were more commonly detected in patients who succumbed to the illness. Elevated C-reactive protein (CRP) and procalcitonin levels within the first five days of treatment were significantly associated with both mortality and the presence of bacteremia. Neither leukocyte count nor absolute neutrophil count at the time of diagnosis showed a significant association with mortality. However, prolonged duration of neutropenia, bloodstream infections, catheter-related bloodstream infections, and the presence of pneumonia emerged as significant risk factors for mortality.

Conclusion: CRP and procalcitonin levels were observed to have both prognostic and diagnostic value. Additionally, resistant Gram-negative bacterial growth was more frequently detected in the blood cultures of patients who did not survive. Further studies are needed to develop new treatment algorithms.

Keywords: Febrile neutropenia, C-reactive protein, procalcitonin, mortality.

Oksuzoglu H, Mert D, Iskender G, Ulu Demirci N, Ertek M. Evaluation of C-reactive protein and procalcitonin as a mortality indicator in febrile neutropenic patients. Pam Med J 2025;18:384-395.

Öz

Giriş: FN, kemoterapötiklerin komplikasyonlara, maliyet artışı ve mortaliteye yol açabilen yan etkisidir. Enfeksiyon hastalıklarının sadece %30-50'sinde ateşin kaynağı ve mikrobiyolojik etkenler saptanabilmektedir. Bu nedenle enfeksiyonu gösterebilecek, spesifik, yüksek etkinlikli ve hızlı belirteçlere ihtiyaç duyulmaktadır. Bu nedenle çeşitli biyomarkerlar araştırılmakta ve günümüzde halen kullanılmaktadır. Çalışmamızda da nötropenik ateşte CRP ve prokalsitoninin tanısal, prognostik kullanılabileceği ve FN ataklarının klinik, laboratuvar özelliklerinin, kültürde üreyen mikroorganizmaların araştırılması amaçlanmıştır.

Gereç ve yöntem: Retrospektif, vaka – kontrol çalışması olarak yürütülen bu çalışma 3 Eylül 2018-25 Mayıs 2022 tarihleri arasında hematoloji, kemik iliği transplantasyon ve tıbbi onkoloji kliniğinde FN sebebiyle tedavi gören 18 yaş ve üstü hastalarda gelişen 748 FN atağı irdelendi. Hastane bilgi yönetim sistemi taranarak uygun olan hastalar veri formuna kaydedilerek The Package for Social Sciences 26.0 (SPSS 26.0) aracılığıyla analiz edildi.

Hasan Öksüzoğlu, M.D. Department of Infectious Diseases and Clinical Microbiology, Kastamonu Training and Resarch Hospital, Kastamonu, Türkiye, e-mail: hasanoksuzoglu29@gmail.com (https://orcid.org/0000-0002-9627-8765) (Corresponding Author)

Duygu Mert, M.D. Department of Infectious Diseases and Clinical Microbiology, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye, e-mail: drduygumert@hotmail.com (https://orcid.org/0000-0002-6810-2199)

Gülşen İskender, Assoc. Prof. Department of Infectious Diseases and Clinical Microbiology, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye, e-mail: golshan1669@hotmail.com (https://orcid.org/0000-0001-7619-1366)

Nagihan Ulu Demirci, M.D. Department of Infectious Diseases and Clinical Microbiology, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye, e-mail: drnagihanulu@gmail.com (https://orcid.org/0009-0008-4748-9735)

Mustafa Ertek, Prof. Department of Infectious Diseases and Clinical Microbiology, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye, e-mail: mustafa.ertek@saglik.gov.tr (https://orcid.org/0000-0002-9397-8432)

Bulgular: Çalışmaya dahil edilen FN hastalarından 118 tanesinde mortalite gelişmişken, 630 tanesinde mortalite gelişmemiştir. Mortalite grubunda yaş ortalaması 51,6, mortalite olmayan grupta ise 50,5 bulunmuştur. Hastaların %47,9'unu kadın, %52,1'ini erkek hastalar oluşturmaktadır. Hastaların %67,2'sinin hematolojik malignitesi mevcuttu. FN ataklarında bakteriyemi oranı %36,5 bulundu ve en sık saptanan mikroorganizma E. coli olmasına rağmen P. aeruginosa ve A. baumannii üremesi olanlar mortalitesi olanlarda daha yüksek saptanmıştır. Tedavinin ilk beş gününde bakılan CRP ve prokalsitonin değeri mortalite ve bakteriyemisi olan hastalarda yüksek bulunmuştur. Tanı anında lökosit ve nötrofil sayısının mortaliteye etkisi olmadığı saptandı. Nötropeni süresi uzaması, kandolaşım enfeksiyonu, kateter ilişkili kandolaşım enfeksiyonu ve pnömoni varlığı mortalite açısından risk faktörü olarak saptanmıştır.
Sonuç: CRP ve prokalsitonin değerlerinin mortalite göstergesi olarak değerlendirildiği çalışmamızda hem prognostik hem de tanısal olarak kullanılabileceği öngörülmüştür. Mortalitesi olan hastalarda dirençli gram-negatif mikroorganizmaların daha hakim olduğu saptanmış olup yeni tedavi algoritmalarının geliştirilmesi için daha çok çalışmaya gereksinim duyulmaktadır.

Anahtar kelimeler: Febril nötropeni, C-reaktif protein, prokalsitonin, mortalite.

Öksüzoğlu H, Mert D, İskender G, Ulu Demirci N, Ertek M. Febril nötropenik hastalarda C-reaktif protein ve prokalsitoninin mortalite göstergesi olarak değerlendirilmesi. Pam Tıp Derg 2025;18:384-395.

Introduction

Intensive and high-dose chemotherapy leads to infectious complications in cancer patients, posing significant challenges in clinical management. Bacterial and fungal infections are the primary causes of morbidity and mortality in these patients [1, 2]. Inflammation and infection often progress with subtle clinical signs and symptoms, making early detection difficult. In neutropenic patients, fever is frequently the sole manifestation of infection.

In 30-50% of patients, fever is attributed to infections classified as clinical or microbiological; however, the causative agent remains unidentified in the remaining cases. Therefore, there is a need for specific, highly effective, and rapid markers for early infection detection.

C-reactive protein (CRP) is frequently used for this purpose, as it serves as a key indicator of the inflammatory response to infection [3-5]. Additionally, procalcitonin has been reported to be useful in detecting bacterial infections in neutropenic patients [6].

This study aimed to assess the effectiveness of CRP and procalcitonin in the early detection of infection and mortality by comparing their quantitative values at the onset of febrile neutropenia (FN) and during treatment.

Materials and methods

A total of 572 patients who were followed up in the Hematology Clinic, Bone Marrow Transplantation Unit, and Medical Oncology Clinic for febrile neutropenia (FN) between September 3, 2018, and May 25, 2022, were included in the study. These patients experienced a total of 748 FN episodes.

This study was reviewed and approved by the Ethics Committee of Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital (approval date: 24.02.2021, approval number: 2021-02/1045).

Patients with a single body temperature measurement of ≥38.3°C or a body temperature of ≥38.0°C persisting for more than one hour, along with an expected neutrophil count of ≤500 cells/mm³ or a neutrophil count falling below 500 cells/mm³ within 24-48 hours, were included in the study with a diagnosis of febrile neutropenia (FN).

Patient data were collected by reviewing the hospital information management system and recorded in a standardized form. The form included the following variables: name and surname, age, gender (female or male), underlying malignancy [hematological malignancy or solid organ tumor (with/without metastasis)], presence of comorbid diseases, specific comorbid conditions [diabetes mellitus (DM), hypertension (HT), coronary artery disease (CAD), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD)], mortality status (yes or no), CRP and procalcitonin levels on days 0, 3, 5, 7, 10, 14, and 21, fever measurement, fever duration, duration of neutropenia, treatment duration, history of antibiotic use in the past three months (yes or no), blood culture growth (yes or no), identified pathogens in blood culture, and antibiogram results.

Statistical analysis

Statistical analyses were performed using the Statistical Program for Social Sciences (IBM SPSS Statistics 26.0) package program.

The normality of the data distribution was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk tests. In descriptive analyses, mean and standard deviation were used for continuous variables, while categorical variables were presented as frequencies and percentages. When parametric test assumptions were not met, the Mann-Whitney U test was applied for continuous data comparisons. For categorical data, Pearson's chi-square, Fisher's exact, or Fisher-Freeman-Halton tests were used to compare groups.

This study utilized advanced statistical and machine learning methods to identify predictors of mortality in patients with febrile neutropenia. Key variables, including fever duration, CRP change rate, neutropenia duration, and additional clinical factors such as blood culture results and comorbidities, were analyzed for their association with mortality. To conduct logistic regression analysis, CRP and procalcitonin values on days 7, 10, 14, and 21 were included for both mortality and non-mortality groups. Multiple imputation was applied to estimate missing values and standardize all measurements, as approximately half of the sample had missing data for these variables.

Statistical analyses, including boxplots and histograms, revealed significant differences in the distribution of key predictors between mortality and non-mortality groups. Machine learning models, such as Gradient Boosting and XGBoost, were evaluated for predictive performance, with the Random Forest model achieving the highest ROC-AUC score (0.86). All analyses were conducted using Python, with a significance threshold set at $p<0.05$.

Results

A total of 572 patients were included in the study. Hematological malignancies were identified in 348 patients (60.8%), while solid organ tumors were detected in 224 patients (39.2%). Among patients with hematological malignancies, 155 (44.5%) were female, and 193 (55.5%) were male. In the solid organ tumor group, 126 (56.25%) were female, and 98 (43.75%) were male.

In total, 748 febrile neutropenia (FN) episodes occurred among the 572 patients. Among those with hematological malignancies, the highest number of FN episodes was observed in patients with acute leukemia.

Mortality occurred in 118 patients (20.6%) during a febrile neutropenia (FN) episode. Patients were categorized into two groups: FN with mortality and FN without mortality. No significant differences were observed between the groups regarding age, underlying malignancy, presence of comorbidities, diabetes mellitus (DM), hypertension (HT), or chronic obstructive pulmonary disease (COPD) (Table 1).

The proportion of male patients was significantly higher in the mortality group than in the non-mortality group ($\chi^2=4.425$, $p=0.035$). Additionally, the presence of metastases in patients with solid organ malignancies was significantly more common in the mortality group compared to the non-mortality group ($\chi^2=7.308$, $p=0.007$). The prevalence of coronary artery disease (CAD) ($p<0.001$) and chronic renal failure (CRF) ($p=0.007$) was also significantly higher in the mortality group (Table 1).

CRP and procalcitonin values on days 0, 3, 5, 7, 10, 14, and 21 were significantly higher in the mortality group compared to the non-mortality group ($p<0.001$ for all) (Table 2).

Fever levels, fever duration, and neutropenia duration were significantly higher in the mortality group compared to the non-mortality group ($p<0.001$ for all). However, there was no significant difference between the groups in terms of treatment duration ($p=0.900$) (Table 3).

The rate of antibiotic use in the past three months was significantly higher in the mortality group than in the non-mortality group ($\chi^2=42.174$, $p<0.001$) (Table 3).

FN patients were categorized into two groups based on the presence or absence of bacterial growth in blood cultures. These groups were compared in terms of CRP and procalcitonin levels on days 0, 3, 5, 7, 10, 14, and 21.

Table 1. Comparison of socio-demographic characteristics and underlying diseases between groups

	FN group with mortality (N=118)	Non-mortality FN group (N=630)	Statistics	
			χ^2 or U	*p*
Age, mean (SD)	51.6 (16.8)	50.5 (16.3)	3601.0[1]	0.590
Gender, n (%)				
Female	46 (39.0%)	312 (49.5%)	4.425[2]	0.035*
Male	72 (61.0%)	318 (50.5%)		
Malignancy, n (%)				
Hematological malignancy	81 (68.6%)	422 (67.0%)	0.124[2]	0.724
Solid organ tumor	37 (31.4%)	208 (33.0%)		
With metastases	26 (22.0%)	96 (15.2%)	7.308[2]	0.007*
Without Metastases	11 (9.3%)	112 (17.7%)		
Comorbidity, n (%)	49 (41.5%)	235 (37.3%)	0.753[2]	0.386
Diagnosis of DM	31 (26.3%)	143 (22.7%)	0.711[2]	0.399
Diagnosis of HT	25 (21.2%)	150 (23.8%)	0.382[2]	0.537
Diagnosis of CAD	23 (19.5%)	45 (7.1%)	18.338[2]	<0.001*
Diagnosis of CRF	9 (7.6%)	15 (2.4%)	-[3]	0.007*
Diagnosis of COPD	5 (4.2%)	20 (3.2%)	-[3]	0.575
None	69 (58.5%)	395 (62.7%)		

FN: febrile neutropenia, DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease CRF: chronic renal failure COPD: chronic obstructive pulmonary disease, 1- Mann-Whitney U, 2- Pearson Chi-Square, 3 Fisher Exact Test; *p<0.05

Table 2. Comparison of CRP and procalcitonin values between groups

	FN group with mortality	Non-mortality FN group	Statistics	
	Mean. (SD)		U[1]	*p*
CRP (mg/L)				
(n=118-630)	201.3 (118.5)	149.5 (89.9)	27548.5	<0.001*
Day 3 (n=103-622)	204.3 (108.8)	115.6 (83.4)	16291.5	<0.001*
Day 5 (n=87-543)	187.4 (72.4)	76.5 (72.4)	8748.0	<0.001*
Day 7 (n=75-462)	187.9 (106.0)	56.5 (63.8)	4752.0	<0.001*
Day 10 (n=60-333)	214.0 (121.0)	45.6 (60.0)	1862.5	<0.001*
Day 14 (n=43-175)	216.6 (121.6)	39.8 (55.6)	491.0	<0.001*
Day 21 (n=25-32)	216.5 (80.7)	32.1 (30.3)	6.0	<0.001*
Procalsitonin (mcg/L)				
Day 0 (n=106-489)	15.4 (25.7)	6.4 (15.0)	17817.0	<0.001*
Day 3 (n=77-409)	19.1 (32.2)	4.5 (12.5)	8236.5	<0.001*
Day 5 (n=68-339)	11.3 (21.6)	2.2 (7.7)	5698.5	<0.001*
Day 7 (n=59-293)	13.8 (25.0)	0.6 (1.5)	3160.5	<0.001*
Day 10. (n=45-187)	19.8 (42.5)	0.3 (1.1)	889.5	<0.001*
Day 14 (n=37-112)	12.5 (20.9)	0.3 (1.3)	173.0	<0.001*
Day 21 (n=22-19)	21.9 (26.3)	0.1 (0.1)	0.0	<0.001*

FN: febrile neutropenia, CRP: C-reactive protein, 1- Mann-Whitney U; *p<0.05

Table 3. Comparison of the groups in terms of fever value, duration of fever, duration of neutropenia, duration of treatment and antibiotic use in the last three months

	FN group with mortality	Non-mortality FN group	Statistic	
			χ^2 veya U	*p*
Fever (ºC), mean. (SD)	39.1 (0.4)	38.7 (0.3)	20923.0[1]	<0.001*
Duration of fever (days), mean. (SD)	6.7 (5.7)	2.2 (1.6)	12421.5[1]	<0.001*
Neutropenia duration, mean. (SD)	9.0 (7.4)	4.4 (3.8)	22433.5[1]	<0.001*
Duration of treatment, mean. (SD)	11.4 (7.9)	10.4 (4.8)	36902.0[1]	0.900
Antibiotic use in the last three months, n (%)				
Yes	92 (78%)	286 (45.4%)	42.174[2]	<0.001*
No	26 (22%)	344 (54.6%)		

FN: febrile neutropenia, 1- Mann Whitney U, 2- Pearson Chi-Square; *p<0.05

CRP values were significantly higher in the FN group with blood culture growth compared to the FN group without growth on days 0 (p<0.001), 3 (p<0.001), 5 (p<0.001), 10 (p=0.037), and 14 (p=0.022). However, no significant difference was observed between the groups for CRP values on days 7 (p=0.129) and 21 (p=0.598) (Table 4).

Procalcitonin values were significantly higher in the FN group with blood culture growth compared to the group without growth on days 0 (p<0.001), 3 (p<0.001), 5 (p<0.001), 7 (p=0.001), and 21 (p=0.047). However, no significant difference was observed between the groups in terms of CRP values on days 10 (p=0.617) and 14 (p=0.332) (Table 4).

When comparing microorganisms identified in blood cultures, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, coagulase-negative *Staphylococci*, *Enterococcus faecium*, and *Candida albicans* were found at significantly higher rates in the FN group with mortality than in the FN group without mortality (χ^2=51.334, p<0.001) (Table 5).

The presence of at least one antibiotic-resistant microorganism in blood cultures was significantly higher in the FN group with mortality compared to the FN group without mortality (χ^2=4.093, p=0.043) (Table 6).

Subsequently, CRP and procalcitonin values on days 0, 3, 7, 14, and 21, which significantly predicted mortality, were analyzed using the backward conditional method. The most significant model identified CRP on days 3, 10, 14, and 21, and procalcitonin on days 0, 5, 10, and 21 as key predictors. The obtained model was statistically significant (p<0.001) and explained 53.4% of the variance in mortality outcomes (R^2=0.534).

The Random Forest algorithm was used to determine the relative importance of variables in predicting mortality. The top predictors identified were fever duration (25%), CRP change rate (15%), and neutropenia duration (13%). Additional variables, including CRP levels, procalcitonin levels, blood culture results indicating resistant Gram-negative bacteria (7%), comorbidities (6%), and prior antibiotic use (5%), also demonstrated significant associations with increased mortality risk. Age (4%) and male sex (3%) contributed relatively less but remained consistent predictors of mortality (Table 7, Figure 1).

Table 4. Comparison of CRP and procalcitonin values according to the growth status in blood culture of the groups

	FN group with growth in blood culture	FN group without growth in blood culture	Statistic	
	Mean. (SD)		U[1]	*p*
CRP (mg/L)				
Day 0 (n=475-273)	175.5 (90.5)	147.5 (98.9)	50689.0	<0.001*
Day 3 (n=461-264)	157.0 (98.4)	111.7 (85.2)	43194.5	<0.001*
Day 5 (n=385-245)	110.5 (95.3)	79.9 (80.2)	36285.5	<0.001*
Day 7 (n=306-231)	77.5 (84.9)	72.7 (83.8)	32637.5	0.129
Day 10. (n=205-188)	66.0 (96.5)	76.2 (92.6)	16929.5	0.037*
Day 14 (n=109-109)	66.1 (99.3)	83.2 (103.5)	4873.0	0.022*
Day 21 (n=29-28)	123.8 (115.2)	102.3 (103.1)	373.0	0.598
Procalcitonin (mcg/L)				
Day 0 (n=354-241)	13.5 (22.8)	4.3 (11.8)	25694.5	<0.001*
Day 3 (n=281-205)	11.0 (23.5)	3.7 (11.6)	17285.0	<0.001*
Day 5 (n=236-171)	5.2 (13.7)	2.6 (10.0)	14445.5	<0.001*
Day 7 (n=188-164)	4.2 (15.1)	1.6 (6.4)	12232.0	0.001*
Day 10 (n=118-114)	6.2 (27.1)	2.1 (9.0)	6470.5	0.617
Day 14 (n=72-77)	2.9 (9.1)	3.8 (13.9)	2516.5	0.332
Day 21 (n=19-22)	19.4 (27.7)	3.0 (5.1)	133.0	0.047*

FN: febrile neutropenia, CRP: C-reactive protein,1- Mann Whitney U; *p<0.05

Table 5. Comparison of pathogens grown in blood culture between groups

Blood culture	FN group with mortality (N=66)	Non-mortality FN group (N=207)	Statistic	
	n (%)		(χ^2)[1]	*p*
Escherichia coli	23 (34.8%)	100 (48.3%)		
Klebsiella pneumoniae	14 (21.2%)	29 (14.0%)		
Pseudomonas aeruginosa	9 (13.6%)	6 (2.9%)		
Acinetobacter baumannii	6 (9.1%)	2 (1.0%)		
Stenotrophomonas maltophilia	0 (0.0%)	1 (1.5%)		
Coagulase negative staphylococci	4 (6.1%)	53 (25.6%)		
Staphylococcus aureus	2 (3.0%)	6 (2.9%)	51.334	<0.001*
Enterococcus faecium	3 (4.5%)	1 (0.5%)		
Streptococcus pyogenes	0 (0.0%)	1 (0.5%)		
Candida albicans	3 (4.5%)	0 (0.0%)		
Candida krusei	1 (1.5%)	1 (0.5%)		
Burkholderia cepacia	0 (0.0%)	1 (0.5%)		
Enterobacter cloacae	0 (0.0%)	6 (2.9%)		

FN: febrile neutropenia, 1 Fisher-Freeman-Halton Test; *p<0.05

Table 6. Comparison of the presence of resistant microorganisms in blood culture between groups

	FN group with mortality	Non-mortality FN group	Statistic	
	n (%)		$(\chi^2)^1$	*p*
Resistant microorganism in blood culture	41 (62.1%)	99 (47.8%)	4.093	0.043*

FN: febrile neutropenia, 1 Pearson Chi-Square; *: $p<0.05$

Table 7. Comprehensive predictor summary table for mortality

Predictor	Impact on Mortality	Relative Importance (Random Forest)
Fever Duration	Longer fever duration is associated with higher mortality risk	25
CRP Change Rate	Slower reductions or increases in CRP levels indicate higher mortality risk	15
Neutropenia Duration	Prolonged neutropenia duration increases the risk of mortality	13
CRP	Elevated CRP levels are associated with higher mortality	10
Procalcitonin	Higher procalcitonin levels are linked to severe infections and mortality	8
Blood Culture Results	Presence of resistant Gram-negative bacteria increases mortality risk	7
Comorbidities	Chronic comorbidities, such as diabetes or chronic kidney disease, are associated with higher mortality	6
Prior Antibiotic Use	Prior antibiotic use within three months increases the likelihood of resistant infections and mortality	5
Age	Older age is associated with higher mortality risk	4
Gender	Male sex is linked to increased mortality risk	3

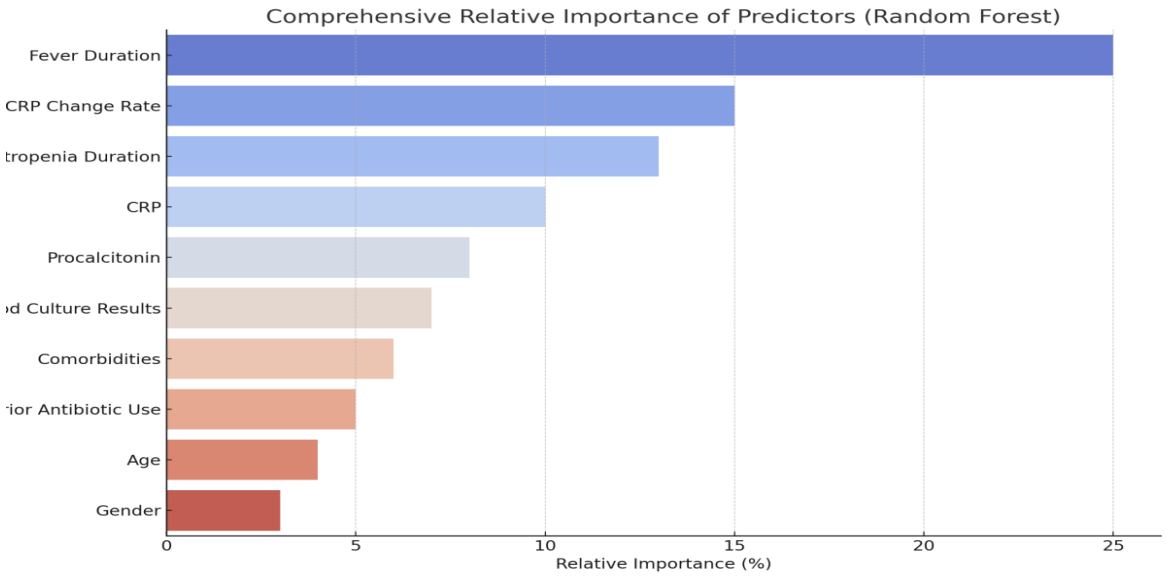

Figure 1. Relative importance of predictors (Random Forest)

Discussion

Among all patients, 20.6% (118) died due to FN. The mortality rate was 16.5% (37) among patients with solid organ tumors and 23.3% (81) among those with hematological malignancies.

In a multicenter study by Kuderer et al. [7], the mortality rate was reported as 11% among 55,276 FN patients. Similarly, Ghosh et al. [8] found an FN-related mortality rate of 19.5% in patients with hematological malignancies. In contrast, Du et al. [9] reported a lower mortality rate of 4.6%, while Hatamabadi et al. [10], in a study focusing on patients with solid organ tumors, found a mortality rate of 5.3%. The variability in mortality rates observed in this study and the literature is likely influenced by factors such as malignancy type, chemotherapy regimens, comorbid conditions, and the specific microorganisms identified in culture.

In this study, male gender, coronary artery disease (CAD), and chronic renal failure (CRF) were found to be significant predictors of FN-related mortality. In a study by Hatamabadi et al. [10], advanced age and the presence of additional comorbidities were associated with increased mortality; however, gender was not found to be a significant factor. Kuderer et al. [7] reported that the presence of comorbidities increased mortality by 2.8%. Similarly, in a study by Lyman et al. [11] involving 5,990 FN patients, being over 65 years of age and having comorbid conditions were significant predictors of mortality. Additionally, Hosmer et al. [12] found that age over 65, as well as the presence of CAD, CRF, and COPD, were associated with increased mortality risk. While comorbidities alone may not directly impact mortality, the findings of this study suggest that they are strongly associated with mortality in elderly patients.

This study found that CRP and procalcitonin levels were consistently elevated on all measured days in the FN group with mortality. In a study by Reyes Mondragon et al. [13], a procalcitonin level above 0.46 ng/mL was identified as an effective predictor of septic shock and mortality. Additionally, CRP has been recognized as an important prognostic marker in FN patients [14]. A study investigating the etiology of fever in patients with hematological malignancies in northern India reported that elevated CRP was a better predictor than procalcitonin for malignancy-related fever [15]. Conversely, in a study conducted on FN patients admitted to the emergency department, procalcitonin was found to be a superior predictor of mortality compared to CRP [16]. Similarly, the findings of this study indicate that both CRP and procalcitonin levels were significantly higher in the FN group with mortality, reinforcing their prognostic value.

Prolonged neutropenia in FN episodes increases the risk of complications. In previous studies, the mean duration of neutropenia was reported as 12 days in FN cases with mortality in one study and 15 days in another [17, 18]. In this study, the mean duration of neutropenia in the mortality group was found to be nine days. In contrast, one study reported a shorter mean neutropenia duration of 3.3 days in the mortality group [19]. However, the same study found that prolonged neutropenia was significantly associated with increased mortality risk [19]. Similarly, in this study, the duration of neutropenia was longer in the FN group with mortality, reinforcing its role as a critical factor in patient outcomes.

In a study by Mert et al. [17], prolonged antibiotic therapy was not found to be a significant factor in mortality; however, antibiotic use in the last three months was significantly higher in the mortality group. Similarly, in this study, the duration of antibiotic therapy was not associated with mortality, whereas prior antibiotic use in the last three months was significantly more common in the FN group with mortality.

In this study, the presence of bloodstream infections was significantly higher in FN patients who did not survive. Similarly, a study involving 85 patients who underwent bone marrow transplantation found bloodstream infections to be a significant predictor of mortality [20]. In a study conducted in Lebanon covering 177 FN episodes between 1995 and 2001, the risk of mortality was reported to be higher in patients with positive blood culture results [21]. Another study also indicated that mortality rates were elevated in patients with confirmed bloodstream infections [22]. Additionally, Feld et al. [23] highlighted the high mortality rate associated with bloodstream infections and emphasized the need for new treatment strategies.

In this study, CRP levels measured on days 0, 3, and 5 were found to be higher in the group with positive blood culture growth. Similarly, procalcitonin levels were significantly elevated in the same group on days 0, 3, 5, and 7. In a study by Ruokonen et al. [6], procalcitonin was reported to be a specific marker in febrile neutropenic episodes; however, it was not considered a highly sensitive marker for detecting infections.

One study reported that serum CRP levels were more sensitive than procalcitonin in detecting bacteremia; however, its specificity was low [24]. Another study found that CRP levels were higher than procalcitonin in cases of neutropenic fever of unknown origin [15]. Additionally, a separate study suggested that the combined use of procalcitonin and lectin-binding protein may have diagnostic value in detecting bacteremia [25]. In this study, the observation that CRP and procalcitonin levels were elevated in patients with bacteremia during the early days of FN episodes suggests their potential diagnostic utility in predicting bacteremia.

Escherichia coli was the most frequently isolated pathogen in blood cultures, followed by *Klebsiella pneumoniae*. The prevalence of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Candida albicans* was significantly higher in the mortality group. Similarly, in a study by Wang et al. [20] investigating FN episodes, *E. coli* was the most commonly identified pathogen in blood cultures. Another study conducted in FN patients also reported *E. coli* as the most abundant microorganism [26]. Additionally, a separate study found that *A. baumannii*, *Enterococcus* spp., and *C. albicans* were associated with higher mortality rates [27]. In a multicenter study, *P. aeruginosa* bacteremia was reported to have the highest mortality rate among patients with hematological malignancies and was linked to poor prognosis [28]. Furthermore, in a study examining FN episodes in 589 acute leukemia patients, *E. coli* was the most frequently detected pathogen in blood cultures [29]. The same study also found that *P. aeruginosa* and *Enterococcus* spp. were associated with higher mortality rates [29].

In this study, high resistance rates to carbapenem and other antibiotic groups were observed among all microorganisms isolated from blood cultures. Consequently, treatment failure and mortality rates were significantly higher in patients with resistant bacterial infections.

This study provides valuable insights into the predictors of mortality in febrile neutropenia patients by integrating machine learning methods with clinical variables. The Random Forest model identified fever duration, CRP change rate, and neutropenia duration as the most significant factors in mortality risk stratification [4, 7].

Fever duration emerged as the strongest predictor of mortality, with prolonged fever indicating poor infection control and an increased risk of adverse outcomes [1, 7]. Similarly, the CRP change rate was identified as a crucial marker of treatment response, with slower reductions or persistent increases in CRP levels being strongly associated with mortality [4, 6]. Regular monitoring of CRP levels may facilitate the early identification of high-risk patients, allowing for timely intervention [3, 5].

Neutropenia duration, as an indicator of ongoing immunosuppression, was identified as another key predictor of mortality. Longer durations were associated with increased complications and mortality, underscoring the need for targeted interventions in high-risk patients [8, 13]. Additionally, blood culture results-particularly the presence of resistant Gram-negative bacteria-were strongly linked to mortality. This finding highlights the critical importance of implementing targeted antibiotic therapies based on local microbiological profiles to improve patient outcomes [20, 21].

Comorbidities such as diabetes and chronic kidney disease, along with prior antibiotic use within the last three months, were associated with an increased risk of mortality [11, 19]. Additionally, age and male sex were identified as moderate predictors, supporting their inclusion in risk assessment models [12, 22].

The Random Forest model demonstrated superior performance compared to other models, providing balanced predictions and

a clear ranking of variable importance [2, 9]. This study highlights the potential of machine learning models in integrating clinical and microbiological variables to enhance mortality prediction in febrile neutropenia patients. However, validation through larger datasets and prospective studies is essential to ensure the generalizability and accuracy of these findings.

Strengths and limitations of the study

One of the key strengths of this study is its large sample size, with a total of 748 FN episodes analyzed. CRP and procalcitonin levels, fever measurements and duration, neutropenia duration, treatment duration, and blood culture results were systematically evaluated on days 0, 3, 5, 7, 14, and 21.

The primary limitation of this study is its retrospective design, which prevented precise determination of the exact day of mortality during FN episodes. More consistent and generalizable findings could be achieved through prospective studies that specifically assess mortality timing in this context.

In conclusion, CRP and procalcitonin levels were found to be significantly higher during the first five days of FN episodes in the mortality group, suggesting their potential utility as prognostic markers. However, their specific predictive value for mortality remains uncertain. Instead, their sequential decline following treatment may serve as an indicator of treatment response, while persistently elevated or increasing levels in the following days may help predict mortality.

Additionally, CRP and procalcitonin demonstrated diagnostic value in FN episodes, beyond their prognostic role. Notably, CRP and procalcitonin levels measured within the first five days were significantly higher in cases of bacteremia, further supporting their use in clinical decision-making.

In this study, the bacteremia rate was 36.5%, with *Escherichia coli* being the most frequently isolated microorganism. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were identified in the blood cultures of patients in the mortality group, all exhibiting multidrug resistance. A notable increase in Gram-negative bacterial infections was observed during FN episodes.

To optimize treatment strategies, healthcare facilities should routinely monitor local microorganism profiles and antibiotic resistance patterns, ensuring that empirical antibiotic therapy is updated accordingly. Identifying risk factors for mortality in FN episodes will contribute to the development of more effective treatment protocols. Further studies are needed to refine these protocols based on evolving clinical conditions, pathogen profiles, and antibiogram patterns.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Declaration of competing interest: The authors have no relevant financial or non-financial interests to disclose.

Authors contributions: H.O. and D.M. constructed the main idea and hypothesis of the study. M.E. and G.I. developed the theory and arranged/edited the material and method section. N.U.D. has done the evaluation of the data in the Results section. Discussion section of the article was written by H.O. D.M. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Südhoff T, Giagounidis A, Karthaus M. Evaluation of neutropenic fever: value of serum and plasma parameters in clinical practice. *Chemotherapy*. 2000;46(2):77-85. doi:10.1159/000007259
2. Feld R. Multinational cooperation in trials and guidelines dealing with febrile neutropenia. *Int J Antimicrob Agents*. 2000;16(2):185-187. doi:10.1016/s0924-8579(00)00240-5
3. Fleischhack G, Kambeck I, Cipic D, Hasan C, Bode U. Procalcitonin in paediatric cancer patients: its diagnostic relevance is superior to that of C-reactive protein, interleukin 6, interleukin 8, soluble interleukin 2 receptor and soluble tumour necrosis factor receptor II. *Br J Haematol*. 2000;111(4):1093-1102. doi:10.1046/j.1365-2141.2000.02458.x
4. Massaro KS, Costa SF, Leone C, Chamone DA. Procalcitonin (PCT) and C-reactive protein (CRP) as severe systemic infection markers in febrile neutropenic adults. *BMC Infect Dis*. 2007;7:137. Published 2007 Nov 22. doi:10.1186/1471-2334-7-137

5. Schuttrumpf S, Binder L, Hagemann T, Berkovic D, Trumper L, Binder C. Utility of procalcitonin concentration in the evaluation of patients with malignant diseases and elevated C-reactive protein plasma concentrations. *Clin Infect Dis*. 2006;43(4):468-473. doi:10.1086/505394
6. Ruokonen E, Nousiainen T, Pulkki K, Takala J. Procalcitonin concentrations in patients with neutropenic fever. *Eur J Clin Microbiol Infect Dis*. 1999;18(4):283-285. doi:10.1007/s100960050277
7. Kuderer NM, Dale DC, Crawford J, Cosler LE, Lyman GH. Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. *Cancer*. 2006;106(10):2258-2266. doi:10.1002/cncr.21847
8. Ghosh S, Chakraborty M, Samanta S, et al. Analysis of blood stream infections, antibiograms and clinical outcomes in haematological patients with febrile neutropenia: data from a tertiary care haematology institute in India. *Ann Hematol*. 2021;100(2):395-403. doi:10.1007/s00277-020-04324-8
9. Du X, Min J, Shah CP, Bishnoi R, Hogan WR, Lemas DJ. Predicting in-hospital mortality of patients with febrile neutropenia using machine learning models. *Int J Med Inform*. 2020;139:104140. doi:10.1016/j.ijmedinf.2020.104140
10. Hatamabadi H, Arhami Dolatabadi A, Akhavan A, Safari S. Clinical Characteristics and Associated Factors of Mortality in Febrile Neutropenia Patients; a Cross Sectional Study. *Arch Acad Emerg Med*. 2019;7(1):39. Published 2019 Jul 27.
11. Lyman GH, Michels SL, Reynolds MW, Barron R, Tomic KS, Yu J. Risk of mortality in patients with cancer who experience febrile neutropenia. *Cancer*. 2010;116(23):5555-5563. doi:10.1002/cncr.25332
12. Hosmer W, Malin J, Wong M. Development and validation of a prediction model for the risk of developing febrile neutropenia in the first cycle of chemotherapy among elderly patients with breast, lung, colorectal, and prostate cancer. *Support Care Cancer.* 2011;19(3):333-341. doi:10.1007/s00520-010-0821-1
13. Reyes Mondragón AL, Cantú Rodríguez OG, Garza Acosta AC, et al. Performance of serum procalcitonin as a biochemical predictor of death in hematology patients with febrile neutropenia. *Blood Cells Mol Dis*. 2021;90:102586. doi:10.1016/j.bcmd.2021.102586
14. Şahin S, Gencer S, Dogan M, Demirhan G, Özer S. Febril Nötropenik Olgularımızda C-Reaktif Proteinin İnfeksiyon ve Mortalite Göstergesi Olarak İncelenmesi. *Flora*. 2009;14(2):72-80.
15. Halder R, Seth T, Chaturvedi PK, et al. Comparison of CRP and procalcitonin for etiological diagnosis of fever during febrile neutropenia in hematology patients- an experience from a tertiary care center in Northern India. *Blood Cells Mol Dis*. 2020;84:102445. doi:10.1016/j.bcmd.2020.102445
16. Coyne CJ, Castillo EM, Shatsky RA, Chan TC. Procalcitonin as a Predictive Tool for Death and ICU Admission among Febrile Neutropenic Patients Visiting the Emergency Department. *Medicina (Kaunas)*. 2022;58(8):985. Published 2022 Jul 23. doi:10.3390/medicina58080985
17. Mert D, Ceken S, Iskender G, et al. Epidemiology and mortality in bacterial bloodstream infections in patients with hematologic malignancies. *J Infect Dev Ctries*. 2019;13(8):727-735. Published 2019 Aug 31. doi:10.3855/jidc.11457
18. Demirel A, Tabak F, Ar MC, et al. Secondary Infections in Febrile Neutropenia in Hematological Malignancies: More Than Another Febrile Neutropenic Episode. *Turk J Haematol*. 2015;32(3):243-250. doi:10.4274/tjh.2013.0422
19. Özden M, Denk A, Demirdağ K, Elkıran T. Febril nötropenik olgular ve risk faktörlerinin değerlendirilmesi. *Mediterr J Infect Microb Antimicrob*. 2013;2:3.
20. Wang L, Wang Y, Fan X, Tang W, Hu J. Prevalence of Resistant Gram-Negative Bacilli in Bloodstream Infection in Febrile Neutropenia Patients Undergoing Hematopoietic Stem Cell Transplantation: A Single Center Retrospective Cohort Study. *Medicine (Baltimore)*. 2015;94(45):e1931. doi:10.1097/MD.0000000000001931
21. Kanafani ZA, Dakdouki GK, El Chammas KI, Eid S, Araj GF, Kanj SS. Bloodstream infections in febrile neutropenic patients at a tertiary care center in Lebanon: a view of the past decade. *Int J Infect Dis*. 2007;11(5):450-453. doi:10.1016/j.ijid.2006.12.008
22. Horasan ES, Ersoz G, Tombak A, Tiftik N, Kaya A. Bloodstream infections and mortality-related factors in febrile neutropenic cancer patients. *Med Sci Monit*. 2011;17(5):CR304-CR309. doi:10.12659/msm.881773
23. Feld R. Bloodstream infections in cancer patients with febrile neutropenia. *Int J Antimicrob Agents*. 2008;32(Suppl1):S30-S33. doi:10.1016/j.ijantimicag.2008.06.017
24. Prat C, Sancho JM, Dominguez J, et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL-6 and IL-8 as a diagnostic marker of infection in patients with febrile neutropenia. *Leuk Lymphoma*. 2008;49(9):1752-1761. doi:10.1080/10428190802258956

25. García de Guadiana Romualdo L, Español Morales I, Cerezuela Fuentes P, et al. Value of lipopolysaccharide binding protein as diagnostic marker of infection in adult cancer patients with febrile neutropenia: comparison with C-reactive protein, procalcitonin, and interleukin 6. *Support Care Cancer*. 2015;23(7):2175-2182. doi:10.1007/s00520-014-2589-1

26. Lakshmaiah KC, Malabagi AS, Govindbabu, Shetty R, Sinha M, Jayashree RS. Febrile Neutropenia in Hematological Malignancies: Clinical and Microbiological Profile and Outcome in High Risk Patients. *J Lab Physicians*. 2015;7(2):116-120. doi:10.4103/0974-2727.163126

27. Kara Ali R, Surme S, Balkan II, et al. An eleven-year cohort of bloodstream infections in 552 febrile neutropenic patients: resistance profiles of Gram-negative bacteria as a predictor of mortality. *Ann Hematol*. 2020;99(8):1925-1932. doi:10.1007/s00277-020-04144-w

28. Aleissa MM, Gonzalez Bocco IH, Zekery Saad S, et al. The Relationship Between Antibiotic Agent and Mortality in Patients With Febrile Neutropenia due to Staphylococcal Bloodstream Infection: A Multicenter Cohort Study. *Open Forum Infect Dis*. 2022;9(8):ofac306. Published 2022 Jun 20. doi:10.1093/ofid/ofac306

29. Garcia Vidal C, Cardozo Espinola C, Puerta Alcalde P, et al. Risk factors for mortality in patients with acute leukemia and bloodstream infections in the era of multiresistance. *PLoS One*. 2018;13(6):e0199531. Published 2018 Jun 28. doi:10.1371/journal.pone.0199531

Research Article
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1630111

Awareness of salt consumption in outpatients admitted to the Nephrology clinic

Nefroloji kliniğine ayaktan başvuran hastalarda tuz tüketimi farkındalığı

Davut Akın

Posted date:30.01.2025 Acceptance date:27.03.2025

Abstract

Purpose: Excess salt consumption contributes significantly to the development of kidney disease, hypertension and heart disease and makes their control difficult. The aim of this study was to investigate the daily salt consumption levels and awareness of salt consumption in outpatients attending a nephrology clinic.

Materials and methods: This descriptive cross-sectional study was conducted between January 10, 2024 and March 10, 2024. Data were collected from individuals aged 18 years and older who were admitted to Pamukkale University Nephrology clinic as outpatients and who agreed to participate in the study by face-to-face questionnaire method. The Beliefs about Dietary Compliance Scale was used to assess salt consumption habits.

Results: The mean age of the 211 patients who participated in the study was 58.94±16.84 years. It was determined that 82.5% (n=174) of the participants knew the recommended daily salt amount as 5 grams and 66.8% of them consumed their meals with little or no salt. Salt was mostly consumed as table salt (63.3% n=133), followed by natural salt found in foods (30.3% n=64) and least from prepared foods (6.6% n=14). The mean score of the benefit subscale was 29.3±2.7 and the mean score of the barrier subscale was 19.4±4.2.

Conclusion: A large proportion (82.5%) of the patients admitted to the Nephrology Clinic as outpatients stated that they were aware of and complied with the daily salt consumption of 5 gr recommended by the guidelines. However, this awareness does not result in a sufficient behavioral change in daily life.

Keywords: Kidney diseases, hypertension, amount of salt in the diet, salt intake.

Akın D. Awareness of salt consumption in outpatients admitted to the Nephrology clinic. Pam Med J 2025;18:398-405.

Öz

Amaç: Fazla tuz tüketimi, böbrek hastalığı, hipertansiyon ve kalp hastalığı gelişimine önemli ölçüde katkıda bulunur ve bunların kontrolünü zorlaştırır. Bu çalışmanın amacı, nefroloji kliniğine ayaktan başvuran hastaların günlük tuz tüketim düzeylerini ve tuz tüketimi konusundaki farkındalıklarını araştırmaktır.

Gereç ve yöntem: Çalışma tanımlayıcı kesitsel tipte olup 10 Ocak 2024 ile 10 Mart 2024 tarihleri arasında Pamukkale Üniversitesi Nefroloji kliniğine ayaktan başvuran, 18 yaş ve üzeri, çalışmaya katılmayı kabul eden bireylerden veriler, yüz yüze gerçekleştirilen anket yöntemiyle toplanmıştır. Tuz tüketim alışkanlıklarını değerlendirmek için Diyet Uyumuna İlişkin İnançlar Ölçeği kullanılmıştır.

Bulgular: Çalışmaya katılan 211 hastanın yaş ortalaması 58,94±16,84'tür. Katılımcıların %82,5'inin (n=174) önerilen günlük tuz miktarını 5 gram olarak bildiği ve %66,8'inin yemeklerini az tuzlu ya da tuzsuz olarak tükettiği belirlenmiştir. Tuz, en fazla sofra tuzu (%63,3, n=133) olarak tüketilirken, bunu gıdalarda doğal olarak bulunan tuz (%30,3, n=64) ve en az hazır gıdalardaki tuz (%6,6, n=14) takip etmiştir. Diyete Uyum Hakkındaki İnançlar Ölçeği yarar alt ölçeğinin ortalama puanı 29,3±2,7 ve engel alt ölçeğinin ortalama puanı 19,4±4,2'dir.

Sonuç: Nefroloji kliniğine ayaktan başvuran hastaların büyük bir kısmı (%82,5) kılavuzlarda önerilen günlük 5 gr tuz tüketiminin farkında olduklarını ve buna uyduklarını belirtmiştir. Ancak bu farkındalık günlük yaşamda yeterli bir davranış değişikliğine yol açmamaktadır.

Anahtar kelimeler: Tuz alımı değişikliği, böbrek hastalıkları, hipertansiyon, diyette tuz miktarı.

Akın D. Nefroloji kliniğine ayaktan başvuran hastalarda tuz tüketimi farkındalığı. Pam Tıp Derg 2025;18:398-405.

Davut Akın, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Internal Medicine, Denizli, Türkiye, e-mail: dakin@pau.edu.tr (https://orcid.org/0000-0002-9567-7940) (Corresponding

Introduction

Current international guidelines for the treatment of kidney disease recommend dietary and lifestyle modifications to delay progression and reduce disease-specific mortality in addition to standard chronic kidney disease (CKD) therapy [1]. Reducing dietary sodium intake is considered an important treatment modality in the management of patients with kidney disease, hypertension, and heart disease. Although there are intercontinental and regional differences in the world, the average sodium consumption among adults in Türkiye is approximately 18 g per day [2], which is more than three times the amount recommended by the World Health Organization. Regarding salt consumption, guidelines recommend a dietary intake of less than 5 g per day of sodium chloride (NaCl), commonly known as table salt, which is equivalent to 2 g of sodium (Na+) [3, 4]. The rationale behind this recommendation is based on observations that salt restriction in CKD patients reduces blood pressure (BP) and proteinuria [5, 6], two key factors for CKD progression. Salt restriction also prevents glomerular hyperfiltration and potentiates the renoprotective response to RAS-i [7]. Recognizing that many developed and developing countries share this scenario and that reducing dietary sodium intake would have a significant impact on hypertension, cardiovascular disease, and kidney disease, the World Health Organization (WHO) has requested that food companies reduce the sodium content in their products and that governments introduce regulatory approaches if recommended sodium intake levels cannot be achieved through voluntary actions.

In this study, we aimed to determine the nutritional habits of outpatients applying to the nephrology outpatient clinic, their level of knowledge about how much salt they consume per day, how much salt they should consume, how much they adapt what they know to their daily life, where they get the salt they consume, whether they pay attention to the amount of salt in the foods they take from outside, the effects of salt on their health, their level of knowledge, and their awareness.

Materials and methods

The study is a descriptive cross-sectional study. It consisted of patients who were 18 years of age or older, literate, able to read and write, and capable of understanding and accepting the questionnaire. These patients applied to the Pamukkale University Nephrology outpatient clinic between January 10, 2024, and March 10, 2024. Prior to the study, approval was obtained from the Pamukkale University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee with the board decision dated January 9, 2023, and numbered E-60116787-020-474865. A questionnaire form was administered to the patients included in the study through a face-to-face interview technique.

In the questionnaire form, age, gender, educational status, place of residence, smoking status, having a disease other than kidney disease, reason for applying to the nephrology clinic, dialysis, kidney transplantation status, family history of kidney disease, amount of salt that should be consumed daily, how you consume your meals, where you eat, adding salt to food, The following questions were asked: the use of salt shaker at the dinner table, the type of salt used, where he/she gets salt from the most, eating salt-free in social settings, requesting coloring according to the amount of salt in the menu in the restaurant, checking the label for the amount of salt, the effect of having color-based labels on the amount of salt in foods on food selection, and how he/she cooked the food. In addition, the Beliefs about Dietary Compliance Scale (BDCS) was used to assess salt consumption habits.

Beliefs about Dietary Compliance Scale (BDCS): This scale, which was developed by Bennett et al. [8] and validity and reliability study was conducted by Oğuz et al. [9] in 2010, consists of 12 questions and two sub-dimensions: benefits (items 1-5, 11, 12) and barriers (items 6-10). Item 2 has a reverse coding. The possible score in the benefit sub-dimension ranges between 7-35 and in the barrier sub-dimension between 5-25. A high score in the benefit subscale indicates that the perceived benefit in compliance with the diet based on salt restriction is high, while a high score in the barrier subscale indicates that the

perceived barriers in compliance with the diet are high. In the study conducted by Oğuz et al. [9] in heart failure patients, Cronbach's alpha was 0.71 for the benefit subscale and 0.58 for the barrier subscale. Similarly, in this study, Cronbach's alpha for the benefit subscale was 0.61 and 0.68 for the barrier subscale.

Data were analysed using IBM SPSS Statistics 25 (Armonk, NY: IBM Corp.) software. Descriptive statistics were given as number and percentage for categorical variables, arithmetic mean and standard deviation for continuous variables. Kolmogorov Smirnov and Shapiro-Wilk tests were performed to evaluate normal distribution. Kruskal-Wallis Analysis of Variance and Mann-Whitney U test were used to evaluate the differences between the groups. A p value <0.05 was accepted as a statistically significant result.

Results

The study included 211 patients. The mean age of the participants was 58.94±16.84 (min: 19 max: 92) and 53.7% were female. Hypertension 64.5% (n=136), diabetes 31.3% (n=66), hyperlipidemia 15.6% (n=33), heart disease 19.9% (n=42), rheumatologic disease 10.4% (n=22) and cancer diagnosis 6.6% (n=14) (Table 1).

It was determined that 82.5% (n=174) of the participants knew the recommended daily salt amount to be 5 grams and 66.8% of them consumed their meals with little or no salt. 94.8% (n=200) of the participants consumed their meals at home and 5.2% (n=11) consumed their meals outside. Salt was mostly consumed as table salt (63.3% n=133), followed by natural salt found in foods (30.3% n=64) and least from prepared foods (6.6% n=14). 82.9% (n=175) of the participants stated that color-based labels indicating the salt content of foods according to their grade could influence their food preferences. In contrast, 17.1% (n=36) of the participants stated that such labels would not have any effect on food choices (Table 2).

The mean score of the benefit subscale of the dietary adaptation scale was 29.3±2.7 (min: 11 max: 32) and the mean score of the barrier subscale was 19.4±4.2 (min: 5 max: 25). There was no difference between the mean scores of the benefit and barrier subscales of the BDCS in terms of gender, but the mean score of the benefit subscale was significantly higher in married participants compared to single participants (p=0.427; p=0.653, p=0.003, respectively). Table 3 shows the comparisons of some variables of the participants with the benefit and barrier subscales of the BDCS.

Table 1. Demographic features of the participants

	n	%
Gender		
Woman	121	57.3
Male	90	42.7
Marital status		
Married	166	78.7
Single	45	21.3
Education status		
Primary education	123	58.3
High school	40	19.0
University/ Master's degree	48	22.7
Place of residence		
Rural	87	41.2
Urban	124	58.8

Table 1. Demographic features of the participants (continued)

	n	%
Smoking status		
Yes	51	24.2
No	160	75.8
Chronic disease status other than kidney disease		
Yes	176	83.4
No	35	16.6
Complaint of presentation to the nephrology outpatient clinic		
Kidney disease	44	20.9
Protein leakage	19	9.0
Kidney stone	9	4.3
Kidney disease	47	22.2
Nephrological disease control	92	43.6
Patient receiving dialysis		
Yes	11	5.2
No	200	94.8
Kidney transplant patient		
Yes	1	0.5
No	210	99.5
Kidney disease in the family		
Yes	62	29.4
No	149	70.6

Table 2. Characteristics of participants salt use

	n	%
The amount of salt to be consumed daily		
5 g (~1 teaspoon silica)	174	82.5
15 gr	29	13.7
20 gr	7	3.3
50 g and above	1	0.5
How you consume your meals		
No Salt	62	29.4
Lightly salted	79	37.4
Normal salty	60	28.4
Salty	10	4.7
Do you add salt by tasting the food?		
Yes	143	64.9
No	68	32.2

Table 2. Characteristics of participants salt use (continued)

	n	%
Do you use a salt shaker at the dinner table?		
I use it all the time	20	9.5
I use it most of the time	28	13.3
Sometimes I use it	41	19.4
I rarely use it	39	18.5
I never use it	83	39.3
Salt type		
Table salt	163	77.3
Sea salt	7	3.3
Rock salt	26	12.3
Himalayan salt	7	3.3
Lodized salt	8	3.8
Do you try to eat salt-free in social situations?		
Always	58	27.5
Mostly	31	14.7
Sometimes	38	18.0
Rarely	42	19.9
Never.	42	19.9
Would you like the menu in a restaurant to be colored according to the amount of salt?		
Yes	179	84.8
No	32	15.2
Can you check the label for the amount of salt?		
Always	72	34.1
Mostly	32	15.2
Sometimes	36	17.1
Rarely	24	11.4
Never	47	22.3
How do you cook your food?		
*I cook salty and don't add salt at the table	70	33.2
*I use tomato paste with high salt content while cooking and do not add salt at the table	75	35.5
*I cook with salt and add salt at the table	12	5.7
*I cook without salt and I don't add salt at the table	54	25.6

Table 3. Comparison of Beliefs about Dietary Compliance Scale with some variables of the participants

	Benefit score	Test value	Barrier score	Test value
Variables	**A.M±SD**	***p***	**A.M±SD**	***p***
Gender				
Woman	29.1±3.0	Z=-.795	19.2±4.7	Z=-.449
Male	29.6±2.3	*p*=0.427	19.73.5	*p*=0.653
Education status				
Primary Education (1)	29.8±2.0	kwh=8.417	19.9±3.9	kwh=3.316
High School (2)	29.5±2.4	*p*=0.015	18.9±4.2	*p*=0.191
University (3)	28.1±4.0	(1-3)	18.6±4.9	
Marital status				
Married	29.7±2.0	Z=-3.007	19.5±4.1	Z=-.135
Single	27.9±4.2	*p*=0.003	19.2±4.9	*p*=0.893
Smoking status				
Yes	29.1±3.6	Z=-0.299	18.9±4.4	Z=-0.913
No	29.4±2.4	*p*=0.765	19.6±4.2	*p*=0.361
Place of residence				
Rural area	29.5±2.2	Z=-.154	19.2±4.4	Z=-.494
City center	29.2±3.1	*p*=0.878	19.5±4.1	*p*=0.621
Additional Chronic disease				
Yes	29.5±2.7	Z=-2.057	19.4±4.2	Z=-.295
No	28.6±2.9	*p*=0.040	19.5±4.7	*p*=0.768
Dialysis				
Yes	29.6±2.0	Z=-0.030	19.9±3.6	Z=-0.184
No	29.3±2.8	*p*=0.976	19.4±4.3	*p*=0.854
Kidney disease in the family				
Yes	29.3±3.4	Z=-0.324	18.5±4.0	Z=-1.766
No	29.3±2.4	*p*=0.746	19.8±4.3	*p*=0.077

AM: Arithmetic mean, SD: Standard deviation, Z: Mann Whitney U test, kwh: Kruskal Wallis Analysis of Variance; *$p<0.05$

Discussion

In this study, it was observed that the majority (82.5%) of the outpatients admitted to the nephrology outpatient clinic were aware of and complied with the salt consumption of 5 g/day recommended by WHO and nephrology guidelines. In the study, 66.8% of the participants consumed unsalted and low-salt foods. This is considerably below the 18 g/day salt consumption in the general population in the SALTÜRK study conducted in Türkiye in 2010 [2]. The possible reason for this may be that the awareness of patients applying to the nephrology outpatient clinic is higher, and the awareness of salt consumption has increased over the years. This study shows that awareness of salt consumption is generally high, but this awareness does not translate into sufficient behavioral change in practice.

In this study, the mean score of the benefit subscale of the BDCS was 29.3±2.7 and the mean score of the barrier subscale was 19.4±4.2. In a study conducted on hypertension patients, the benefit subscale of the BDCS was found to be 23.44±5.50 and the barrier subscale of the BDCS was found to be 12.9±3.59 [10].

Similarly, in a study conducted in hypertension patients, the mean of the benefit sub-dimension of the BDCS was found to be 27.42±3.95 and the barrier sub-dimension of the BDCS was found to be 11.82±4.82 [11]. In a study conducted on renal patients undergoing regular hemodialysis in a dialysis center, the mean scores of the benefit subscale were found to be 26.71±5.78 and the mean scores of the barrier subscale were found to be 14.59±3.65 [12]. In this study, the mean scores related to the benefit and barrier subscales of the BDCS were found to be higher than the studies in the literature. This difference may be due to the differences in the awareness and attitude levels of the participants towards salt restriction and the clinical characteristics of the study group. In addition, this suggests that perceptions and barriers to salt restriction may vary in different patient groups or in the presence of comorbidities.

In contrast to the results of Oğuz et al. [10] and Zengin and Oren [12], who did not observe a significant difference in marital status, our study indicates that married individuals scored significantly higher on the benefit subscale. In married individuals, elevated “benefit” subscale scores may stem from factors such as spousal/familial support, ease of meal preparation and sharing at home, and a heightened sense of health responsibility. Such family support can enhance dietary adherence by offering both motivational and practical assistance.

In conclusion, it can be said that outpatients attending the nephrology clinic are aware of the 5 grams of salt consumption per day recommended by nephrology guidelines and WHO to slow the progression of diseases and to control them. However, although awareness of salt consumption is generally high, it is observed that this awareness is not sufficiently transformed into behavioral change.

Acknowledgment: I would like to thank my Special Study Module students Semiha Boyaci, Suheda Cavda, Mevlude Doke, and Sude Nur Kalkan for their contributions to the article.

Funding: None.

Conflict of interest: No conflict of interest was declared by the author.

References

1. Stevens PE, Levin A. Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* 2013;158(11):825-830. doi:10.7326/0003-4819-158-11-201306040-00007
2. Erdem Y, Arici M, Altun B, et al. The relationship between hypertension and salt intake in Turkish population: SALTURK study. *Blood Press.* 2010;19(5):313-318. doi:10.3109/08037051003802541
3. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser.* 2003;916. Available at: https://iris.who.int/bitstream/handle/10665/42665/WHO_TRS_916.pdf. Accessed 31.01.2025
4. Levin A, Stevens P. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl.* 2013;3:1-150. doi:10.1038/kisup.2012.73
5. McMahon EJ, Campbell KL, Bauer JD, Mudge DW. Altered dietary salt intake for people with chronic kidney disease. *Cochrane Database Syst Rev.* 2015;(2):CD010070. Published 2015 Feb 18. doi:10.1002/14651858.CD010070
6. Vogt L, Waanders F, Boomsma F, de Zeeuw D, Navis G. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. *J Am Soc Nephrol.* 2008;19(5):999-1007. doi:10.1681/ASN.2007060693
7. D’Elia L, Rossi G, Schiano di Cola M, Savino I, Galletti F, Strazzullo P. Meta-Analysis of the Effect of Dietary Sodium Restriction with or without Concomitant Renin-Angiotensin-Aldosterone System-Inhibiting Treatment on Albuminuria. *Clin J Am Soc Nephrol.* 2015;10(9):1542-1552. doi:10.2215/CJN.09110914
8. Bennett SJ, Milgrom LB, Champion V, Huster GA. Beliefs about medication and dietary compliance in people with heart failure: an instrument development study. *Heart Lung.* 1997;26(4):273-279. doi:10.1016/s0147-9563(97)90084-4
9. Oğuz S, Enç N, Yiğit Z. Kronik kalp yetersizliği olan hastalar için inanç ve uyum ölçeklerinin Türkçeye uyarlanması. *Türk Kardiyol Dern Arş.* 2010;38(7):480-485.
10. Oğuz S, Yanmış S, Yılmaz B, Atman R. Hipertansiyon hastalarının ilaç ve diyet tedavisine uyum düzeyleri. *Kardiyovasküler Hemşirelik Dergisi*. 2019;10(21):1-7. doi:10.5543/khd.2019.63644

11. Oğuz S, Yetim M, Yalçın Ö, Ünalan YE, Çamcı G. Hipertansiyonlu bireylerde hastalık algısının tedavi ve diyet uyumuna etkisi. *Cumhuriyet Hemşirelik Dergisi*. 2017;5(2):75-83.
12. Zengin N, Ören B. Hemodiyaliz hastalarının tuzdan kısıtlı diyete uyumlarının incelenmesi. *Nefroloji Hemşireliği Dergisi.* 2015;10(2):39-49.

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1589977

The presence of a palpable mass is an independent predictor of microinvasion in Ductal Carcinoma in situ of the Breast

Meme Duktal Karsinoma in situ hastalarında mikroinvazyonun bağımsız bir öngörücüsü olarak palpabl kitle varlığı

Sevda Yılmaz, Taha Yasin Baran, Utku Özgen, Yeliz Arman Karakaya, Alim Akdağ

Posted date:22.11.2024 Acceptance date:26.11.2024

Abstract

Purpose: Breast cancer is the most common cancer among women worldwide. Ductal Carcinoma in situ (DCIS) is one of the most common types of these cancers. Factors associated with microinvasion still need to be investigated.

Materials and methods: In this retrospective study, we analyzed data from 70 female patients diagnosed with DCIS and managed at a tertiary center between 2011 and 2024. Demographic parameters, clinicopathological characteristics, and immunohistochemical findings of the patients were examined in comparison with their microinvasion status.

Results: 70 female patients with Ductal Carcinoma in situ, with a median age of 51 years, were investigated. Among these patients, 17 cases (24.3%) had microinvasion. Compared with demographic parameters, only BMI was associated with microinvasion status. Patients with higher BMI had lower risk of microinvasion (p=0.038). The presence of a palpable mass was significantly higher in patients with microinvasion (p=0.001), suggesting a potential link between tumor palpability and invasion.

Immunohistochemical analysis demonstrated associations between hormone receptor status, HER2 expression, and microinvasion. The presence of HER2 was significantly associated with microinvasion (p=0.026). Multivariate analysis however, revealed that tumor palpability was the only independent factor associated with microinvasion status (Odds Ratio: 5.233; 1.339-20.455; p=0.017).

Conclusion: The presence of a palpable mass emerged as the only independent factor associated with microinvasion in DCIS.

Keywords: DCIS, palpable mass, microinvasion, breast cancer, sentinel iymph node.

Yilmaz S, Baran TY, Ozgen U, Arman Karakaya Y, Akdag A. The presence of a palpable mass is an independent predictor of microinvasion in Ductal Carcinoma in situ of the Breast. Pam Med J 2025;18:406-415.

Öz

Amaç: Meme kanseri, Dünya genelinde kadınlar arasında en sık görülen kanser türüdür. Duktal Karsinoma İn Situ (DKIS), bu kanserlerin en yaygın türlerinden biridir. Mikroinvazyonla ilişkili faktörlerin daha fazla araştırılması gerekmektedir.

Gereç ve yöntem: Bu retrospektif çalışmada, 2011-2024 yılları arasında bir üçüncü basamak sağlık merkezinde DKIS tanısı almış ve tedavi edilmiş 70 kadın hastanın verileri analiz edildi. Hastaların demografik parametreleri, klinikopatolojik özellikleri ve immünohistokimyasal bulguları, mikroinvazyon durumlarına göre karşılaştırıldı.

Bulgular: Ortalama yaşı 51 olan 70 kadın hasta incelendi. Bu hastalar arasında 17 vakada (%24,3) mikroinvazyon tespit edildi. Demografik parametreler arasında sadece vücut kitle indeksi (VKİ) mikroinvazyon durumu ile ilişkili bulundu. Daha yüksek VKİ'ye sahip hastalarda mikroinvazyon riski daha düşük olarak saptandı (p=0,038). Mikroinvazyonu olan hastalarda ele gelen kitlenin varlığı anlamlı derecede daha yüksekti (p=0,001). Bu durum, tümörün palpabilitesi ile invazyon arasında potansiyel bir bağlantıyı düşündürmektedir. İmmünohistokimyasal analizler, hormon reseptör durumu, HER2 ekspresyonu ve mikroinvazyon arasında ilişkiler olduğunu ortaya koydu. HER2 varlığı, mikroinvazyon ile anlamlı şekilde ilişkili bulundu (p=0,026). Ancak, çok değişkenli analizler sonucunda, tümör palpabilitesinin mikroinvazyon durumuyla ilişkili tek bağımsız faktör olduğu belirlendi (Odds Ratio: 5,233; 1,339-20,455; p=0,017).

Sonuç: DCIS'de, ele gelen kitlenin varlığı, mikroinvazyonla ilişkili tek bağımsız faktör olarak öne çıkmıştır.

Sevda Yılmaz, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of General Surgery, Denizli, Türkiye, e-mail: syilmaz_md@hotmail.com (https://orcid.org/0000-0002-1309-0805)

Taha Yasin Baran, M.D. Ardahan State Hospital, Department of General Surgery, Ardahan, Türkiye, e-mail: tybaran@hotmail.com (https://orcid.org/0000-0002-2933-7251) (Corresponding Author)

Utku Özgen, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of General Surgery, Denizli, Türkiye, e-mail: dr_utkuozgen@yahoo.com (https://orcid.org/0000-0002-6481-1473)

Yeliz Arman Karakaya, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Medical Pathology, Denizli, Türkiye, e-mail: ykarakaya@pau.edu.tr (https://orcid.org/0000-0002-6669-9972)

Alim Akdağ, Ph.D. Pamukkale University Faculty of Medicine, Department of General Surgery, Denizli, Türkiye, e-mail: aakdag@pau.edu.tr (https://orcid.org/0000-0002-0363-0759)

Anahtar kelimeler: DKIS, palpabl kitle, mikroinvazyon, meme kanseri, sentinel lenf nodu.

Yılmaz S, Baran TY, Özgen U, Arman Karakaya Y, Akdağ A. Meme Duktal Karsinoma in situ hastalarında mikroinvazyonun bağımsız bir öngörücüsü olarak palpabl kitle varlığı. Pam Tıp Derg 2025;18:406-415.

Introduction

Ductal Carcinoma in Situ (DCIS), also referred to as intraductal carcinoma, is a non-invasive breast cancer defined by the proliferation of abnormal epithelial cells confined within the basal membrane. The diagnosis changes to invasive breast cancer when the basal membrane is disrupted, making DCIS a recognized precursor to invasive breast carcinoma.

Breast cancer is the leading cancer type among women globally, with DCIS representing 20-25% of newly diagnosed breast cancers in the United States. The increasing prevalence of DCIS diagnoses parallels the growing use of screening mammography, as a majority of cases are first identified through this method. In contrast, prior to the widespread implementation of mammographic screening, DCIS accounted for less than 5% of newly diagnosed breast cancer cases [1, 2].

DCIS associated with invasive carcinoma measuring ≤1 mm is classified as “DCIS with microinvasion” (DCIS/microinvasion) rather than invasive breast carcinoma. Studies indicate that DCIS with microinvasion accounts for less than 1% of all breast cancers, and the limited number of cases has resulted in an incomplete understanding of its prognostic significance [3].

There are five histopathological subgroups of DCIS: comedo, cribriform, micropapillary, papillary, and solid DCIS. DCIS commonly exhibits a variety of these histopathological structures. Micropapillary, cribriform, and papillary DCIS are considered low-grade lesions, while solid and comedo DCIS are high-grade and show an increased risk of progressing to invasive carcinoma [4].

The treatment of DCIS typically involves a combination of surgery, radiation therapy, and hormone therapy. Treatment decisions are guided by factors such as tumor stage, histopathological findings (including classification, hormone receptor status, and the presence of microinvasion), as these influence recurrence risk. Surgical options for DCIS include breast-conserving surgery (BCS) followed by radiotherapy or mastectomy, with hormone therapy recommended for hormone receptor-positive cases. Both approaches have been shown to provide equivalent long-term survival outcomes. During mastectomy, sentinel lymph node biopsy (SLNB) may be performed to assess axillary metastasis. SLNB is commonly carried out using blue dye (e.g., isosulfan blue) or radioactive colloid [5-7].

High-grade DCIS with large tumors, suspected microinvasion, and solid or cribriform patterns may warrant SLNB. DCIS with microinvasion occurs in 0.68-2.4% of all breast cancer cases and approximately in 14% of DCIS cases. Because DCIS is by definition non-invasive, it is not expected to spread to axillary lymph nodes. Data from the NSABP B24 trials indicate that the rate of axillary recurrence in DCIS, independent of treatment, is 0.36 per 1000 cases. Therefore, SLNB may be deemed unnecessary in DCIS cases [8-11].

Factors potentially associated with microinvasion in DCIS patients include age, tumor size, histopathological features (such as grade, comedonecrosis, ER/PR receptor status, and HER2 expression), and axillary lymph node metastasis. These are also important clinicopathological indicators for breast cancer prognosis [12].

This study explores the relationship between clinical and demographic factors and microinvasion among DCIS patients.

Materials and methods

Approval for the study was obtained from the Pamukkale University Hospital Non-Invasive Clinical Research Ethics Committee (number 18 dated 22/10/2024, E-60116787-020-604967). All stages of the project were conducted in accordance with the Helsinki Declaration. Patient data used in the study were obtained from the University Hospital’s electronic information system and pathology archive. 70 female patients who were treated for

Ductal Carcinoma in situ at the Breast Surgery Clinic between December 2011 and December 2024, and whose data were fully accessible through the hospital's electronic PACS system, were included in the study. The data were reviewed retrospectively. The patients included in the study were evaluated for medical history, sociodemographic data, type of surgical treatment, pathological results of sentinel lymph node biopsy (SLNB), and histopathological features of DCIS (histologic subtype, nuclear grade, presence of comedo necrosis, tumor diameters, histopathological grade, presence of microinvasion, hormone receptor status, HER2 status, Ki-67 proliferation index percentage).

The estrogen receptor (ER) and progesterone receptor (PR) statuses of the patients were determined from immunohistochemistry results, with a value of ≥1% considered positive. The HER2 status was obtained from immunohistochemistry or fluorescence in situ hybridization (FISH) results. Tumors classified as FISH positive with IHC 3+ and IHC 2+ were categorized as HER2 positive; tumors that were FISH negative, IHC 1+, or IHC 2+ were classified as HER2 negative. No experimental studies were conducted. Molecular subtyping was performed according to the latest St. Gallen Consensus in 2013 [13]. The classifications are as follows:

- Luminal A (ER positive, PR >20%, HER2 negative, and Ki-67 index <20%),
- Luminal B/HER2 negative (ER positive, PR <20%, HER2 negative, and Ki-67 index >20%),
- Luminal B/HER2 positive (ER positive, any PR, HER2 positive, and any Ki-67 index),
- HER2 positive (ER negative, PR negative, HER2 positive, and any Ki-67 index),
- Triple negative (ER negative, PR negative, HER2 negative, and any Ki-67 index).

Statistical analysis

Data analysis was conducted using the software SPSS 21.0 (SPSS Inc., Chicago, IL), which was used for the statistical analysis. Continuous variables were assessed for normality using visual inspection and the Shapiro-Wilk test. Variables following a normal distribution were reported as mean (SD), while categorical variables were presented as frequencies and percentages. Normally distributed continuous variables were compared using the Student's t-test, and 95% confidence intervals (CIs) were calculated for differences in means. Differences between categorical variables were evaluated using the Chi-square test. A *p*-value of less than 0.05 was considered statistically significant. Multivariable analysis was conducted using a logistic regression model to evaluate the independent effects of different factors.

Results

Among 70 female Ductal Carcinoma in situ patients, 17 (24.3%) cases had microinvasion. The demographical characteristics of the patients are presented in Table 1. Among these parameters, only BMI was associated with microinvasion status. Patients with higher BMI had a lower risk of microinvasion (p=0.038).

Diagnostic methods and treatment

The study comprised 70 patients, categorized into two groups: those without microinvasion (n=53) and those with microinvasion (n=17).

Tru-cut biopsy was performed on 22 patients (31.4%), evenly distributed between those without microinvasion (n=11) and those with microinvasion (n=11). Excisional biopsy was conducted on 18 patients (25.7%), with 16 patients in the non-microinvasion group and 2 in the microinvasion group. Wire-guided breast biopsy was utilized in 30 patients (42.8%), including 26 without microinvasion and 4 with microinvasion.

Sentinel lymph node biopsy (SLNB) was performed on all 70 patients, with the following results: 67 patients (95.7%) were SLNB-negative, including 51 in the non-microinvasion group and 16 in the microinvasion group. SLNB positivity (1+ nodes) was identified in 2 patients (2.8%), both from the non-microinvasion group, and none in the microinvasion group. SLNB positivity (2+ nodes) was observed in 1 patient (1.4%) from the microinvasion group, with none in the non-microinvasion group. The difference in SLNB outcomes between the groups was not statistically significant (p=0.152).

The surgical methods employed for the 70 patients were categorized based on microinvasion status: those without microinvasion (n=53) and those with microinvasion (n=17). The distribution of procedures was as follows:

- Simple mastectomy was performed in 48 patients (68.5%), including 35 without microinvasion and 13 with microinvasion.
- Breast-conserving surgery was conducted in 12 patients (17.1%), with 11 in the non-microinvasion group and 1 in the microinvasion group.
- Mastectomy with immediate prosthesis was carried out in 6 patients (8.5%), of whom 5 were without microinvasion and 1 with microinvasion.
- Modified radical mastectomy was performed in 4 patients (5.7%), with 2 in each group.

The differences in surgical methods between the groups were not statistically significant (p=0.322) (Table 2).

Table 1. Demographic parameters of Ductal Carcinoma *in situ* Patients with or without Microinvasion

	Total patients (n=70)	Without Microinvasion (n=53)	With Microinvasion (n=17)	*p* value	Test value
Age (years)	51±10.6	51.6 ± 10.4	49.3 ± 11.8	0.445[a]	t=0.768
BMI (kg/m²)	27.5±5.1	28.3±5.3	25.3±4.12	0.038*[a]	t=2.118
Age of menarge	12.7±1.8	12.7±1.16	12.5±3.1	0.655[a]	t=0.449
Number of deliveries	1.9±0.8	2.0±0.8	1.7±0.7	0.202[a]	t=1.290
Age of first pregnancy	23.0±5.4	23.1±5.6	23.1±4.7	0.998[a]	t=-0.003
Breastfeeding history					
No (n=7)	7 (10%)	4 (57.1%)	3 (42.9%)	0.349[b]	X=1.459
Yes (n=63)	63 (90%)	49 (77.8%)	14 (22.2%)		
Breastfeeding duration (months)	21.8±13.8	22.3±14.1	19.9±12.9	0.535[a]	t=0.624
Smoking status					
Nonsmoker	63 (90%)	48 (76.2%)	15 (23.8%)	1.0[b]	X=0.078
Ever smoked	7 (10%)	5 (71.4%)	2 (28.6%)		
Oral contraceptive use					
Never used	59 (84.3%)	44 (74.6%)	15 (25.4%)	1.0[b]	X=0.264
Used	11 (15.7%)	9 (81.8%)	2 (18.2%)		
Menopausal status					
Premenopausal	32 (45.7%)	24 (75.0%)	8 (25.0%)	0.089[b]	X=0.016
Postmenopausal	38 (54.3%)	29 (76.3%)	9 (23.7%)		
Hormone therapy status					
Never used	67 (95.7%)	50 (74.6%)	17 (25.4%)	1.0[b]	X=1.005
Used	3 (4.3%)	3 (5.7%)	0 (0.0%)		

*p<0.05; BMI: Body Mass Index, [a]Student's t-test, [b]Chi-square test; Continuous parameters are presented as mean (standard deviation) categorical parameters are presented in number of cases (percentage of the case)

Table 2. Surgical management in Ductal Carcinoma in situ patients

Surgical Methods	Total patients (n=70)	Without Microinvasion (n=53)	With Microinvasion (n=17)
Simple Mastectomy	48 (68.5%)	35	13
Breast-conserving surgery	12 (17.1%)	11	1
Mastectomy and immediate prosthesis surgery	6 (8.5%)	5	1
Modified radical Mastectomy	4 (5.7%)	2	2

[a]Chi-square test

Clinicopathological characteristics

Clinicopathological features of the patients were compared between the two groups according to their microinvasion status. History of breast cancer, BIRADS category, Paget appearance, tumor diameter, and the quadrant of the tumor were similar among patients with and without microinvasion. However, presence of a palpable mass was associated with microinvasion status (Table 3.) Microinvasion was observed in 46.5% and 9.5% of the cases with and without palpable breast mass, respectively (p=0.001).

Immunohistochemical findings

Immunohistochemical analysis demonstrated associations between hormone receptor status, HER2/neu (Cerb2) expression, and microinvasion. Among these patients, 37 (52.9%) were HER2-negative and 33 (47.1%) were HER2-positive. In the HER2-negative group, 32 patients (86.5%) had no microinvasion, while 5 patients (13.5%) had microinvasion. In the HER2-positive group, 21 patients (63.6%) had no microinvasion, whereas 12 patients (36.4%) had microinvasion. The presence of HER2 was significantly associated with microinvasion (p=0.026) (Table 4).

Table 3. Clinicopathological features of the patients with or without Microinvasion

	Total patients (n=70)	Without Microinvasion (n=53)	With Microinvasion (n=17)	*p* value	Test value
Family history					
No family history	58 (82.9%)	44 (75.9%)	14 (24.1%)	1.000[a]	X=0.004
With family history	12 (17.1%)	9 (75.0%)	3 (25.0%)		
BIRADS mammography result					
0 No family history	9 (12.9%)	8 (88.9%)	1 (11.1%)		
1 With family history	2 (2.9%)	1 (50.0%)	1 (50.0%)		
2	7 (10.0%)	5 (71.4%)	2 (28.6%)	0.348[a]	X=5.590
3	1 (1.4%)	1 (100.0%)	0 (0.0%)		
4	46 (65.7%)	36 (78.3%)	10 (21.7%)		
5	5 (7.1%)	2 (40%)	3 (60.0%)		
Palpable mass					
No	42 (60.0%)	38(90.5%)	4(9.5%)	0.001*[a]	X=12.444
Yes	28(40.0%)	15(53.6%)	13(46.4%)		
Paget appearance					
No	60 (85.7%)	45 (75.0%)	15 (25.0%)	1.000[a]	X=0.117
Yes	10 (14.3%)	8 (80.0%)	2 (20.0%)		
Tumor diameter	14.5±11.1	13.4±9.3	17.8±15.5	0.161[a]	t=-1.418

Table 3. Clinicopathological features of the patients with or without Microinvasion (continued)

Quadrant of the Tumor					
UOQ	48 (68.6%)	34 (70.8%)	14 (29.2%)		
UIQ	9 (12.9%)	9 (100.0%)	0 (0.0%)		
LOQ	5 (7.1%)	3 (60.0%)	2 (40.0%)	0.268[a]	X=5.192
LIQ	3 (4.3%)	3 (100.0%)	0 (0.0%)		
Retroareolar	5 (7.1%)	4 (80.0%)	1 (20.0%)		
Tumor Grade					
1	6 (8.6%)	6 (100.0%)	0 (100.0%)		
2	48 (68.6%)	36 (75.0%)	12 (25.0%)	0.307[a]	X=2.360
3	16 (22.9%)	11 (68.8%)	5 (31.2%)		
Tumor Grade					
≤2	54 (77.1%)	42 (77.8%)	12 (22.2%)	0.513[a]	X=0.547
3	16 (22.9%)	11 (68.8%)	5 (31.2%)		
Multicentricity					
No	48 (68.6%)	36 (75.0%)	12 (25.0%)	0.837[a]	X=0.042
Yes	22 (31.4%)	17 (77.3%)	5 (22.7%)		
Ductal type Without comedo patern	26 37.1%)	17 (65.4%)	9 (34.6%)	0.121[a]	X=2.400
With comedo patern	44 62.9%)	36 (81.8%)	8 (18.2%)		

*p<0.05, [a]Chi-square test; Continuous parameters are presented as mean (standard deviation); categorical parameters are presented in number of cases (percentage of the case); BIRADS: Breast-Imaging Reporting and Data System, UOQ: Upper Outer Quadrant
UIQ: Upper Inner Quadrant, LOQ: Lower Outer Quadrant, LIQ: Lower Inner Quadrant

Table 4. Comparison of Immunhistochemistry results of the patients between Ductal Carcinoma *in situ* Patients with or without Microinvasion

	Total patients (n=70)	**Without Microinvasion (n=53)**	**With Microinvasion (n=17)**	***p* value**	***Test value***
ER					
Negative	17 (24.3%)	11 (64.7%)	6 (35.3%)	0.329[a]	X=1.480
Positive	53 (75.7%)	42 (79.2%)	11 (20.8%)		
PR					
Negative	27 (38.6%)	18 (66.7%)	9 (33.3%)	0.162[a]	X=1.957
Positive	43 (61.4%)	35 (81.4%)	8 (18.6%)		
HER2/neu (Cerb2)					
Negative	37 52.9%	32 86.5%	5 13.5%	0.026*[a]	X=4.953
Positive	33 47.1%	21 63.6%	12 36.4%		
Luminal Type					
Luminal A type	16 (22.9%)	15 (93.8%)	1 (6.2%)		
Luminal B type	37 (52.9%)	27 (73%)	10 (27%)	0.222[a]	X=4.395
Her2/Neu positive	10 (14.3%)	6 (60%)	4 (40%)		
Triple negative	7 (10%)	5 (71.4%)	2 (28.6%)		
Ki 67 staining					
<20 %	33 (47.1%)	26 (78.8%)	7 (21.2%)	0.571[a]	X=0.321
20 %	37 (52.9%)	27 (73%)	10 (27%)		

*p<0.05; ER: Estrogen Receptor, PR: Progesterone Receptor, [a]Chi-square test; Continuous parameters are presented as mean (standard deviation); categorical parameters are presented in number of cases (percentage of the case)

BMI, presence of palpable mass, and Cerb2 status, which are the parameters that were found to be significantly associated with microinvasion in univariate comparison, were analyzed in logistic regression analysis. This analysis revealed that the presence of a palpable mass was the only independent predictor of microinvasion in Ductal Carcinoma in situ of the breast (Odds Ratio:5.233, 95% CI:1.339-20.455, p=0.017) (Table 5).

Table 5. Logistic regression analysis for predicting microinvasion in Ductal Carcinoma *in situ* Patients

Parameters	B	Standard error	Odds ratio	95% confidence interval for the odds ratio	*p* value
Constant	0.114	2.123	1.120		0.957
BMI	-0.100	0.076	0.905	0.780-1.051	0.190
Cerb2 status	0.974	0.672	2.649	0.709-9.891	2.649
Palpable mass	1.655	0.696	5.233	1.339-20.455	0.017*

BMI: Body Mass Index

Discussion

This study analyzed 70 female patients with Ductal Carcinoma in Situ (DCIS), with a median age of 51 years. Microinvasion was identified in 17 patients (24.3%). Univariate analysis showed that BMI, the presence of a palpable mass, and HER2/neu (Cerb2) positivity were associated with microinvasion. However, multivariate analysis identified the presence of a palpable mass as the only independent factor associated with microinvasion.

In DCIS patients, identifying features that help predict prognosis is crucial for determining the appropriate treatment strategy. Microinvasion is associated with a worse prognosis, influencing decisions regarding surgical methods, the need for sentinel lymph node biopsy, and postoperative follow-up. Clinicopathological tests that raise suspicion of microinvasion are particularly valuable in guiding these decisions. Patient demographic data and histopathological evaluations of tumors provide insights into the likelihood of microinvasion. In both DCIS and DCIS-Mi patients, larger tumor size, higher nuclear grade, hormone receptor (HR) negativity, and HER2 overexpression are associated with recurrence and poor prognosis [14].

The findings obtained in our study showed that Her2 positivity and palpable mass increased the risk of microinvasion. In addition, we think that the risk of microinvasion may be lower in patients with higher BMI. However, the factors previously associated with microinvasion in the literature, such as the presence of axillary metastasis, the presence of comedonecrosis, ER, PR negativity, were not found to be significantly related to microinvasion in our study. On the other hand, our results showed that higher BMI was associated with lower microinvasion risk, which this finding, consistent with the literature, shows that larger body size is not associated with known adverse features of DCIS, such as larger tumor size, higher nuclear grade, or the presence of necrosis. In contrast, some studies linked obesity to invasive breast cancer [15, 16]. Our findings interestingly suggest that the presence of a palpable mass is an independent predictor of microinvasion risk in DCIS cases.

In the study conducted by Canbay et al. [17], SLNB positivity was found to be high (5.8%) in pure DCIS cases. The probability of transformation into invasive cancer was found to be higher in patients with SLNB positivity, palpable mass, and tumor diameter >3 cm. When we examined the SLNB results, 4 patients (5.71%) were found to be SLNB-positive, consistent with the findings of Canbay et al. [17] However, this rate is lower than that reported by Diaz Casas et al. [18]. SLNB may be performed as part of surgery in DCIS patients suspected to be at risk for invasive carcinoma.

In a study by Magnoni et al. [19] involving 257 patients with microinvasive Ductal Carcinoma in situ (DCIS) who underwent sentinel lymph node biopsy (SLNB), 226 patients (87.9%) had negative sentinel lymph nodes (SLNs), while 31 patients (12.1%) were found to have metastatic SLNs. After a median follow-up period of 11 years, only one recurrence was observed in the 15 patients with positive SLNs who did not undergo axillary lymph node dissection. No recurrences were reported among the 16 patients with positive SLNs who underwent axillary dissection. In our treatment group, 4 patients (5.7%) underwent modified radical mastectomy, which also includes axillary dissection; 2 of them were in the non-microinvasive group and 2 were in the microinvasive group. In our study, only 3 out of 17 patients with microinvasive DCIS demonstrated SLNB positivity. Although SLNB positivity in DCIS patients was not statistically significant in our findings, based on current literature, we recommend performing SLNB in DCIS patients to assess potential lymph node involvement. SLNB positivity is considered an important indicator of poor prognosis and recurrence risk.

In a study by Champion et al. [20], utilizing a large sample from a national cancer database, it was observed that DCIS with microinvasiveness was more likely to be ER-negative, PR-negative, HER2-positive, and of higher grade compared to invasive breast cancers. These findings suggest that DCIS with microinvasiveness exhibits more aggressive characteristics. In the literature, estrogen and progesterone receptor expression rates in DCIS are reported to range from 60% to 81% [21]. Similarly, in our study, ER expression was found to be 75%, and PR expression was 61%. However, no significant relationship was identified between hormone receptor expression and microinvasion in our study.

HER2 overexpression was identified as the only significant predictor of invasive disease, suggesting that HER2 expression plays a key role in the progression of DCIS lesions to invasive carcinoma [22]. HER2 status in DCIS has been associated with an increased risk of recurrence, with HER2-positive DCIS often presenting as larger lesions. HER2 positivity is also linked to a higher likelihood of progression to invasive carcinoma. In a study by Roses et al. [23], similarly, in our study, HER2 positivity was found to be significantly higher in the DCIS group with microinvasion.

Yu et al. [24] investigated the relationship between molecular subtypes and microinvasion, reporting that tumors were larger and had higher nuclear grades in DCIS patients with microinvasion. They also found that microinvasion rates were lower in luminal-like tumors but higher in ERBB2+ and basal-like DCIS. Similarly, in our study, the risk of microinvasion was significantly increased in the HER2-positive group. However, we did not observe a significant correlation between molecular subtypes and microinvasion, likely due to the relatively small sample size in our study. We believe that subgroup analyses in larger cohorts could provide more robust and meaningful insights.

Upon evaluating histopathological subtypes, comedo carcinoma is reported as the most common histological subtype in DCIS with microinvasion [25]. In our study, the comedo pattern was detected in 62.9% of cases, aligning with the literature, while its detection rate in the microinvasive group was 18.2%. This suggests that, despite being a common subtype, the comedo pattern is not a definitive factor in the relationship with microinvasion.

Maffuz et al. [26] demonstrated in their study that the rate of microinvasion was higher in DCIS patients with tumors larger than 2.5 cm compared to those with smaller tumors. They also reported that such lesions often included various high-grade histological subtypes and were associated with comedonecrosis, palpable masses, and nipple discharge. Similarly, a study by Lagios et al. [27] found that the incidence of microinvasion was 29% in tumors larger than 26 mm, compared to 2% in tumors smaller than 25 mm. However, in our study, we found that the palpability of the mass, rather than its diameter, was independently associated with microinvasion. This may be due to changes in tissue characteristics that occur when microinvasion begins in a sufficiently large tumor, leading to the formation of a palpable mass. Therefore, we believe that the presence of a palpable mass in clinical practice should serve as a warning sign for microinvasion in the management of DCIS patients. DCIS

with microinvasion is biologically more aggressive and carries a higher risk of potential metastatic disease compared to DCIS without microinvasion. Current literature also indicates that disease-free survival rates are longer in patients without a microinvasive component [14]. Thus, close monitoring of patients with DCIS microinvasion, particularly those presenting with a palpable mass as highlighted in our study, is crucial.

In a recent study by Balac et al. [28], which compared the rates of progression to invasive cancer between patients with palpable DCIS and those diagnosed with DCIS through imaging, no significant difference was found between the two groups. Although our study does not focus on the long-term risk of recurrence or progression to invasive cancer, it has shown that patients with palpable DCIS may have a higher likelihood of harboring microinvasion, which is associated with poor prognosis. We believe our findings could contribute to the existing literature on this subject.

The limitations of our study include its retrospective design and the relatively small patient cohort.

In conclusion, this study highlights the complex interplay between demographic, clinicopathological, and immunohistochemical factors in assessing microinvasion risk in DCIS. The observed association between lower BMI and reduced microinvasion risk, along with the significance of HER2 positivity, offers a foundation for targeted risk assessment in DCIS management. Notably, the presence of a palpable mass emerged as the only independent factor associated with microinvasion, suggesting that physical examination may provide valuable prognostic information prior to surgery. Further prospective studies with larger patient cohorts are warranted to confirm these findings.

Funding: None.

Acknowledgment: We also owe a special thanks to Prof. Dr. Ömer Tolga Güler for his contributions to our study.

Authors contributions: S.Y. and T.Y.B. constructed the main idea and hypothesis of the study. A.A. was involved in the postoperative follow-up of the patients and the data collection part of the study. U.O. has done the evaluation of the data in the Results section. The discussion section of the article was written by S. Y., T.Y. B. and U.O.

Y.A.K. performed the histopathological examinations of the patients. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Tomlinson Hansen SE, Khan M, Cassaro S. Breast Ductal Carcinoma in situ. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.statpearls.com. Accessed February 27, 2023
2. Kerlikowske K. Epidemiology of Ductal Carcinoma in situ. *J Natl Cancer Inst Monogr*. 2010;2010(41):139-141. doi:10.1093/jncimonographs/lgq027
3. Shiino S, Quinn C, Ball G, et al. Prognostic significance of microinvasion with Ductal Carcinoma in situ of the breast: a meta-analysis. *Breast Cancer Res Treat*. 2023;197(2):245-254. doi:10.1007/s10549-022-06800-3
4. Stanciu Pop C, Nollevaux MC, Berlière M, et al. Morphological intratumor heterogeneity in Ductal Carcinoma in situ of the breast. *Virchows Arch*. 2021;479(1):33-43. doi:10.1007/s00428-021-03040-6
5. Wapnir IL, Dignam JJ, Fisher B, et al. Long-term outcomes of invasive ipsilateral breast tumor recurrences after lumpectomy in NSABP B-17 and B-24 randomized clinical trials for DCIS. *J Natl Cancer Inst*. 2011;103(6):478-488. doi:10.1093/jnci/djr027
6. Lyman GH, Giuliano AE, Somerfield MR, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. *J Clin Oncol*. 2005;23(30):7703-7720. doi:10.1200/JCO.2005.08.001
7. Parker C, Damodaran S, Bland KI, Hunt KK. The breast. In: Brunicardi FC, Andersen DK, Billiar TR, et al., editors. Schwartz's principles of surgery. 11th ed. New York, NY: McGraw-Hill Education; 2019.
8. Boler DE, Cabioglu N, Ince U, Esen G, Uras C. Sentinel lymph node biopsy in pure DCIS: is it necessary? *ISRN Surg*. 2012;2012:394095. doi:10.5402/2012/394095
9. Julian TB, Land SR, Fourchotte V, et al. Is sentinel node biopsy necessary in conservatively treated DCIS? *Ann Surg Oncol*. 2007;14(8):2202-2208. doi:10.1245/s10434-007-9353-4
10. Aksoy SÖ, Sevinç Aİ, Güray M, Balcı P. Duktal karsinoma in situ'da sentinel lenf nodu biyopsisi gerekli midir? *DEU Tıp Derg*. 2017;31(3):147-151. doi:10.5505/deutip.2017.50023

11. Watanabe Y, Anan K, Saimura M, et al. Upstaging to invasive Ductal Carcinoma after mastectomy for Ductal Carcinoma in situ: predictive factors and role of sentinel lymph node biopsy. *Breast Cancer*. 2018;25(6):663-670. doi:10.1007/s12282-018-0871-7
12. Ozkan Gurdal S, Cabioglu N, Ozcinar B, et al. Factors predicting microinvasion in Ductal Carcinoma in situ. *Asian Pac J Cancer Prev*. 2014;15(1):55-60. doi:10.7314/apjcp.2014.15.1.55
13. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2019;30(8):1194-1220.
14. Jia H, Zhao P, Chen Z, et al. Clinicopathological characteristics and prognostic analysis of tumor-infiltrating lymphocytes (TILs) in Ductal Carcinoma in situ (DCIS) and DCIS with microinvasion (DCIS-Mi) of the breast. *Breast Cancer Res Treat*. 2022;193(1):111-120. doi:10.1007/s10549-022-06553-z
15. Kuerer HM, Lari SA, Arun BK, et al. Biologic features and prognosis of Ductal Carcinoma in situ are not adversely impacted by initial large body mass. *Breast Cancer Res Treat*. 2012;133(3):1131-1141. doi:10.1007/s10549-012-1999-3
16. Porter GA, Inglis KM, Wood LA, et al. Effect of obesity on presentation of breast cancer. *Ann Surg Oncol*. 2006;13(3):327-332. doi:10.1245/ASO.2006.03.049
17. Canbay E, Cercel A, Aydoğan F, Gazioğlu E, Ferahman M, Kocael A, Aydın Ö, Calay Z, Çelik V. Indications for sentinel lymph node biopsy in patients with Ductal Carcinoma in situ. *Basic and Clinical Sciences.* 2013;2(2):47-56. doi:10.12808/bcs.v2i2.25
18. Diaz Casas SE, Serrano Muñoz WA, Buelvas Gómez NA, et al. When is Sentinel Lymph Node Biopsy Useful in Ductal Carcinoma In Situ? The Experience at a Latin American Cancer Center. *Cureus*. 2021;13(7):e16134. doi:10.7759/cureus.16134
19. Magnoni F, Massari G, Santomauro G, et al. Sentinel lymph node biopsy in microinvasive ductal carcinoma in situ. *Br J Surg*. 2019;106(4):375-383. doi:10.1002/bjs.11079
20. Champion CD, Ren Y, Thomas SM, et al. DCIS with Microinvasion: Is It In Situ or Invasive Disease?. *Ann Surg Oncol*. 2019;26(10):3124-3132. doi:10.1245/s10434-019-07556-9
21. Daly MB. Tamoxifen in Ductal Carcinoma in situ. *Semin Oncol*. 2006;33(6):647-649. doi:10.1053/j.seminoncol.2006.08.019
22. Van Bockstal MR, Wesseling J, Lips EH, Smidt M, Galant C, van Deurzen CHM. Systematic assessment of HER2 status in Ductal Carcinoma in situ of the breast: a perspective on the potential clinical relevance. *Breast Cancer Res*. 2024;26(1):125. doi:10.1186/s13058-024-01875-w
23. Roses RE, Paulson EC, Sharma A, et al. HER-2/neu overexpression as a predictor for the transition from in situ to invasive breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2009;18(5):1386-1389. doi:10.1158/1055-9965.EPI-08-1101
24. Yu KD, Wu LM, Liu GY, et al. Different distribution of breast cancer subtypes in breast Ductal Carcinoma in situ (DCIS), DCIS with microinvasion, and DCIS with invasion component. *Ann Surg Oncol*. 2011;18(5):1342-1348. doi:10.1245/s10434-010-1407-3
25. Silver SA, Tavassoli FA. Mammary Ductal Carcinoma in situ with microinvasion. *Cancer*. 1998;82(12):2382-2390. doi:10.1002/(sici)1097-0142(19980615)82:12<2382::aid-cncr12>3.0.co;2-l
26. Maffuz A, Barroso Bravo S, Nájera I, Zarco G, Alvarado Cabrero I, Rodríguez Cuevas SA. Tumor size as predictor of microinvasion, invasion, and axillary metastasis in Ductal Carcinoma in situ. *J Exp Clin Cancer Res*. 2006;25(2):223-227.
27. Lagios MD, Margolin FR, Westdahl PR, Rose MR. Mammographically detected Ductal Carcinoma in situ. Frequency of local recurrence following tylectomy and prognostic effect of nuclear grade on local recurrence. *Cancer*. 1989;63(4):618-624. doi:10.1002/1097-0142(19890215)63:4<618::aid-cncr2820630403>3.0.co;2-j
28. Balac N, Tungate RM, Jeong YJ, et al. Is palpable DCIS more aggressive than screen-detected DCIS?. *Surg Open Sci*. 2022;11:83-87. doi:10.1016/j.sopen.2022.12.002

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1565193

Revitalizing traditional carotid endarterectomy methods: a comprehensive review of primary closure techniques

Karotis endarterektomide geleneksel yöntemlerin canlandırılması: primer kapatma tekniklerinin kapsamlı bir incelemesi

Oğuz Arslantürk, Emrah Keskin

Posted date:11.10.2024 Acceptance date:16.12.2024

Abstract

Purpose: Carotid endarterectomy (CEA) remains essential for the treatment of carotid artery disease; however, primary closure techniques have recently gained interest. This study investigated the outcomes, efficacy, and safety of primary closure compared with conventional methods.

Materials and methods: A retrospective analysis of 130 patients who underwent CEA with primary closure at our clinic between January 2018 and April 2024 was performed. All surgeries followed a standardized procedure under general anesthesia. Data on demographics, surgical time, cross-clamp time, mortality, morbidity, and length of hospital stay were collected. Follow-up evaluations were performed 1, 6, and 12 months post-surgery.

Results: The study included 130 patients, 90 males (69%) and 40 females (31%), with an average age of 69.54±5.42 years. Restenosis occurred in five patients (3%) at 12 months, all of whom were asymptomatic. Three patients experienced transient ischemic attacks (TIA) postoperatively, which fully resolved within one month. Importantly, no early mortality was recorded during the one-month follow-up period.

Conclusion: Primary closure in CEA demonstrates promising outcomes, with low rates of restenosis and postoperative complications and no early mortality. These findings highlight primary closure as a potentially advantageous approach for CEA. Further research and prospective trials are necessary to validate and optimize this technique and ultimately improve patient outcomes in carotid artery disease management.

Keywords: Carotid endarterectomy, primary closure, surgical techniques, restenosis.

Arslanturk O, Keskin E. Revitalizing traditional carotid endarterectomy methods: a comprehensive review of primary closure techniques. Pam Med J 2025;18:418-424.

Öz

Amaç: Karotid endarterektomi (KEA), karotid arter hastalığının tedavisinde önemini korurken, son zamanlarda birincil kapatma tekniklerine olan ilgi artmıştır. Bu çalışma, geleneksel yöntemlerle karşılaştırıldığında birincil kapatmanın sonuçlarını, etkinliğini ve güvenliğini araştırmaktadır.

Gereç ve yöntem: Ocak 2018 ile Nisan 2024 tarihleri arasında kliniğimizde primer kapatma ile KEA uygulanan 130 hastanın retrospektif analizi yapıldı. Tüm ameliyatlar genel anestezi altında standart bir prosedür izledi. Hastaların demografik özellikleri, ameliyat süresi, kros klemp süresi, mortalite, morbidite ve hastanede kalış süresiyle ilgili veriler toplandı. Takip değerlendirmeleri ameliyattan 1, 6 ve 12 ay sonra gerçekleştirildi.

Bulgular: Çalışmaya 90 erkek (%69) ve 40 kadın (%31) olmak üzere toplam 130 hasta dahil edildi. Hastaların ortalama yaşı 69,54±5,42 yıldı. Takiplerin 12. ayında, asemptomatik olarak 5 hastada (%3) restenoz gelişti. Postoperatif dönemde 3 hastada geçici iskemik atak (TIA) görüldü ve bu ataklar bir ay içinde tamamen düzeldi. Önemli olarak, bir aylık takip süresi boyunca hiçbir erken ölüm vakası bildirilmedi.

Sonuç: KEA'da primer kapatma tekniği, düşük restenoz ve postoperatif komplikasyon oranları ile erken mortalite olmaması bakımından umut verici sonuçlar ortaya koymaktadır. Bu bulgular, primer kapatma tekniğinin KEA'da avantajlı bir yaklaşım olabileceğini göstermektedir. Yöntemin doğrulanması ve optimize edilmesi amacıyla daha fazla araştırma ve prospektif çalışmalar gereklidir, böylece karotis arter hastalığının yönetiminde hasta sonuçları iyileştirilebilir.

Anahtar kelimeler: Karotis endarterektomi, primer kapatma, cerrahi teknikler, restenoz.

Arslantürk O, Keskin E. Karotis endarterektomide geleneksel yöntemlerin canlandırılması: primer kapatma tekniklerinin kapsamlı bir incelemesi. Pam Tıp Derg 2025;18:418-424.

Oğuz Arslantürk, Asst. Prof. Department of Cardiovascular Surgery, Zonguldak Bülent Ecevit University, Faculty of Medicine, Zonguldak, Türkiye, e-mail: dr.arslanturk@gmail.com (https://orcid.org/0000-0002-4047-9656) (Corresponding Author)

Emrah Keskin, Assoc. Prof. Department of Neurosurgery, Zonguldak Bülent Ecevit University, Faculty of Medicine, Zonguldak, Türkiye, e-mail: emrah.keskin@beun.edu.tr (https://orcid.org/0000-0001-5326-741X)

Introduction

Carotid artery disease (CAD) is an important cause of ischemic stroke worldwide. Carotid endarterectomy is one of the most effective treatment modalities for both symptomatic and asymptomatic patients [1]. Intervention in carotid stenosis (endarterectomy or stenting depending on the clinical situation) is generally recommended in symptomatic patients with stenosis greater than 70% and in selected asymptomatic low-risk patients and symptomatic patients with stenosis between 50-69% [2]. Carotid endarterectomy (CEA) is the surgical removal of atherosclerotic plaques deposited in the carotid artery and has long been recognized as the gold standard treatment [3]. Over the years, various innovations have been made in CEA techniques to improve the safety and outcomes of surgery. Among these innovations, the method of arterial closure remains an important research topic. A correct closure technique plays an important role in maintaining in hemodynamic stability of the vessel; however, the optimal surgical closure method during CEA is still the focus of controversy. Patch closure is widely preferred because of its potential to reduce the risk of restenosis, but its favorable effect on the hemodynamic profile has been questioned [4]. The primary closure technique involves direct suturing of the arterial wall without the use of a patch and offers advantages such as shortening the operation time, reducing the complexity of the procedure, and avoiding the use of foreign material [5]. However, questions regarding the risk of restenosis and long-term efficacy of this method are among the factors limiting its widespread use. This study aimed to evaluate the safety and efficacy of CEA with primary closure and to demonstrate its potential advantages over conventional closure techniques. By analyzing surgical and postoperative data from 130 patients treated in our clinic, we aimed to strengthen the evidence for primary closure and to examine the potential role of this technique in the management of carotid artery disease.

Materials and methods

This study retrospectively analyzed data from 130 patients who underwent CEA using the primary closure method at our clinic between January 2018 and April 2024. The study protocol was in accordance with the ethical rules of the Declaration of Helsinki, and approval was obtained from the Ethics Committee of the Zonguldak Bülent Ecevit University Faculty of Medicine Application and Research Hospital (date: 02/10/2024 and decision number: 2024/17).

In this retrospective study, specific exclusion criteria were applied to enhance the reliability and accuracy of results. First, patients who had previously undergone surgical interventions, such as CABG or carotid artery stenting, were not included in the study. Additionally, patients who had patches used for artery closure during surgery were excluded, as this study only examined cases performed using the primary closure method. Patients who did not complete the 12-month follow-up period after surgery or were lost to follow-up during the monitoring period were also excluded from the evaluation.

Patients with serious comorbidities that could negatively affect surgical outcomes (such as advanced heart failure, kidney failure, or cancer) were excluded from the study because they could potentially affect the surgical results of this group. Additionally, patients diagnosed with active infection or inflammatory disease during or after surgery were excluded from the study.

Patient selection

The patients included in the study were symptomatic or asymptomatic patients with >70% stenosis detected in the carotid artery using Doppler ultrasonography or other imaging methods. Additionally, patients who were symptomatic and had stenosis between 50-69% were also included. Demographic data (age, sex, and comorbidities) and clinical information were obtained retrospectively from hospital records.

Surgical protocol

All surgical procedures were performed under general anesthesia by an experienced vascular surgeon using standard techniques. Patients underwent a standard preoperative evaluation, and the risk assessment for surgical suitability was completed by the anesthesia team. Patients were positioned in the supine position on the operating table, and the head was slightly extended to best expose the surgical field.

During the operation, the neck area of the patient was cleaned with antiseptic solutions and isolated with sterile drapes. A surgical incision was made along the sternocleidomastoid muscle, typically parallel to the medial border. The carotid artery triangle was carefully exposed, and the carotid bifurcation, internal and external carotid arteries, and adjacent structures were carefully dissected and isolated. While dissecting along the carotid artery, care was taken to protect critical structures, such as the vagus nervu and hypoglossal nerve.

To remove atherosclerotic plaque, the artery was cross-clamped to stop blood flow and expose the plaque area. During the endarterectomy procedure, the arterial wall was carefully opened, and the atheromatous material was removed. After the plaque was completely removed, the inner surface of the artery was cleaned using a soft spatula. At this stage, careful technique was used to avoid damage to the carotid artery and distal embolization.

After plaque removal, artery closure was performed using a primary closure. Primary closure was performed via direct suturing of the arterial wall without the use of any patch material. A thin monofilament suture material (usually 5-0 or 6-0 polypropylene (Ethicon, Sommerville, NJ) was used during closure. During the suturing process, care was taken to obtain a smooth anastomosis without damaging the hemodynamics of the artery or causing stenosis. During all closure procedures, the distal and proximal arterial structures were carefully protected to ensure adequate blood flow.

After removal of the cross-clamp, blood flow was restored, and hemodynamic stability was assessed. Blood flow in the carotid artery was assessed using Doppler ultrasonography for any obstruction or flow disturbance. After hemostasis was achieved, the surgical field was washed with sterile serum, and the operation was completed by closing the layers. In the postoperative period, patients were closely monitored for neurological status, and intervention was performed in case of any complications. Postoperatively, the patients were admitted to the intensive care unit or surgical ward in accordance with standard follow-up protocols, and postoperative follow-up was performed.

Postoperative follow-up and evaluation

All patients were followed up regularly at 1, 6, and 12 months postoperatively. During follow-up, neurological evaluations were performed, and restenosis was confirmed using Doppler ultrasonography. Postoperative complications include restenosis, transient ischemic attack (TIA), stroke, and mortality. Restenosis was defined as more than 50% stenosis of the carotid artery. In addition, the length of the hospital stay and other postoperative complications were recorded.

Laboratory and imaging tests

Biochemical parameters, such as complete blood count (CBC), electrolyte levels, renal function tests (creatinine and urea), and C-Reactive Protein (CRP) levels, were analyzed preoperatively and postoperatively. Doppler ultrasonography was used to evaluate the presence of restenosis, and cardiac complications were monitored using electrocardiography (ECG) during the postoperative period.

Results

This retrospective study aimed to evaluate the efficacy and safety of primary closure during CEA in 130 patients. The patients were 90 males (69%) and 40 females (31%) with a mean age of 69.54±5.42 years. The demographic data of the patients are shown in Table 1. Asymptomatic restenosis was detected in five patients (3%) at the 12th month of postoperative follow-up, but no clinically significant symptoms were observed in these patients. In addition, three patients (2.3%) developed TIA in the postoperative period, and these episodes resolved completely within one month.

Table 1. Patient characteristics

Characteristic	Values
Mean age at the time of surgery (years)	69.54±5.42
Gender n (%) - Male	90 (69%)
Gender n (%) - Female	40 (31%)
Hypertension n (%)	60 (46%)
Hyperlipidaemia n (%)	45 (35%)
Coronary Artery Disease n (%)	50 (38%)
Atrial Fibrillation n (%)	20 (15%)

At the end of 1 year, one patient (0.8%) developed myocardial infarction and was managed with appropriate cardiovascular therapy. Amaurosis fugax was observed in one patient (0.8%). Furthermore, among surgical complications, hematoma developed in four patients (3%), but none of these patients required revision surgery. Regarding nerve damage, two patients (1.5%) had hypoglossal nerve damage, and one patient (0.8%) had marginal mandibular nerve damage. All nerve injuries showed partial recovery in the months following surgery. The postoperative complications are shown in Table 2. In terms of early mortality, there were no deaths in any of the patients during the first month of follow-up.

Table 2. Postoperative complications

Complications	Values
Restenosis (3%)	5 patients
Transient Ischemic Attack (TIA) (2.3%)	3 patients
Myocardial Infarction	1 patient (after 1 year)
Amarozis Fugax	1 patient
Hematoma	4 patient
Hypoglossal Nerve Damage	2 patient
Marginal Mandibular Nerve Injury	1 patient
Early Mortality	0 patient

Discussions

Carotid endarterectomy is the gold standard surgical procedure for the treatment of carotid artery diseases. CEA is an effective treatment option to prevent recurrent stroke in patients with symptomatic carotid artery stenosis. This effective treatment is associated with stroke and mortality rates of less than 3% [6-9]. These results demonstrate the reliability and clinical preference of this method. However, the differences between surgical techniques and surgeon experience play a decisive role in the postoperative outcomes. Differences between long-standing surgical closure methods are increasingly being discussed in terms of their impact on patient clinical outcomes. In this context, the first comparison of primary carotid artery closure and patch angioplasty dates back to 1964. These early studies provided an important starting point for understanding the effects of artery closure methods on postoperative hemodynamics and complications [10]. In our study, we aimed to evaluate our results using the primary closure technique, which began in the late 19th century.

In the study by Rockman et al. [11], primary closure was reported to have more unfavorable postoperative outcomes than patch closure and eversion techniques. The authors suggested that this method was associated with higher restenosis rates and long-term complication risks. However, in the same study, surgeons, particularly those with little surgical experience, tended to prefer the primary closure method. This causes confusion in the literature regarding the safety and efficacy of this method [11]. In our study, asymptomatic restenosis was detected in five patients (3%) at 1 year. The results obtained in this study suggest that primary closure is a safe option, with low restenosis and postoperative complication rates. However, the better results compared to the findings of Rockman et al. [11] suggest that surgeon experience and patient selection criteria play a critical role in the success rate of this method.

Kapoor et al. [12] analyzed intraoperative EEG data and emphasized that selective shunting with primary closure yielded similar results to routine patch angioplasty and shunting. In particular, this study shows that primary closure may be a neurologically safe option and that this method provides neurologic protection equivalent to routine patch angioplasty. This finding suggests that the primary closure technique may be a safe alternative, particularly when used in combination with selective shunting.

One possible complication of patch closure is pseudoaneurysm development. In a study conducted by Hertzer et al. [13] on 917 cases, it was reported that three patients developed pseudoaneurysms and required surgical repair. Curiously, it has been reported that no such complications were observed in the primary closure group without patching. This finding suggests that the patch closure method should be carefully evaluated for its safety. Although patch application has hemodynamic advantages, the risks of such complications should not be ignored. In our study, no false aneurysm development was observed in patients who underwent primary closure. However, large-scale, long-term studies are necessary for a clearer understanding of the differences between primary and patch closures. Such studies will allow us to better understand the risks of complications associated with surgical techniques and patient outcomes.

Stenting remains a good treatment option for carotid artery stenosis [14-16]. In the literature, several studies have demonstrated that CEA offers a more cost-effective treatment option compared with carotid artery stenting [17, 18]. Although we aimed to differentiate between surgical techniques, the materials used in both stents and different surgical techniques caused a significant cost. In addition to being cost-effective, CEA using the primary closure technique offers successful clinical outcomes in the postoperative period, making this method a more attractive option. This finding supports the idea that primary closure is an advantageous alternative in terms of both economic and clinical outcomes. In a single-center study by Zagzoog et al. [19], it was reported that primary and patch closure techniques have similar risk profiles, and both methods offer equally robust surgical approaches that can be safely applied in CEA procedures. This study suggests that the choice of closure technique can be determined by surgeon preference and patient characteristics and reveals that there is no significant clinical difference between the two methods. In another study, Clagett et al. [20] reported that primary closure significantly reduced the clamp time and increased restenosis (12.9% vs. 1.7%). These results suggest that primary closure may be a more effective option in terms of the operative time and surgical complexity. However, the choice of surgical technique should be based on individual considerations for each patient, and further prospective studies are required.

Primary closure appears to be a safe, low complication rate, and economical option for CEA. When compared with different closure methods in the literature, primary closure has been reported to shorten the surgical time, maintain hemodynamic stability, and accelerate postoperative recovery. However, patient selection, surgeon experience, and the effects of additional surgical techniques should be considered. Although the findings showed that the short-term results of the primary closure technique are favorable, prospective studies based on long-term follow-up data in larger patient populations are necessary. Such studies will allow us to better understand the long-term safety and efficacy of primary closure and will guide the choice of the surgical technique.

Limitations

This study had some limitations owing to its retrospective design. First, retrospective data collection may have introduced potential biases in patient selection and data validation. Second, our patient group represented a single-center population; therefore, the results obtained may not be fully generalizable to the general population. Furthermore, multicenter and prospective studies involving large patient populations are necessary to evaluate the long-term efficacy and safety of primary closure.

In conclusion, this study provides data demonstrating the efficacy and safety of the primary closure technique for CEA. In our study, we observed low complication rates, minimal nerve damage, and limited postoperative events with primary closure. It was also found to be advantageous in terms of surgical time and cost. These findings suggest that primary closure can be used as a safe and effective alternative in selected patient groups. However, multicenter prospective studies are needed to better understand the long-term results of this method and to validate it in different patient populations.

Funding: None.

Authors contributions: O.A. and E.K. constructed the main idea and hypothesis of the study. O.A. and E.K. developed the theory and arranged/edited the material and method section. O.A. has done the evaluation of the data in the Results section. The discussion section of the article was written by O.A. and E.K. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Brott TG, Hobson RW 2nd, Howard G, et al. Stenting versus endarterectomy for treatment of carotid-artery stenosis [published correction appears in N Engl J Med. 2010 Jul 29;363(5):498] [published correction appears in N Engl J Med. 2010 Jul 8;363(2):198]. *N Engl J Med*. 2010;363(1):11-23. doi:10.1056/NEJMoa0912321
2. Grotta JC. Clinical practice. Carotid stenosis. *N Engl J Med*. 2013;369(12):1143-1150. doi:10.1056/NEJMcp1214999
3. Howell SJ. Carotid endarterectomy. *Br J Anaesth*. 2007;99(1):119-131. doi:10.1093/bja/aem137
4. Byrne J, Feustel P, Darling RC 3rd. Primary closure, routine patching, and eversion endarterectomy: what is the current state of the literature supporting use of these techniques?. *Semin Vasc Surg*. 2007;20(4):226-235. doi:10.1053/j.semvascsurg.2007.10.006
5. Cheng I, Vyas KS, Velaga S, Davenport DL, Saha SP. Outcomes of Carotid Endarterectomy with Primary Closure. *Int J Angiol*. 2017;26(2):83-88. doi:10.1055/s-0037-1601053
6. Naylor AR, Sayers RD, McCarthy MJ, et al. Closing the loop: a 21-year audit of strategies for preventing stroke and death following carotid endarterectomy. *Eur J Vasc Endovasc Surg*. 2013;46(2):161-170. doi:10.1016/j.ejvs.2013.05.005
7. Rerkasem A, Orrapin S, Howard DP, Rerkasem K. Carotid endarterectomy for symptomatic carotid stenosis. *Cochrane Database Syst Rev*. 2020;9(9):CD001081. Published 2020 Sep 12. doi:10.1002/14651858.CD001081.pub4
8. North American Symptomatic Carotid Endarterectomy Trial Collaborators, Barnett HJM, Taylor DW, et al. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med*. 1991;325(7):445-453. doi:10.1056/NEJM199108153250701
9. Hobson RW 2nd, Weiss DG, Fields WS, et al. Efficacy of carotid endarterectomy for asymptomatic carotid stenosis. The Veterans Affairs Cooperative Study Group. *N Engl J Med*. 1993;328(4):221-227. doi:10.1056/NEJM199301283280401
10. Norton LW, Spencer FC. Long-term comparison of vein patch with direct suture. Technique of anastomosis of small arteries. *Arch Surg.* 1964;89:1083-1088. doi:10.1001/archsurg.1964.01320060151027
11. Rockman CB, Halm EA, Wang JJ, et al. Primary closure of the carotid artery is associated with poorer outcomes during carotid endarterectomy. *J Vasc Surg*. 2005;42(5):870-877. doi:10.1016/j.jvs.2005.07.043
12. Kapoor R, Evins AI, Marcus J, Rigante L, Kubota M, Stieg PE. Selective Patch Angioplasty and Intraoperative Shunting in Carotid Endarterectomy: A Single-Center Review of 141 Procedures. *Cureus*. 2015;7(10):e367. Published 2015 Oct 28. doi:10.7759/cureus.367
13. Hertzer NR, Beven EG, O'Hara PJ, Krajewski LP. A prospective study of vein patch angioplasty during carotid endarterectomy. Three-year results for 801 patients and 917 operations. *Ann Surg*. 1987;206(5):628-635. doi:10.1097/00000658-198711000-00013
14. White CJ. Carotid artery stenting. *J Am Coll Cardiol*. 2014;64(7):722-731. doi:10.1016/j.jacc.2014.04.069

15. International Carotid Stenting Study investigators, Ederle J, Dobson J, et al. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis (International Carotid Stenting Study): an interim analysis of a randomised controlled trial [published correction appears in Lancet. 2010 Jul 10;376(9735):90. Nasser, H-C [corrected to Nahser, H-C]]. *Lancet*. 2010;375(9719):985-997. doi:10.1016/S0140-6736(10)60239-5
16. Bonati LH, Dobson J, Featherstone RL, et al. Long-term outcomes after stenting versus endarterectomy for treatment of symptomatic carotid stenosis: the International Carotid Stenting Study (ICSS) randomised trial. *Lancet*. 2015;385(9967):529-538. doi:10.1016/S0140-6736(14)61184-3
17. Sternbergh WC 3rd, Crenshaw GD, Bazan HA, Smith TA. Carotid endarterectomy is more cost-effective than carotid artery stenting. *J Vasc Surg*. 2012;55(6):1623-1628. doi:10.1016/j.jvs.2011.12.045
18. Kilaru S, Korn P, Kasirajan K, et al. Is carotid angioplasty and stenting more cost effective than carotid endarterectomy?. *J Vasc Surg*. 2003;37(2):331-339. doi:10.1067/mva.2003.124
19. Zagzoog N, Elgheriani A, Attar A, et al. Comprehensive comparison of carotid endarterectomy primary closure and patch angioplasty: A single-institution experience. *Surg Neurol Int*. 2022;13:1. Published 2022 Jan 5. doi:10.25259/SNI_1013_2021
20. Clagett GP, Patterson CB, Fisher DF Jr, et al. Vein patch versus primary closure for carotid endarterectomy. A randomized prospective study in a selected group of patients. *J Vasc Surg*. 1989;9(2):213-223. doi:10.1067/mva.1989.vs0090213

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1649652

Comparison of serum fibroblast growth factor 23 (FGF23) levels and Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) parameters in home hemodialysis and center hemodialysis

Ev hemodiyalizi ve merkez hemodiyalizinde serum fibroblast büyüme faktörü 23 (FGF23) seviyeleri ve Kronik Böbrek Hastalığı-Mineral ve Kemik Bozukluğu (CKD-MBD) parametreleri üzerindeki etkilerinin karşılaştırılması

Davut Akın, Mevlüt Çeri, Gülşah Gündoğdu, Mehmet Batmazoğlu, Evrim Bozkaya, Hasan Akça

Posted date:02.03.2025 Acceptance date:25.03.2025

Abstract

Purpose: This study aims to compare serum fibroblast growth factor 23 (FGF23) levels and chronic kidney disease-mineral and bone disorder (CKD-MBD) parameters in patients undergoing home hemodialysis (HHD) and center hemodialysis (CHD).

Materials and methods: A total of 42 patients over 18 years old who had been receiving dialysis treatment for at least six months were included in the study. The patients were divided into two groups: HHD (n=17) and CHD (n=25). Demographic data, dialysis duration, biochemical parameters (serum phosphorus, calcium, parathyroid hormone (PTH), albumin, hemoglobin, FGF23, etc.), and medication use were recorded. Serum FGF23 levels were measured using an enzyme-linked immunosorbent assay (ELISA) method from blood samples taken before midweek HD sessions. Frequency distributions were expressed as percentages and compared using the chi-square test.

Results: The Kt/V values were higher in the HHD group (2.4±0.1 vs. 1.6±0.1), while serum phosphorus levels were lower (4.1±1.1 vs. 5.0±1.2 mg/dL). Although FGF23 levels were lower in the HHD group, the difference was not statistically significant (383±423 vs. 441±480 pg/mL, p=0.05). Erythropoietin (Epo) usage was significantly lower in the HHD group (47% vs. 92%, p=0.001).

Conclusion: HHD was associated with better phosphorus control and a reduced requirement for EPO compared to CHD. The lower FGF23 levels in HHD suggest that this modality may offer advantages in CKD-MBD, cardiovascular outcomes, and mortality. However, these findings need to be supported by prospective studies involving larger patient populations.

Keywords: FGF23, home hemodialysis, center hemodialysis, chronic kidney disease, dialysis.

Akin D, Ceri M, Gundogdu G, Batmazoglu M, Bozkaya E, Akca H. Comparison of serum fibroblast growth factor 23 (FGF23) levels and Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) parameters in home hemodialysis and center hemodialysis. Pam Med J 2025;18:426-430.

Öz

Amaç: Bu çalışma, ev hemodiyalizi (HHD) ve merkez hemodiyalizi (CHD) tedavisi altındaki hastalarda serum fibroblast büyüme faktörü 23 (FGF23) seviyelerini ve Kronik Böbrek Hastalığı-Mineral ve Kemik Bozukluğu (CKD-MBD) parametrelerini karşılaştırmayı amaçlamaktadır.

Gereç ve yöntem: Çalışmaya 18 yaş üstü, en az 6 aydır diyaliz tedavisi alan 42 hasta dahil edildi. Hastalar, HHD (n=17) ve CHD (n=25) olmak üzere iki gruba ayrıldı. Demografik veriler, diyaliz süresi, biyokimyasal parametreler (serum fosfor, kalsiyum, parathormon (PTH), albümin, hemoglobin, FGF23 vb.) ve ilaç kullanımı kaydedildi. Serum FGF23 seviyeleri, haftanın ortasında HD seansı öncesi alınan kan örneklerinden ELISA yöntemi ile ölçüldü. Gruplar arasındaki frekans dağılımları yüzdeli olarak ifade edildi ve ki-kare testi ile karşılaştırıldı.

Bulgular: HHD grubunda Kt/V değerleri daha yüksek (2,4±0,1 vs. 1,6±0,1) ve serum fosfor seviyeleri daha düşük (4,1±1,1 vs. 5,0±1,2 mg/dL) bulundu. FGF23 seviyeleri HHD grubunda daha düşük olmasına rağmen, iki grup arasında istatistiksel olarak anlamlı fark tespit edilmedi (383±423 vs. 441±480 pg/ml, p=0,05). EPO kullanımı HHD grubunda belirgin şekilde daha düşük bulundu (%47 vs. %92, p=0,001).

Davut Akın, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Nephrology, Denizli, Türkiye, e-mail: dakin@pau.edu.tr (https://orcid.org/0000-0002-9567-7940) (Corresponding Author)

Mevlüt Çeri, Prof. Pamukkale University Faculty of Medicine, Department of Nephrology, Denizli, Türkiye, e-mail: mevlutceri@gmail.com (https://orcid.org/0000-0002-2276-5157)

Gülşah Gündoğdu, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Physiology, Denizli, Türkiye, e-mail: ggundogdu@pau.edu.tr (https://orcid.org/0000-0002-9924-5176)

Mehmet Batmazoğlu, Specialist M.D. Denizli State Hospital, Clinic of Nephrology, Denizli, Türkiye, e-mail: drbatmazoglu@hotmail.com (https://orcid.org/0009-0001-8296-9629)

Evrim Bozkaya, Specialist M.D. Denizli State Hospital, Clinic of Nephrology, Denizli, Türkiye, e-mail: evrimoskay@yahoo.com (https://orcid.org/0009-0002-4894-4759)

Hasan Akça, Research Assistant, Pamukkale University Faculty of Medicine, Department of Physiology, Denizli, Türkiye, e-mail: hasanakca@pau.edu.tr (https://orcid.org/0000-0001-8782-9724)

Sonuç: HHD, CHD'ye kıyasla daha iyi fosfor kontrolü ve daha düşük EPO gereksinimi ile ilişkilendirildi. FGF23 seviyelerinin HHD'de daha düşük olması, bu modalitenin CKD-MBD, kardiyovasküler sonuçlar ve mortalite açısından avantaj sağlayabileceğini düşündürmektedir. Ancak, bu bulguların daha geniş hasta gruplarında prospektif çalışmalar ile desteklenmesi gerekmektedir.

Anahtar kelimeler: FGF23, ev hemodiyalizi, merkez hemodiyalizi, kronik böbrek hastalığı, diyaliz.

Akın D, Çeri M, Gündoğdu G, Batmazoğlu M, Bozkaya E, Akça H. Ev hemodiyalizi ve merkez hemodiyalizinde serum fibroblast büyüme faktörü 23 (FGF23) seviyeleri ve Kronik Böbrek Hastalığı-Mineral ve Kemik Bozukluğu (CKD-MBD) parametreleri üzerindeki etkilerinin karşılaştırılması. Pam Tıp Derg 2025;18:426-430.

Introduction

Over the past 10-15 years, home hemodialysis (HHD) has emerged as an alternative modality, particularly with nocturnal dialysis or extended dialysis session durations [1]. Various studies have demonstrated that HHD improves serum phosphorus control, reduces phosphate binder requirements, and allows for a more liberalized diet [2-4].

Hemodialysis is the most commonly used renal replacement therapy that significantly improves the prognosis of end-stage renal disease (ESRD) patients [5]. Conventional in-center hemodialysis (CHD) is performed three times a week, with each session lasting four hours, while HHD is performed at least three times per week with a session duration of at least six hours. Peritoneal dialysis (PD) is also an alternative dialysis modality [6]. Globally, 89% of dialysis patients undergo HD, while 11% receive PD. However, mortality rates among CHD patients remain relatively high, with all-cause mortality being seven times higher and cardiovascular mortality eight times higher than in the general population [7].

In parallel, fibroblast growth factor 23 (FGF23) has been recognized as a key regulator of phosphate and vitamin D metabolism over the past decade. FGF23, a potent calcium and phosphorus regulator, is produced by osteoblasts and osteocytes. It reduces phosphorus levels by promoting phosphate excretion and inhibiting 1.25(OH)2D3 formation [8]. In the early stages of chronic kidney disease (CKD), FGF23 secretion is upregulated to maintain phosphate balance, but this subsequently leads to decreased 1.25(OH)2D3 synthesis and triggers secondary hyperparathyroidism [9]. As kidney function deteriorates, the ability to promote phosphate excretion declines due to a reduction in functional nephrons, and abnormally elevated FGF23 becomes a uremic toxin strongly associated with adverse clinical outcomes [10].

FGF23 levels are extremely high in dialysis patients [11], correlating with serum phosphorus concentrations and increasing with enteral phosphate intake and vitamin D sterol treatment [12]. Although serum phosphorus concentration has long been associated with cardiovascular disease and mortality in dialysis patients, FGF23 has also been identified as an independent predictor of cardiovascular disease and mortality in both the general and dialysis populations [13].

Given that elevated FGF23 is linked to increased cardiovascular and all-cause mortality [4], we hypothesized that HHD could lower serum FGF23 levels by altering CKD-MBD parameters, potentially reducing mortality. However, the impact of different dialysis modalities on circulating FGF23 levels, underlying regulatory mechanisms, and potential clinical benefits remains unclear. This retrospective study aimed to investigate whether FGF23 levels were lower in CHD versus HHD patients and whether this difference could improve patient prognosis by comparing serum FGF23 levels, CKD-MBD-related parameters, hemoglobin, and albumin levels between these two dialysis modalities.

Materials and methods

Study design and population

This retrospective study includes patients aged 18 years or older receiving HHD, who remained on this treatment modality for at least six months between 2015 and July 2024, as well as patients undergoing CHD at the center. Patients with malignancies, severe infections, or advanced cardiac or hepatic failure, as well as those not meeting the inclusion criteria, were excluded. Consequently, a total of 42 patients who met the criteria were included in the study.

CHD Group: Underwent HD three times per week, each session lasting four hours, with a blood flow rate of 200-360 mL/min and a dialysate flow rate of 500 mL/min.

HHD Group: Underwent HD three times per week, with sessions lasting at least six hours, a blood flow rate of 200-300 mL/min, and a dialysate flow rate of 300-500 mL/min.

All HD patients used polysulfone membranes (1.5-2.1 m²), and anticoagulation was maintained using heparin or low-molecular-weight heparin.

Ethics approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval date: 21.01.2025/02, file no: E-60116787-020-643233).

Demographic data, dialysis duration, and primary ESRD causes were collected from the Pamukkale University Faculty of Medicine and Electronic medical records (e-Nabız). Biochemical parameters, including calcium (Ca), phosphorus (P), hemoglobin (Hb), albumin (Alb), ferritin, 25-hydroxyvitamin D, intact parathyroid hormone (iPTH), and Kt/V, were recorded. Serum intact FGF23 levels were measured using the enzyme-linked immunosorbent assay (ELISA) kit (E0059Hu, BT Lab, Shanghai, China) on blood samples collected before midweek dialysis sessions (typically before the second treatment session of the week). Samples were obtained according to a standardized schedule to ensure consistency and were drawn from the arteriovenous fistula (or central venous catheter, if applicable) immediately prior to heparin administration. Blood samples were collected in 2 mL biochemical tubes, centrifuged at 3.500 rpm for 20 minutes at room temperature within 15 minutes of collection, and the resulting sera were stored at -80°C until the day of analysis.

Statistical analysis

All continuous data were expressed as mean ± standard deviation (SD). Independent sample t-tests were used to analyze differences between the two groups. Frequency distributions were expressed as percentages and compared using the chi-square test. A $p<0.05$ was considered statistically significant.

Results

A total of 42 dialysis patients were included (HHD: n=17, CHD: n=25). The mean ages were 47.8±12.8 and 53.1±11.9 years for the HHD and CHD groups, respectively. The dialysis duration was significantly longer in the HHD group (106.6±48.7 vs. 48.1±44.5 months) (Table 1).

Regarding erythropoietin (EPO) use, 47% (n=8) of HHD patients and 92% (n=23) of CHD patients used EPO, with a statistically significant difference between the two groups ($p=0.001$). No significant differences were observed in the use of iron supplements, phosphate binders, calcimimetics, or vitamin D (Table 2).

Table 1. Biochemical values in patients undergoing treatment with home and center-based hemodialysis

	Home Hemodialysis (n=17)	Central hemodialysis (n=25)	Test value	*p* value
Age	47.8±12.8	53.1±11.9	t=-1.360	*p*=0.182
Dialysis Duration (months)	106.6±48.7	48.1±44.5	t=3982	*p*=0.0001*
KT/V	2.39±0.1	1.6±0.1	t=6.647	*p*=0.0001*
Hemoglobin(gr/dL)	12.1±1.5	11.1±1.5	t=2.001	*p*=0.052
Albumin (g/L)	39.4±5.9	38.5±3.3	t=0.570	*p*=0.572
Calcium (mg/dL)	8.8±0.5	9.0±1.0	t=-0.533	*p*=0.597
Phosphorus (mg/dL)	4.1±1.1	5.0±1.2	t=-2.368	*p*=0.023*
PTH (ng/L)	510±444	340±292	t=1.497	*p*=0.142
Vitamin D (µg/L)	14.7±13.2	12.7±13.8	t=-0.471	*p*=0.640
FGF23 (pg/ml)	383±423	441±480	t=-0.397	*p*=0.694

Kt/V: Dialysis adequacy index, PTH: Parathyroid Hormone, FGF23: fibroblast growth factor 23, t:Independent sample t-tests, *: $p<0.05$

Table 2. Medications used in patients undergoing home hemodialysis and center hemodialysis

Treatment	Home hemodialysis (n=17)	Central hemodialysis (n=25)	Test value	*p* value
Vitamin D use	6 (35%)	16 (64%)	χ^2=3.343	*p*=0.067
Phosphate binder use	11 (65%)	18 (72%)	χ^2=0.252	*p*=0.616
Erythropoietin Use	8 (47%)	23 (92%)	χ^2=10.572	*p*=0.001*
Iron use	6 (35%)	15 (60%)	χ^2=2471	*p*=0.116

χ^2: chi-square test, *: *p*<0.05

Discussion

Consistent with previous studies, urea clearance (Kt/V) was found to be higher in HHD. This higher clearance was associated with significantly lower serum phosphorus levels and reduced requirements for EPO and phosphate binders, suggesting improved mortality outcomes [14]. Furthermore, this study found that serum FGF23 levels were lower in the HHD group, which may serve as a bridge linking CKD-MBD to left ventricular hypertrophy and all-cause mortality. While multiple studies have focused on regulating high FGF23 levels in dialysis patients, their results have been inconsistent. Increased phosphate load is well-known to stimulate FGF23 secretion [15], but the molecular mechanism remains unclear. Although vitamin D plays a positive regulatory role in FGF23 expression, in this study, despite lower vitamin D use in HHD patients, phosphate binder use was lower in the CHD group.

While multiple studies have explored the regulation of elevated FGF23 levels in dialysis patients, their findings remain inconsistent. Increased phosphate load is well known to stimulate FGF23 secretion [15], though the precise molecular mechanisms remain unclear. Vitamin D plays a positive regulatory role in FGF23 levels. Although no statistically significant difference was observed between dialysis modalities, the trend of lower FGF23 levels in HHD may be attributed to reduced phosphate load. Interestingly, despite lower vitamin D usage in the HHD group, phosphate binder use was significantly lower in CHD patients.

PTH is another potential regulator of FGF23 in CKD, as both PTH and FGF23 levels tend to increase in patients with impaired renal function. Lavi Moshayoff et al. [16] suggested that PTH might upregulate FGF23 expression in vitro, though other studies have failed to confirm a direct regulatory effect. In this study, PTH levels were higher in HHD patients, whereas FGF23 levels were lower, a finding that contrasts with the CHD group.

Another significant result of this study was the significantly lower EPO requirement in the HHD group. Similar to findings by Ok et al. [17], we observed a reduced need for EPO in HHD patients. Although iron use was also lower in HHD patients, this difference did not reach statistical significance.

This study has several limitations. Firstly, the retrospective design of the study. Secondly, the relatively small sample size reduces the power to detect differences between the two dialysis modalities, particularly in terms of FGF23 levels. Thirdly, the fact that this study was conducted as a single-center study. Lastly, FGF23 levels were measured only once, and changes over time were not evaluated. Larger, multicenter, and prospective studies are needed to validate these findings.

In conclusion, in this study, although no statistically significant difference in FGF23 levels was observed between HHD and CHD patients, FGF23 levels were lower in the HHD group. This finding suggests that HHD may provide better phosphorus control and reduce phosphate binder medication use. Additionally, the significantly lower EPO requirement in the HHD group indicates potential benefits of this dialysis modality on erythropoiesis. However, given the study's small population and retrospective design, these findings need to be validated by larger, randomized controlled trials.

Funding: None.

Authors contributions: D.A. has constructed the main idea and hypothesis of the study. M.C. and G.G. developed the theory and arranged the material and method section. M.B., E.B. and H.A., have done the evaluation of the data in the Results section. Discussion section of the article. Written by D.A., M.C., M.B., E.B. and H.A. reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Trinh E, Chan CT. The Rise, Fall, and Resurgence of Home Hemodialysis. *Semin Dial*. 2017;30(2):174-180. doi:10.1111/sdi.12572
2. Lacson E Jr, Xu J, Suri RS, et al. Survival with three-times weekly in-center nocturnal versus conventional hemodialysis. *J Am Soc Nephrol*. 2012;23(4):687-695. doi:10.1681/ASN.2011070674
3. Ok E, Duman S, Asci G, et al. Comparison of 4- and 8-h dialysis sessions in thrice-weekly in-centre haemodialysis: a prospective, case-controlled study. *Nephrol Dial Transplant*. 2011;26(4):1287-1296. doi:10.1093/ndt/gfq724
4. Mucsi I, Hercz G, Uldall R, Ouwendyk M, Francoeur R, Pierratos A. Control of serum phosphate without any phosphate binders in patients treated with nocturnal hemodialysis. *Kidney Int*. 1998;53(5):1399-1404. doi:10.1046/j.1523-1755.1998.00875.x
5. Goodkin DA, Bragg Gresham JL, Koenig KG, et al. Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *J Am Soc Nephrol*. 2003;14(12):3270-3277. doi:10.1097/01.asn.0000100127.54107.57
6. Bugeja A, Dacouris N, Thomas A, et al. In-center nocturnal hemodialysis: another option in the management of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4(4):778-783. doi:10.2215/CJN.05221008
7. de Jager DJ, Grootendorst DC, Jager KJ, et al. Cardiovascular and noncardiovascular mortality among patients starting dialysis. *JAMA*. 2009;302(16):1782-1789. doi:10.1001/jama.2009.1488
8. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiol Rev*. 2012;92(1):131-155. doi:10.1152/physrev.00002.2011
9. Shimada T, Urakawa I, Yamazaki Y, et al. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun*. 2004;314(2):409-414. doi:10.1016/j.bbrc.2003.12.1021
10. Galitzer H, Ben Dov IZ, Silver J, Naveh Many T. Parathyroid cell resistance to fibroblast growth factor 23 in secondary hyperparathyroidism of chronic kidney disease. *Kidney Int*. 2010;77(3):211-218. doi:10.1038/ki.2009.464
11. Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med*. 2008;359(6):584-592. doi:10.1056/NEJMoa0706130
12. Nakanishi S, Kazama JJ, Nii Kono T, et al. Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. *Kidney Int*. 2005;67(3):1171-1178. doi:10.1111/j.1523-1755.2005.00184.x
13. Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA*. 2011;305(23):2432-2439. doi:10.1001/jama.2011.826
14. Lacson E Jr, Wang W, Lester K, Ofsthun N, Lazarus JM, Hakim RM. Outcomes associated with in-center nocturnal hemodialysis from a large multicenter program. *Clin J Am Soc Nephrol*. 2010;5(2):220-226. doi:10.2215/CJN.06070809
15. Ferrari SL, Bonjour JP, Rizzoli R. Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. *J Clin Endocrinol Metab*. 2005;90(3):1519-1524. doi:10.1210/jc.2004-1039
16. Lavi Moshayoff V, Wasserman G, Meir T, Silver J, Naveh Many T, PTH.increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol*. 2010;299:F882-889. doi:10.1152/ajprenal.00360.2010
17. Ok E, Duman S, Asci G, et al. Comparison of 4- and 8-h dialysis sessions in thrice-weekly in-centre haemodialysis: a prospective, case-controlled study. *Nephrol Dial Transplant*. 2011;26(4):1287-1296. doi:10.1093/ndt/gfq724

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1652305

Evaluation of S100B, NSE, MBP and GFAP serum levels in children with attention deficit hyperactivity disorder

Dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda serum S100B, NSE, MBP ve GFAP düzeylerinin değerlendirilmesi

Hatice Çelik Yıldırım, Bürge Kabukçu Başay, Egem Burcu Ünal

Posted date:07.03.2025 Acceptance date:27.03.2025

Abstract

Purpose: This study aimed to investigate serum levels of S100B protein, Neuron Specific Enolase (NSE), Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) that reflect glial and neural structure. To date, these proteins have been studied in neuropsychiatric diseases and relation with neuronal damage has been shown. However, there is no study that simultaneously evaluates the serum levels of these proteins in children with ADHD and compares them with healthy controls.

Materials and methods: 44 children with ADHD and 37 healthy volunteers participated in the study. They were selected from children and adolescents aged 6-18 years who had no history of other medical or psychiatric diseases and had not used psychotropic drugs for the last six months. Levels of proteins were assessed by enzyme-linked immunosorbent assay (ELISA).

Results: Serum levels of S100B, NSE and GFAP were found to be statistically significantly higher in the ADHD group than in the control group (p=0.012, p=0.000, p=0.001, respectively). No significant difference was found in the intergroup comparison for MBP (p=0.181).

Conclusion: Increased levels of S100B, NSE, GFAP may be an indicator of neuronal or glial changes in ADHD. Future studies combining serial measurements of these biochemical proteins with genetics and neuroimaging data are needed to evaluate the possible role of glial and neuronal damage in the etiopathogenesis of ADHD.

Keywords: ADHD, glia, astrocyte, neuroinflammation.

Celik Yildirim H, Kabukcu Basay B, Unal EB. Evaluation of S100B, NSE, MBP and GFAP serum levels in children with attention deficit hyperactivity disorder. Pam Med J 2025;18:432-443.

Öz

Amaç: Bu çalışmanın amacı glial ve nöral yapıyı yansıtan S100B proteini, Nöron Spesifik Enolaz (NSE), Miyelin Bazik Protein (MBP) ve Glial Fibriller Asidik Protein (GFAP) serum düzeylerini araştırmaktır. Bugüne kadar bu proteinler nöropsikiyatrik hastalıklarda çalışılmış ve nöronal hasarla ilişkisi gösterilmiştir. Ancak bu proteinlerin serum düzeylerini DEHB'li çocuklarda eş zamanlı olarak değerlendirerek sağlıklı kontroller ile karşılaştıran bir çalışma mevcut değildir.

Gereç ve yöntem: Çalışmaya 44 DEHB'li ve 37 sağlıklı gönüllü katımıştır. Katılımcılar, başka tıbbi veya psikiyatrik hastalık öyküsü olmayan ve son altı aydır psikotropik ilaç kullanmayan 6-18 yaş arası çocuk ve gençler arasından seçilmiştir. Serum protein seviyeleri enzim-bağlı immünosorbent yöntemi (ELISA) ile değerlendirilmiştir.

Bulgular: S100B, NSE ve GFAP serum düzeyleri, DEHB grubunda kontrol grubundan istatistiksel olarak anlamlı düzeyde yüksek bulunmuştur (sırasıyla p=0,012, p=0,000, p=0,001). MBP için gruplar arası karşılaştırmada anlamlılık düzeyinde bir farklılık saptanmamıştır (p=0,181).

Sonuç: S100B, NSE, GFAP'nin yükselmiş düzeyleri DEHB'deki nöronal veya glial değişikliklerin bir göstergesi olabilir. DEHB etiyopatogenezinde glial ve nöronal hasarın olası rolünü değerlendirmek için bu biyokimyasal proteinlerin seri ölçümlerini genetik ve nörogörüntüleme verileriyle birleştiren gelecek çalışmalara ihtiyaç vardır.

Anahtar kelimeler: DEHB, glia, astrosit, nöroinflamasyon.

Çelik Yıldırım H, Kabukçu Başay B, Ünal EB. Dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda serum S100B, NSE, MBP ve GFAP düzeylerinin değerlendirilmesi. Pam Tıp Derg 2025;18:432-443.

Hatice Çelik Yıldırım, M.D. Manisa City Hospital, Department of Child and Adolescent Psychiatry, Manisa, Türkiye, e-mail: drhaticecelik1987@gmail.com (https://orcid.org/0009-0009-6151-7242)

Bürge Kabukçu Başay, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Denizli, Türkiye, e-mail: burgekabukcu@yahoo.com (https://orcid.org/0000-0003-4124-2340) (Corresponding Author)

Egem Burcu Ünal, M.D. Adana City Training and Research Hospital, Department of Physiology, Adana, Türkiye, e-mail: egemburcu@hotmail.com (https://orcid.org/0000-0002-9499-2316)

Introduction

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by inattention, hyperactivity and impulsivity. It has a multifactorial etiology with a no single cause. Mainly biological causes play a role in the etiology of ADHD and some environmental and psychosocial adversities have relation with the disorder [1, 2]. In a meta-analysis, ADHD was found to be one of the most heritable psychiatric disorders with an average heritability of 76% [3].

As Farone stated, the etiology and pathogenesis of ADHD is not clearly defined, so more valid diagnosis would be welcomed [4]. Biomarkers are supposed to be good candidates at this point. A biomarker refers to a characteristic that can be objectively measured and assessed to indicate normal biological functions, disease-related processes, or the body's response to a therapeutic treatment [5]. Although various biomarkers related to neurophysiology, neurochemistry, neuroimaging, and genetics have been identified with small to moderate effects in ADHD, a definitive biomarker for its diagnosis has not yet been established [6].

Brain-specific proteins such as S100B (S100Beta), NSE (Neuron Specific Enolase), GFAP (Glial Fibrillary Acidic Protein), MBP (Myelin Basic Protein) are not found in other tissues and can be easily measured in the blood. These proteins may provide information about the active status of brain regions with structural and functional damage, the severity of the disease, and the prognosis of the patient [7].

The S100B protein family is responsible for protein phosphorylation, cell growth and change, regulation of transcription factors and enzymes, enzyme activities, inflammatory cell response, and Ca+2 metabolism [8]. The increase in S100B in serum in traumatic brain injury, Down Syndrome, Alzheimer's Disease, manic attacks, and schizophrenia is generally associated with neural toxicity, and this has led some researchers to call it as "CRP of the brain" [9]. S100B overexpression has been detected in children with cerebral palsy and developmental delay [10]. It has been also stated that S100B may be involved in the pathogenesis of psychiatric diseases related to its neurotrophic effect [11]. It has been also found to be elevated in autism spectrum disorder (ASD) [12, 13]. A study involving 360 ADHD-probands reported that S100B moderates the relationship between low birth weight and hyperactivity and impulsivity symptoms [14]. Another study reported that maternal smoking history was related with increased S100B levels in ADHD children [15]. Liu et al. [16] reported a positive association between S100B levels and ADHD symptoms in a group of ADHD children with lead exposure.

NSE (Neuron Specific Enolase) increases in blood and cerebrospinal fluid (CSF) secondary to structural damage in neurons. It is secreted from neuroendocrine cells in the central nervous system and the periphery [17]. Since it is involved in ATP synthesis, it is of vital importance for the excitability of the neuronal membrane [18]. NSE has been investigated in many chronic neurological diseases. It has been proven to be useful in the diagnosis of various conditions, including traumatic brain injury, epilepsy, intracerebral hemorrhage, ischemic stroke, Alzheimer's disease, Creutzfeldt-Jakob syndrome, delirium, and Guillain-Barré syndrome." [19]. Wiener et al. [20] investigated NSE in bipolar disorder and depression patients and reported lower levels than controls. The authors mentioned the role of NSE in energy metabolism and emphasized more permanent and irreversible damage by disease progression leading to loss of neurons and brain volume. They attributed reduced serum levels of NSE to diminished synaptic connectivity and impaired structural plasticity that is present in patients with mood disorders. ASD patients have been researched in regard to NSE levels, and increased levels [21, 22] or no difference [23] has been reported in the ASD groups in comparison to controls. Regarding ADHD, the only case-control study researching serum NSE levels included internet addicted child and adolescent patients, and the ADHD group had higher levels of NSE with S-100B [24].

GFAP and MBP, which show neuronal, astrocytic and oligodendroglia damage in neuropsychiatric diseases, are other potential neuromarkers [8]. MBP is in the myelin sheath and is synthesized only by oligodendrocytes and Schwann cells during active demyelination. In the presence of neuroglial damage MBP

levels increase in serum [25]. Its increment levels in some neurological diseases such as Multiple Sclerosis provides valuable information about the severity of the disease [25]. GFAP is an acidic cytoskeleton protein and a basic intermediate filament in neurons [26]. It has high sensitivity for the status of the neuroglial structure and is important in the central nervous system neurodegeneration and damage [26]. GFAP was again found to be high and clinically significant in children diagnosed with infantile autism and it has been stated that elevated levels of GFAP suggest gliosis and nonspecific brain damage in children with autism [27]. A case-control study in ADHD diagnosed patients found also higher levels of GFAP compared to controls [28].

Although S100B has been studied in ADHD, albeit in small numbers, there are very scarce case-control studies on NSE, GFAP and MBP. This study aimed to measure these markers in ADHD-diagnosed children and adolescents, and to compare the marker levels with healthy controls. We hypothesized that the levels of S100B, NSE, GFAP and MBP would have increased in ADHD group in comparison to healthy subjects. Secondly, we aimed to search the relation of these markers with each other in ADHD patients and with neurocognitive test results. Our second hypothesis was that biomarker levels would be positively correlated with each other and would show a significant relationship with neurocognitive test results.

Materials and methods

The research was conducted at the Department of Child and Adolescent Psychiatry, Pamukkale University Faculty of Medicine, between November 2018 and June 2019. The current study is a cross-sectional case-control study comparing serum S100B, NSE, MBP and GFAP levels of ADHD-diagnosed children and adolescents with healthy controls.

Within the scope of the study, children and adolescents between the ages of 6 and 18 who applied to Pamukkale University Faculty of Medicine Child and Adolescent Psychiatry Outpatient Polyclinics with ADHD symptoms were evaluated. A total of 44 voluntary children and adolescents who were diagnosed with ADHD according to Diagnostical Statistical Manual of Mental Disorders-V (DSM-V) diagnostic criteria, and met the inclusion criteria for the study were recruited for the study to form the ADHD group. From the same outpatient polyclinics 37 voluntary similar ages of children who were not diagnosed with any psychiatric disorder composed the control group.

Having received psychotropic medication within the last 6 months, having an infection history and any medication use within the last week, having a chronic medical disease/ continuous medication use, having symptoms that would suggest mental retardation clinically, being unwilling to participate the study, not being able to complete the necessary evaluations and tests, or the participant or their parent giving up participating in the study were accepted as exclusion criteria for both groups. For the ADHD group, having a diagnosis of a psychiatric disorder other than ADHD, and for the control group, having been diagnosed with any psychiatric disorder according to DSM-V diagnostic criteria at the end of the clinical assessment were accepted as exclusion criteria.

Participants who agreed to participate in the study and their parents were clinically interviewed, study scales were filled in, computer-based psychometric assessment tests were applied, and 10 cc of venous blood was taken for laboratory analysis.

In the clinical evaluation phase, DSM-V based psychiatric interviews were conducted to evaluate ADHD and other possible psychiatric diagnoses with all children and adolescents and their families in ADHD and control groups. During the interview, the sociodemographic data form, and the Turgay DSM-IV Based Child and Adolescent Disruptive Behaviors Disorders Screening and Rating Scale were filled out by the parents. In addition, two computer based neurocognitive tests; The Psychology Experiment Building Language (PEBL)-Berg's Wisconsin Card Sorting Test which is the short computer version of the Wisconsin Card Sorting Test, and the Stroop Victoria test were applied to the children and adolescents during the interview. All interviews and test applications were completed by the same researcher.

All children, adolescents and their families participating in the study were informed about the study in accordance with the Declaration of Helsinki. Written consent was obtained from both parents and children. Before the study, approval was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee in the meeting dated 29.05.2018 and numbered 11, with the number 601167787-020/40438 (additional ethical approval for a correction application was received in the meeting dated 21.01.2020 and numbered 02, with the number 601167787-020/10336). This study was supported by Pamukkale University Scientific Research Projects Commission with the decision numbered 2018TIPF038.

Study instruments

Turgay DSM-IV based child and adolescent disruptive behaviors disorders screening and rating scale

Turgay DSM-IV Based Child and Adolescent Disruptive Behaviors Disorders Screening and Rating Scale was developed by Turgay (1994) [29], based on DSM-IV diagnostic criteria. It consists of 9 items for inattentiveness, 6 items for hyperactivity, 3 items for impulsivity, 8 items for oppositional defiant disorder, and 15 items for conduct disorder. Each item has four options ranging from none to a lot. The validity and reliability study of the scale was conducted by Ercan et al. [30].

Wisconsin Card Sorting Test (WCST)

It was created by Grant and Berg in 1948 [31]. and developed by Heaton in 1981 [32]. Turkish standardization was made by Karakaş [33]. The WCST is a frontal lobe test associated with attention, feature identification, working memory, executive functions, conceptualization, abstract thinking, and especially deperseveration. It assesses the individual's response to changing conditions and the problem-solving strategies.

WCST consists of two decks of cards, each consisting of 64 response cards, and 4 stimulus cards. Each card contains shapes (plus, circle, star, and triangle) in different colors (red, yellow, blue, and green) and quantities (one, two, three, and four). The task that the individual must do in WCST is to match the response cards with the stimulus cards that he/she deems appropriate. In the test, the number of completed categories, the total number of correct answers, the total number of errors, the total number of perseverative responses, the total number of perseverative errors, the number of nonperseverative errors, the unique error, the number of trials used to complete the first category, the learning to learn score, the number of conceptual level responses, and the percentage of conceptual level responses are calculated by the computer program.

Stroop color and word test, victorian version

Originally developed by Stroop (1935) [34], various forms of the test have subsequently been developed. The Turkish standardization was made by Karakaş [33] and Kilic et al. [35]. There are many different versions of the Stroop test, including the Victorian version used in this study. The Stroop test measures the ability to change perceptual setups in response to changing demands and under a "disruptive stimulus"; the ability to suppress a habitual behavior pattern and engage in an unusual behavior; and additionally focused attention. The Stroop test provides a timed measure of selective attention and cognitive flexibility.

The test is composed of Part D (dots, shapes), W (words, neutral/non-colored words) and C (colors). The time to complete the tasks and the number of errors made are recorded by the PEBL program. The first and second parts of the test are used to measure cognitive speed, while the third part of the test is used to measure response inhibition. The computer program (PEBL)-based administration of the test requires the participant to correct the error if an error is made before moving on to the next item. This is reflected in the completion time of the test. The test was administered to children and adolescents in the consultation room of the outpatient clinic via the computer program (PEBL).

Biochemical evaluation

Ten cc of venous blood was taken from the antecubital vein of participants after 12 hours of fasting. The samples were kept at room temperature for approximately 15 minutes in the Physiology Laboratories of Pamukkale

University Faculty of Medicine and then centrifuged at 7240 rpm for 6 minutes. The samples obtained were stored at -80 °C until the evaluation time for S100B, NSE, GFAP and MBP levels. The study was conducted with YLBiont ELISA kits (Shanghai YL Biotech Co., Ltd).

In the laboratory analysis phase, first, all collected samples and kits were brought to room temperature. After the standard solutions of the kits, Chromagen A-B, and antigen and antibody containing chemicals were prepared; standards and samples were placed in the wells in the plate. Then, the samples were colored by following the steps explained in the manual. After the color formation was observed, the absorbance values of the wells at 450 nanometers (nm) were read and the results were recorded. Concentrations were calculated using the serum absorbance values. The values were in nanogram/milliliter (ng/ml) units, except for MBP. MBP was in picogram/ml.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences version 18 (SPSS Inc., Chicago, IL, USA). Pearson Chi-square test (X^2) was used to compare categorical data. Shapiro–Wilk test was used to evaluate whether the distribution of continuous variables was normal. Homogeneity of variances was determined by Levene test. In comparison of two groups of numerical values, Student t test was used if continuous variables showed normal distribution, and Mann-Whitney U test was used if they did not show normal distribution. In examining the relationship between continuous variables, Pearson correlation test was used for those with normal distribution and Spearman correlation test for those without normal distribution. Statistical significance value was accepted as $p<0.05$.

Results

In the ADHD group, 10 (22.73%) participants were female and 34 (77.27%) were male; in the control group, 15 (40.54%) participants were female and 22 (59.46%) were male. There was no statistically significant difference between the ADHD group and the control group in terms of gender ($p=0.084$). The mean age of the ADHD group was 9.95±3.09 years, and the mean age of the control group was 10.61±0.92 years. No statistically significant difference was found between the two groups in terms of age ($p=0.292$).

The ADHD and control groups were compared in terms of the biochemical parameters analyzed in the study. Accordingly, the mean S100B values of the children in the ADHD group were found to be 33.89±16.07 ng/L, and the mean S100B values of the children in the control group were found to be 26.05±4.43 ng/L. When the ADHD and control groups were compared in terms of S100B values, it was seen that S100B values of the ADHD-diagnosed participants were higher than the values of control group; and a statistically significant difference was recorded ($p=0.012$).

The mean NSE values of the ADHD group were found to be 3.73±1.37 ng/ml, and the mean NSE values of the control group were found to be 2.73±0.71 ng/ml. When the ADHD and control groups were compared in terms of NSE values, it was seen that the NSE values of the ADHD group were higher than the control group, and a statistically significant difference was found between them ($p<0.001$).

The mean GFAP values of the ADHD group were determined as 1.33±0.56 ng/ml, and the mean of the control group was determined as 1±0.25 ng/ml. When the ADHD and control groups were compared in terms of GFAP values; it was seen that the values of the ADHD group were higher, and a statistically significant difference was present ($p=0.01$).

The mean serum MBP values of the ADHD group were determined as 644.91±395.87 ng/L, and the mean MBP values of the control group were determined as 539.48±246.29 ng/L. When the ADHD and control groups were compared in terms of MBP values; although the values of the ADHD-diagnosed participants were higher than the control group, no statistically significant difference was found ($p=0.181$) (Table 1).

Table 1. Comparison of ADHD and Control Groups in terms of S100B, NSE, GFAP and MBP values

	ADHD Group		Control Group		*z*	*p*
	Mean±SD	**Med (min-max)**	**Mean±SD**	**Med (min-max)**		
S100B	33.89±16.07	28 (21.47-7.17)	26.05±4.43	25.55 (16.96-37.26)	-2.503	0.012*
NSE	3.73±1.37	3.44 (1.89-8.23)	2.73±0.71	2.74 (0-4.25)	-3.958	0.000*
GFAP	1.33±0.56	1.11 (0.51- 3.15)	1±0.25	0.94 (0.57-1.75)	-3.337	0.001*
MBP	644.91±395.87	519.75 (277.1-2143)	539.48±246.29	494.6 (303.9-1349)	-1.337	0.181

z: Mann Whitney U test, *p<0.05 is statistically significant, Med: median, min: minimum, max: maximum, SD: standard deviation
S100B: S100 Beta, NSE: neuron specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

When the relation between biochemical parameters in the ADHD group were examined, positive correlations ranging from small to large levels (statistically significant r values ranged between 0.3 and and 0.7; r values between 0.2 and 0.3 were accepted as low, between 04. and 0.5 as medium and greater than 0.6 were accepted as large correlataions) were detected. Table 2 presents the relationship between S100B, NSE, GFAP and MBP values.

Table 2. Correlations between S100B, NSE, GFAP and MBP values measured in the ADHD group

		S100B	**NSE**	**GFAP**	**MBP**
S100B	**r**	1.00	0.765[a]	0.551[a]	0.324[b]
	p		0.000*	0.000*	0.032*
NSE	**r**		1.000	0.487 [a]	0.392[a]
	p			0.001*	0.009*
GFAP	**r**			1.000	0.080 [a]
	p				0.605
MBP	**r**				1.000
	p				

[a]: Spearman correlation test, [b]: Pearson correlation test, *p<0.05 is statistically significant, S100B: S100Beta, NSE: neuron specific enolase
GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

Within the scope of the study, Wisconsin card sorting test (WCST) and Stroop Victoria tests were applied to ADHD and control group of participants; and the possible correlations between test and subtest results and biochemical analysis values were investigated. Accordingly, the neurocognitive test analysis results of the ADHD group were generally unfavorable in comparison to the control group; and some of the findings were at statistical significance level (Table 3). No clinically important medium or large correlation was found between the neurocognitive test results and S100B, NSE, GFAP and MBP values. Table 4 presents the statistically significant correlations in groups.

Table 3. Comparison of ADHD and Control Groups in terms of Stroop Victoria and Wisconsin Card Sorting Test (WCST) results

	ADHD Group		Control Group			
WCST	**Mean±SD**	**Med (min-max)**	**Mean±SD**	**Med (min-max)**	***z***	***p***
Number of completed categories	2.02±1.5	2 (0-5)	3.06±1.48	4 (0-5)	-2.906	0.004*
Total number of correct answers	43.98±19.4	42 (17-100)	49.61±15.64	51 (23-100)	-2.265	0.023*
Total number of errors	28.47±13.35	27 (8-64)	18.27±9.7	14 (7-41)	-3.461	0.001*
Total number of perseverative responses	22.12±14.7	21 (0-58)	23±11.43	22 (0-52)	-0.252	0.801
Total number of perseverative errors	12.81±10.39	10 (0-38)	11.61±8.19	9 (0-39)	-0.283	0.777
Number of non-perseverative errors	15.28±12.04	12 (2-47)	6.67±6.53	4 (0-28)	-3.911	0.000*
Unique error	5.35±7.23	2 (0-26)	1.52±1.8	1 (0-8)	-2.424	0.015*
Number of trials used to complete the first category	16.09±11.94	13 (0-59)	15.12±10.88	11 (0-55)	-1.041	0.298
Learning to learn score	4.27±5.85	4.12 (-7.95-14.5)	4.35±5.7	3.1 (-1.96- 23.8)	-1.440	0.150
Number of conceptual level responses	35.53±20.05	36 (7-87)	43.58±16.43	44 (13-91)	-2.207	0.027*
Percentage of conceptual level responses	47.87±22.05	50 (10.9-84.4)	63.81±17.45	68.8 (20.3-84.4)	-3.188	0.001*
Stroop test						
Part D time	84.23±43.71	74.44 (26.03-210.77)	68.77±29.29	62.67 (30.17-146.81)	-1.359	0.034*
Part D errors	27.79±6.36	26 (24-60)	25.73±1.63	25 (24-31)	-0.732	0.336
Part W time	78.9±51.34	71.91 (22.78-296.61)	54.14±25.51	48.26 (30.1-148.7)	-2.734	0.001*
Part W errors	26.79±3.97	25 (24-41)	25.97±2.3	25 (24-34)	-0.032	0.859
Part C time	97.19±63.68	77.5 (24.68-311.65)	71.75±41.48	62.28 (36.15-228.63)	-1.867	0.012*
Part C errors	29.58±6.69	27 (24-59)	27.61±3.98	26 (24-42)	-0.936	0.256

z=Mann Whitney U test, **p*<0.05 is statistically significant, WCST: Wisconsin Card Sorting Test, Med: median, min: minimum, max: maximum SD: standard deviation

Table 4. Significant correlations between biochemical parameters and neurocognitive test results in the study groups

	r	*p*
Whole group		
NSE- WCST non-perseverative errors	0.309	0.007*
GFAP- WCST number of completed categories	-0.271	0.018*
GFAP- WCST number of correct answers	-0.239	0.037*
GFAP- WCST number of conceptual level responses	-0.249	0.030*
MBP- WCST learning to learn score	0.353	0.032*
MBP-Stroop Part C errors	-0.242	0.036*
ADHD group		
S100B- WCST learning to learn score	0.327	0.032*
Control group		
MBP- WCST perseverative errors	-0.369	0.035*

r: Spearman correlation test, **p*<0.05 is statistically significant, WCST: Wisconsin Card Sorting Test, S100B: S100 Beta
NSE: neuron specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

Discussion

Neuronal tissue specific proteins such as S100B, NSE, GFAP, MBP can be easily measured in the blood and provide information about the active status of brain regions with structural and functional damage, the severity of the disease, and the prognosis of the patient [7]. The levels of these proteins have been investigated in many chronic neurological diseases and some psychiatric diseases. In the literature, there are very limited studies on these markers conducted with ADHD patients and these markers have not been studied collectively in ADHD. In our study, it was hypothesized that S100B, NSE, GFAP, MBP levels would be increased in children and adolescents with ADHD compared to the control group, and this hypothesis was confirmed in other proteins except MBP. In addition, we analyzed the interrelation among these biomarkers in ADHD patients and recorded a medium or large relationship in most assessments. It is known that neurocognitive test performances of individuals with ADHD are generally lower than the healthy controls. Based on this, it was thought that increased marker levels may be associated with unfavorable test results, but no meaningful evidence was obtained in this direction. Future studies with larger samples may shed light on this issue. The results of biochemical parameters are discussed below in light of literature information.

The S100B protein family is mainly found in astrocytes and secreted by these cells [8]. Elevations of S100B in peripheral body fluids in various neurological and psychiatric conditions are attributed to brain damage or dysfunction and increased blood-brain barrier permeability [36]. A recent study reviewed researches that investigated peripheral S100B levels in psychiatric disorders; schizophrenia, depressive disorder, bipolar disorder, ASD and ADHD. The authors concluded that mostly elevated S100B levels across disorders had been reported but the results are inconsistent and alterations in S100B peripheral levels do not seem to be disease specific [37].

There are very limited studies in literature comparing S100B levels between ADHD and control cases. A study by Oades et al. [38] compared 21 ADHD medication naive cases with 21 healthy controls and reported no marked group differences in levels of S100B. On the contrary, a recent study by Ouadih Moran et al. [39] reported higher S100 B levels in medication-naive ADHD children and adolescents than the healthy controls, which is parallel to our result. The authors proposed that this finding of elevated S100B levels in the ADHD group strengthened

the hypothesis of glial damage within the mechanisms preceding the catecholaminergic disruption responsible for ADHD [39]. Another recent study also reported that elevated S100B levels in children and adolescents with ADHD and internet addiction compared to healthy controls, and S100B was correlated with low sleep quality [24]. Our findings support these two studies' reports of elevated S100B in the ADHD groups. As a neurodevelopmental disorder, in ADHD, increased levels of a well-known peripheral brain damage marker, may be an indication of neurotoxic processes that take role in etiopathogenesis.

Another neuronal cell-specific biomarker, NSE, is localized in the cytoplasm of the neuron and increases in CSF and serum in case of neuronal damage [40]. NSE, has not been studied effectively in the ADHD field. A study that included children with traumatic brain injury reported an association between higher scores on inattention, hyperactivity/impulsivity and executive functioning scales [41]. The only case-control study included internet addicted ADHD patients and found elevated NSE levels in comparison to healthy controls [24]. ASD, being another neurodevelopmental disorder, has had relatively more NSE research compared to ADHD, although it is still scarce. A retrospective study examining newborn blood samples from children with autism reported increased NSE levels compared to healthy controls [42]. Two more recent studies showed increased levels of NSE levels in ASD children compared to controls [21, 22], in contrast to the study by Esnafaoglu et al. [23] which reported no difference. Although there are very limited numbers of studies investigating NSE levels in neurodevelopmental disorders, our findings support the increased levels of NSE in ADHD diagnosed children and adolescents.

GFAP is a major part of astrocyte intermediate filaments [6]. Astrocytes, a type of glial cells, are cells that play many roles in brain development, healthy survival of neurons, and brain homeostasis. GFAP mainly provides resistance of brain tissue to mechanical stress and plays role in astrocyte functions such as cell migration and motility. In the presence of damage in the central nervous system, there are changes in the morphology and function of astrocytes, and astrocytes increase GFAP expression in response to neuronal damage [43]. GFAP, such as S100B, is an established indicator of astrogliosis in neuropathology [36]. A recent review study assessed GFAP in neurological disorders including ASD and suggested elevated GFAP levels as a valuable body fluid biomarker in the evaluation of different neurological diseases [44]. In regard to ADHD, animal [45, 46] and human studies have been conducted [28, 47]. A case-control study by Cetin et al. [28] found higher GFAP levels in the ADHD group than the control group, which is parallel to our study result [35]. Collectively, the study results point out astroglial involvement in rat models of ADHD or increased levels of GFAP in ADHD patients. Our study finding of elevated GFAP in ADHD group of children supports the astroglail dysfunction in ADHD.

MBP is in the myelin sheath. It is found in both the central and peripheral nervous systems. It increases in serum only during active demyelination and in the presence of neuroglial damage [6]. Elevation in MBP levels in serum is not expected while myelination continues, however, MBP becomes detectable in the serum in cases of demyelinating processes and disruption of the blood brain barrier [25]. DTI (diffusion tensor imaging) studies have shown that in the white matter of ADHD patients, there is delayed maturation and microstructural anomalies [48]. Shaw et al. [49] reported in their DTI study conducted with adult ADHD patients that white matter dysfunction was present only in the group with ongoing symptoms; white matter was alike with the control group in ADHD patients whose symptoms remitted in adulthood. Considering that ADHD is a neurodevelopmental disorder that presents symptoms starting from childhood, and it does not exhibit clinical features that are progressive and progress with biological destruction (such as in the demyelinating process in multiple sclerosis); it seems understandable that there was no statistically significant increase in MBP levels -an indicator of a demyelinating process-, between the ADHD and control groups in our study. No study evaluating MBP levels in ADHD is found in the literature. A very recent study reported that the level of myelin autoantibodies in the context of autoimmunity did not predict the diagnosis of ADHD [50].

In our study, possible inter-relations of neuron-specific proteins were also assessed among ADHD children and adolescents. Accordingly, it was found that the protein couples except GFAP-MBP were moderately or highly correlated with each other. This finding can be considered as a consistency of the other results of the study and a support for the neurobiological basis of ADHD.

As a last point, we compared the neurocognitive performance of ADHD patients with healthy controls with WCST and Stroop tests. As expected, ADHD children showed worse performance than the controls in general. However, we could not find a meaningful association between neurocognitive scores and biochemical markers, although some correlations were recorded. Future studies with greater sample sizes may provide evidence for possible relations.

When evaluating the results of our study, it is necessary to consider some limitations. The case and control groups consisted of relatively small numbers of children and adolescents. Although systemic diseases were excluded with detailed anamnesis, acute phase reactant or disease marker measurements such as CRP, sedimentation were not assessed. In addition, although clinically normal IQ participants were included in both groups, intelligence was not measured with a psychometric test and the groups were not matched in terms of intelligence score. It would be useful to consider the ADHD subtypes separately. The fact that the ADHD group was composed of medication naïve children and adolescents and that additional psychiatric and medical diseases were excluded in both groups were the strengths of the study.

Conclusively, we suggest that the increase in S100B, NSE and GFAP levels may reflect microglialor astroglial changes and as well presence of neuroinflammation in ADHD. To more accurately evaluate the possible role of glial and neuronal tissue in the etiopathogenesis of ADHD; further studies with serial measurements that combine genetic research and neuroimaging findings are required. The data to be obtained may contribute to a holistic understanding of the etiopathogenesis of ADHD.

Acknowledgement: Pamukkale University Scientific Research Projects Commission supported the specialty thesis project with the decision numbered 2018TIPF038. The study authors would like to thank Pamukkale University Scientific Research Projects Commission. They would also like to thank all the children and adolescents who participated in the study and their parents.

Funding: None.

Authors contributions: H.C.Y. and B.K.B. have constructed the main idea and hypothesis of the study. They developed the theory and arranged/edited the material and method section. H.C.Y. collected the study data and has done the evaluation of the data with E.B.U. in the Results section. E.B.U. conducted the laborotory analysis and contributed to Materials and Methods section. Discussion section of the article is written by H.C.Y. and B.K.B.

B.K.B. and H.C.Y. reviewed, corrected and approved the study. In addition, all authors discussed the entire study and approved the final version.

Conflicts of interest: The study authors have no conflict of interest.

References

1. Biederman J. Attention-deficit/hyperactivity disorder: a selective overview. *Biol Psychiatry*. 2005;57(11):1215-1220. doi:10.1016/j.biopsych.2004.10.020
2. Faraone SV, Asherson P, Banaschewski T, et al. Attention-deficit/hyperactivity disorder. *Nat Rev Dis Primers*. 2015;1:15020. Published 2015 Aug 6. doi:10.1038/nrdp.2015.20
3. Polanczyk GV, Casella EB, Miguel EC, Reed UC. Attention deficit disorder/hyperactivity: a scientific overview. *Clinics (Sao Paulo)*. 2012;67(10):1125-1126. doi:10.6061/clinics/2012(10)01
4. Faraone SV, Bonvicini C, Scassellati C. Biomarkers in the diagnosis of ADHD--promising directions. *Curr Psychiatry Rep*. 2014;16(11):497. doi:10.1007/s11920-014-0497-1
5. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95. doi:10.1067/mcp.2001.113989
6. Mehta T, Mannem N, Yarasi NK, Pradeep C, Bollu PC. Biomarkers for ADHD: the Present and Future Directions. *Curr Dev Disord Rep*. 2020;7:85-92. doi:10.1007/s40474-020-00196-9

7. Lamers KJ, Vos P, Verbeek MM, Rosmalen F, van Geel WJ, van Engelen BG. Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients. *Brain Res Bull*. 2003;61(3):261-264. doi:10.1016/s0361-9230(03)00089-3
8. Donato R. Intracellular and extracellular roles of S100 proteins. *Microsc Res Tech*. 2003;60(6):540-551. doi:10.1002/jemt.10296
9. Sen J, Belli A. S100B in neuropathologic states: the CRP of the brain?. *J Neurosci Res*. 2007;85(7):1373-1380. doi:10.1002/jnr.21211
10. Park ES, Park CI, Choi KS, Choi IH, Shin JS. Over-expression of S100B protein in children with cerebral palsy or delayed development. *Brain Dev*. 2004;26(3):190-196. doi:10.1016/S0387-7604(03)00126-8
11. Manev H, Manev R. S100B: an old neurotrophic factor with putative new roles in psychiatric illnesses. *J Psychiatr Res*. 2001;35(6):347-350. doi:10.1016/s0022-3956(01)00039-5
12. Al Ayadhi LY, Mostafa GA. A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. *J Neuroinflammation*. 2012;9:54. Published 2012 Mar 16. doi:10.1186/1742-2094-9-54
13. Shaker NM, Taha GR, Kholeif H, Sayed NM, El Sheikh MM, Abulmagd ML. Serum levels of S100b, interleukin-6 and anti-transglutaminase Ii IgA as immune markers in a sample of egyptian children with autistic spectrum disorders. *Autism Open Access*. 2016;6:1-8. doi:10.4172/2165-7890.1000191
14. Smith TF, Anastopoulos AD, Garrett ME, et al. Angiogenic, neurotrophic, and inflammatory system SNPs moderate the association between birth weight and ADHD symptom severity. *Am J Med Genet B Neuropsychiatr Genet*. 2014;165B(8):691-704. doi:10.1002/ajmg.b.32275
15. Oades RD. An exploration of the associations of pregnancy and perinatal features with cytokines and tryptophan/kynurenine metabolism in children with attention-deficit hyperactivity disorder (ADHD). *Atten Defic Hyperact Disord*. 2011;3(4):301-318. doi:10.1007/s12402-011-0062-2
16. Liu W, Huo X, Liu D, Zeng X, Zhang Y, Xu X. S100β in heavy metal-related child attention-deficit hyperactivity disorder in an informal e-waste recycling area. *Neurotoxicology*. 2014;45:185-191. doi:10.1016/j.neuro.2014.10.013
17. Marangos PJ, Schmechel DE. Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Annu Rev Neurosci*. 1987;10:269-295. doi:10.1146/annurev.ne.10.030187.001413
18. Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: What are we measuring?. *Neurosci Biobehav Rev*. 2016;68:460-473. doi:10.1016/j.neubiorev.2016.05.009
19. Isgrò MA, Bottoni P, Scatena R. Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. *Adv Exp Med Biol*. 2015;867:125-143. doi:10.1007/978-94-017-7215-0_9
20. Wiener CD, Jansen K, Ghisleni G, et al. Reduced serum levels of neuron specific enolase (NSE) in drug-naïve subjects with major depression and bipolar disorder. *Neurochem Res*. 2013;38(7):1394-1398. doi:10.1007/s11064-013-1036-x
21. Stancioiu F, Bogdan R, Dumitrescu R. Neuron-Specific Enolase (NSE) as a Biomarker for Autistic Spectrum Disease (ASD). *Life (Basel)*. 2023;13(8):1736. Published 2023 Aug 13. doi:10.3390/life13081736
22. Ayaydın H, Kirmit A, Çelik H, Akaltun İ, Koyuncu İ, Bilgen Ulgar Ş. High Serum Levels of Serum 100 Beta Protein, Neuron-specific Enolase, Tau, Active Caspase-3, M30 and M65 in Children with Autism Spectrum Disorders. *Clin Psychopharmacol Neurosci*. 2020;18(2):270-278. doi:10.9758/cpn.2020.18.2.270
23. Esnafoglu E, Ayyıldız SN, Cırrık S, et al. Evaluation of serum Neuron-specific enolase, S100B, myelin basic protein and glial fibrilliary acidic protein as brain specific proteins in children with autism spectrum disorder. *Int J Dev Neurosci*. 2017;61:86-91. doi:10.1016/j.ijdevneu.2017.06.011
24. Demirci E, Tastepe N, Gul MK, Ozmen S, Kilic E. S100B and Neuron-Specific Enolase Levels as Brain Injury Biomarkers in Internet Addiction: Effect of Sleep. *Pediatr Neurol*. 2023;149:93-99. doi:10.1016/j.pediatrneurol.2023.08.029
25. Whitaker JN. Myelin basic protein in cerebrospinal fluid and other body fluids. *Mult Scler*. 1998;4(1):16-21. doi:10.1177/135245859800400105
26. Petzold A. The prognostic value of CSF neurofilaments in multiple sclerosis at 15-year follow-up. *J Neurol Neurosurg Psychiatry*. 2015;86(12):1388-1390. doi:10.1136/jnnp-2014-309827
27. Ahlsén G, Rosengren L, Belfrage M, et al. Glial fibrillary acidic protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders. *Biol Psychiatry*. 1993;33(10):734-743. doi:10.1016/0006-3223(93)90124-v
28. Cetin I, Bulut H, Simsek S. Examination of the Neuroplastic Biomarker Levels in Attention Deficit Hyperactivity Disorder. Asian J Biochem. 2017;12:1-8. doi:10.3923/ajb.2017.1.8
29. Turgay A. *Disruptive behavior disorders: child and adolescent screening and rating scales for children, adolescents, parents and teachers.* West Bloomfield (Michigan): Integrative Therapy Institute Publication; 1994.

30. Ercan ES, Amado S, Somer O, Çıkoğlu S. Development of a test battery for the assessment of attention deficit hyperactivity disorder. *Turkish J of Child and Adolesc Mental Health*. 2001;8:132-144.

31. Grant DA, Berg EA. Wisconsin Card Sorting Test [Database record]. *APA PsycTests*. 1948. doi:10.1037/t31298-000

32. Heaton RK. *A manual for the Wisconsin Card Sorting Test*. Odessa, FL: Psychological Assessment Resources;1981.

33. Karakaş S. *BİLNOT Bataryası El Kitabı: Nöropsikolojik Testler için Araştırma ve Geliştirme Çalışmaları* (1. Ed.). Ankara: Dizayn Ofset;2004.

34. Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of experimental psychology*, 1935;18(6):643-662. doi:0.1037/h0054651

35. Kilic G, İlden Kockar A, Irak M, Şener S, Karakaş S. The standardizatıon study of the stroop test TBAG form in chıldren between 6-11 years of age. *Turkish J of Child and Adolesc Mental Health*. 2002;9(2):86-99.

36. Janigro D, Mondello S, Posti JP, Unden J. GFAP and S100B: What You Always Wanted to Know and Never Dared to Ask. *Front Neurol*. 2022;13:835597. Published 2022 Mar 21. doi:10.3389/fneur.2022.835597

37. Kozlowski T, Bargiel W, Grabarczyk M, Skibinska M. Peripheral S100B Protein Levels in Five Major Psychiatric Disorders: A Systematic Review. *Brain Sci*. 2023;13(9):1334. Published 2023 Sep 16. doi:10.3390/brainsci13091334

38. Oades RD, Dauvermann MR, Schimmelmann BG, Schwarz MJ, Myint AM. Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism--effects of medication. *Behav Brain Funct*. 2010;6:32. Published 2010 Jun 9. doi:10.1186/1744-9081-6-32.

39. Ouadih Moran M, Muñoz Hoyos A, D'Marco L, Molina Carballo A, Seiquer I, Checa Ros A. Is S100B Involved in Attention-Deficit/Hyperactivity Disorder (ADHD)? Comparisons with Controls and Changes Following a Triple Therapy Containing Methylphenidate, Melatonin and ω-3 PUFAs. *Nutrients*. 2023;15(3):712. Published 2023 Jan 31. doi:10.3390/nu15030712

40. Royds JA, Timperley WR, Taylor CB. Levels of enolase and other enzymes in the cerebrospinal fluid as indices of pathological change. *J Neurol Neurosurg Psychiatry*. 1981;44(12):1129-1135. doi:10.1136/jnnp.44.12.1129

41. Wilkinson AA, Dennis M, Simic N, et al. Brain biomarkers and pre-injury cognition are associated with long-term cognitive outcome in children with traumatic brain injury. *BMC Pediatr*. 2017;17(1):173. Published 2017 Jul 24. doi:10.1186/s12887-017-0925-6

42. Lv MN, Zhang H, Shu Y, Chen S, Hu YY, Zhou M. The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. *Transl Neurosci*. 2016;7(1):6-11. Published 2016 Feb 18. doi:10.1515/tnsci-2016-0002

43. Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol*. 2015;32:121-130. doi:10.1016/j.ceb.2015.02.004

44. Heimfarth L, Passos FRS, Monteiro BS, Araújo AAS, Quintans Júnior LJ, Quintans JSS. Serum glial fibrillary acidic protein is a body fluid biomarker: A valuable prognostic for neurological disease - A systematic review. *Int Immunopharmacol*. 2022;107:108624. doi:10.1016/j.intimp.2022.108624

45. Stevens HE, Scuderi S, Collica SC, Tomasi S, Horvath TL, Vaccarino FM. Neonatal loss of FGFR2 in astroglial cells affects locomotion, sociability, working memory, and glia-neuron interactions in mice. *Transl Psychiatry*. 2023;13(1):89. Published 2023 Mar 11. doi:10.1038/s41398-023-02372-y

46. Lim SY, Mah W. Abnormal Astrocytosis in the Basal Ganglia Pathway of Git1(-/-) Mice. *Mol Cells*. 2015;38(6):540-547. doi:10.14348/molcells.2015.0041

47. Nowak MK, Ejima K, Quinn PD, et al. ADHD May Associate With Reduced Tolerance to Acute Subconcussive Head Impacts: A Pilot Case-Control Intervention Study. *J Atten Disord*. 2022;26(1):125-139. doi:10.1177/1087054720969977

48. Chen L, Hu X, Ouyang L, et al. A systematic review and meta-analysis of tract-based spatial statistics studies regarding attention-deficit/hyperactivity disorder. *Neurosci Biobehav Rev*. 2016;68:838-847. doi:10.1016/j.neubiorev.2016.07.022

49. Shaw P, Sudre G, Wharton A, Weingart D, Sharp W, Sarlls J. White matter microstructure and the variable adult outcome of childhood attention deficit hyperactivity disorder. *Neuropsychopharmacology*. 2015;40(3):746-754. doi:10.1038/npp.2014.241

50. Tezcan ME, Ekici F, Ugur C, et al. Do specific myelin autoantibodies and increased cerebral dopamine neurotrophic factor in the context of inflammation predict the diagnosis of attention deficit hyperactivity disorder in medication-free children?. *Brain Behav Immun*. 2025;124:125-136. doi:10.1016/j.bbi.2024.11.026

Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1655923

Efficacy of induction therapy and its impact on the development of delayed graft function in kidney transplant recipients: a single-center retrospective analysis

Böbrek nakli hastalarında indüksiyon tedavisinin etkinliği ve gecikmiş greft fonksiyonu gelişimine etkisi: tek merkezli retrospektif bir analiz

Davut Akın, Onur Birsen, Ergun Mete, Mevlüt Çeri, Utku Özgen, Murat Özban

Posted date:11.03.2025 Acceptance date:26.03.2025

Abstract

Purpose: This study aimed to evaluate the impact of two different induction therapies-Basiliximab and ATG-Fresenius (ATG-F)-on early outcomes and the development of delayed graft function (DGF) in kidney transplant recipients from living or deceased donors at a single center.

Matherials and methods: A total of 33 patients over 18 years old who underwent kidney transplantation at a single center between February 2022 and February 2025 were analyzed. Body mass index (BMI), demographic data, transplant characteristics, and complications were recorded. DGF was defined as requiring dialysis within the first seven postoperative days. Statistical analyses were performed using the t-test or Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables, with $p<0.05$ considered significant.

Results: The mean age of recipients was 39.8±11.9 years; there were 10 females and 23 males. The mean BMI was 24.5±3 kg/m². Sixteen patients (14/16 Basiliximab) received living-donor kidneys, while 17 (17/17 ATG-F) received deceased-donor kidneys. Cold ischemia time was 1.1±0.2 hours for living-donor grafts versus 11.6±2.0 hours for deceased donors ($p=0.001$). Similarly, Pre-transplant dialysis duration was significantly longer in deceased-donor recipients (2.5±2.6 vs. 9.4±4.8 years, $p=0.001$). Overall, 30.3% (10/33) of patients developed DGF, predominantly in those with longer ischemia and dialysis times.

Conclusion: In deceased-donor kidney transplant recipients, prolonged cold ischemia and pre-transplant dialysis duration increase the incidence of DGF. The use of ATG-F in patients with high immunologic risk appears to be beneficial and is consistent with the existing literature. However, the limited sample size makes it difficult to clarify the impact of induction therapy on DGF; therefore, larger prospective studies are needed.

Keywords: Kidney transplant, end-stage renal disease, hemodialysis.

Akin D, Birsen O, Mete E, Ceri M, Ozgen U, Ozban M. Efficacy of induction therapy and its impact on the development of delayed graft function in kidney transplant recipients: a single-center retrospective analysis. Pam Med J 2025;18:446-452.

Öz

Amaç: Bu çalışma, tek merkezde canlı veya kadavradan böbrek nakli yapılan hastalarda iki farklı indüksiyon tedavisinin-Basiliksimab ve ATG-Fresenius (ATG-F)-erken dönem sonuçları ve gecikmiş greft fonksiyonu (DGF) gelişimi üzerindeki etkilerini değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Şubat 2022 ile Şubat 2025 tarihleri arasında, tek bir merkezde böbrek nakli yapılan 18 yaş üzeri toplam 33 hasta retrospektif olarak incelendi. Vücut kitle indeksi (VKİ), demografik veriler, nakil özellikleri ve komplikasyonlar kaydedildi. DGF, transplantasyonu takiben ilk yedi gün içinde diyalize ihtiyaç duyulması şeklinde tanımlandı. Sürekli değişkenler için normal dağılıma göre t-testi veya Mann-Whitney U testi, kategorik değişkenler için ise Ki-kare testi kullanıldı. $p<0,05$ istatistiksel olarak anlamlı kabul edildi.

Bulgular: Hastaların yaş ortalaması 39,8±11,9 yıl olup; 10'u kadın, 23'ü erkekti. Ortalama VKİ 24,5±3 kg/m² idi. Canlı verici grubundaki 16 hastanın 14'üne Basiliksimab, kadaverik gruptaki 17 hastanın tamamına ATG-F uygulanmıştır. Soğuk iskemi süresi, canlı vericili greftlerde 1,1±0,2 saat; kadavradan alınan greftlerde ise 11,6±2,0 saat olarak bulunmuştur ($p=0,001$). Benzer şekilde, transplantasyon öncesi diyaliz süresi de kadavra verici alıcılarında anlamlı derecede daha uzundu (2,5±2,6 yıla karşı 9,4±4,8 yıl; $p=0,001$).

Davut Akın, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Internal Medicine, Denizli, Türkiye, e-mail: dakin@pau.edu.tr (https://orcid.org/0000-0002-9567-7940) (Corresponding Author)

Onur Birsen, Prof. Pamukkale University Faculty of Medicine, Department of General Surgery, Denizli, Türkiye, e-mail: dronurbirsen@gmail.com (https://orcid.org/0000-0001-6064-5693)

Ergun Mete, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of Medical Microbiology, Denizli, Türkiye, e-mail: ergunmete@pau.edu.tr (https://orcid.org/0000-0002-0854-2440)

Mevlüt Çeri, Prof. Pamukkale Universıty Faculty of Medicine, Department of Internal Medicine, Denizli, Türkiye, e-mail: mevlutceri@gmail.com (https://orcid.org/0000-0002-2276-5157)

Utku Özgen, Assoc. Prof. Pamukkale University Faculty of Medicine, Department of General Surgery, Denizli, Türkiye, e-mail: dr_utkuozgen@yahoo.com (https://orcid.org/0000-0002-6481-1473)

Murat Özban, Prof. Denizli Private Health Hospital, General Surgery Department, Denizli, Türkiye, e-mail: mozban@pau.edu.tr (https://orcid.org/0000-0003-4974-8442)

Genel olarak hastaların %30,3'ünde (10/33) DGF gelişmiş olup, bu hastalarda iskemi ve diyaliz süreleri anlamlı derecede daha uzundu.
Sonuç: Kadavradan böbrek nakli yapılan hastalarda uzamış soğuk iskemi ve transplantasyon öncesi diyaliz süresi, DGF gelişme insidansını artırmaktadır. Yüksek immünolojik riske sahip hastalarda ATG-F kullanımının faydalı olabileceği mevcut literatür ile uyumludur. Ancak, sınırlı hasta sayısı, indüksiyon tedavisinin DGF üzerindeki etkisini netleştirmeyi güçleştirmektedir; bu nedenle daha geniş prospektif çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Böbrek nakli, son dönem böbrek hastalığı, hemodiyaliz.

Akın D, Birsen O, Mete E, Çeri M, Özgen U, Özban M. Böbrek nakli hastalarında indüksiyon tedavisinin etkinliği ve gecikmiş greft fonksiyonu gelişimine etkisi: tek merkezli retrospektif bir analiz. Pam Tıp Derg 2025;18:446-452.

Introduction

Chronic kidney disease (CKD), which has become a significant public health problem worldwide, is an important cause of morbidity and mortality [1]. After end-stage renal disease (ESRD) develops, renal replacement therapy becomes necessary, and kidney transplantation is currently the most preferred replacement approach [2]. Kidney transplantation improves patients' quality of life, reduces treatment costs, and increases patient survival. Significant advances in immunosuppressive therapy in recent years have considerably improved graft and patient survival rates [3, 4], yet graft rejection remains a major complication both in the early and late postoperative periods [5].

Immunosuppressive regimens are used to prevent rejection. Rejection episodes have an adverse effect on graft survival and are important determinants of graft failure. Antilymphocyte biological agents for induction therapy are widely used immediately before, during, or after the kidney transplant operation. Commonly used induction therapies include T lymphocyte-depleting agents (rabbit or horse anti-thymocyte globulin [rATG], alemtuzumab) or a non-depleting IL-2 receptor antagonist (IL2RA) (basiliximab), high-dose calcineurin inhibitors, and high-dose methylprednisolone [6, 7].

The first randomized studies demonstrated that both rATG and IL2RA induction reduce early acute rejection. They also recommended routine use of IL2RA induction as first-line therapy after renal transplantation while reserving ATG for high-risk cases. In patients at high immunological risk, ATG induction can reduce the relative risk of acute rejection by almost 50% compared to IL2RA [8].

A general trend toward increased use of induction therapy in solid organ transplantation (except liver transplantation) has been observed over the past two decades [4]. The use of rATG in induction therapy has also risen. However, the optimal regimen and duration of immunosuppression remain unknown [5].

For deceased donor kidney transplants, induction therapy has become more clearly established, while its use in living donor kidney transplantation is still somewhat debated. Living donor transplantation is generally considered less risky than deceased donor transplantation [6], but there are also studies showing opposite results.

The transplant recipient's immunological status, HLA mismatches, panel-reactive antibody (PRA) levels, donor-specific antibodies (DSA), donor age, etiology of chronic kidney disease, and cold ischemia time all play a role in the selection and dosing of induction therapy. Cost, availability, and side effects are also important considerations. Basiliximab and ATG-Fresenius (ATG-F, also known as Grafalon) have long been used as induction agents at our transplant center. In addition, all patients routinely receive high-dose methylprednisolone, calcineurin inhibitors, and mycophenolic acid (or mycophenolate mofetil). The aim of this study was to present the outcomes of kidney transplant induction therapy.

Materials and methods

Sample selection

This retrospective study included patients over the age of 18 who received a kidney transplant from a living or deceased donor at the PAUTF University Hospital transplant center between February 2022 and February 2025. Patients with missing data and pediatric cases were excluded.

Data collection

For all kidney transplant recipients, we gathered the following data from the hospital's electronic records: age, sex, body mass index (BMI), cause of ESRD, comorbidities, prior dialysis type and duration, the time from starting renal replacement therapy to transplantation, donor age and type (living or deceased), cold ischemia time, immunological profiles, and the initial immunosuppressive therapy regimen. Postoperative medical complications were noted, including DGF, leukopenia, neutropenia, severe neutropenia, thrombocytopenia, the day on which serum creatinine fell below three times its baseline, and discharge creatinine levels.

DGF was defined as the need for hemodialysis within seven days following kidney transplantation. Leukopenia was defined as WBC <3000/mm^3. Neutropenia was defined as an absolute neutrophil count <1500 cells/μL, and severe neutropenia as <500 cells/μL.

Immunosuppressive therapy regimen

Two main groups were formed based on induction therapy with Basiliximab or ATG-F.

Basiliximab group: Given to low-immunological-risk patients with PRA <20% (if <70 years old) or <50% (if ≥70 years old) and no DSA, dosed at 20 mg immediately before and four days after kidney transplantation.

ATG-F group: Given to high-risk patients (factors such as higher PRA, immunological mismatch, etc.). All patients also received standard induction and maintenance immunosuppressive therapy: corticosteroids, mycophenolic acid (or mycophenolate mofetil), and a calcineurin inhibitor (tacrolimus).

Ethics approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval date: 04.02.2025/03, approval no: E-60116787-020-643233).

Statistical analysis

All statistical analyses were performed using the SPSS 27.0 software. Normality of distribution was assessed with the Kolmogorov-Smirnov test. Descriptive statistics for continuous variables were expressed as mean ± standard deviation for normally distributed data or as median (minimum-maximum) for non-normally distributed data. Categorical variables were expressed as frequency and percentage. For group comparisons, we used the independent two-sample t-test for normally distributed variables and the Chi-square independence test for categorical data. Statistical significance was set at $p<0.05$.

Results

Thirty-three patients who underwent kidney transplantation were included in the study. Their demographic characteristics are shown in Table 1. The cohort included 10 women and 23 men, with a mean age of 39.8±11.9 years and a mean BMI of 24.5±3 kg/m^2. When classified by donor type, 16 patients (48.5%) received kidneys from living donors, and 17 (51.5%) from deceased donors. DGF was observed in 10 patients (30.3%). Factors affecting the development of DGF are shown in Table 2.

Among both living-donor and deceased-donor recipients, the most common blood group was A Rh(+) (33.3%). No statistically significant relationship was found between blood group and DGF (p=0.757, cs=1.184). When recipients were evaluated according to tissue matching:

In terms of induction therapy, Basiliximab was used in 14 of the living-donor transplants and ATG in 2 (12.5%). All deceased-donor recipients received ATG induction. For maintenance immunosuppression, all recipients received corticosteroids + tacrolimus + mycophenolate mofetil.

Table 1. Demographic characteristics of patients receiving living-donor or deceased-donor kidney transplants

		Donor		*p*
		Living donor	**Deceased donor**	
Recipient Sex (n / %)	**Female**	3 (18.8%)	7 (43.8%)	0.127 (cs=2.327)
	Male	13 (81.3%)	9 (56.3%)	
Recipient age (year)	**Mean ± S.D**	38.25±14.63	41.41±8.94	0.456 (t=-0.744)
	Med (IQR)	38.5 (24-49.5)	40 (36-49)	
	min-max	18-67	24-56	
BMI (kg/m²)	**Mean ± S.D**	24.06±4.34	25.02±3.23	0.472 (t=-0.729)
	Med (IQR)	24.2 (20.5-27.95)	25.4 (22.05-28.05)	
	min-max	15-30	20 - 29.1	
Cold ischemia time	**Mean ± S.D**	0.61±0.23	11.61±2	0.0001* (z=-4.911)
	Med (IQR)	0.55 (0.5-0.57)	11.52 (10.38-13.39)	
	min-max	0.4-1.15	8.17-15.2	
Pre-transplant dialysis duration (year)	**Mean ± S.D**	2.53±2.6	9.41±4.85	0.0001* (z=-4.321)
	Med (IQR)	2 (1-3)	8 (6-11.5)	
	min-max	0-11	3-22	
Donor age (year)	**Mean ± S.D**	43±10.05	41.94±18.76	0.84 (t=0.204)
	Med (IQR)	41.5 (36.25-52.5)	46 (22-58)	
	min-max	25-60	14-70	
Donor sex (n / %)	**Female**	3 (18.8%)	5 (29.4%)	0.688 γ
	Male	13 (81.3%)	12 (70.6%)	

*p<0.05 statistically significant, S.D: Standard Deviation, Med (IQR): Median (25^{th}-75^{th} percentiles), z: Mann Whitney U test
t: Independent samples t test, cs: Chi-square test, γ: Fisher exact test, BMI: Body Mass Index

Table 2. Factors affecting patients requiring dialysis in the first week after transplantation (DGF)

		No DGF	DGF	*p*
Recipient age (year)	**Mean ± S.D**	38.57±13.22	42.9±8.12	0.346 (t=-0.956)
	Med (IQR)	38 (30-50)	41 (37.75-49.25)	
	min-max	18-67	29-56	
Donor age (year)	**Mean ± S.D**	40.48±12.62	47±19.3	0.256 (t=-1.158)
	Med (IQR)	39 (33-51)	53 (25-61.25)	
	min-max	18-60	14-70	
BMI	**Mean ± S.D**	23.78±3.96	26.34±2.72	0.073 (t=-1.855)
	Med (IQR)	24 (20.5-27.8)	26.95 (24.95-28.48)	
	min-max	15-30	20-29.1	
Cold ischemia time	**Mean ± S.D**	3.92±5.23	11.71±2.04	0.0001* (z=-3.338)
	Med (IQR)	0.55 (0.5-10.28)	11.75 (10.23-13.43)	
	min-max	0.4-14.48	8.58-15.2	
Pre-transplant dialysis duration (year)	**Mean ± S.D**	3.76±3.28	11.4±4.99	0.0001* (z=-3.755)
	Med (IQR)	3 (2-5)	10.5 (7.75-14.75)	
	min-max	0-12	6-22	
PRA Class I	**negative**	20 (87%)	10 (100%)	0.536 γ
	pozitive	3 (13%)	0 (0%)	
PRA Class II	**negative**	22 (95.7%)	9 (90%)	0.521 γ
	pozitive	1 (4.3%)	1 (10%)	
HLA matches	**0**	2 (8.7%)	0 (0%)	0.082 (cs=9.78)
	1	6 (26.1%)	2 (20%)	
	2	5 (21.7%)	7 (70%)	
	3	5 (21.7%)	1 (10%)	
	4	3 (13%)	0 (0%)	
	6	2 (8.7%)	0 (0%)	
A	**0**	10 (45.5%)	8 (88.9%)	0.047* (cs=6.119)
	1	9 (40.9%)	1 (11.1%)	
	2	3 (13.6%)	0 (0%)	
B	**0**	9 (40.9%)	3 (33.3%)	0.41 (cs=1.781)
	1	11 (50%)	6 (66.7%)	
	2	2 (9.1%)	0 (0%)	
DR	**0**	7 (31.8%)	0 (0%)	0.017* (cs=8.102)
	1	9 (40.9%)	8 (88.9%)	
	2	6 (27.3%)	1 (11.1%)	
Donor	**Living**	16 (69.6%)	0 (0%)	0.0001* γ
	Deceased	7 (30.4%)	10 (100%)	
Nephrectomy	**Left**	16 (69.6%)	3 (30%)	0.057
	Right	7 (30.4%)	7 (70%)	
ATG	**0**	14 (60.9%)	0 (0%)	0.001* γ
	1	9 (39.1%)	10 (100%)	
Basilixumab	**0**	8 (34.8)	10 (100%)	0.0001* γ
	1	15 (65.2)	0 (0%)	

*p<0.05 statistically significant, S.D: Standard Deviation, Med (IQR): Median (25^{th}-75^{th} percentiles), z: Mann Whitney U test
t: Independent samples t test, cs: Chi-square test, γ: Fisher exact test, BMI: Body Mass Index, PRA: Panel Reactive Antibody
HLA: Human Leukocyte Antigen, ATG: Antithymocyte Globulin

Discussion

In this study, we examined early outcomes and the impact on DGF of different induction therapies used in patients who received kidney transplants from living or deceased donors. Our findings showed that 30.3% of the overall patient group developed DGF, particularly among those who received kidneys from deceased donors and had longer cold ischemia time and pre-transplant dialysis durations. The literature similarly reports that prolonged cold ischemia and pre-transplant dialysis duration increase the risk of not only graft dysfunction but also graft loss [3, 4]. This accumulation of high immunological and clinical risk may render the kidney more vulnerable in the postoperative adaptation period.

In the present study, Basiliximab was generally chosen for living-donor transplants, whereas ATG-F was used in deceased-donor transplants that were considered higher risk (prolonged pre-transplant dialysis duration, potential immunological mismatches, etc.). The frequent observation of DGF in the ATG-F group does not imply that ATG-F alone causes DGF; rather, it suggests that these recipients already presented more unfavorable baseline clinical and immunological conditions. Therefore, with the limited number of patients and the retrospective design of our study, drawing definitive conclusions about a direct effect of the induction regimen on DGF etiology is challenging. However, previous studies also emphasize the effectiveness of T-lymphocyte-depleting agents (e.g., rATG, ATG-F) in reducing the incidence of acute rejection in high-risk patients [6-8].

Our study observed that patients who developed DGF had a slightly higher mean BMI, although it did not reach statistical significance, making it difficult to draw firm conclusions on the role of BMI in DGF. Similarly, age and certain immunological parameters (HLA matches, PRA status) showed some influence on DGF but did not reach statistical significance. However, previous reports have noted that advanced age, high BMI, or insufficient HLA matching could negatively affect graft function [3, 5]. The trends observed in our study may not have achieved statistical significance due to the small sample size and heterogeneity of the study population.

In conclusion, our findings demonstrate that extended cold ischemia and pre-transplant dialysis duration are major determinants of DGF in deceased-donor kidney recipients. In living-donor recipients, giving basiliximab to those with a lower immunological risk profile might be associated with fewer early DGF events. Nonetheless, it is important to note that such differences may largely stem from the distinct baseline clinical and immunological risk profiles between the two recipient groups. Prospective, large-sample, and multicenter studies are needed to more clearly elucidate the impact of different induction strategies on DGF and long-term graft survival. Such research will provide valuable data for reducing early complications and identifying optimal immunosuppressive approaches in high-risk patient populations.

Funding: None.

Authors contributions: D.A. developed the main idea and hypothesis of the study. O.B. and E.M. contributed to the study design and the materials and methods section. U.O., D.A., and M.O. performed the data evaluation in the results section. The discussion section of the article was written by D.A., E.M., M.C., O.B., U.O., and M.O., who all reviewed, corrected, and approved the final version. Additionally, all authors discussed the entire study and approved the final version.

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

References

1. Francis A, Harhay MN, Ong ACM, et al. Chronic kidney disease and the global public health agenda: an international consensus. *Nat Rev Nephrol.* 2024;20(7):473-485. doi:10.1038/s41581-024-00820-6
2. Registry of The Nephrology. Dialysis and Transplantation in Turkey. Registry 2021. Ministry of Health and Turkish Society of Nephrology Joint Report. In: Ateş K. Seyahi N. Koçyiğit İ. eds. Ankara: Miki Matbaacılık San. ve Tic. Ltd. Şti.: 2022.
3. Guimarães J, Araújo AM, Santos F, Nunes CS, Casal M. Living-donor and deceased-donor renal transplantation: Differences in early outcome -- a single-center experience. *Transplant Proc.* 2015;47(4):958-962.
4. Ayar Y, Ersoy A, Ocakoglu G, et al. Risk factors affecting graft and patient survivals after transplantation from deceased donors in a developing country: a single-center experience. *Transplant Proc.* 2017;49(2):270-277.

5. Matas AJ, Gillingham KJ, Humar A, Dunn DL, Sutherland DE, Najarian JS. Immunologic and nonimmunologic factors: different risks for cadaver and living donor transplantation. *Transplantation.* 2000;69(1):54-58. doi:10.1097/00007890-200001150-00011
6. Kasiske BL, Zeier MG, Chapman JR, et al. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. *Kidney Int.* 2010;77(4):299-311. doi:10.1038/ki.2009.377
7. Danovitch GM. Handbook of Kidney Transplantation. 6. baskı. Philadelphia: Lippincott Williams & Wilkins; 2017.
8. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D. Thymoglobulin Induction Study Group. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N Engl J Med.* 2006;355(19):1967-1977. doi:10.1056/NEJMoa060068

Case Report
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1517473

Differentiation of dissociative psychosis, delirious mania, and unspecified dissociative disorder in an adolescent case

Bir ergen olguda dissosiyatif psikoz, deliryöz mani ve tanımlanmamış dissosiyatif bozukluğun ayrımı

Asya Çankaya, Ali Evren Tufan

Posted date:17.07.2024 Acceptance date:07.10.2024

Abstract
Dissociative psychosis is, it is a condition that usually occurs after a trauma when psychotic and dissociative symptoms occur together. In this process, disorganized behaviors increase, insight is lost, and hallucinations become worse. It usually results in sudden improvement over a period of several days to several weeks, and most patients remain amnesic into the dissociative psychosis phase. A sixteen-year-old female adolescent was brought in with severe manic and psychotic complaints that started suddenly a week ago. In the mental status examination, disorganized associations thought content, and auditory and somatic hallucinations were particularly striking. During the hospitalization process, passivity, delusional delusions of misidentification, and disorganized speech and behavior were also observed. The adolescent, whose complaints regressed in the fourth week, was amnesic during the episode. The differential diagnosis and treatment process of dissociative psychosis, delirious mania and unspecified dissociative disorder in a patient whose medications were discontinued after discharge and no relapse was observed is presented.

Keywords: Psychosis, dissociation, adolescent, mood disorder.

Cankaya A, Tufan AE. Differentiation of dissociative psychosis, delirious mania, and unspecified dissociative disorder in an adolescent case. Pam Med J 2025;18:454-458.

Öz
Disosiyatif psikoz; genellikle travma sonrası, psikotik ve disosiyatif bulguların bir arada orataya çıkmasıyla oluşan bir tablodur. Bu süreçte dezoganize davranışlar artar, iç görü kaybolur, varsanılar ağırlaşır. Genellikle birkaç günden birkaç haftaya kadar uzayan bir sürede aniden düzelme ile sonuçlanmaktadır ve çoğu hasta dissosiyatif psikoz dönemine amnezik kalmaktadır. On altı yaşındaki, kız ergen bir hafta önce aniden başlayan şiddetli manik ve psikotik yakınmaları ile getirildi. Ruhsal durum muayenesinde; dağınık çağrışımları, düşünce içeriği, işitsel ve somatik halüsinasyonları özellikle dikkat çekiciydi. Yatış sürecinde edilgenlik, sanrısal yanlış tanıma sanrılarının, dezorganize konuşma ve davranışlarının da olduğu gözlendi. Dördüncü haftada yakınmaları gerileyen ergen epizoda amnezikti. Taburculuk sonrası ilaçları kesilen ve nüks görülmeyen olguda disosiyatif psikoz, deliryöz mani, belirlenmemiş disosiyatif bozukluk ayırıcı tanı ve tedavi süreci sunulmuştur.

Anahtar kelimeler: Psikoz, disosiasyon, ergen, duygu durum bozukluğu.

Çankaya A, Tufan AE. Bir ergen olguda dissosiyatif psikoz, deliryöz mani ve tanımlanmamış dissosiyatif bozukluğun ayrımı. Pam Tıp Derg 2025;18:454-458.

Introduction

Hallucinatory experiences and delusions during adolescence are commoner than once thought. These symptoms can also be seen in disorders other than psychotic ones, including mood disorders, anxiety, attention-deficit/hyperactivity disorder, autism spectrum disorders, post-traumatic stress disorder (PTSD), and related conditions along with different psychopathologies or medical conditions [1]. Current studies have led to an increasing recognition of adolescents at risk for developing psychosis [1, 2]. These youth may have sub-threshold, heterogeneous symptoms (“prodromal symptoms”) and may progress to more severe disorders, including schizophrenia, during follow-up. As a result of studies on this

Asya Çankaya, Research Asst. Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Child and Adolescent Mental Health and Disorders, Bolu, Türkiye, e-mail: asyaafacan@gmail.com (https://orcid.org/0009-0006-3272-7142) (Corresponding Author)

Ali Evren Tufan, Prof. Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Child and Adolescent Mental Health and Disorders, Bolu, Türkiye, e-mail: tevrenus@yahoo.com (https://orcid.org/0000-0001-5207-6240)

subject, “Attenuated Psychosis Syndrome” was introduced in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [3]. Although dissociative disorders, post-traumatic spectrum disorders, and psychotic disorders are evaluated in separate diagnostic categories in DSM-5, current opinion suggests that dissociative and psychotic symptoms may partially overlap and dissociative processes may mediate between early traumas and later developing psychotic symptoms [4].

“Dissociative Psychosis” (DP) is not included in current diagnostic systems and describes a syndrome that is widely used, especially among psychiatrists of European origin [5, 6]. It is reported that the syndrome is not culture-dependent but occurs suddenly and unexpectedly after stressors that may vary depending on culture, can last for a short time (<3 weeks), and patients with good premorbid functionality may develop amnesia for the episode. Although the duration may vary, the relationship between the traumatic experience and dissociative characteristics is relatively constant. In cases, changes in the level of consciousness and pre-conscious phenomena may be observed, symptom severity and form may fluctuate, and although the appearance may resemble schizophrenia, mania, or organic cognitive disorders, the absence of affective blunting and a good prognosis is characteristic [5, 6]. It was thought that this diagnosis could explain the sudden recovery and amnesic period seen in our case. “Delirious mania” (“Bell mania”) is also a relatively little-known neuropsychiatric syndrome and is characterized by sudden onset delirium, mania, psychosis, and catatonic symptoms [7]. It is independent of general medical conditions, substance use, and other mental disorders and can be seen in up to approximately 15.0% of acute manic cases. Its severity may progress with delays in diagnosis and treatment, but it is reported to respond well to high-dose lorazepam and electroconvulsive therapy (ECT) [7]. In our case, the presence of findings suggestive of delirium in addition to manic symptoms may indicate delirium mania. However, this diagnosis could not be finalized because our case did not explain the amnesic period. In this study, we aimed to present the differential diagnosis and treatment process of rare but clinically similar dissociative psychosis, delirium mania, and unspecified dissociative disorder in an adolescent female patient and to help the diagnosis and treatment process of cases with uncertain diagnosis.

Case

A sixteen-year-old, 11th-grade female was brought to the clinic with complaints of “talking to herself in unintelligible speech, laughing, inability to spend time alone and not being able to recognize people around her”. It was learned that her complaints started suddenly about a week ago, her communication with the environment decreased about six months before the application, she reported a change in her religious beliefs after visiting a relative two months ago, her appetite was reduced, and she lost approximately 15 kg/month. From the past medical history, it was learned that she was a baby born with IVF, that she had no problems before, during and after birth, that she was a temperamentally docile baby, that her motor-mental development stages occurred on time, that she had no chronic medical diseases and no previous psychiatric applications. In the family history, it was determined that the mother had received treatment for a diagnosis of bipolar disorder Type I (BPI) and had been in remission for a long time and that her grandmother had periodic tantrums and self-harming behavior but had never received treatment.

The mental status examination observed that the care of the adolescent girl, who showed age-appropriate physical development and partially communicated with the interviewer, decreased. It was observed that she alternately displayed defensive and regressive attitudes and made partial eye contact. Her orientation was fluctuating, and her consciousness was clear. Spontaneous - voluntary attention - concentration, working memory - short-term memory were evaluated as impaired and fluctuating. Long-term memory could not be evaluated. The speech was fast, disorganized, and pressured. The thought process is tangential/ circumstantial with loose associations, flights of ideas, the incoherence (“purple, orange, thank you”), neologisms (“shine is a code between me and my lover”, “I explain by coding”), perseverations and thought blocks. The thought content was poor. Delusions were compatible with her mood, unsystematic, bizarre, somatic (“she is pregnant”), erotomanic (“she has a lover”), grandiose (“only she can

hear her lover"), persecutory ("they are trying to poison her"). Perceptual examination revealed auditory and somatic hallucinations consistent with mood. Affect was inappropriate and labile, mood was labile and exuberant. Psychomotor agitation, stereotypy, and mannerisms were observed. Sleep and appetite were decreased while libido was increased. Insight and judgment were impaired. She was admitted to the inpatient service to clarify her diagnosis and arrange treatment.

No organic pathology, intoxication, or withdrawal findings were detected in evaluations, including pediatric and pediatric neurology consultations, physical examination, laboratory tests including autoantibodies, electroencephalography (EEG), and cranial MRI. Young Mania (YMRS), Positive and Negative Affect Scale (PANSS), and Clinical Global Monitoring (CGI) scale scores [8] were 30 (significantly manic), 113 (Positive:41, Negative:72, General:113) and 6 ("Seriously ill"), respectively. As a result of the history, examination, and evaluations, she was preliminarily diagnosed with Brief Psychotic Disorder (Dissociative Psychosis), BP-I (Manic Episode, Delirious Mania), Unspecified Dissociative Disorder according to DSM-5 criteria and was started on risperidone 1 mg/ day, alprazolam 1 mg/ day, sodium valproate 250 mg/day treatment. Risperidone, alprazolam, and sodium valproate (VPA) doses were gradually increased to 4 mg/day, 1.5 mg/ day, and 1000 mg/day in the second week.

During her stay, she continued to display regressive behaviors (e.g., wanting to hold the doctor's hand while talking), labile affect, vague persecutory (rejecting the food served in favor of packaged foods), passivity ("What I write is written in blue"), delusional misidentification/ Fregoli/ Capgras delusions ("She disguised herself as Arif", to the doctor: "you can also be Arif" and "You are my mother") [9], disorganized speech and behavior were observed.

In the first week of hospitalization, the VPA level was 100 µg/mL, and due to complaints of pain and nausea, valproate treatment was reduced to 250 mg/day, and risperidone was increased to 6 mg/day. YMRS, PANSS, and CGI scores [8] in the second week of hospitalization were 13 (insignificant manic symptoms), 93 (Positive:29, Negative:64, General:93), and 4 ("moderately ill"), respectively. On the twentieth day, the alprazolam treatment of the adolescent, whose complaints continued, was discontinued, and treatment with lorazepam 2 mg/day and olanzapine 5 mg/day was started, gradually increasing the latter to 10 mg/day. In the fourth week of hospitalization, the adolescent's complaints were significantly reduced, and she reported for the first time that her complaints started after she had a row with a few friends she met online but had no recollection of what came after. The adolescent was discharged in the fifth week with risperidone 6 mg/day, olanzapine 10 mg/day, VPA 250 mg/day, lorazepam 1 mg/day, and biperiden 1 mg/day. YMRS, PANSS, and CGI scores [8] at discharge were 0, 0 (Positive:0, Negative:0, General:0), and 2 ("borderline mentally ill"), respectively. Lorazepam and valproate treatments were discontinued for the adolescent, who had no symptoms other than subthreshold depressive and anxiety complaints a week after discharge; olanzapine treatment was continued while risperidone was reduced to 3 mg/day.

In the second follow-up interview, the adolescent reported that she still did not remember the hospitalization period, that she had converted to another religion against her will, and that this might disturb her and cause her complaints. The risperidone dose was increased to 6 mg/day again, and 1 mg/day of biperiden was added. Then, the anxiety symptoms experienced in social communication were addressed in visits via cognitive-behavioral methods. Scores for Post-traumatic Stress Response Scale (PTSTS), Beck Depression Inventory (BDI), and Adolescent Dissociation Scale (A-DES) at the sixth visit were 55 (clinically significant PTSD symptoms), 32 (severe depressive symptoms), and 6.4 (dissociative disorder level symptoms), respectively. However, other than the argument with her peers, she recollected no significant trauma. Therapy sessions, therefore, are aimed at reducing anxiety, increasing assertiveness, and assuming age-appropriate responsibilities.

At the eighth interview, no active complaints were detected. Scores of 0, 0, and 2 were obtained from the YMRS, PANSS, and CGI scales [8], respectively. The adolescent, who was followed weekly until the eighth interview, was evaluated first every two weeks and then once a month for the next nine months. Since

the symptoms did not recur, all medications were tapered and discontinued. She is still free of symptoms a month after cessation of treatment.

Discussion

In this study, the differential diagnosis and treatment process of DP, delirious mania, and unspecified dissociative disorder in an adolescent female is presented. Schizoaffective disorder was ruled out due to a lack of significant psychotic symptoms lasting at least two weeks in the absence of mood symptoms and the absence of complaints after cessation of treatment. Substance abuse was ruled out with history and laboratory evaluations. PTSD with psychotic and dissociative symptoms was ruled out due to lack of exposure to actual or threatened death, serious injury, or sexual violence. In DSM-5, dissociative disorders occur with the division of functions such as consciousness, memory, identity, affect, perception, body schema, motor control, and behavior, which form a whole under normal conditions and are defined as dissociative identity disorder (DID), dissociative amnesia, depersonalization/derealization disorder, other specified and unspecified dissociative disorders [3]. In the presented case, DID was excluded due to impaired reality testing, the presence of additional symptoms, lack of two or more personality states, and recurrent episodes of dissociative amnesia. In the case, during the follow-up period, an inability to remember autobiographical information that could not be explained by normal forgetfulness, consistent with localized and selective dissociative amnesia, was observed, but the adolescent did not report subjective distress due to this symptom, and no dysfunction was detected. The differential diagnosis can also consider other specified dissociative disorders (acute dissociation in response to stressful events). For this diagnosis, symptoms typically last less than a month, sometimes emerging within hours/days of the stressor, level of consciousness changes with depersonalization/derealization, time is perceived as slowed with macropsia and similar perceptual disorders, microamnesias, transient somnolence and/or loss of sensory-motor functions can be seen [1]. In our case, this diagnosis was excluded because the duration of the symptoms was longer than one month; symptoms included hallucinations, delusions, and impaired reality testing. Delirious mania is ruled out due to the absence of catatonic symptoms worsening over time, psychotic/dissociative symptoms being independent of mood-related symptoms, and the relatively low dose of lorazepam used [7]. Dissociative psychosis is, it is a condition that usually occurs after a trauma when psychotic and dissociative symptoms occur together. In this process, disorganized behaviors increase, insight is lost, and hallucinations become worse. It usually results in sudden improvement over a period of several days to several weeks, and most patients remain amnesic into the dissociative psychosis phase [10]. DP was considered due to the sudden development of symptoms, short duration, good premorbid functionality, post-episode amnesia, association with traumatic experience, absence of affective blunting, and the presence of noisy, waxing-and-waning, heterogeneous symptoms [5, 6]. Although social withdrawal and changes in religious beliefs in our patient could also be considered prodromal symptoms before schizophrenia or attenuated psychosis syndrome, the follow-up period does not support this view. Although PTSD was ruled out due to a lack of significant traumatic experiences, according to the adolescent's Report, it should be borne in mind that she had dissociative amnesia during the period of hospitalization. This may suggest that there might be other traumatic experiences for which she is currently amnesic, and those may resurface during longer follow-up.

The presented case may inform the clinicians in keeping the diagnosis of DP in mind among adolescent patients in whom dissociative, psychotic, and affective symptoms suddenly and dramatically emerge after a stressor.

Acknowledgment: I would like to thank Dr. Süleyman Çankaya for his support during the patient follow-up process.

Consent to participate: Written consent was obtained from the patient and her family.

Funding: We do not have any financiers.

Authors contributions: A.C. wrote the article under the supervision of A.E.T. In addition, the authors discussed the entire study and approved the final version.

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

References

1. Stevens JR, Prince JB, Prager LM, Stern TA. Psychotic disorders in children and adolescents: a primer on contemporary evaluation and management. *Prim Care Companion CNS Disord*. 2014;16(2):PCC.13f01514. doi:10.4088/PCC.13f01514
2. Raballo A, Poletti M, Preti A. Editorial Perspective: Psychosis risk in adolescence - outcomes, comorbidity, and antipsychotics. *J Child Psychol Psychiatry*. 2022;63(2):241-244. doi:10.1111/jcpp.13438
3. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Publishing Inc.; 2013. doi:10.1176/appi.books.9780890425596
4. Chiu CD, Li DJ, Hsieh YC, et al. Linking childhood trauma and dissociation to psychotic symptoms in major depressive disorder, bipolar disorders, and schizophrenia: A transdiagnostic examination using patient and clinician ratings. *Psychol Trauma*. 2024;16(Suppl 1):S242-S249. doi:10.1037/tra0001584
5. Devillé C, Moeglin C, Sentissi O. Dissociative disorders: between neurosis and psychosis. *Case Rep Psychiatry*. 2014;2014:425892. doi:10.1155/2014/425892
6. Van der Hart O, Witztum E. Dissociative psychosis: Clinical and theoretical aspects. In: Moskowitz A, Schäfer I, Dorahy MJ, eds. Psychosis, Trauma and Dissociation: Emerging Perspectives on Severe Psychopathology. 1st ed. West Sussex, UK: John Wiley & Sons Ltd.; 2008:255-269. doi:10.1002/9780470699652.ch18
7. Jacobowski NL, Heckers S, Bobo WV. Delirious mania: detection, diagnosis, and clinical management in the acute setting. *J Psychiatr Pract*. 2013;19(1):15-28. doi:10.1097/01.pra.0000426324.67322.06
8. Aydemir Ö. Psikiyatride değerlendirme araçları: özellikleri, türleri, kullanımı. In: Aydemir Ö, Köroğlu E, eds. Psikiyatride Kullanılan Klinik Ölçekler. Ankara, Türkiye: Hekimler Yayın Birliği; 2007.
9. Ashraf N, Antonius D, Sinkman A, Kleinhaus K, Malaspina D. Fregoli syndrome: an underrecognized risk factor for aggression in treatment settings. *Case Rep Psychiatry*. 2011;2011:351824. doi:10.1155/2011/351824
10. Taşkın Uyan T, Hocaoğlu Ç. DSM 5'te Yer Almayan Bir Tanı: Dissosiyatif Psikoz - İki Olgu Sunumu. 1. Uluslararası Ergen ve Erişkin Klinik Psikiyatri Kongresi; 2016.

Review

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1467619

Histological structure and functional properties of the tunica albuginea of the ovary

Ovaryum tunika albugineasının histolojik yapısı ve fonksiyonel özellikleri

Hale Yetgin, Murat Serkant Ünal, Cihan Kabukçu, Ahmet Çevik Tufan

Posted date:17.04.2024 Acceptance date:02.07.2024

Abstract

Just below the surface epithelium of the ovary is the tunica albuginea, which is a tight irregular connective tissue structure that gives the tissue its white color and contains fibroblast cells. Tunica albuginea, which is more resistant to environmental factors, contains fewer cells and is rich in collagen fibers, is observed as the niche of preantral follicles. It has been observed that fibroblasts forming collagen fibers provide the development of follicles with the paracrine factors and cytokines they secrete and function as a nourishing cell layer. After the graaf follicle forms the corpus luteum, fibroblasts in the adjacent tunica albuginea proliferate and the thickness of this structure increases. The tunica albuginea undergoes frequent renewal due to the corpus luteum structures formed in rats. When the corpus luteum is formed, new capillaries and venules are formed in the adjacent tunica albuginea. This structure may be a suitable model for investigating the migration of cells from the bone marrow to the ovary via vessels. It can be suggested that mesenchymal cells and very small embryonic-like stem cells (VSELs), which show pluripotent stem cell characteristics, may migrate from the bone marrow to the tunica albuginea through vascular structures and that the bone marrow may be the source of these cells, which have been previously shown to be present in the ovary. The aim of this review is to examine the effects of the tunica albuginea on the development of follicles and the dynamic structure of the ovary.

Keywords: Ovary, tunica albuginea, collagen fibers, follicle, corpus luteum.

Yetgin H, Unal MS, Kabukcu C, Tufan AC. Histological structure and functional properties of the tunica albuginea of the ovary. Pam Med J 2025;18:460-473.

Öz

Ovaryumda yüzey epitelinin hemen altında dokuya beyaz rengini veren, sıkı düzensiz bir bağ doku yapısında olan ve içinde fibroblast hücrelerini içeren tunika albuginea bulunur. Çevresel etkenlere daha dirençli olan, az sayıda hücre içeren ve kollajen liflerden zengin olan tunika albuginea preantral foliküllerin nişi olarak gözlenmektedir. Kollajen lifleri oluşturan fibroblastların salgıladıkları parakrin faktörlerle ve sitokinlerle foliküllerin gelişimlerini sağladıkları ve besleyici hücre tabakası gibi fonksiyon gördükleri izlenmiştir. Graaf folikülün korpus luteumu oluşturmasından sonra ise hemen bitişiğindeki tunika albugineadaki fibroblastların prolifere olduğu ve bu yapının kalınlığının arttığı izlenmiştir. Ratlarda oluşan korpus luteum yapıları nedeniyle tunika albuginea sık sık yenilenmeye uğramaktadır. Korpus luteum oluştuğu zaman bitişiğindeki tunika albugineada yeni kapillerler ve venüller meydana gelmektedir. Bu yapı; hücrelerin kemik iliğinden, damarlarla ovaryuma migrasyonlarının araştırılması için uygun bir model olabilir. Mezenkimal hücrelerin ve pluripotent kök hücre özelliği gösteren çok küçük embriyonik benzeri kök hücrelerin (VSELs) vasküler yapılar aracılığıyla kemik iliğinden tunika albugineaya gelebileceği ve ovaryumda bulundukları daha önce gösterilen bu hücrelerin kaynağının kemik iliği olabileceği ileri sürülebilir. Bu derlemedeki amacımız tunika albugineanın foliküllerin gelişimine ve ovaryumun dinamik yapısı üzerine olan etkilerini incelemektir.

Anahtar kelimeler: Ovaryum, tunika albuginea, kollajen lifler, folikül, korpus luteum.

Yetgin H, Ünal MS, Kabukçu C, Tufan AÇ. Ovaryum tunika albugineasının histolojik yapısı ve fonksiyonel özellikleri. Pam Tıp Derg 2025;18:460-473.

Hale Yetgin, Ph.D. Department of Histology and Embryology, Pamukkale University, Faculty of Medicine, Denizli, Türkiye, e-mail: haleapaydn@gmail.com (https://orcid.org/0009-0005-9153-8221) (Corresponding Author)

Murat Serkant Ünal, Asst. Prof. Department of Histology and Embryology, Pamukkale University, Faculty of Medicine, Denizli, Türkiye, e-mail: serkantunal72@gmail.com (https://orcid.org/0000-0003-1992-7909)

Cihan Kabukçu, Assoc. Prof. Pamukkale University, Faculty of Medicine, Department of Obstetrics and Gynecology, Denizli, Türkiye, e-mail: cihank@pau.edu.tr (https://orcid.org/0000-0003-3331-5714)

Ahmet Çevik Tufan, Prof. Department of Histology and Embryology, University of Health Sciences, Gülhane Faculty of Medicine, Ankara, Türkiye, e-mail: ahmetcevik.tufan@sbu.edu.tr (https://orcid.org/0000-0002-5920-0475)

Introduction

Ovarian histology

The ovary is a pinkish-white oval-shaped organ located on either side of the uterus. The ovary is defined as an exocrine gland because of gamete production and an endocrine gland because of hormone secretion [1]. The surface of the ovary is lined with single layer cubic epithelium and pseudo-multilayer epithelium in some areas and flat epithelium in some areas. The surface epithelium is rich in cytokeratin (CK) 7, 8, 18 and 19 and has intercellular desmosomes and tight junctions. Under the surface epithelium is a basal membrane consisting of two layers. One layer is the basal lamina made by epithelial cells. The second layer is the reticular lamina formed by fibroblasts [2-4]. Just below the basal lamina is the tunica albuginea, a tight connective tissue structure that gives the ovary a whitish colour (Figure 1, 2) [1, 4].

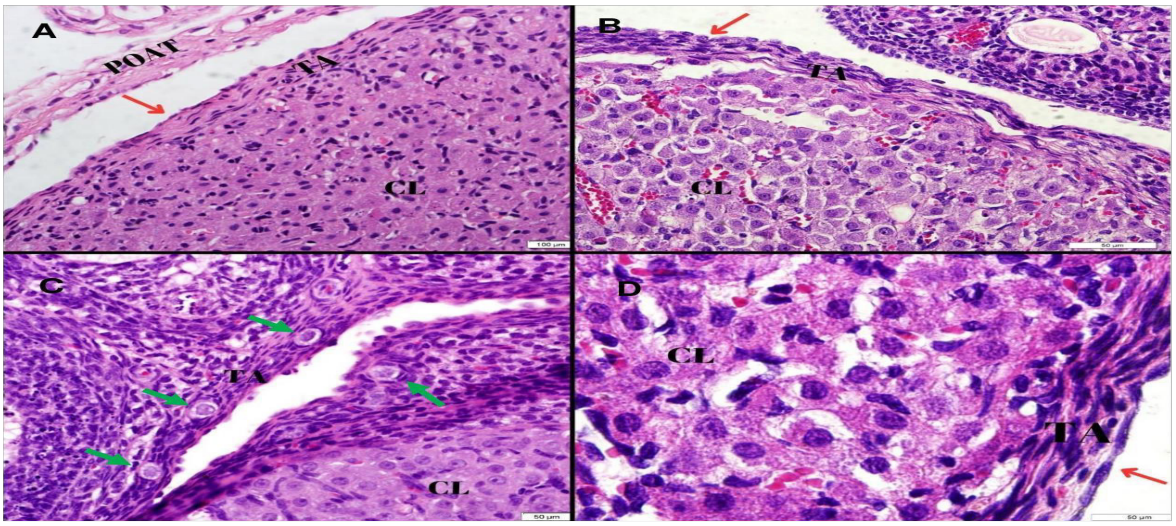

Figure 1. A-B: Tunica albuginea and surface epithelium adjacent to the corpus luteum, C: Primordial follicles within the tunica albuginea, D: Tunica albuginea and surface epithelium adjacent to the corpus luteum, green arrow indicates primordial follicle, red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum, POAT: Periovarian Adipose Tissue, Magnification (A, B, C: 400X; D: 1000X)

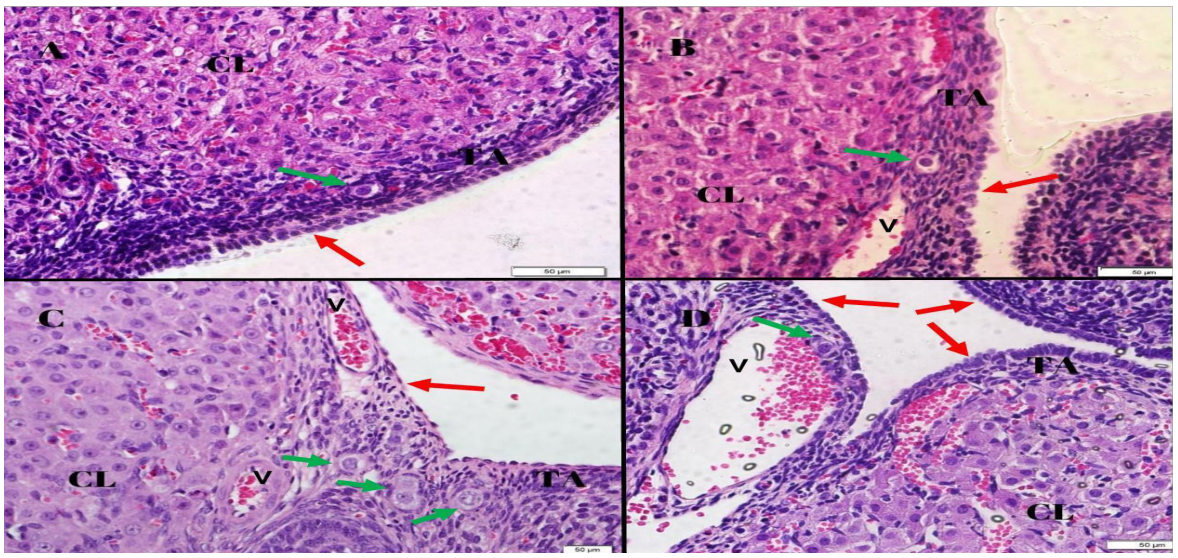

Figure 2. A-B-C-D: The tunica albuginea adjacent to the corpus luteum and primordial follicles within it. Green arrow indicates primordial follicle, red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum V: Vascular Structures, Magnification (A, B, C: 400X; D: 1000X)

The surface epithelium of the ovary shows a highly dynamic structure. The surface of the ovary expands or narrows due to changes in the size of the follicles and corpus luteum. In addition, when the follicle wall ruptures during ovulation, repair occurs in the tunica albuginea and surface epithelium. This repair is thought to occur by the proliferation of surface epithelial cells with stem cell properties [4]. In the studies carried out at advanced ages, it is observed that the surface epithelium of the ovary turns into a multi-level flat epithelium and its structure becomes irregular. In some cases, after ovulation some of the ovarian surface epithelium becomes invaginated towards the cortex and continues along with some tunica albuginea and is called an inclusion cyst. In studies with neonatal rat ovary, it has been observed that the surface epithelium has an discontinuous basal membrane and consists of multiple cell layers. It is then observed that a continuous basal membrane forms under a pseudostratified surface epithelium. In the following process, it is observed that the surface epithelium is organized as a single-layer structure and a continuous basal membrane is formed [5]. Ovarian follicles are surrounded by the basal lamina, a specialized form of extracellular matrix (ECM) that separates the granulosa cells from the stroma. This basal lamina is surrounded by a network of different macromolecules such as collagen IV, laminin, nidogen and perlecan produced by stromal cells and granulosa cells. Type IV collagen is the most abundant component in the basal lamina [6]. The ovary consists of two parts, the cortex and medulla layers, which have different histologic features. While these two layers can be easily distinguished in ovaries in the early reproductive period, these differences begin to diminish in later periods. The connective tissue in the tunica albuginea is an irregular tight connective tissue containing fibroblasts and fibrocyte cells, whereas the medulla is a loose connective tissue. In addition to fibroblast and fibrocyte cells, which are less abundant in the medulla, hilar cells, adipocytes, macrophages and vascular structures are present. Spiral arteries and arterioles are located parallel to the surface epithelium and tunica albuginea of the ovary. Branches of this artery extend into the cortex and medulla. It has been shown that periovarian adipose tissue is located immediately adjacent to the surface epithelium in the rats and there is a fibrous thin sheath between them (Figure 3-5).

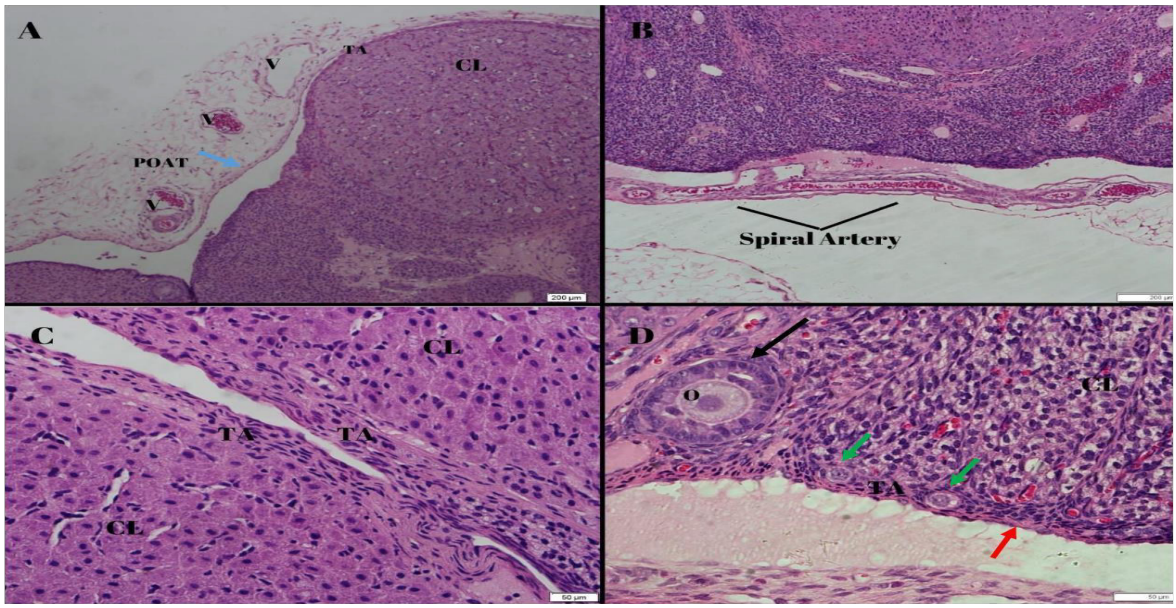

Figure 3. A: Tunica albuginea and surface epithelium adjacent to the corpus luteum, B: Spiral artery extending parallel to the cortical layer of the ovary, C: Tunica albuginea and surface epithelium adjacent to the corpus luteum, D: Tunica albuginea adjacent to the regressed corpus luteum and primordial and primary follicles within it. Blue arrow indicates fibrous sheath, green arrow indicates primordial follicle, black arrow indicates primary follicle, red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum O: Oocyte, Magnification (A, B: 200X; C, D: 400X)

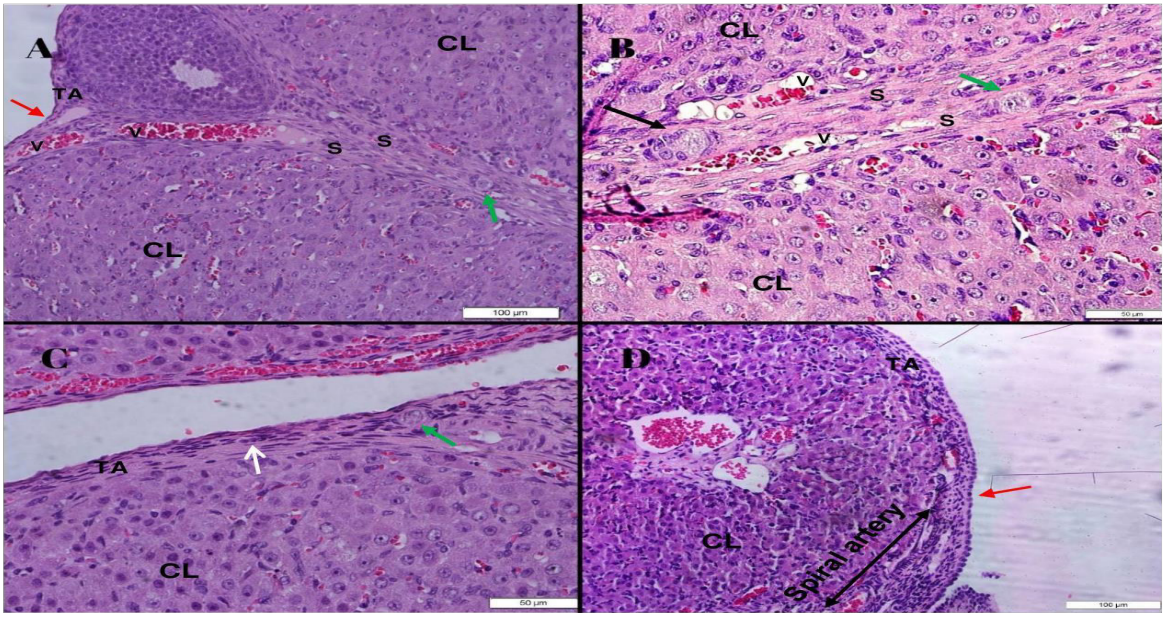

Figure 4. A-B-C-D: The tunica albuginea adjacent to the corpus luteum and the primordial and primary follicles within it. Green arrow indicates primordial follicle, black arrow indicates primary follicle, red arrow indicates ovarian surface epithelium, white arrow indicates germ cells

TA: Tunica Albuginea CL: Corpus Luteum V: Vascular structures S: Septa, the outer part of the corpus luteum containing fibroblast cells and collagen fibers, Magnification (A, D: 200X; B, C: 400X)

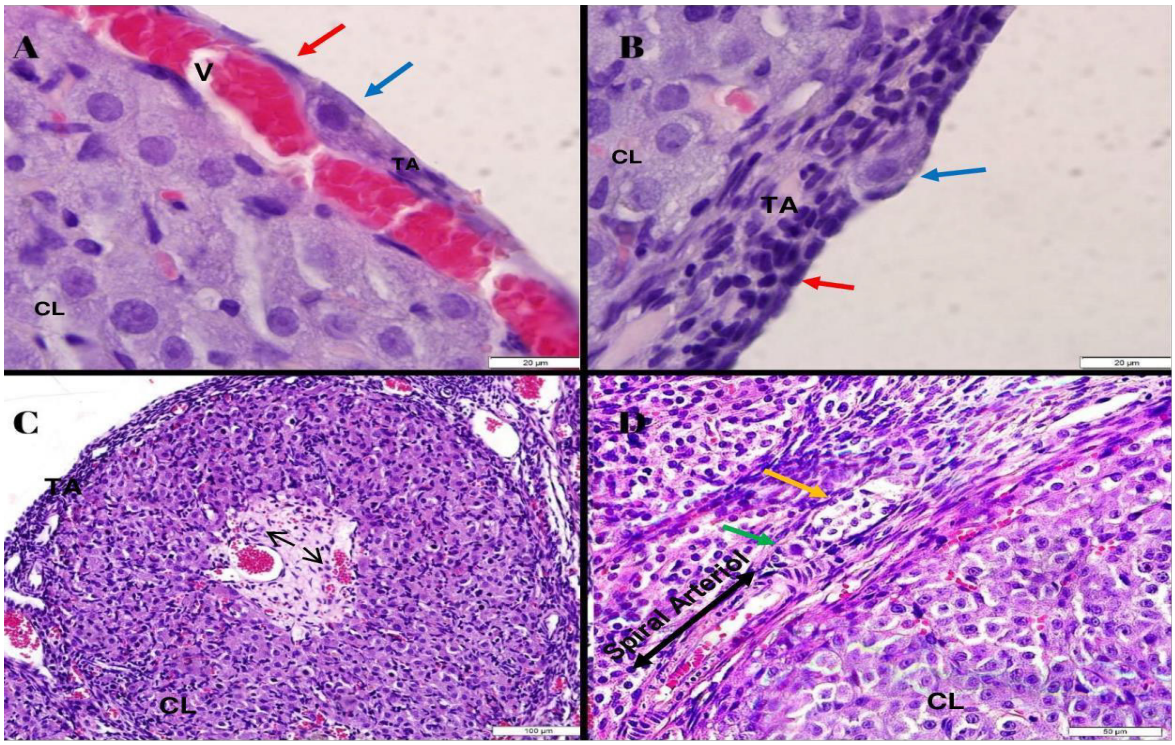

Figure 5. A-B: Granulosa lutein cells eccentrically located in the tunica albuginea adjacent to the corpus luteum, C: Macrophages seen in the regressed corpus luteum, D: Spiral arteriole, primordial follicle and rete ovarii adjacent to the tunica albuginea of the corpus luteum. Blue arrow indicates granulosa lutein cell, black arrow indicates macrophage, red arrow indicates ovarian surface epithelium and yellow arrow indicates rete ovarii

TA: Tunica Albuginea CL: Corpus Luteum V: Vascular structures, Magnification (A, B: 1000X; C: 200X; D: 400X)

The area where blood vessels and nerves enter the ovary is called hilus. Information on the hilar cells located in the ovarian hilus is limited. Hilar cells are often found in clusters associated with the nerve trunk. Hilar cells are found in the inner cortex and often in the medulla. They have been reported to synthesize and secrete androgens in response to luteinizing hormone (LH) stimulation, although their physiologic role is not well defined. Hyperplasia of these cells has been shown to play a role in virilization in postmenopausal women (Figure 6, 7) [1, 7].

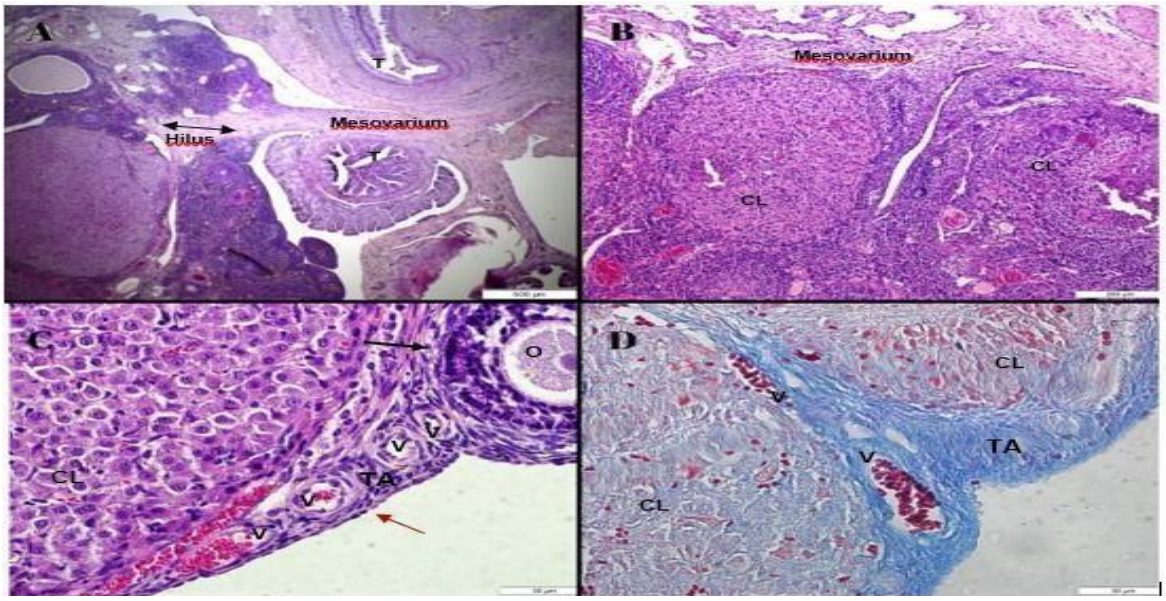

Figure 6. A-B: Adjacency of the tunica albuginea, cortex and mesovarium of the ovary, C: Venule and arteriole structures seen in the tunica albuginea adjacent to the corpus luteum, D: Tunica albuginea adjacent to the corpus luteum (Masson Trichrome staining). Black arrow indicates secondary follicle, red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum O: Oocyte T: Tuba Uterina V: Vascular structures, Magnification (A : 40X; B: 200X; C, D: 400X)

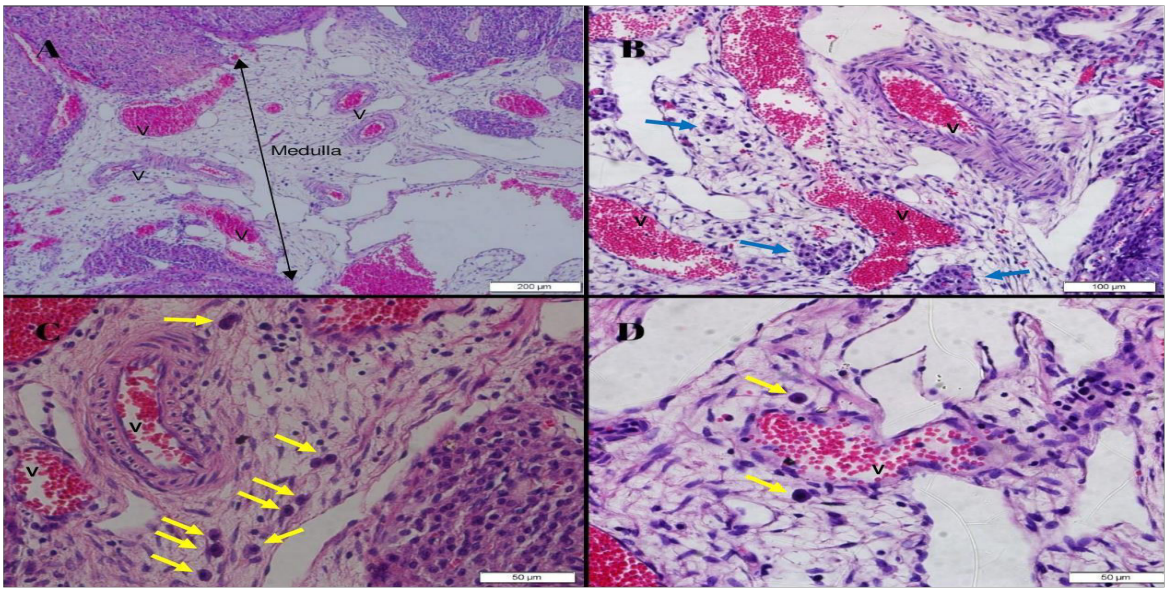

Figure 7. A-B-C-D: The medulla of the ovary is a loose connective tissue layer containing vascular structures, adipocytes, fibroblasts, macrophages and hilar cells. Blue arrow indicates hilar cells and yellow arrow indicates macrophages

V: Vascular structures, Magnification (A: 40X; B: 200X; C, D: 400X)

The ovarian stroma contains ovarian-specific components including immune cells, blood vessels, nerves and lymphatic vessels, as well as the ovarian surface epithelium, tunica albuginea, intraovarian rete ovarii, hilar cells, stem cells and most of the incompletely characterized stromal cells, including fibroblast-like, spindle-shaped and interstitial cells. Studies have demonstrated that cortical stromal cells actively differentiate into theca cells in the presence of granulosa cells [8, 9].

The immune cells that support the physiological processes of the ovary are macrophages, B lymphocytes, T lymphocytes, natural killer cells (NK), dendritic cells, neutrophils, eosinophils, and mast cells [7].

Macrophages are the most abundant immune system cell community in the ovary. The ovary contains different types of macrophages, proinflammatory (M1) and anti-inflammatory (M2). IL4 and IL13, cytokines that induce M2 macrophage differentiation, are increased in the ovarian stroma in association with obesity and ageing. Increased proportions of M2 macrophages, monocyte-derived macrophages and multinucleated macrophages were found in mouse ovarian ageing. There is emerging evidence that macrophages play a role in ovarian dysfunction, particularly in polycystic ovary syndrome (PCOS), premature ovarian failure and endometriosis. Studies have shown infiltration of the ovary by both the yolk sac and fetal liver-derived macrophages during embryonic development. Bone marrow-derived monocytes have been shown to contribute to ovarian macrophages after birth. Consequently, both embryonic-derived and bone marrow-derived marrow-derived macrophages contribute to different ovarian macrophage subpopulations in adults [10, 11]. In the regressed corpus luteum, capillary endothelial cells, which do not express cytokeratin, recruit macrophages to this region (Figure 7, 5).

Embryology of the ovary

In mammals, primordial germ cells (PGC) were first identified in the posterior intestinal (hindgut) epithelium of the embryo and were assumed to originate from the yolk sac wall. The yolk sac originates from the extraembryonic endoderm differentiated from the hypoblast, whereas the hindgut develops from the embryonic endoderm differentiated from the epiblast. Therefore, PGCs were thought to originate from the hypoblast, but later studies showed that PGCs originate from the epiblast. In humans, PGCs are observed in the hindgut epithelium of the 3-week-old embryo. At 4 weeks, PGCs reach the dorsal mesentery and begin their migration. By 6 weeks of age, almost all PGCs have reached the gonads.

Unlike mammals, avian PGCs do not cross the hindgut epithelium to reach the dorsal mesentery but instead are incorporated into the extraembryonic vascular network and pass into the peripheral circulation to reach the dorsal mesentery [12, 13].

Although the reconstitution of adult ovarian blood vessels by angiogenesis is crucial for the regulation of female reproduction, studies on this process are limited. Long-term blockade of angiogenesis with drugs that specifically block the vascular endothelial growth factor (VEGF) signaling pathway has been shown to reduce ovarian follicle depletion and delay ovarian senescence, leading to prolonged reproductive life span in older females [14].

Human gonad development begins in the same way in males and females, with the embryonic urogenital ridge (gonadal ridge) containing the mesonephric duct and mesonephric tubules. The initially undifferentiated human embryonic gonad contains four elements: (a) the coelomic epithelium of the urogenital ridge; (b) the underlying mesenchyme; (c) the mesonephros; and (d) primordial germ cells (PGCs).

Sex differentiation of the undifferentiated human gonad into the ovary or testis is recognized morphologically by the appearance of testis- or ovary-specific features. In the male embryo, testicular cords appear at approximately 7 weeks of gestation. In the female embryo, ovary-specific features appear at 8 weeks of gestation: (a) a cortical region containing ovigerous cords and (b) a medullary region also containing cords. The ovigerous cords are separated from the stroma by a basal lamina [15-17].

In the human ovary at 10 weeks of gestation, PGCs mitotically proliferate to form germ cell nests. Moreover, these germ cell nests are evolutionarily conserved in males and females of insects, frogs, rodents and vertebrates.

Mitotic proliferation results in the formation of interconnected clusters of oogonia. The oogonia then enter meiotic prophase I, during which they are called oocytes, and stop at this stage of meiosis. The germ cell nests then begin to fragment, during which the majority of oocytes are lost through apoptotic cell death, while the remaining oocytes are surrounded by pregranulosa cells, forming primordial follicles. Primordial follicles switch to primary follicles formed by oocytes surrounded by single-layer cuboidal granulosa cells. When multilayered granulosa cells surround an oocyte, secondary follicles emerge. More developed antral and preovulatory follicular stages occur postnatally (Figure 8, 9) [15, 18, 19].

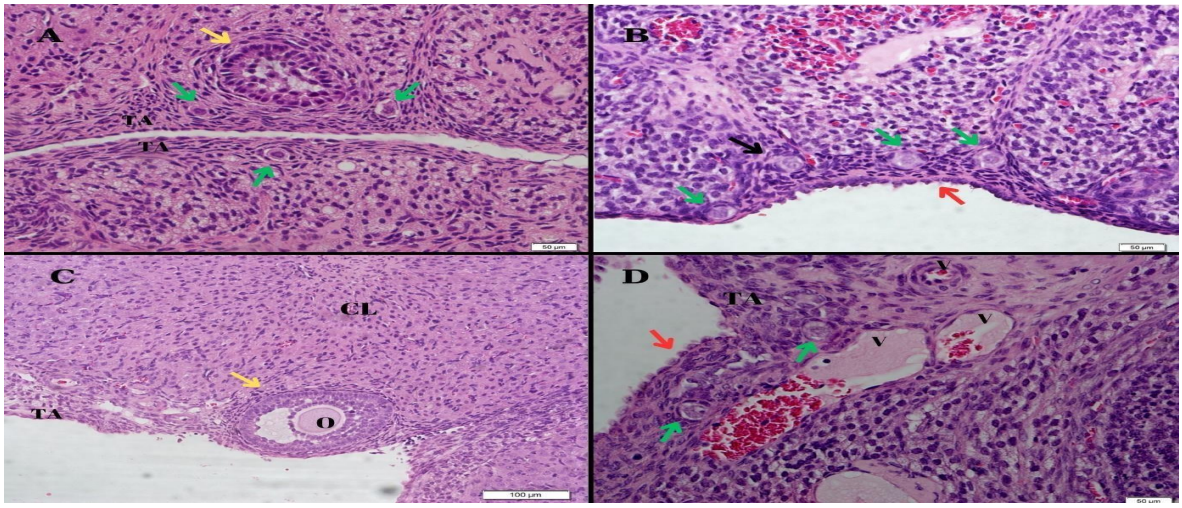

Figure 8. A: Tunica albuginea and primordial follicles within it, B: Primordial and primary follicles within the tunica albuginea, C: Secondary follicle within the corpus luteum, D: Primordial follicles within the tunica albuginea. Green arrow indicates primordial follicle, orange arrow indicates secondary follicle and red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum O: Oocyte V: Vascular structures Magnification (A, B, D: 400X; C: 200X)

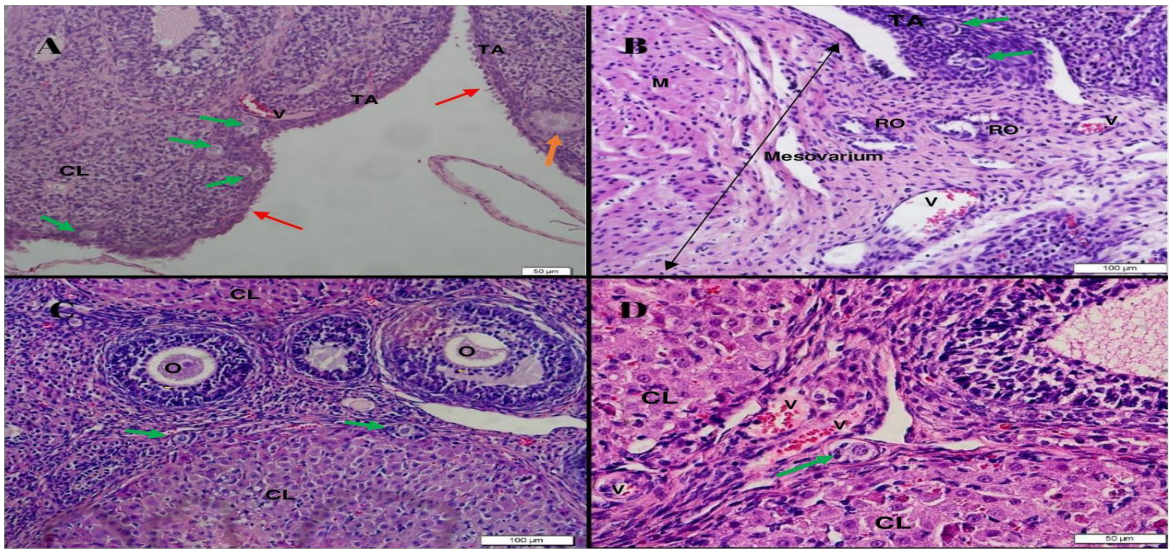

Figure 9. A: Tunica albuginea and primordial follicles within it, B: Mesovarium adjacent to tunica albuginea and primordial follicles within it, C-D: Tunica albuginea adjacent to corpus luteum and primordial follicles within it. Green arrow indicates primordial follicle, orange arrow indicates secondary follicle and red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum O: Oocyte V: Vascular structures, Magnification (A: 40X; B, C: 200X; D: 400X)

During the development of the ovary, ovarian follicles are divided into two different groups. The first group, called medullary follicles, disappears before puberty. The second group, or cortical follicles, remain fixed until puberty. Along with puberty, 15-20 primary follicles are monitored in each menstrual cycle, but as a result, one oocyte matures in the dominant follicle. The primary oocyte completes meiosis I during this maturation, and the haploid is composed of a secondary oocyte and a nonfunctional polar body. The meiosis stops at the metaphase II stage and ends with fertilization by the sperm [20, 21].

Fetal ovarian histology and tunica albuginea

Unlike the adult ovary, the fetal ovary is long and flat in shape. The basic cellular components of the ovary are the surface epithelium, stroma and its derivatives, germ cells and granulosa cells that will surround the germ cells. The surface epithelium consists of a single layer of cuboidal to pseudostratified cells without a distinct basement membrane. In contrast to the adult ovary, the stroma of the fetal ovary is very scanty, because 90% of the cortex is composed of germ cells. At 21 weeks of gestation, the surface epithelium of the fetal ovary appears as a single layer of cuboidal cells without a distinct basement membrane. Throughout the fetal period, the tunica albuginea appears to develop significantly at 28 weeks [22].

The tunica albuginea is formed from the mesonephric stroma after the ovarian surface epithelium is formed. Given the proximity to the surface epithelium, it may be possible that factors from surface epithelial cells influence the transformation of adjacent stromal cells into fibrous tunica [23].

Structural characteristics of the tunica albuginea of the ovary

The tunica albuginea is a fiber-rich, hypocellular, tight, irregular, avascular layer of connective tissue located beneath the surface of the ovary, consisting mainly of structural collagens and other extracellular proteins (collagen type I, collagen type 4, laminin, fibronectin, decorin, versican) [7, 23, 24]. It serves as a protective layer for the ovary. The tunica albuginea undergoes remodelling after ovulation [7].

Structural and physical properties of testicular tunica albuginea

The testicle is enclosed in a capsule consisting of 3 layers:

1. Tunica vasculosa (innermost)
2. Tunica albuginea (center)
3. Tunica vaginalis (outside)

The tunica vasculosa forms a vascular plexus.

Tunica albuginea forms an abundance of collagen fiber. Tunica vaginalis provides the connection between the peritoneal cavity and the scrotum [25].

Tunica albuginea is located in the central region of the testicular capsule and separates the capsule into the inner and outer regions. This layer of fibroblasts, elastin fibers, collagen fibers (arranged in an organized wavy appearance), ectopic leydig cells, mast cells, blood vessels, nerve endings, etc, it is composed of myofibroblasts and an dense connective tissue composed of smooth muscle cells. The outer zone contains abundant myofibroblasts, while the inner zone is rich in smooth muscle cells. In the transition zone between the two, mast cells, ectopic leydig cells, myelin-free nerve fibers and blood vessels are present. Previous findings that tunica albuginea exerts contractile properties raise the possibility that it may have physiological functions;

1. transport of sperm produced in the testicle to the epididymis
2. maintenance of interstitial pressure within the testis
3. control of blood flow through the testis

The human testicle finds a large amount of various contraction elements in the tunica albuginea. Complex and regionalized contraction regulated by cyclic guanosine monophosphate (cGMP)-mediated processes has important physiological roles, such as the continuous support of sperm transport within the rete testis [26, 27].

Structural differences of ovarium and testis tunica albuginea

The ovarian tunica albuginea is not as thick as the testicular tunica albuginea [23]. The ovary tunica albuginea shows a variable thickness ranging from almost undetectable to over 50 μm [28]. The thickness of testicular tunica albuginea varies with age, with younger males increasing from 400-450 μm to over 900 μm in older men [29].

There is a correlation between the density and thickness of the ovarian tunica albuginea and the age of the patient. It is observed that the area forming the ovarian tunica albuginea in elderly individuals is expanding and becoming more compact. As a result, with the formation of fibrosis areas under the tunica albuginea and the increase in the total volume of dense connective tissue under the surface of the ovary, the thickness of this area can be traced to increased [30].

The tunica albuginea of the ovaries contains more collagen, abnormal changes have been observed in tunica albuginea, such as polycystic ovary syndrome (PCOS), in which it exhibits a thicker property and also increases the thickness of cortical and subcortical stroma, which may adversely affect folliculogenesis and ovulation [23].

In polycystic ovary disease, bilateral ovaries expand due to a large number of follicle cysts. Since there is no ovulation, the surface of the ovary is smooth and white in color. Beneath the thickened tunica albuginea are numerous cysts and atrophic secondary follicles [31]. Although hormonal therapy is often administered, third-line therapy may be surgical in patients with resistance to pharmacological treatments [32]. Thus, ovulation is ensured without the physical barrier created by the pre-existing thickened tunica albuginea of the ovary [31].

Changes to the tunica albuginea during ovulation

Tunica albuginea is rich in collagen fibers and undergoes post-ovulation reconstruction. As the ovarian follicles examined by electron microscopy reach the preovulatory stage, a decrease in the presence of collagen fiber bundles in the apex of the follicles (the ruptured part in ovulation) has been observed. This deterioration is parallel to the increase in apical fibroblasts with rich cytoplasm and lysosome-like granules, which are estimated to contain collagenases for the breakdown of tunica albuginea [7].

A microscopic study with electrons suggests that in human follicles some of the collagen fibre bundles in the tunica albuginea and theca externa disappear before ovulation, and biochemical methods show that collagen fibre synthesis is present at the follicle apex. Tunica albuginea in the follicular apex region has been shown to contain less collagen than tunica albuginea in areas without follicles [33].

After ovulation in man, changes in the region-dependent collagen fiber content are observed in the tunica albuginea. The tunica albuginea in the apical region of the follicles contains less collagen fiber than in follicle-free areas, and the percentage of collagen fiber dissolved in acetic acid with pepsin is lower. These results show that collagen fibers are broken down in the apical tunica albuginea. Significantly low collagen fiber content within the tunica albuginea follicular wall resulting from local high collagenase activity has been claimed to be able to facilitate more stigma formation by reducing strain in the albuginea in the tunica [34].

Studies in rabbits have shown that the collagen layers in the tunica albuginea, which cover the theca layer inside, around and over, form the tensile strength of the follicle wall. A study in the Rat ovary pointed out that tunica albuginea contains a rich amount of interstitial collagen types I and III. Studies in rats and cows have shown that type IV collagen is present in both of the basal lamina that separates the cell layers from theca interna and granulosa, and the basal lamina between the surface epithelium of the ovary and tunica albuginea. These results show a difference in the composition between the surface epithelium basal lamina of the ovary and the theca /granulosa basal lamina [33].

Matrix metalloproteinase-1 (MMP-1) and Matrix metalloproteinase-3 (MMP-3) are proteolytic enzymes that play a role in reshaping the extracellular matrix of the ovary during the menstrual cycle. Compared to tunica albuginea from follicle-free areas, less MMP-1 is detected in the apical wall of atretic follicles. These data are associated with low MMP-1 concentrations

of atresia in the apical wall of the follicle, indicating that both MMPs play an important role in the final stage of atresia [35].

In ovulatory follicular rupture studies, it is observed that connective tissue degeneration starts at the ovarian surface and progresses towards the follicular wall. The proteolytic enzymes released from the ovary epithelium degenerate the tunica albuginea and the underlying theca layer, weakening the apical follicular wall.

Plasmin (fibrinolysin) is derived from zymogen plasminogen by enzymatic activation. There are two types of plasminogen activators (PA), urokinase (u) and tissue (t) types, in vertebrates. The increase in the biosynthesis of plasmin in the apical and adjacent tunica albuginea of preovulatory follicles in sheep has been attributed to the secretion of uPA by the surface epithelial cells of the ovary. It has been found that theca and granulosa cells secrete uPA and tPA in rodents.

With the secretion of plasminogen activator (PA), a localized increase is observed in the level of plasmin. As a result, collagenases are activated and the tumour necrosis factor (TNF) in theca cells is released. TNF causes collagen degradation by inducing the gene expression of MMP-1 and MMP-2 [36].

The contractions of the tunica albuginea in the ovary facilitate oocyte and fluid movements, ovulation in the ovary [37, 38]. The study in cattle showed increased gene expression of G-protein-coupled receptor (GPCR) and cyclic adenosine monophosphate (cAMP) in tunica albuginea compared to stroma. The bovine ovary suggests that the tunica albuginea may be contractile, as in the fish-aquarium tunica and the mammalian testicle tunica albuginea. In addition, granulosa cells of preovulatory follicles express endothelin 2, causing ovarian contraction [38].

The relationship of the ovarium surface epithelium with tunika albuginea

The surface epithelium of the ovary is connected to the basal membrane, which continues with the underlying tunica albuginea with collagen fibrils [38]. The mesenchymal cells in the human ovary tunica albuginea express cytokeratin (CK) and can differentiate into surface epithelial cells through the mesenchymal-epithelial transition [39, 40]. Bipotent mesenchymal cells in the tunica albuginea layer can form granulosa cells and germ cells in the ovary cortex. The mesenchymal cells in the adult ovary tunica albuginea can differentiate into the surface epithelium of the ovary by mesenchymal-epithelial passage. These cells may have the ability to become granulosa or germ cells depending on the effects of the stromal cells (microenvironment). The result is that tunica albuginea fibroblasts, which exhibit a transient CK immune expression, have been shown to differentiate into surface epithelial cells through the mesenchymal-epithelial transition process. This process can result in the formation of surface epithelial channels and cords in the ovary cortex, or by differentiation into the surface epithelial cells of the ovary, depending on the factors not yet explained [28].

The fibroblasts in tunica albuginea support the development of follicles with the paracrine factors they secrete and function as a nourishing cell layer. The thickening or degeneration of tunica albuginea in diseases such as chemotherapy or polycystic ovary syndrome can affect the development of follicles. Chemotherapy agents may also cause premature ovarian failure, in which loss of primordial follicles is seen [41, 42].

In ovulatory follicular rupture studies, it is observed that connective tissue degeneration starts at the ovarian surface and progresses towards the follicular wall. (Figure 10). A week after the transplantation of mesenchymal stem cells marked green fluorescence protein (GFP) into the femoral bones of rats as intramedullary, the study of these cells by fluorescence microscopy of ovarian tissue in the tunica albuginea, they have been shown to disperse into the follicles and more often the corpus luteum (Figure 11). In one study, cells isolated from the ovarian and testicular tissues by explant culture technique showed fibroblast-like morphology (Figure 12).

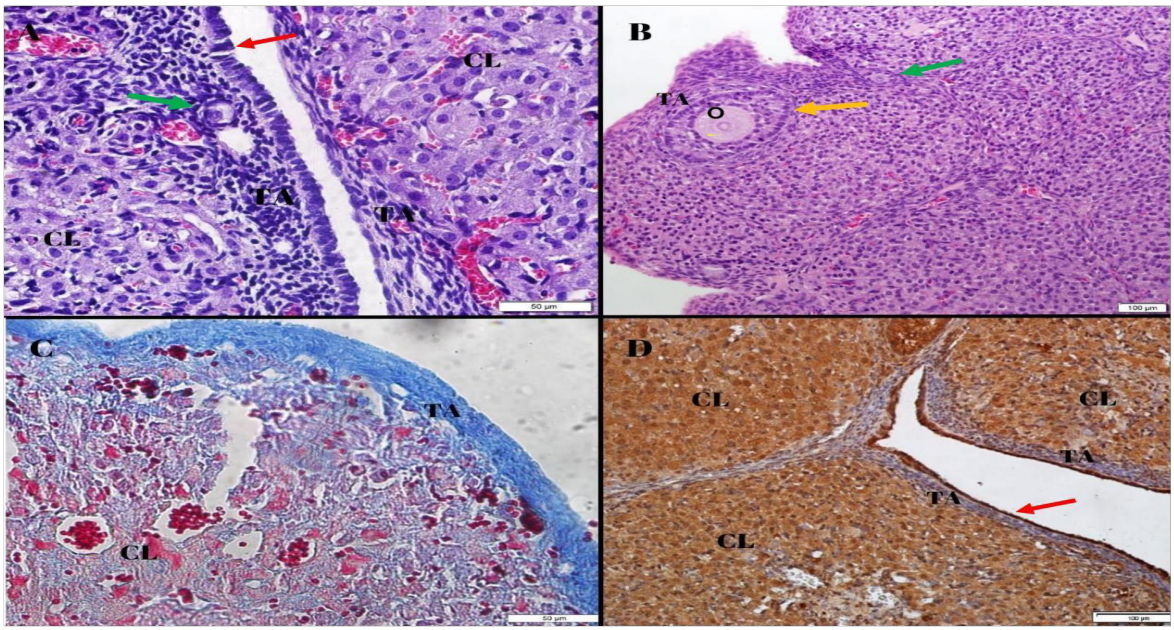

Figure 10. A: Tunica albuginea adjacent to the corpus luteum and primordial follicles within it, B: Secondary follicle within the corpus luteum and primordial follicle within the tunica albuginea, C: Tunica albuginea adjacent to the corpus luteum (Masson Trichrome staining), In rats treated with, D: Cyclophosphamide chemotherapy, the tunica albuginea of the corpus luteum reacted less with caspas-3 reactivity, an apoptotic marker, than granulosa lutein and theca lutein cells. Green arrow indicates primordial follicle, orange arrow indicates secondary follicle, red arrow indicates ovarian surface epithelium

TA: Tunica Albuginea CL: Corpus Luteum O: Oocyte, Magnification (A, C: 400X B, D: 200X)

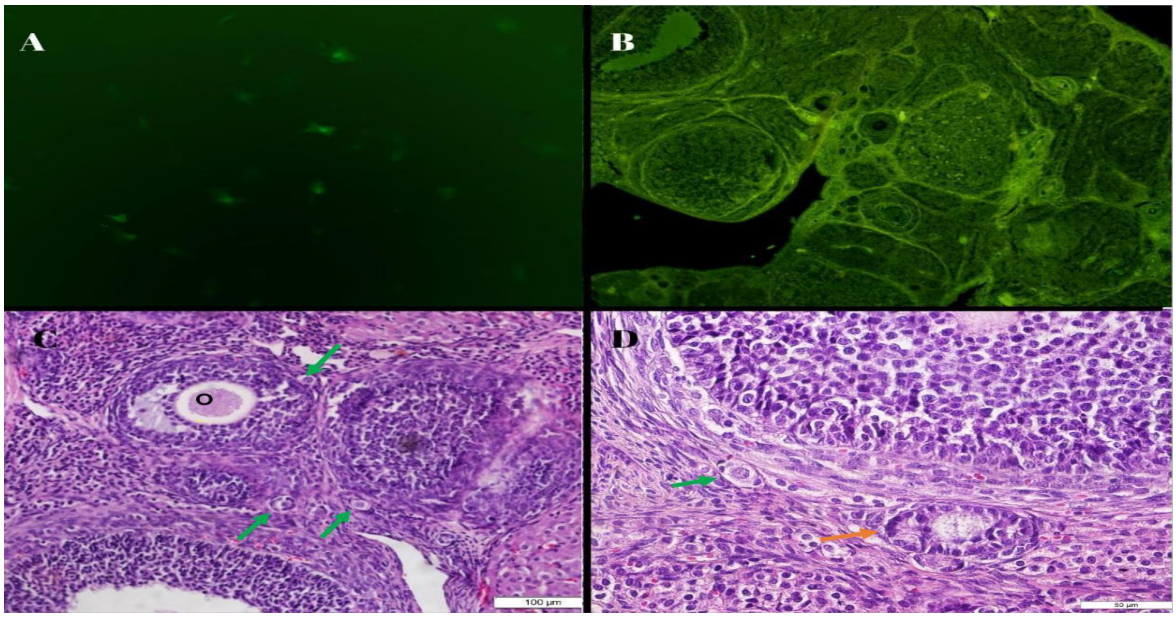

Figure 11. A-B: Fluorescence microscope view of the distribution of GFP-linked mesenchymal stem cells intramedullaryly injected into the femur bone into the tunica albuginea, cortex and medulla of the ovary, C-D: Primordial follicles seen between fibroblasts in the theca interna and theca externa layer of the secondary and graaf follicle. Green arrow indicates primordial follicle and orange arrow indicates secondary follicle

O: Oocyte, Magnification (C: 200X D: 400X)

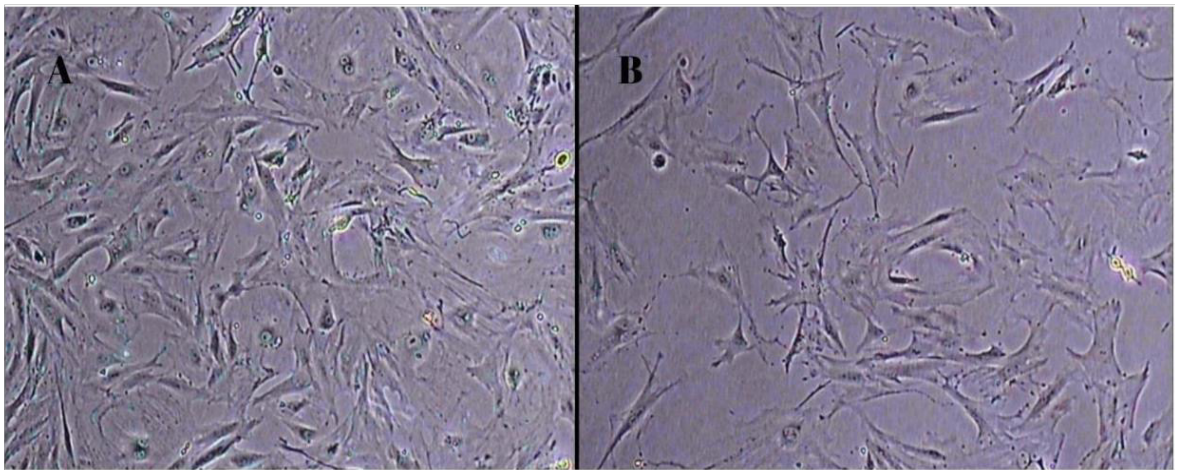

Figure 12. A-B: Fibroblast-like cells isolated from explant culture of ovarian and testicular tissues (Magnification: 100X)

Another study showed that tunica albuginea, adjacent to the corpus luteum, which occurs from the Graaf follicle after ovulation, has been reconstructed. Concentric collagen fibers, which form the tunica albuginea adjacent to the ever-expanding corpus luteum, were previously reported to have a 1-2-row sequence, followed by a regression of 5-6 rows and thickened. It has also been suggested that corpus luteum structures that reach quite large volumes may cause them to alter localizations of the preantral follicles in particular and may direct them to atresia by affecting their development [43].

As a result, the tunica albuginea may be a suitable model for the study of the migration of pluripotent and multipotent stem cells from the bone marrow to the ovary via vascular structures. After the formation of the corpus luteum, numerous capillaries and venules form in the adjacent tunica albuginea. During the reproductive period, proliferating fibroblasts differentiate from mesenchymal cells in the stroma. In addition, mesenchymal cells can come from the bone marrow through vascular structures and differentiate into fibroblasts in these regions. Fibroblasts in the tunica albuginea also undergo continuous renewal due to estrous cycles. Studies have shown that a large number of Very small embryonic like stem cells (VSELs) are present in ovarian tissue. The potential of these cells to migrate from the bone marrow through vascular structures to form follicles in the tunica albuginea could be investigated. Fibroblasts in various layers of the corpus luteum, antral follicles and atretic follicles create a niche for the development of primordial follicles in different locations of the ovary. Thus, in addition to the fibroblasts in the tunica albuginea, new septa-like regions are formed in which preantral follicles can develop. Further studies are needed to investigate the contribution of the tunica albuginea to the development of follicles and the dynamic structure of the ovary, the structure and functions of which have not been sufficiently investigated so far.

Funding: None.

Authors contributions: M.S.U. have constructed the main idea and hypothesis of the study. M.S.U., H.Y., C.K. and A.C.T. developed the theory and edited/evaluation the introduction and results section. Written by H.Y., M.S.U., C.K. and A.C.T. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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

References

1. Özgünen T, Polat S, Balcıoğlu E. Güncel Fizyoloji Histoloji Embriyoloji Çalışmaları,Ovaryum Histolojisi, Ankara: Akademisyen Kitabevi, 2019;113-124. Available at: https://books.akademisyen.net/index.php/akya/catalog/view/1223/1226/26056. Accessed January 22, 2024
2. Menter DG, Dubois RN. Prostaglandins in cancer cell adhesion, migration, and invasion. *Int J Cell Biol*. 2012;2012:723419. doi:10.1155/2012/723419
3. Ünal MS, Seçme M. Ovaryum yüzey epiteli primordial folikül ve primer folikül öncüsü yapılara farklılaşıyor mu? *Cukurova Med J.* 2022;47:1256-1262. doi:10.17826/cumj.1134852

4. Hartanti MD, Hummitzsch K, Bonner WM, Bastian NA, Irving-Rodgers HF, Rodgers RJ. Formation of the Bovine Ovarian Surface Epithelium during Fetal Development. *J Histochem Cytochem*. 2020;68(2):113-126. doi:10.1369/0022155419896797
5. Rajah R, Glaser EM, Hirshfield AN. The changing architecture of the neonatal rat ovary during histogenesis. *Dev Dyn*. 1992;194(3):177-192. doi:10.1002/aja.1001940303
6. Philippart C, Masciangelo R, Camboni A, Donnez J, Dolmans MM. Basal lamina characterization in frozen-thawed and long-term grafted human prepubertal ovarian tissue. *Reprod Biomed Online*. 2021;42(5):859-869. doi:10.1016/j.rbmo.2021.02.012
7. Kinnear HM, Tomaszewski CE, Chang AL, et al. The ovarian stroma as a new frontier. *Reproduction*. 2020;160(3):R25-R39. doi:10.1530/REP-19-0501
8. Orisaka M, Tajima K, Mizutani T, et al. Granulosa cells promote differentiation of cortical stromal cells into theca cells in the bovine ovary. *Biol Reprod*. 2006;75(5):734-740. doi:10.1095/biolreprod.105.050344
9. Orisaka M, Tajima K, Tsang BK, Kotsuji F. Oocyte-granulosa-theca cell interactions during preantral follicular development. *J Ovarian Res*. 2009;2(1):9. Published 2009 Jul 9. doi:10.1186/1757-2215-2-9
10. Jokela H, Lokka E, Kiviranta M, et al. Fetal-derived macrophages persist and sequentially maturate in ovaries after birth in mice. *Eur J Immunol*. 2020;50(10):1500-1514. doi:10.1002/eji.202048531
11. Umehara T, Winstanley YE, Andreas E, et al. Female reproductive life span is extended by targeted removal of fibrotic collagen from the mouse ovary. *Sci Adv*. 2022;8(24):eabn4564. doi:10.1126/sciadv.abn4564
12. Hen G, Sela-Donenfeld D. "A narrow bridge home": The dorsal mesentery in primordial germ cell migration. *Semin Cell Dev Biol*. 2019;92:97-104. doi:10.1016/j.semcdb.2018.08.010
13. Himelreich Perić M, Takahashi M, Ježek D, Cunha GR. Early development of the human embryonic testis. *Differentiation*. 2023;129:4-16. doi:10.1016/j.diff.2022.07.001
14. Xu X, Mu L, Li L, et al. Imaging and tracing the pattern of adult ovarian angiogenesis implies a strategy against female reproductive aging. *Sci Adv*. 2022;8(2):eabi8683. doi:10.1126/sciadv.abi8683
15. Overland MR, Li Y, Derpinghaus A, et al. Development of the human ovary: Fetal through pubertal ovarian morphology, folliculogenesis and expression of cellular differentiation markers. *Differentiation*. 2023;129:37-59. doi:10.1016/j.diff.2022.10.005
16. Hummitzsch K, Irving Rodgers HF, Schwartz J, Rodgers RJ. Development of the Mammalian Ovary and Follicles. *The Ovary.* 2019:71-82. doi:10.1016/B978-0-12-813209-8.00004-2
17. Sawyer HR, Smith P, Heath DA, Juengel JL, Wakefield SJ, McNatty KP. Formation of ovarian follicles during fetal development in sheep. *Biol Reprod*. 2002;66(4):1134-1150. doi:10.1095/biolreprod66.4.1134
18. Islam MR, Ichii O, Nakamura T, et al. Developmental Changes of the Ovary in Neonatal Cotton Rat (*Sigmodon hispidu*s). *Front Physiol*. 2021;11:601927. Published 2021 Jan 13. doi:10.3389/fphys.2020.601927
19. Brieño Enríquez MA, Faykoo Martinez M, Goben M, et al. Postnatal oogenesis leads to an exceptionally large ovarian reserve in naked mole-rats. *Nat Commun*. 2023;14(1):670. Published 2023 Feb 21. doi:10.1038/s41467-023-36284-8
20. McKey J, Anbarci DN, Bunce C, Ontiveros AE, Behringer RR, Capel B. Integration of mouse ovary morphogenesis with developmental dynamics of the oviduct, ovarian ligaments, and rete ovarii. *Elife*. 2022;11:e81088. Published 2022 Sep 27. doi:10.7554/eLife.81088
21. Virant Klun I. Postnatal oogenesis in humans: a review of recent findings. *Stem Cells Cloning*. 2015;8:49-60. Published 2015 Mar 20. doi:10.2147/SCCAA.S32650
22. Ernst LM, Ruchelli ED, Carreon CK, Huff DS. Color Atlas of human fetal and neonatal histology. Switzerland: 2nd Ed, Springer Nature, 2019; 175-176. Available at https://books.google.com.tr/books?id=3WOsDwAAQBAJ&pg=PA105&hl=tr&source=g bs_toc_r&cad=2#v=onepage&q&f=false. Accessed February 2, 2024
23. Chandra PK, Atala AA. Encyclopedia of Tissue Engineering and Regenerative Medicine. *Genitourinary System* 2019;361-379. doi:10.1016/B978-0-12-801238-3.65846-4
24. Heeren AM, van Iperen L, Klootwijk DB, et al. Development of the follicular basement membrane during human gametogenesis and early folliculogenesis. *BMC Dev Biol*. 2015;15:4. Published 2015 Jan 21. doi:10.1186/s12861-015-0054-0
25. Tullington JE, Blecker N. Pelvic Trauma. StatPearls Publishing, 2023.
26. Middendorff R, Müller D, Mewe M, Mukhopadhyay AK, Holstein AF, Davidoff MS. The tunica albuginea of the human testis is characterized by complex contraction and relaxation activities regulated by cyclic GMP. *J Clin Endocrinol Metab*. 2002;87(7):3486-3499. doi:10.1210/jcem.87.7.8696
27. Raad G, Massaad V, Serdarogullari M, et al. Functional histology of human scrotal wall layers and their overlooked relation with infertility: a narrative review. *Int J Impot Res*. 2023;35(5):428-438. doi:10.1038/s41443-022-00573-5
28. Bukovsky A, Caudle MR, Svetlikova M, Upadhyaya NB. Origin of germ cells and formation of new primary follicles in adult human ovaries. *Reprod Biol Endocrinol*. 2004;2:20. Published 2004 Apr 28. doi:10.1186/1477-7827-2-20

29. Nistal M, Paniagua R. Urologic Surgical Pathology. Saunders, 2nd ed, 2008; 614-755. doi:10.1016/B978-0-323-01970-5.50014-2 Available at: https://www.sciencedirect.com/science/article/pii/B9780323019705500142. Accessed March 11, 2024
30. Ferré Pujol P, Otsuki J, Funahashi H, Nakatsuka M. The Thickness and Density of the Ovarian Tunica Albuginea Increases with Age in Transgender Patients. *Reprod Sci*. 2021;28(5):1339-1346. doi:10.1007/s43032-020-00390-5
31. Ross MH, Pawlina W, Histoloji Konu Anlatımı ve Atlas 6.ed, Ankara: Lippincott Williams & Wilkins, 2013;837-839.
32. Della Corte L, Boccia D, Palumbo M, et al. Is There Still a Place for Surgery in Patients with PCOS? A Review. *Life (Basel)*. 2023;13(6):1270. Published 2023 May 28. doi:10.3390/life13061270
33. Lind AK, Weijdegård B, Dahm Kähler P, Mölne J, Sundfeldt K, Brännström M. Collagens in the human ovary and their changes in the perifollicular stroma during ovulation. *Acta Obstet Gynecol Scand*. 2006;85(12):1476-1484. doi:10.1080/00016340601033741
34. Postawski K, Rechberger T, Skorupski P, Jakowicki JA. Extracellular matrix remodelling within the normal human ovarian capsule. *Eur J Obstet Gynecol Reprod Biol*. 1996;67(2):173-177. doi:10.1016/0301-2115(96)02468-2
35. Bogusiewicz M, Rechberger T, Jakimiuk AJ, Skorupski P, Jakowicki JA, Postawski K. Evaluation of matrix metalloproteinases-1 and -3 concentrations in the tunica albuginea, the apical wall of atretic follicles and the corpus luteum of normal human ovaries. *Gynecol Endocrinol*. 2000;14(1):25-31. doi:10.3109/09513590009167656
36. Murdoch WJ, McDonnel AC. Roles of the ovarian surface epithelium in ovulation and carcinogenesis. *Reproduction*. 2002;123(6):743-750. doi:10.1530/rep.0.1230743
37. Piccinno M, Zupa R, Corriero A, et al. In vitro effect of isotocin on ovarian tunica albuginea contractility of gilthead seabream (Sparus aurata L.) in different reproductive conditions. *Fish Physiol Biochem*. 2014;40(4):1191-1199. doi:10.1007/s10695-014-9915-x
38. Hummitzsch K, Hatzirodos N, Macpherson AM, Schwartz J, Rodgers RJ, Irving-Rodgers HF. Transcriptome analyses of ovarian stroma: tunica albuginea, interstitium and theca interna. *Reproduction*. 2019;157(6):545-565. doi:10.1530/REP-18-0323
39. Bukovsky A, Caudle MR, Svetlikova M, Wimalasena J, Ayala ME, Dominguez R. Oogenesis in adult mammals, including humans: a review. *Endocrine*. 2005;26(3):301-316. doi:10.1385/ENDO:26:3:301
40. Bukovsky A, Svetlikova M, Caudle MR. Oogenesis in cultures derived from adult human ovaries. *Reprod Biol Endocrinol*. 2005;3:17. Published 2005 May 5. doi:10.1186/1477-7827-3-17
41. Çil N, Mete GA. The effect of adipose-derived mesenchymal stem cell treatment on mTOR and p-mTOR expression in ovarian damage due to cyclophosphomide. *Reprod Toxicol*. 2021;103:71-78. doi:10.1016/j.reprotox.2021.06.003
42. Kabasakal G, Tural E, Ünal MS. Deneysel overyan yetmezliklerde mezenkimal kök hücrelerin ovaryum dokusuna etkisi. KTD. Nisan 2023;24(2):249-253. doi:10.18229/kocatepetip.849512
43. Ünal MS, Tan S, Seçme M. Changes in structure during the corpus luteum's formation. Pam Med J. April 2024;17(2):285-301. doi:10.31362/patd.1383988